finalreport

Project code: AHW.057

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Date published: June 2005

ISBN: 1 74036 797 9

PUBLISHED BY
Meat & Livestock Australia
Locked Bag 991
NORTH SYDNEY NSW 2059

Calf Scours in Southern

Beef Enterprises Phase 2

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

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Abstract

The first phase of this study determined that calf scours is a significant and time-consuming understand disease problem for many beef enterprises, and that producers and veterinarians poorly the predisposing factors, causative agents, and appropriate management of calf scours in a cow calf enterprises. The aim of the current study was to put in place pathways that ensure consistent and scientific advice is provided to beef farmers, by all levels of extension, on the prevention, investigation, treatment and management of calf scours. The emphasis of the project was on calves aged 0 to 6 weeks in pasture based suckler beef enterprises. We also targeted standardisation of appropriate and affordable diagnostic investigation protocols on farms and laboratory testing in veterinary diagnostic laboratories. This study includes a comprehensive literature review that outlines the latest research and opinions on all aspects of scours in neonatal beef calves and a series of best practice information modules targeted at veterinarians and farmers. It also details options for extension of this information and identifies areas for further research and product development to facilitate the prevention of calf scours in southern Australian suckler enterprises. Research is urgently required to elucidate the epidemiology of calf scours in these enterprises.

Executive summary

The aims of this project were a direct result of the initial “Calf Scours in Southern Australia” project AHW.026 which demonstrated calf scours is a significant and time-consuming disease problem in southern beef production. Producer and veterinarian surveys conducted in that study demonstrated that producers and veterinarians poorly understand the predisposing factors, causes and management of calf scours in cow calf enterprises. A whole of industry approach was recommended to ensure clear and consistent advice, together with structured systems to minimise the impact of this problem.

The aim of the current study was to put in place pathways that will ensure consistent and scientific advice is provided to beef farmers, by all levels of extension, on the prevention, investigation and management of calf scours. The emphasis of the project was on calves aged 0 to 6 weeks in pasture based cow-calf enterprises. We also targeted standardisation of appropriate and affordable diagnostic investigation protocols on farms and laboratory testing in veterinary diagnostic laboratories.

A comprehensive literature review was carried out of refereed published research relating to calf scours in beef and dairy enterprises. Whilst significant research has been done on neonatal calf diarrhoea there are still many areas that are poorly understood. Moreover there is virtually no published Australian research pertaining to the prevention and control of calf scours in suckler beef herds.

There is a lot of research on the contribution of individual aetiological agents, but there are few studies that consider all aspects of this complex disease and attempt to quantify the significance of the different predisposing factors. Molecular biology is resulting in significant advances in laboratory techniques for the diagnosis of pathogens, however there are some enteropathogenic viruses for which a commercially affordable diagnostic test has yet to be developed and consequently their contribution to the aetiology of neonatal calf diarrhoea is unknown. Although there is a reasonable understanding of the epidemiology of the respective pathogens there is little knowledge of the significance of environmental reservoirs of pathogens relative to the role of subclinically infected animals within a herd.

An active area of research is the development of oral electrolyte solutions, and best practice methods of intravenous administration of fluids, and this review will result in improved recommendations for treatment of scouring calves. There is a lot of research into factors that influence colostrum quality, however the trials are often small and the information contradictory, and it is not known how significant FPT is in outbreaks of neonatal calf diarrhoea in beef herds in Southern Australia.

There is a paucity of information on the prevention of calf scours in pasture based herds. Where research has been carried out it has often been in much colder climates such as Canada. Recommendations can be made using adaptations of these techniques and knowledge of the epidemiology of specific pathogens, however it is difficult to rank the impact of these suggestions.

The knowledge gained from this study was used to compile a set of standard recommendations to prevent calf scours in beef cattle. The veterinary documents address the prevention, investigation, treatment and control of calf scours. The farmer documents address the aetiology and prevention of scours, and a best practice approach to calf scour outbreaks. Extension of this information will be addressed by the next phase of this project. These documents were subject to

peer review by a panel of veterinarians and farmers respectively. The response was in general favourable, and changes have been made to the documents as directed by the reviewers.

The initial phase of the project determined that diagnosis of calf scours is frustrating, because it is relatively expensive, results are not guaranteed, and when pathogens are isolated the appropriate advice and interpretation is sometimes inconsistent. Moreover a high proportion of investigations were not diagnostic. This could be attributed to intermittent shedding of a pathogen, inappropriate sampling, sample processing or diagnostic testing. Many laboratory submissions only included testing for a subset of the major pathogens. There was also a variation in the availabilities of diagnostic tests between laboratories and a reasonable variation in the methodologies used.

Consequently we facilitated inter-laboratory discussion aimed at improving and standardising protocols for the diagnosis of the aetiological agents and reporting of results. The response from the laboratories was favourable and a document with recommendations from the meeting was circulated. There is a requirement for the quality assurance of any standardised diagnostic protocol to be audited by NATA or SCAHLS. It is in the interest of the industry as an end user to encourage the need to invest in this process, but it should be recognised that currently there are no financial incentives for the laboratories to adopt such a process.

There also needs to be continued encouragement of the development and validation of emerging laboratory diagnostic techniques, both to enable better diagnosis of the known aetiological agents and to determine the role of specific pathogens shown to contribute to the aetiology of calf scours overseas. One potential advance in the diagnosis of calf scours is the recent availability of calf side faecal dipsticks. This would allow for rapid diagnosis of the common aetiological agents. However, independent testing of the tests has not been conducted so the sensitivity and specificity in Australian conditions requires validation.

The industry needs to consider the importance of retrospective laboratory data to establish the prevalence and economic impact of a disease, as well as for monitoring any change in the prevalence of a specific presentation that may facilitate the identification of unknown disease. If laboratory data is determined to be an important factor in ongoing research and the biosecurity of the beef industry then negotiations need to be carried out with veterinary laboratories to support databases and fund the required data entry.

The literature review allowed us to identify and prioritise areas where further research is required. Notably research is needed to address the lack of epidemiological information in pasture based systems in Australia. There are also specific diagnostic areas that need addressing that relate to the role of specific pathogens in the aetiology of calf scours and the development and standardisation of impending diagnostic techniques.

Whilst a series of recommendations have been produced as part of this project, they are based on research conducted overseas where cattle are raised under different husbandry systems, therefore it would be appropriate to conduct applied studies in Australia to validate the recommendations. The first phase of this project determined a need for case control studies to determine key management strategies for the Australian beef industry, and this recommendation is supported by the current study.

The literature review, farmer, and veterinarian documents produced provide the industry with best management practices for the prevention, control, diagnosis and treatment of calf scours. Veterinarians benefit from this project as they will have easy access to clear and current protocols to use with their clients experiencing a scour problem, hence increasing their service to

their clients. However the main beneficiaries of this project will be beef producers across southern Australia who are experiencing difficulties with scouring calves.

The workshop with laboratory personnel has hopefully already resulted in an improvement in diagnostics where required. Before farmers and veterinarians can benefit from this project it is necessary to present the information in a style that will provide maximum impact. It is planned to present the veterinary information at the Australian Cattle Veterinarian conferences during 2005. While it is possible that seminars for producers could be held in targeted locations to coincide with the upcoming autumn calving period, increased impact is likely to be achieved by designing learning modules that can be incorporated into existing extension programs, such as Beefcheque and the Herd Health and Welfare module of “More Beef from Pastures”.

The major achievement of this project has been the production of clear and consistent information on the prevention, diagnosis, treatment and control of calf scours for veterinarians and producers. The presentation and dissemination of this information is the obvious next step. However it is unlikely that this in itself will be the whole solution to the problem that the industry faces. Increased knowledge of the epidemiology of calf scours in southern Australia is essential, as is continued encouragement of the development and validation of emerging laboratory diagnostic techniques.

List of Acronyms and abbreviations

ANZ	Australia and New Zealand
BCV	Bovine coronavirus
BVD	Bovine Viral Diarrhoea virus
EBL	Enzootic bovine leukosis
ELISA	Enzyme-linked immunosorbent assay
EM	Electron Microscopy
ETEC	Enterotoxigenic *E. coli*
FPT	Failure of passive transfer
IV	Intravenous
LPS	Lipopolysaccharide
MIC	Minimum inhibitory concentration
NATA	National Association of Testing Authorities
NCD	Neonatal calf diarrhoea
PCR	Polymerase chain reaction
P.O.	Per Os
RNA	Ribonucleic Acid
RT-PCR	Reverse transcriptase-polymerase chain reaction
SCAHLS	Sub-Committee on Animal Health Laboratory Standards
SDP	Standard Diagnostic Protocol

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1 Background to project and the industry context

This project was a direct result of the recommendations from the initial "Calf Scours in Southern Australia" project AHW.026. That study demonstrated that neonatal calf scours is a significant and time-consuming disease problem on many of the properties surveyed and that on those properties the mean cost of calf scours to the enterprise was $18.70 per breeding cow. Significantly the majority of producers that responded to the surveys were from suckler beef enterprises as opposed to calf rearing/vealer enterprises. It determined that producers and veterinarians poorly understand the predisposing factors, causes and management of calf scours in cow calf enterprises. A whole of industry approach was recommended to ensure clear and consistent advice, together with structured systems to minimise the impact of this problem.

The specific recommendations from phase 1 were as follows:

1) Establish key management strategies at the herd level to minimise calf scours

2) Develop a scoring system that will improve definition of the problem on farm

3) Recommend appropriate, affordable and reliable standard diagnostic procedures and pathways on farm and at the laboratory, including establishment of the role of post mortems

4) Recommend standards for the interpretation and reporting of laboratory results

5) Establish coordination between industry and state agriculture departments to specifically investigate and establish diagnoses for outbreaks where there is a high mortality rate (suggested level greater than 5%)

6) Investigate the role of enteropathogenic attaching and effacing *E. coli* in calf scours in Australia

7) Establish appropriate treatment protocols

8) Extension of strategies.

The final recommendation was an industry recommendation on improving disease surveillance. It was noted that during collation of information from veterinary laboratories many submissions could not be included because not enough information was available. Effective and efficient disease surveillance should be a major priority for the Australian animal export industries and this deficiency should be addressed at the veterinary and laboratory level.

The bulk of these recommendations were incorporated into the current project.

2 Project objectives

The overall outcome for the current project is to ensure consistent and scientific advice is provided to beef producers, by all levels of extension, on the prevention, investigation and management of calf scours. Presentation and delivery of this information will be carried out in phase 3 of this project. The emphasis of this project will be on calves aged 0 to 6 weeks in pasture based cow-calf enterprises.

The principle objectives for phase 2 were as follows:

1. Determine scientific and repeatable principles for the prevention, investigation and management of calf scours relevant to the southern cow calf industry that can be disseminated to laboratories, veterinarians and producers

2. Describe in outline the way this information should be delivered to key groups

3. Liaise and coordinate with pathologists in all relevant veterinary laboratories and determine an agreed process for laboratory diagnosis of reporting of calf scour results

4. Facilitate a reliable system for surveillance of future results through all southern veterinary laboratories

5. Identify and report to MLA areas where further research is required in the prevention, control and treatment of calf scours in southern Australian cow calf systems.

3 Methodology

A comprehensive literature review was carried out of published refereed research relating to neonatal calf diarrhoea in beef and dairy enterprises. The topics reviewed were:

a) Prevention and control of neonatal calf diarrhoea

The topics covered included transmission and shedding of all relevant pathogens, factors affecting survival of all relevant pathogens, risk factors associated with neonatal calf diarrhoea/calf mortality, colostrum management and supplementation in cow calf operations, management of calves at birth, management of herd in outbreak, management of paddocks – rotation, minimising contamination etc and efficacy of current vaccines available in Australia and overseas.

b) Diagnostic pathways

The topics covered were information to be included in history (mainly derived from section on prevention and control), selection of appropriate calves to test, appropriate tests, appropriate sample type and quantity of calves to sample and the interpretation of laboratory results.

c) Treatment of neonatal calf diarrhoea

The topics covered included appropriate formulations of oral and intravenous electrolytes, electrolyte products available in Australia, the role of antibiotics, appropriate antibiotics available in Australia, and the management of sick calves in suckler beef enterprises.

Abstracts from over 1500 references were studied to compile a comprehensive list of references that required detailed examination. References from this list that contained significant information were included in the literature review.

The literature review was then used to prepare information to use in extension packages for veterinarians and producers. Where proven scientific strategies were not available, solutions were based on sound scientific principles and practical information from veterinarians working in the field. These packages currently contain the information that is necessary to present to these groups. The exact format for extension will be decided in the next phase of this project and the packages need to be appropriately edited and presented to provide maximum impact.

The information that was prepared for veterinarians covers prevention, diagnosis, control and treatment of neonatal calf diarrhoea. The information for producers addresses the aetiology and prevention of neonatal calf diarrhoea, and a best practice approach to calf scours outbreak.

The prepared documents were then submitted to peer review. The content and practicality of application of the veterinary documents was reviewed by 8 veterinarians with extensive experience in southern Australian beef suckler enterprises and/or with scouring calves. These veterinarians were based in New South Wales, South Australia, Victoria and Western Australia. The producer documents were reviewed in a workshop attended by producers from 8 suckler beef enterprises, as well as 5 veterinarians independent from the project authors and representatives of MLA. The content and clarity of the papers as well as the practicality of implementing the recommendations was evaluated. Recommendations for the extension of this information to the industry were then determined.

The information derived from the literature review was also used to prepare a discussion paper for veterinary laboratories on best practice diagnostic technique. This was circulated and then a meeting was convened with a representative from each veterinary laboratory involved in the diagnosis of calf scours. Current best practice methods for the diagnosis of calf scours both on the farm and in the laboratory were discussed and a paper outlining these was circulated to the participating laboratories.

Finally the literature review gave the comprehensive insight into the current understanding of neonatal calf diarrhoea in suckler beef enterprises, and also allowed us to determine where there were gaps in the knowledge. A document was compiled recommending areas of further research and product development required to progress prevention, control and diagnosis of neonatal calf diarrhoea in southern Australian beef enterprises.

4 Results and discussion

4.1 Objective 1: Determining scientific and repeatable principles for the prevention, investigation and management of calf scours

4.1.1 Literature review of the prevention, aetiology, diagnosis, treatment and control of neonatal calf diarrhoea in beef calves

Calf diarrhoea is a multifactorial disease of complex aetiology. Almost inevitably an outbreak of calf diarrhoea reflects an overwhelming challenge with pathogens from a heavily contaminated environment, severe environmental or other conditions that impair immune function in calves, rather than the introduction of a virulent pathogen. The presence of enteric pathogens does not ensure the occurrence of disease. Occasionally outbreaks reflect the introduction of a particularly virulent pathogen into a naïve population. Numerous variables influence pathogen survival and host immunity hence there is no single simple "out of the box" disease prevention remedy. The basic premises of disease prevention and control are to reduce pathogen build up and exposure, optimise host immunity, and maintain biosecurity. As the resources and facilities differ between farms, implementation involves tailoring resources and management to adopt these principles working within the constraints and resources of the specific farming operation.

A detailed review of the relevant literature is provided in Appendix 1. The main objective of the review was to collate and summarise current thinking on the prevention, diagnosis, control and treatment of neonatal calf diarrhoea in cow calf operations. The review found that whilst significant research is occurring in some areas relating to neonatal calf diarrhoea, there are still many areas that are poorly understood, or where there is conflicting information. Moreover there is virtually no published Australian research pertaining to the prevention and control of calf scours in suckler beef herds. Recommendations can be made by adapting these techniques and applying knowledge of the epidemiology of the aetiological agents, however it is difficult to rank the impact of these suggestions.

In summary the review has identified new developments in the diagnosis and treatment of neonatal calf diarrhoea that can be extended into the Australian industry. More significantly, it has identified areas of research from overseas that need clarification here in Australia – for example the significance of milk clotting times could explain the regional and seasonal variation in the incidence of neonatal calf diarrhoea. It has clearly demonstrated that ongoing research is required into the prevention of neonatal calf diarrhoea in pasture based suckler beef enterprises.

4.1.2 Documents for Veterinarians

A series of four documents were prepared to form the basis of material for provision of information on calf scours to veterinarians. These documents are included in Appendix 2, and described briefly below.

1. Prevention of calf scours

The objective of this paper was to provide information on preventive strategies that can be applied to properties with a history of neonatal calf diarrhoea. Many of these are good management practices that will also promote general calf health and reduce the risk of transmitting diseases such as Johne's disease. Although practices can be applied generically without a diagnosis, it is advisable to have a thorough knowledge of the property and the calf scour problems they have experienced.

The paper focuses on achievable management changes for the majority of beef enterprises, but most effective ways of achieving these goals will vary between management systems. Some preventive strategies will require a major change in how cows are managed and may be more time consuming. Producers are most likely to be interested in preventive strategies if they have experienced a calf scour outbreak.

2. Investigation of calf scours

Calf scours needs to be approached as an enterprise level problem as there are risk factors common across all pathogens. It is important to identify environmental, management and nutritional factors that are contributing to the problem, failure to do so will limit the effectiveness of disease prevention efforts. Pinpointing the aetiological agent(s) may be more difficult, however submission of a moderate number of samples for an appropriate diagnostic protocol should allow determination of the more common aetiological agents on a property. This knowledge is important to establish the appropriate treatment for affected calves, but greater benefit will be gained from recommending appropriate management changes to control the current outbreak and prevent recurrence in subsequent calving seasons.

This paper details the steps required for a thorough investigation of a calf scours outbreak. It includes a comprehensive list of risk factors, and describes a logical process and the specimens required to establish the pathogens contributing to the problem.

3. Control of a calf scours outbreak

In order to target specific control measures it is useful to have an established aetiology. However in many cases initial control measures will have to be taken in the absence of this. This paper details strategies that can be used to assist in the control of a calf scours outbreak

4. Treatment of the scouring beef calf

This paper has comprehensive detail on medical management of the scouring calf, including correction of fluid, electrolyte, metabolic, and acid base derangements, prevention or treatment of sepsis, and general supportive treatment. The practicalities of treating scouring calves on commercial enterprises are also discussed.

4.1.3 Documents for Producers

A series of three documents were also prepared to form the basis of material for provision of information on calf scours to producers. These documents are included in Appendix 3, and described briefly below.

1. Why do calves get scours?

This document provides a general overview of the clinical presentation, epidemiology, predisposing causes and pathogens associated with calf scours.

2. Prevention of calf scours

Calves develop scours when they ingest a large dose of infective agent and/or have a lowered resistance to disease. In order to prevent a calf scours problem it is necessary to manage your cows to prevent a build-up of causative agent on the farm and to optimise the health of pregnant dam and subsequently the newborn calf. There are many ways to manage a farm and there are also many ways to minimise the risk of calf scours. It is not possible to make generic

recommendations that will suit all farms and all management systems. This document describes the best management strategies for prevention of calf scours. The implementation of these strategies will depend on the resources and restraints of each producer.

3. Approach to a calf scours outbreak

Exposure to the agents that cause calf scours is a normal part of “growing up” for a calf and almost every property will have a couple of calves that have sticky white or yellow diarrhoea around their tail. One or two calves that are scouring but remain bright and continue suckling are not a problem, although it is advisable to observe them daily to ensure rapid treatment if they do become sick. However when calves become sick and require treatment, or start dying, it is important to rapidly put in place control procedures and effectively treat the scouring calves. This document details triggers for action, management strategies for control and effective protocols for on farm treatment.

4.2 Objective 2: Describe in outline the way this information should be delivered to key groups

4.2.1 Overall Delivery of information

A website should be set up to provide basic information on the project and have the farmer leaflets available for download.

4.2.2 Delivery of information to veterinarians:

Reference information

In order to maximise the impact of this information, and so that it can be easily referenced in the future, key sections of the literature review should be submitted to publication to the Australian Veterinary Journal (or the Australian Cattle Veterinarian).

Suggested sections would include

- ✓ The aetiology of NCD
- ✓ Risk factors for NCD in pasture based beef calves
- ✓ Recognised methods of prevention of NCD
- ✓ Current best practice diagnostic methods

Because a comprehensive review of oral electrolyte therapy has been recently published in the Australian Cattle Veterinarian, there is probably no requirement for publication of any treatment sections.

Information leaflets

The four leaflets on prevention, control, diagnosis and treatment should be appropriately edited and presented to provide maximum impact and published and distributed to cattle veterinarians. Prior to publication, a second review of these documents by the current review panel would be advisable to ensure readability and a clear understanding of the information. One effective method of ensuring dissemination to the appropriate veterinarians is to distribute them to members of the Australian Cattle Veterinarians. This is likely to be the best way to achieve clear separation between these leaflets and the farmer information. It is possible that they could also be available for download from the AVA/AACV website. The production of audio or audiovisual information on CD should also be considered.

Promotional presentations:

The program and leaflets can be “launched” at the Australian Veterinary Association conference in May. A “Calf scours day” is planned for the Australian Association of Cattle Veterinarians conference in the Barossa in October (see attached program)

4.2.3 Delivery of information to producers:

Information leaflets

The 3 leaflets entitled “Why do calves get scours”, “Prevention of calf scours” and “Approach to a calf scours outbreak” should be appropriately edited and presented to provide maximum impact.

Prior to publication, a second review of these documents by a farmer review panel would be advisable to ensure readability and a clear understanding of the information. Publications should be advertised through the appropriate MLA publications such as Feedback and the MLA website available to MLA members from the publications department. They could also be disseminated as articles in MLA publication or the rural press. The production of audio or audiovisual information on CD should also be considered.

Promotional presentations:

The information should be developed into a presentation format that can be slotted into existing farmer workshops such as Beefcheque and the Herd Health and Welfare module of “More Beef from Pastures”. There may also be a demand for specific workshops in ares with a particular problem. Training information should also be developed for advisers prior to presenting the material. The most important concept that needs to be disseminated to producers is that neonatal calf diarrhoea is not a disease with a quick fix. There are many predisposing factors and prevention and control may require significant management changes and prior planning. Often these strategies will fit well with good nutritional and reproductive management techniques currently being promoted within the industry.

4.2.4 Delivery of information to other extension providers:

Information leaflets

Whilst no leaflets have been specifically designed for other extension providers, most of the information required is in the leaflets for veterinarians or producers, and it is probable that the farmer documents are also an appropriate resource for this group. If separate information is required this should be compiled and appropriately edited and presented to provide maximum impact. It is very important that extension officers are aware of the program and the availability of leaflets for farmers. Promotional material for these leaflets should be sent to the known list of extension officers and advisers.

4.2.5 Maintaining the impetus

The current understanding of many aspects of neonatal calf diarrhoea is likely to change as further research is published. Moreover, as in any industry there is likely to be change in the personnel involved, specifically with new graduate veterinarians and producers entering the industry. It is important that the information modules produced by the current study are revised and updated at least every 5 years in order to continue to have an impact on the industry.

4.3 Objective 3: To liaise and coordinate with pathologists in all relevant veterinary laboratories and determine an agreed process for laboratory diagnosis of reporting of calf scour results

A meeting was held on 4th November 2004 with key laboratory personnel from all laboratories that process a significant number of bovine submissions. There was lively discussion on many aspects of the diagnosis of calf scours resulting in an agreed protocol for best practice diagnosis. The minutes of the meeting and a practitioner guideline on best practice diagnosis of calf scours that was circulated after the meeting are included in appendix 4.

4.3.1 Discussion and recommendations for Objective 3

It is important to ensure high and consistent standards for the diagnosis and reporting of neonatal calf diarrhoea in Australian veterinary laboratories. The circulation of best practice recommendations is a positive step to promote best practice diagnostic techniques in the field and facilitate the development of a standard diagnostic protocol (SDP).

The response from the laboratories to the suggestions of the meeting was favourable, but continued encouragement from industry is required to ensure the development of the SDP. It was suggested that work should be done to incorporate the diagnosis of calf scours as an ANZ SDP as determined by the Sub-Committee on Animal Health Laboratory Standards (SCAHLS). This may be a protracted process, but a discussion paper was to be tabled for the next SCAHLS meeting.

Any SDP should be accompanied by an external auditing process that involves proficiency testing. Currently whilst laboratories have standard audited procedures for specific diagnostic techniques, it is not mandatory that all tests carried out within the laboratory are NATA accredited. NATA proficiency testing for veterinary laboratories is aimed at auditing a specific accredited technique as opposed to the laboratory's ability to correctly diagnose a pathogen on samples that may require a series of tests in different disciplines.

In many states there is only one veterinary laboratory that processes the bulk of the production animal specimens, and consequently there is little market incentive to carry out such testing. It is in the interest of the industry as an end user to encourage the need to invest in an audit, but it should be recognised that currently there are no financial incentives for the laboratories to adopt such a process.

If it is not possible to incorporate the diagnosis of calf scours as an ANZ SDP under SCAHLS, the possibility of a quality assurance process audited by NATA should be investigated. There is likely to be benefit in working with other production animal industry bodies, especially Dairy Australia, as well as state government departments to encourage the development of audited SDPs for a range of production animals diseases.

4.4 Objective 4: To facilitate a reliable system for surveillance of future results through all southern veterinary laboratories

Phase 1 of this project involved substantial collation of information from veterinary laboratories and in many cases submissions could not be included because not enough information was available. Moreover in all cases the original submission forms had to be accessed to confirm epidemiological and breed information making data collection slow and expensive. Some laboratories did not have a good enough retrieval system to make collation of any reliable data cost effective.

The problem was three-fold

- ✓ Veterinarians are not providing enough information when submitting samples
- ✓ Some submission forms do not request appropriate information. In the current study the focus was the beef industry and some laboratory forms do not have the proviso to differentiate breed or industry
- ✓ Laboratories are using different computer systems, with different and often incomplete information stored on them, and varying abilities to export this information into other formats.

Effective and efficient disease surveillance should be a major priority for the Australian animal export industries. For this to occur standardisation of submission forms for production animals is essential, together with recognition by veterinarians of the importance of providing epidemiological details. This needs to be combined with effective and complete recording in a standard format across all veterinary laboratories to allow for the rapid and efficient collation of national disease data.

These objectives were discussed at the meeting with laboratory personnel. Whilst all of these objectives are achievable they could only occur at a cost. This is due to the nature of the veterinary laboratory system in Australia, where both private and government funded laboratories are processing specimens from beef cattle. Data input and databases that facilitate easy retrieval of the data are an additional expense to the laboratories, and unless industry is willing to pay for this some veterinary laboratories have no incentive to record this information so that it can be easily retrieved.

Consequently the industry needs to consider the importance of retrospective laboratory data to establish the prevalence and economic impact of a disease, as well as for monitoring any change in the prevalence of a specific presentation that may facilitate the identification of unknown disease. If laboratory data is determined to be an important factor in ongoing research and the biosecurity of the beef industry then negotiations need to be carried out with veterinary laboratories to support databases and fund the required data entry. It will also be necessary to promote the requirement for specific data on laboratory submission forms to veterinarians in the field.

There is likely to be benefit in working with other production animal industry bodies, especially Dairy Australia on facilitating this requirement, as well as state government veterinary departments.

4.5 Objective 5: To identify areas where further research is required in the prevention, control and treatment of calf scours in southern Australian cow calf systems.

A comprehensive literature review has shown that there has been little Australian published research specifically targeted at prevention or control of calf scours in Australian cow calf operations. There are numerous studies worldwide on the diagnosis and treatment of neonatal calf diarrhoea. Studies to identify risk factors and management procedures to minimise calf scours have been carried out in pasture-based systems in the USA and Canada. Due to the climatic and management differences these may not be directly applicable to Australian conditions.

The aetiology of neonatal calf diarrhoea in southern cow-calf production systems has not been clearly determined. Studies of laboratory data (Gunn 2003) have indicated that the most common pathogens affecting calves with neonatal diarrhoea in Australian beef calves are rotavirus and cryptosporidia. However there are many producers who are unclear as to the cause of their problem and the lack of specific, targeted and quick “fix”. This is likely to be a combination of insufficient diagnostic work-up, poor or insufficient sampling technique, incomplete laboratory testing and laboratory diagnostic techniques with poor sensitivity. It is also likely to be compounded by a poor understanding of the multifactorial nature of the disease, and a lack of knowledge of the predisposing factors.

No systematic survey has been carried out, but there appear to be specific areas that have a much greater problem with neonatal calf diarrhoea (Notably SE South Australia and the far SW of Western Australia), and areas with similar environmental conditions that don’t have a problem (Tasmania). This may be because these areas have a more pro-active and vocal producers, or have management systems that focus more on the individual animals, but the veterinarians in these areas would confirm this observation. However it is possible that there are specific nutritional or geological predisposing factors in these areas.

It would be useful to establish the impact of the problem at a farm and industry level both across southern Australia and in specific geographic areas. This could be achieved by a random survey of MLA members, or a survey of producer groups.

Further research is needed to address the lack of epidemiological information in pasture based systems in Australia, and there are also specific diagnostic areas that need addressing. Whilst a series of recommendations have been produced as part of this project based on overseas research and first principles from the epidemiology of the disease they need validation.

4.5.1 Areas for study where there is no published research relevant to Australia

1. The relationship of calf scours in Australia to

Farm management factors:

- ✓ Soil management, specifically fertiliser type and timing of application in relationship to calving
- ✓ Pasture species and quality
- ✓ Grazing management specifically set stocking vs rotational grazing

- ✓ Nutritional management: nutritional stress supplementary feeding and mineral status

Environmental factors

- ✓ Soil type
- ✓ Climate / meteorological conditions

Other factors

The influence of breed/genetics: specifically Angus and Wagyu

2. Other areas of study

- ✓ Poor milk clotting in cows has been shown to be a cause of calf scours in Scotland, with a nutritional origin. This is a factor that could explain the regional differences in NCD in a similar climate and should be investigated.
- ✓ The significance of FPT as a predisposing cause for calf scours in Australia. If it is a significant factor then further research should be carried out on the predisposing causes.
- ✓ Relationship between mineral deficiencies and colostrum quality
- ✓ The importance of different water supplies as a source of pathogens
- ✓ The significance of Attaching and effacing *E. coli* (AEEC) and Shiga toxin-producing *E. coli* (STEC) in neonatal calf diarrhoea in Australia
- ✓ The significance of environmental sources versus shedding by the dams as the initial source in a calf scours outbreak. Need to know for each pathogen
- ✓ The significance and aetiology of scours associated with cows being fed lush pasture.
- ✓ The significance of toroviruses in Australia
- ✓ Development of standardized diagnostic testing. PCR is likely to be the gold standard, multiplex systems could be developed to reduce cost. These tests would provide a suitable standard for evaluation of calf-side rapid dipstick kits as they become available.
- ✓ Validation of calf-side rapid dipstick kits currently available in Australia

4.5.2 Major areas for validation of recommendations

- ✓ Separation of heifers and cows prior to and at calving
- ✓ Separation of calved cows from calving cows
- ✓ Effect of stocking density and maximum stocking density, especially for nursing groups
- ✓ Effectiveness of providing water troughs (as opposed to letting cattle have access to water courses) to minimise spread
- ✓ Ideal and maximum group size for nursing groups

4.5.3 Possible directions for further study

Many of the above are management and climatic factors. Their significance could be studied with a case control study of an appropriately determined number (using a sample size estimate) of scour outbreaks across multiple areas. Where possible the study should involve producer groups. This investigation should include thorough examination of environment, dam herd, and scour sampling. The study should cover at least 5 sites using well-trained veterinary cooperators (preferably those with Australian College Qualifications in Cattle Medicine, Epidemiology or other higher degrees). There would also be major benefits in collaborating with the Departments of Primary Industry in each state. The principle investigators should submit a fully developed protocol that considers the herd and individual animal level risks and allow for appropriate numbers of faecal samples to be obtained to provide information on the correct number of samples to be taken in routine investigations and sufficient supported necropsies to support the diagnostic processes conducted on the faeces and farm. Standard operating procedures will be needed to provide consistency of approach in all aspects of the trial. A well designed trial collecting the appropriate information and with good diagnostic techniques would also facilitate a much better understanding of the aetiology of calf scours in southern Australia. Some of the other major areas for research, such as the evaluation of the diagnostic kits, the significance of FPT and the effectiveness of milk clotting could be included within this study.

4.5.4 Commercial products that may be of use to Australian farmers

Most of the emphasis of this project should be on the prevention of calf scours, and consequently there is little requirement for commercial products, apart from the evaluation of the calf side diagnostic kits, as they are likely to promote a science based approach to calf scours and reduce the cost of investigations for producers. There is some producer demand for a rotavirus vaccine, this could be a useful tool but is unlikely to be the “quick fix’ that is hoped for. Conducting the case control study first would be appropriate to determine the relative prevalence of rotavirus infections in Australian beef herds. This would provide the data required to conduct an economic analysis regarding the potential benefit of vaccination. Studies of vaccines available overseas show that in lieu of complete protection, the manifestations of passive immunity to bovine rotavirus that are often noted are (1) a delay of a few days in the onset of clinical signs and or (2) a reduced severity of clinical signs, and or (3) a reduction in the length of the period of viral shedding associated with infection. Although there are reports of a positive response in field trials involving bovine rotavirus/bovine rotavirus-coronavirus – vaccinated cows, benefit has not been observed in all trials. A common problem with commercial vaccines on the market in the U.S.A. and Europe is a lack of vaccine specific data supporting efficacy claims. Protection correlates with serum titres, independent studies have sometimes failed to demonstrate effective seroconversion with some products. This problem would be circumvented by the vaccine registration process in Australia which requires demonstration of efficacy.

References

Gunn, A. A. (2003). Calf scours in southern Australia; phase 1 final report, Meat and Livestock Australia.

5 Success in achieving objectives

The main objective for this project was to determine scientific and repeatable principles for the prevention, investigation and management of calf scours relevant to the southern cow calf industry that can be disseminated to laboratories, veterinarians and producers. This was achieved within the limits of current knowledge, however there is no good scientific research into the epidemiology of neonatal calf diarrhoea in Australian suckler beef enterprises and consequently the information produced is also limited. Objectives 2,3 and 5 were fully achieved, but objective 4 is more difficult because of the structure of the veterinary laboratory system in Australia, where both private and government funded laboratories are processing specimens from beef cattle. Data input costs money and unless industry is willing to pay for this some veterinary laboratories have no incentive to record this information so that it can be easily retrieved.

6 Impact on Meat and Livestock industry - now and in five years time

Scours in beef calves has been an ongoing issue for the producers for many years. Little published research has been carried out to date in Australia and no satisfactory strategies to minimise the impact have been demonstrated. The long-term goal of this project is to have a clear and consistent approach to calf scours documented and adopted by producers, advisers, veterinarians and veterinary pathology laboratories. For this to occur the first step is to extend the information compiled in this project to producers, veterinarians and other extension workers involved in the industry. It will also be important to encourage veterinary laboratories to adopt best practice techniques for the diagnosis of neonatal calf diarrhoea. If the study stimulates the recommended research into the epidemiology of calf scours in southern Australian suckler enterprises, then it would be hoped that there would be significant benefits for the affected producers in five years time. The extension information produced should not be static, and for it to have continuing impact it will be necessary to revise and update the packages at least every three to five years.

7 Conclusions and recommendations

The major achievement of this project has been the production of clear and consistent information on the prevention, diagnosis, treatment and control of calf scours for veterinarians and producers. The presentation and dissemination of this information is the obvious next step and we have recommended options for effective extension solutions.

Notably:

- ✓ to achieve a “whole of industry” approach it is important that both veterinarians and non-veterinary extension personnel are aware of the program and the different modules that will be available
- ✓ the information should be available online from appropriate websites to ensure ease of access
- ✓ a training pack must be produced for advisers that wish to incorporate this material into courses for producers

- ✓ The information should be revised and updated at least every 5 years

Increased knowledge of the epidemiology of calf scours in southern beef suckler enterprises is essential. The first phase of this project determined a need for case control studies to determine key management strategies for the Australian beef industry and this has been confirmed by the current study. A thorough epidemiological investigation of an appropriately determined number of scours outbreaks should be supported. The study should involve multiple regions and thoroughly examine a wide range of managemental and environmental variables, and include diagnostic testing of scour samples. Other important areas for further research include:

- ✓ Applied studies in Australia to validate the recommendations of this project determined from first principles of pathogen epidemiology and research conducted overseas.

- ✓ Investigation of the role of milk clotting in the aetiology of calf scours.

- ✓ The significance of FPT as a predisposing cause for calf scours in Australia. If it is a significant factor then further research should be carried out on the predisposing causes, including the relationship between mineral deficiencies and colostrum quality.

- ✓ The significance of Attaching and effacing *E. coli* (AEEC) and Shiga toxin-producing *E. coli* (STEC) in neonatal calf diarrhoea in Australia

- ✓ The significance of toroviruses in Australia

- ✓ Validation of calf-side rapid dipstick kits currently available in Australia

Some or all of these could be nested within the epidemiological study recommended above.

It is important to ensure high and consistent standards for the diagnosis and reporting of neonatal calf diarrhoea in Australian veterinary laboratories. There should be standardised testing protocols for the diagnosis of neonatal calf diarrhoea implemented in veterinary laboratories and the quality assurance of this should be audited by NATA or SCAHLS. It is in the interest of the industry as an end user to encourage the need to invest in this process, but it should be recognised that currently there are no financial incentives for the laboratories to adopt such a process.

There also needs to be continued encouragement of the development and validation of emerging laboratory diagnostic techniques, both to enable better diagnosis of the known aetiological agents and to determine the role of specific pathogens shown to contribute to the aetiology of calf scours overseas.

Finally the industry needs to consider the importance of retrospective laboratory data to establish the prevalence and economic impact of a disease, as well as for monitoring any change in the prevalence of a specific presentation that may facilitate the identification of unknown disease. If laboratory data is determined to be an important factor in ongoing research and the biosecurity of the beef industry then negotiations need to be carried out with veterinary laboratories to support databases and fund the required data entry. It will also be necessary to promote the requirement for specific data on laboratory submission forms to veterinarians in the field.

There is likely to be benefit in working with other production animal industry bodies, especially Dairy Australia, as well as state government veterinary departments to progress both the development of audited standard diagnostic protocols and the support for laboratory databases and the data entry required.

8 Appendices

8.1 Appendix 1: Literature review of the prevention, aetiology, diagnosis, treatment and control of neonatal calf diarrhoea in beef calves

Table of contents for literature review

Introduction

Calf diarrhoea is a multifactorial disease of complex aetiology. Almost inevitably an outbreak of calf diarrhoea reflects an overwhelming challenge with pathogens from a heavily contaminated environment, severe environmental or other conditions that impair immune function in calves, rather than the introduction of a virulent pathogen. The presence of enteric pathogens does not ensure the occurrence of disease. Occasionally outbreaks reflect the introduction of a particularly virulent pathogen into a naïve population. Numerous variables influence pathogen survival and host immunity hence there is no single simple "out of the box" disease prevention remedy. The basic premises of disease prevention and control are to reduce pathogen build up and exposure, optimise host immunity, and maintain biosecurity. As the resources and facilities differ between farms, implementation involves tailoring resources and management to adopt these principles working within the constraints and resources of the specific farming operation.

When addressing the problem of NCD it is important to comprehend the role of the calf as a biological incubator and amplifier.[1] As this paper will detail many pathogens are shed in large numbers not only by clinically ill calves, but also by sub-clinically infected calves and cows. Consequently in the face of an outbreak there can be a very rapid build up of pathogens in the environment.

A study of Australian producers affected by calf diarrhoea indicated that it is a major problem causing high mortality for some producers, yet other producers are unaffected.[2] This mimics data from the USA indicating that neonatal mortalities tend to cluster in individual herds.[3, 4] Moreover veterinarians surveyed in the Australian study commented that different producers were affected each year. Studies from the USA and Canada show a similar picture, and according to Townsend[5] veterinarians should expect between 4% and 16% of their producers to experience an unacceptable level of mortality from NCD each year

The objective of this literary review is to determine the most current thinking on the prevention, diagnosis, control and treatment of neonatal calf diarrhoea in cow calf operations.

Common pathogens and their properties

Pathogens frequently implicated in neonatal calf diarrhoea include viruses, bacteria and protozoa.

Viruses

Rotavirus

Bovine rotavirus is a major cause of NCD in calves less than 1 month of age worldwide[6-11] and an increased prevalence has been noted in suckler beef herds as compared with calf rearing units.[12]

Rotavirus of calves, lambs, kids, pigs, foals, mice and children are morphologically identical. They are classified into seven antigenically distinct serogroups A-F. Rotaviruses from serogroups A, B, and C have been isolated from cattle and serogroup A is the most common cause of diarrhoea in calves. Group B rotaviruses have been isolated from calves and adult cattle, however there is less information available regarding the significance and prevalence of group B rotavirus.[13-17] Group C rotavirus have only been isolated from adult cattle.[16] A range of serotypic diversity and virulence has been reported within serogroup A.[18-20]

Affected calves are generally 5 days to 2 weeks of age, although disease can occur at 24 hours, particularly in colostrum deprived calves.[20-22] There is a variation between strains with some strains only causing disease in newborn calves and more virulent strains producing diarrhoea in calves over 1 month of age[20] Disease has been reported at two to three months of age, although this may have been associated with the introduction and rapid build up of the pathogen at this time.[23] Resistance to infection is not age dependant, but age dependant resistance to clinical disease has been demonstrated.[20, 21, 24] The age at which calves develop resistance varies between strains.

Rotaviruses are shed in the faeces of infected animals and transmission is primarily faecal-oral. Clinical signs occur 1-3 days after infection and last for 5-9 days. Virus excretion commences with the onset of clinical signs and continues for 3-7 days.[20, 25] Subclinical infections are common due to the development of age-dependant resistance to clinical disease. Subclinically affected calves have no difference in the duration of infection or the levels of rotavirus antigen in the faeces but are associated with virus excretion at a later time after inoculation.[20, 21] Three surveys demonstrated rotavirus in a significantly greater proportion of faecal samples from calves with diarrhoea than from unaffected calves.[12, 26, 27] A fourth study confirmed rotavirus infection in a similar proportion of affected and unaffected calves.[8] This latter study was a prospective study of 94 beef farms designed to show the pattern and association of diarrhoea with several enteropathogens. No details were provided on the history and age of the unaffected calves. The former studies were case control studies in diarrhoea outbreaks in both beef and dairy cattle. The unaffected calves in both of these studies were of similar age and with no history of diarrhoea. It is likely that virulent strains of rotavirus will be present in a diarrhoea outbreaks, whereas in a prospective study a wider range of virulence will be detected, and hence a greater proportion of subclinical infections.

Adult cows can be subclinically infected and intermittently shed the virus during pregnancy and especially at parturition.[28-30] Calves from carrier cows have a significantly higher risk of clinical disease and the birth of calves from known carrier cows have been associated with the beginning of an outbreak. Recovered calves can become reinfected and shed virus.[31]

A variable proportion of cows will have colostral antibodies to rotavirus.[32, 33] Antibody levels in milk decline rapidly after calving and calves can become susceptible to infection at one week of age. This decline in antibody levels is thought to be responsible for outbreaks of rotavirus diarrhoea year after year, despite the presence of colostral antibodies in most cows at calving.

Bovine rotaviruses have been shown to experimentally infect dogs and cats that then shed the virus for up to 14 days after infection.[34, 35] The infection was asymptomatic. Cross transmission between dogs and cats and from cats to dogs also occurred. Low amounts of virus are sufficient to determine infection, viral multiplication and excretion in dogs. These two species may play a significant role in the maintenance of this viral infection on a farm. Antibodies to bovine rotavirus have also been found in the faeces of deer, pigs, foxes and rabbits indicating that feral animals are likely to be another source of infection.[36, 37]

The environment may be an important source of infection. Rotaviruses can survive in fresh water for more than 2 weeks at 23 ^{o}C and for months in water or soil < 5 ^{o}C.[38] They are stable in faeces and effluent for up to 9 months and are likely to remain in calving areas from year to year.[39] In soil systems rotavirus survival is prolonged after adsorption to soil surfaces. This limits the movement of organisms through the soil. After rainfall or changes in the organic matter present in the water passing through the soil, viruses desorb from the soil surfaces and move as a burst of infective particles through the soil. Viruses can move great depths through the soil (>30m) to contaminate ground water.

Pathogenesis is the result of enterocyte invasion leading to shortening of the villi in the small intestine. Enterocytes are lost to the gut faster than they can be replaced from the crypts. Intestinal secretions continue but absorption is impaired leading to diarrhoea. Infections are short lived; after destruction of villous epithelial cells, the lack of further target cells halts the infection. It is thought that virulent strains replicate more quickly and infect a larger area of epithelium. The mechanism of age-dependant resistance has not been elucidated.[21] Concurrent infection with ETEC has also been shown to cause clinical signs at a later age than with a single infection of either agent alone.[24]

Clinically calves present with severe watery diarrhoea or copious amounts of yellow or white pasty faeces.[40] Outbreaks can be explosive and associated with a change in the weather. Morbidity and mortality is extremely variable but can be high.

Coronavirus

Bovine coronavirus (BCV) is widely recognised as an important cause of diarrhoea in calves 5 days to 1 month of age, although it is less frequently isolated than rotavirus.[8, 10, 12, 41] The virus has little antigenic variance. Disease can occur at 24 hours, particularly in colostrum deprived calves and has been recorded in calves up to 5 months of age.[22, 42] Clinical disease is associated with adverse weather conditions.[28]

Calves may be infected with BCV by the oral or respiratory route.[25] Faecal shedding commences 3 days after infection and persists for up to a week, nasal shedding can be detected 2 days after infection and persists for 2 weeks. BCV respiratory infections are common and aerosol transmission is important in the epizootiology of enteritis.[42-44] BCV has also been associated with winter dysentery in cattle.[45] Like rotavirus subclinical infection is common, but several large studies have shown a significant association between coronavirus in the faeces and clinical cases of diarrhoea.[8, 12] Studies that failed to show a significant difference only detected a low number of isolates.[26, 27] Age dependent resistance is not documented in calves, but does occur with transmissible gastro-enteritis in pigs.[46] Calves have subclinical persistent or recurrent infections and shed virus in faeces and nasal secretions maintaining a reservoir of disease in the population.[42, 47]

Disease is more common in the winter months and may occur annually on the same farm. It is possible that coronavirus survives in the environment from year to year, however it is a relatively fragile virus and outbreaks will still occur when cows calve onto clean pasture. It is more likely that calves are infected by virus particles shed by persistently infected cows.[45] BCV has been detected in the faeces of more than 70% of clinically normal cows.[30] The rate of virus excretion increases at parturition and in the winter months and calves born to carrier animals have a significantly increased risk of developing diarrhoea.[28, 48]

The pathology of coronavirus is often more severe than rotavirus resulting in a mucohaemorrhagic enterocolitis.[22] The virus infects both the small and large intestine. Virus replication occurs in the surface epithelium, especially in the distal half of the villi, resulting in stunting and fusion of the villi. Immature cells replace epithelial cells and in severe infection there can be areas of complete desquamation. Intestinal secretions continue and absorption is impaired by reduced surface area. Undigested lactose accumulates in the intestinal lumen, often resulting in a secondary bacterial overgrowth as well as an osmotic imbalance that draws more water into the gut. Most infections are self-limiting because the virus rarely attacks crypt epithelial cells.[45] In response to infection the mitotic rate of crypt cells increases producing immature cells that are more resistant to virus infection and that migrate up the villi to replace the damaged cells.

The clinical signs are similar to rotavirus, but the disease may be more severe and protracted. Experimentally yellow diarrhoea develops 48 hours after infection. Calves are initially depressed and anorexic for the acute phase, and may become dehydrated and pyrexic in a severe infection.[45] Severe infections can result in death due to dehydration, acidosis, shock and cardiac failure. Respiratory signs are generally mild and rhinitis, sneezing and coughing may occur. Lesions may be found in the lungs but clinical signs of pneumonia are rare, except when secondary infection occurs. The mortality rate is generally low.

Bovine Viral Diarrhoea virus

Bovine Viral Diarrhoea virus (BVD) can occasionally cause diarrhoea and thrombocytopaenia in young calves outside the confines of the persistently infected disease model.[22, 49, 50] Colostral antibodies generally protect young calves from BVD infection, but disease may occur due to failure of passive transfer or the introduction of novel BVD strains with new cattle or viral mutation in persistently infected home-grown cattle. BVD is also thought to exacerbate infections due to other pathogens.[51] It has also been implicated in necrotic enteritis, an acute enteritis of 7-12 week old beef calves reported in the UK.(see p 39).[52]

Torovirus

Bovine torovirus has been detected worldwide[53-56] and has recently been implicated as an important cause of NCD in the USA and Canada.[57, 58] Initially known as Breda virus, it is part of the *Coronaviridae* family. It has been relatively infrequently reported because it is difficult to recognise by EM and it cannot as yet be grown in cell culture, which has precluded the development of routine immunospecific diagnostic tests for use in faecal samples.[58] Consequently the prevalence and epidemiology of this viral infection is unclear. Laboratory studies using PCR have implicated it as the sole pathogen isolated in between 25-30% of faecal samples from calves with diarrhoea under 6 weeks of age.[57, 58]

It is also found in the faeces and nasal secretions of asymptomatic animals[41, 58] implicating that the epizootiology is likely to be similar to that of rotavirus and coronavirus, with asymptomatic carriers acting as a reservoir of infection within a herd.[28] No studies have been published on its survival in the environment.

It is mainly a disease of calves less than 3 weeks of age with diarrhoea commencing as early as 1-3 days after birth,[55, 56] but clinical signs have been observed up to 10 months of age.[57, 59] Clinically it produces mild to moderate diarrhoea in calves under both experimental and field conditions.[56, 60] The virus infects the small and large intestines affecting differentiating epithelial cells in the crypts of the intestinal villi.[56, 59] Clinical signs developing 24-72 hours after experimental infection.[56] It has also been isolated from the respiratory tract of cattle and associated with respiratory signs in calves at 1 month and 4-6 months of age.[61]

Bovine Enteric Calcivirus

Bovine enteric caliciviruses were first described in the UK as Newbury agents,[62] and were isolated by electron microscopy in 11% of calves with diarrhoea,[12] but until recently little information was available on these viruses. Studies have been limited as there is no cell culture system for bovine caliciviruses and no small animal model for infection.[63] There are 4 families of calicivirus: the vesiviruses, lagoviruses, noroviruses, and sapoviruses.[64] Noroviruses, otherwise known as Norwalk-like viruses, have emerged as the leading worldwide cause of acute non-bacterial gastroenteritis in humans.[65] Calves are the only animals from which noroviruses have been isolated and shown to cause disease in an experimental setting.[63] Consequently

there has been a recent increase in studies of bovine enteric caliciviruses to determine if cattle are a reservoir for Norwalk-like viruses.

Bovine enteric caliciviruses have been classified as noroviruses by phylogenetic analysis. These include the original Newbury viruses from the UK[66, 67] and one from Germany (Jena virus)[63, 68] but other similar strains have also been documented from Holland[69] and the USA.[70, 71] They are all genetically different from human norovirus strains and there is no evidence that they pose a threat to human health.[65]

Several norovirus strains have been shown to cause diarrhoea in colostrum-deprived or gnotobiotic calves.[67, 68, 70] Histopathological lesions are limited to the small intestine, mainly in the duodenum. There is diffuse villous atrophy with exfoliation of villous enterocytes and crypt hypoplasia.[70] Virus excretion occurs 2 days after infection and only persists for 2-3 days with low viral numbers.[67]

Jena virus was detected by ELISA in 9% of 381 faecal samples from diarrhoeic calves in southern Germany and in 99% of 824 serum samples collected from 25 dairy herds around Thuringia, indicating that this virus is endemic in Germany.[63] Norovirus RNA was also detected in faecal samples from 33% of 75 veal farms in Holland.[72] and in 80% of 74 samples from diarrhoeic calves on 20 farms in Michigan and Wisconsin, USA.[71]

Although norovirus infection may prove to be common in cattle to date these viruses have not been associated with severe clinical disease in calves. However this may be due to the short virus excretion time and lack of good diagnostic tests, as opposed to low significance in the aetiology of NCD.

Other Viruses

Astrovirus, adenovirus, parvovirus and picobirnavirus have all been associated with outbreaks of NCD.[73-76] There are few reports involving the latter 3 viruses and their pathogenicity is uncertain. It is likely that astrovirus is not pathogenic.[74]

Bacteria

Escherichia coli

Escherichia coli are part of the normal flora of the bovine gastrointestinal tract. Pathogenic strains of *E. coli* possess virulence attributes that are involved in the pathogenesis of disease. Virulence attributes include adhesins, enterotoxins, and cytotoxins. Pathogenic strains of *E. coli* may be shed by adult cattle with transmission to neonates by the faecal oral route. Sick neonates amplify environmental contamination via prolific faecal shedding. Survival of *E. coli* in the environment is dependent on the environmental conditions (temperature, pH, water of activity, surface porosity, etc) and on the characteristics of the specific serotype. Survival in faeces for 70 days has been reported.[77]

Enterotoxigenic E. coli *(ETEC)*

Enterotoxigenic *E. coli* possess two virulence factors: fimbriae (pili) and enterotoxins. F5 (K99) and or F41 fimbriae mediate adherence and thermolabile (LT) and thermostable (STa and STb) enterotoxins stimulate a secretory response by intestinal crypt cells. Although some bovine origin ETEC produce LT, most strains that cause diarrhoea in neonatal calves produce STa heat stable enterotoxin.[78] The STa enterotoxin and K99 antigen are plasmid-mediated virulence factors. Susceptibility to Enterotoxigenic *E. coli* is age dependent according to the binding specificity of

pilli antigens to immature enterocytes.[79] Disease is typically observed in calves less than 3 days of age, however concurrent infection with rotavirus may extend this window to 7-14 days of age.[80, 81] Intestinal cells of calves greater than two days of age acquire natural resistance to F5 adhesion.[79] Despite this F5 positive *E. coli* have been isolated from healthy 4 – 12 week old calves and F5 positive ETEC are shed in faeces for several weeks following experimental infection of newborn calves.[82]

Enterotoxigenic *E. coli* are non-invasive, hence oral antimicrobial therapy with drugs that have limited gastrointestinal absorption are effective. Vaccination of cows with ETEC bacterins during pregnancy stimulates production of antibodies to fimbrial antigens. Colostral transfer of K99 antigen specific antibodies to calves prior to pathogen exposure is effective at preventing disease caused by this strain. It is important to note however that not all strains of ETEC possess K99 fimbriae.

Attaching and effacing E. coli *(AEEC) and Shiga toxin-producing* E. coli *(STEC)*

Attaching and effacing *E. coli* (AEEC) and Shiga toxin-producing *E. coli* (STEC) have also been identified as causes of diarrhoea and dysentery in calves.[83-85] Disease is mediated by cytotoxic damage to the intestinal mucosa. Lesions may be observed in the ileum, caecum and colon.[86] Attaching and effacing *E. coli* (Vero or HeLa toxin producing) induce a mucohaemorrhagic colitis, with petechial or ecchymotic haemorrhages in the wall of the colon and rectum.[87-89] *E. coli* that carry this toxin often belong to O serogroups 5, 26, 111, and 118.[88, 90] Naturally occurring outbreaks have been reported in 2 day to 4 week old calves.[91] The most common clinical sign is diarrhoea but dysentery, abdominal pain manifested by bruxism, and dehydration are seen in some cases.

Shiga toxin-producing *E. coli* serotypes associated with dysentery in calves include (O5:H-, O26:H11, O111:H-, O113:H21)[92] these serotypes may produce shiga toxins, those that are immunologically similar to the Shiga toxin produced by *Shigella dysenteriae* (Stx1) and those that are immunologically distinct from *Shigella dysenteriae* Shiga toxin (STx2).[93] Bovine STEC produce either STx1, STx2, or both.[94] Attaching and effacing *E. coli* that cause disease and do not produce enterotoxins or shiga toxin are referred to as enteropathogenic *E. coli* (EPEC).

The prevalence of AEEC and STEC in calves and the incidence of disease caused by these strains are not clearly defined as most diagnostic laboratories do not routinely screen for AEEC and STEC. In a study aimed at determining the clinical significance and prevalence of AEEC *E. coli* in Swiss cattle, faecal swabs of 93 cattle from two farms with calf diarrhoea, and of 54 cattle from two similar farms without clinical problems, were screened for AEEC by PCR and colony-blot hybridisation. On average, 21% of all cows were positive for AEEC by PCR, without differences between farms with and without diarrhoea problems. By contrast, AEEC were detected by PCR in 60% of animals younger than 2 years from farms with diarrhoea problems, whereas only 32% of comparable control animals from farms without clinical problems had AEEC. A variety of toxin types, antimicrobial-susceptibility patterns and serotypes were found by colony blot hybridisation, among the AEEC in individual herds. However, there was a significant relationship between the verotoxin 1 type and the presence of antimicrobial resistance and clinical disease in calves. No association could be found between levels of AEEC excretion and the presence of diarrhoea.[95] In another study of *E. coli* faecal isolates from 101 healthy and 114 diarrhoeic calves, more healthy than diarrhoeic calves shed eae and stx positive *E. coli.* However, significantly more of the eae and eae/stx strains from diarrhoeic calves were resistant to one or more antimicrobials. No significant difference was detected among the eae and eae/stx strains from healthy and diarrhoeic calves for enterohemolysin production. Serogroups O-negative, O5, O26, and O111 were predominate among both healthy and diarrhoeic calves.[96]

Salmonella

There are over 2,200 reported serotypes of salmonella yet fewer than 2% of these account for approximately 80 % of the disease reported in livestock.[97] In cattle, over 95% of salmonella associated with disease is in serogroups B, C, D, and E. There is significant homology between the serotypes isolated from livestock, poultry, and humans suggesting all species are exposed to a common pool of salmonella. Epidemiological studies indicate significant transmission of salmonella between species.[98-100] Clinical outbreaks of disease in livestock amplify environmental salmonella contamination. Salmonella can survive for 4 years in dry manure.[101, 102] Survival of salmonella in wet manure is dependent on the method of disposal employed. Salmonella can be eliminated from cattle manure in less than 10 days by composting when temperatures of at least 50-55 ^{o}C are attained for not less than a week.[103] In cold manure salmonella survive for over a year.[103] Mammals, reptiles, birds, and insects may disseminate salmonella within and between production units.[104-109]

Salmonella induces a wide spectrum of disease in cattle of all ages ranging from in-apparent sub-clinical infections to acute fulminant bacteraemia, endotoxemia, and death. The variable manifestations of disease reflect the tissue trophisms of different salmonella serotypes and the influence of challenge dose and host immunity. Common clinical signs associated with "salmonellosis" include fever, diarrhoea, anorexia, depressed mentation, and dehydration. Many of the clinical signs are associated with endotoxaemia. Systemic signs of endotoxemia include, fever, tachypnea, tachycardia, scleral injection, leukopenia / leukocytosis and weakness. Some serotypes particularly *S. typhimurium* have a tendency to induce severe inflammation of the bowel mucosa resulting in dysentery, and passage of fibrin and mucosal casts. Fluid, electrolyte, and protein loss may progress rapidly and become life threatening if not corrected. With severe disease animals rapidly become emaciated due to the catabolic state induced by release of TNF-α. Sequelae occasionally observed following invasive Salmonella infections in neonates include septic osteoarthritis and meningitis.

A small proportion of cattle infected with salmonella remain chronically infected. Chronic salmonella infections in cattle are most commonly observed with *S. dublin,* which is host adapted to cattle. Chronic infections with other salmonella serotypes including *S. typhimurium* (B), *S. ohio* (C)*, S. enteritidis* (D), and *S. muenster* (E) have been reported but appear less common.[110-116] Cattle chronically infected with salmonella often shed salmonellae in milk as well as faeces. The prevalence of salmonella carriers in cattle populations is unknown. In one study 5 of 200 neonatal calves infected with *S. dublin* maintained persistent infections.[117] This estimate is likely to be low as the study was not designed to determine the incidence of salmonella carriers and only 5 of 20 suspect calves were intensively sampled for 6 months to verify infection status.[117] In a herd infected with *S. ohio* (Group C) 7% of the herd maintained chronic infections.[116]

Immunity to salmonella changes rapidly during the first 3 months of life. At 2 weeks of age the LD_{50} for some virulent strains is 10^5,[118] at 6-7 weeks 10^7, and at 12-14 weeks 10^{10}.[119] In contrast, administration of 10^{10} salmonella to 24-28 week old calves failed to induce clinical signs of disease.[119] The numbers cited reflect the influence of age on immunity, but should not be interpreted as absolute. Different age predilections, manifestations of disease, and virulence are observed between salmonella serotypes and between different strains of the same serotype.[120, 121] While adults may serve as carriers and a source of infection of *S. dublin* infection for neonates, disease in adults is less common in mature cattle compared to calves. In contrast, *S. typhimurium* tends to manifest disease in an epidemic manner, causing illness in all age groups.

Calves on endemically infected farms are commonly exposed to salmonella in the first few days of life.[122] Salmonella exposure may occur via salmonella contaminated colostrum or milk, surface contamination of teats and udder, personnel, equipment, or the environment. Chronically infected Salmonella carriers may shed 2.5 x 10^8 salmonella in milk per day (25 kg of milk containing 10^5 salmonella per mL).[115] Feeding utensils and personnel often play a significant role in transmitting salmonella between calves.[123] Salmonella infects the salivary glands and is shed in saliva and nasal secretions.[124, 125] Aerosol is not considered the primary mode of transmission and the risk probably varies by serotype. The risk would be increased in *S. dublin* infections as this causes primary respiratory disease. Adequate cleaning and disinfection of feeding and medicating utensils is necessary to remove salmonella contamination. Salmonella is sensitive to most disinfectants, but removal of contaminating organic debris is imperative as the activity of disinfectants is reduced by the presence of organic matter.[126]

Clostridia

Although clostridia are not commonly considered a major pathogen causing NCD there are a number of reports associating clostridial infections with enteritis and abomasitis.

Clostridium perfringens is the most important cause of clostridial enteric disease in calves. Some types of *C. perfringens* (mainly type A) are consistently recovered from the intestinal tracts of healthy animals and from the environment, while others (types B, C, D, and E) are less common in the intestinal tracts of animals and can occasionally be found in the environment in areas where disease produced by these organisms is enzootic.[127] The species is divided into types on the basis of production of the four major exotoxins α, β, ε, and τ. Type A is defined as strains producing α toxin, type B as strains producing α, β and ε toxins, type C as strains producing α and β toxin, type D as strains producing α and ε toxins, and type E as strains producing α and τ toxins. Disease is usually precipitated by management factors that lead to the proliferation of the organism within the gastrointestinal tract or attenuated digestion of clostridial toxins within the lumen of the alimentary tract.

Gastrointestinal disease syndromes reportedly associated with *C. perfringens* infections include haemorrhagic enteritis and abomasitis in calves.[127-130]

Clostridium perfringens Type A has been associated with acute haemorrhagic abomasitis in neonatal calves. Clinical signs include acute abdominal distension, colic, depression, and sudden death. Onset of clinical signs is rapid, affected animals become anorexic, depressed, or restless. Signs of abdominal discomfort are observed in approximately half of the cases and include treading on the spot and kicking at the abdomen. On physical examination splashing and metallic sounds are heard on succussion of the distended abdomen, passage of a stomach tube fails to relieve the distension. Faecal output is reduced and melaena may be observed. Gross pathology may include abomasal ulcers, abomasitis, and abomasal tympany.[131, 132] *Campylobacter* spp. have also been incriminated in other studies. Histopathologic evaluation of abomasums from 38 affected calves at necropsy revealed 31 contained abundant gram-positive bacteria associated with the damaged abomasal mucosa.[133] Campylobacter-like organisms were demonstrated in 9 and *C. perfringens* in 14 of the 38 cases.[133] Trace mineral deficiencies of copper and/or selenium may also be involved in the pathogenesis of the condition in this region.[134] A decreased prevalence of abomasal tympany and ulceration were reported in neonatal calves from herds having a history of these problems following implementation of a *C. perfringens* vaccination program.[134, 135] In a more recent report enterotoxaemia caused by *C. perfringens* Type A was described in 2–4 month old calves with the condition observed more often in beef calves than dairy calves.[136] The disease was characterised by a high case fatality

rate, sudden deaths, lesions of necrotic and haemorrhagic enteritis of the small intestine and, most often, an absence of other clinical signs.[129]

Clostridium perfringens Type B is not commonly associated with neonatal diarrhoea in calves however there is a report from the United States in 1956.[137]

Clostridium perfringens Type C infections are most frequently observed in neonates less than 10 days of age.[138] Newborn animals are typically most susceptible, perhaps because of ready colonisation of the gut by *C. perfringens* in the absence of well established normal intestinal flora.[127] Alteration of the flora by sudden dietary changes may also be an inciting factor in type C infections. Vigorous, healthy calves develop haemorrhagic, necrotic enteritis and enterotoxaemia, often accompanied by evidence of abdominal pain and neurological signs that may include frenzied bellowing, aimless running, tetany and opisthotonus. Death may be peracute, occasionally without other clinical signs, but may also follow a clinical course of several days.

Campylobacter spp.

The clinical significance of *Campylobacter* spp. in calf scours is inconclusive. *Campylobacter* spp. are part of the normal intestinal flora. Experimental challenge studies demonstrated the capacity of *C. jejuni* to cause enteritis in calves.[139-142] However, there is a paucity of convincing reports that demonstrate a causal association in naturally occurring cases.

Protozoa

Cryptosporidium

Two species of *Cryptosporidium* have been identified in cattle: *C. parvum* in the intestine and *C. andersoni* in the abomasum.[143] The two species have morphologically distinct oocysts and differ genetically.[144] *C. andersoni* is a parasite of calves post weaning and has not been associated with NCD.

C. parvum or a *C. parvum* like organism has been detected in 152 species of mammals including domestic animals, wildlife species and humans.[145] Molecular tools for species differentiation have been used to describe a number of distinct intraspecific variants or subgenotypes, many of which appear to be host specific and could represent distinct species.[143, 146] These genotypes include type 1 that is found in human sources and type 2 that is considered to be zoonotic and can be isolated from bovines and other farm animals, such as sheep and goats.[147]

Transmission is faecal-oral by ingestion of an encysted, sporulated oocyst. Transmission can be direct from host to host, by ingestion of contaminated food or water and probably mechanically via flies.[148] A study of oocyst shedding in experimentally infected neonatal calves demonstrated a prepatent and a patent period ranging from 3±6 and 4±13 days respectively.[149] Oocyst excretion has been described as early as 2 days of age, which means that calves are susceptible for infection during or shortly after birth.[150]

Peak shedding of oocysts occurs between 1 and 3 weeks of age and calves up to 4 months of age are most likely to be actively shedding significant numbers of oocysts.[149-152] Infected calves can shed in excess of 10^6 oocysts g^{-1} of faeces.[149, 153] *C. parvum* oocysts have also been isolated from adult cows with herd prevalence ranging from 7-100%.[151, 154-156] These studies were unable to show any increase in the shedding of oocysts at parturition, but this was recently demonstrated in a dairy cows.[157] Mean shedding intensity reported for adult cows has

ranged from 3-900 oocysts g^{-1} of faeces.[156-158] It is likely that carrier cows are a source of infection for young calves, regardless of whether there is an increase in shedding at parturition. Some studies have observed increased cryptosporidium shedding at certain times of year.[152, 154, 159, 160] However this is most likely to be related to climate and management factors such as calving season.

Other pathogens can be involved and are likely to contribute to the severity of the disease. Affected calves can take 4–6 weeks to recover. Cases of cryptosporidiosis in beef suckler herds have been associated with the introduction of dairy calves to beef herds as replacement calves.[143] High mortality rates have been attributed to lack of herd immunity in seasonal calving herds where the transmission cycle is broken. Neutralising antibodies in colostrum and milk are believed to reduce infectivity by immobilising the parasite, blocking invasion, inhibition of adhesion to host cells or direct cytotoxicity to Cryptosporidium sporozoites.[161] High mortality rates have also been associated with concurrent low levels of selenium, inadequate nutrition, presence of concurrent enteric infections and specific management practices.[143]

Oocysts are stable in faeces for many days at room temperature and the most critical factor affecting oocyst survival is the temperature.[162] Using coefficients of inactivation it was calculated that the time to reach 99.9% inactivation of oocysts at 4 ^{o}C in silt loam was over 4000 days, compared to 336 days in a silty clay loam soil at 30 ^{o}C[163] (see Table 1). However this study used soil that was air-dried at 37 ^{o}C, possibly inactivating many soil bacteria. Hence it represents the potential for oocyst survival under ideal conditions. Another study demonstrated nearly a total inactivation of oocysts in soil and faeces at 25 ^{o}C after 8 weeks and an increased rate of degradation in soil containing natural microorganisms.[164] This study showed that oocysts did survive for longer than 12 weeks at 4 ^{o}C. Oocysts have been shown to survive for weeks in soil under natural conditions at near freezing temperatures until freeze-thaw cycles appeared to inactivate them.[165] Drying of oocysts has been shown to dramatically reduce their viability and infectivity in mice.[166, 167] The susceptibility of oocysts to heat and drying make survival in the environment for long periods of time less likely in many parts of Australia. This was confirmed by a recent study that failed to find oocysts in 20 soil samples from 2 dairy properties with a greater than 30% infection rate in their calves, including samples taken from the calf rearing pens.[168]

Table 1: Inactivation of *C. parvum* oocysts at different temperatures and in different soil types (from Jenkins 2002[163])

Media type	Temperature (°C)	Days to reach 99% inactivation[1]
Silty Clay Loam	4	2302
	20	622
	30	336
Silt Loam	4	4063
	20	2302
	30	1096
Loamy Sand	4	2228
	20	690
	30	634
Water	4	895
	20	231
	30	211

Oocysts can enter watercourses and ground water by direct contact with cows or from run-off of rain or irrigation water from pastures and manure storage areas.[143, 169] *Cryptosporidium* oocysts have been shown to survive in water for at least 12 weeks at 4 °C.[164] Viable oocysts can be found in run-off irrigation water and river water.[170, 171] Oocysts are resistant to chlorination of water and most disinfectants.[143] They have also been shown to survive in silage.[172]

Wildlife may be a significant reservoir for *C. parvum* and act as a method of amplification and infection in the environment. In California the annual grassland and oak woodlands are inhabited by ground squirrels (*Spermophilus beecheyi*) that shed substantial numbers of *C. parvum* oocysts during spring and summer. These oocysts have been shown to have genotypes similar to those obtained from beef calves.[173] Populations of mice and voles on a farm in Warwickshire, UK have also been implicated in the transmission of *C. parvum*.[154] A range of native and introduced Australian mammals are hosts to *C. parvum*.[145] The genotype of many of these *C. parvum* is unknown and phylogenetic analysis is required to elucidate the role of wildlife in the epizootiology of *C. parvum* infections in Australia. *C. parvum* can also be transmitted by flies.[174]

Studies have shown a large variation between herds in the percentage of animals infected and the levels of oocysts shed. In one study involving dairy herds throughout the USA there was a significant difference in the percentage of herds with cryptosporidiosis between regions.[175] Many studies have demonstrated that more than 50% of calves can be infected by *C. parvum* before 30 days,[151, 176] even when the calves are housed in individual pens.[177] However one study on a cow-calf operation in Alberta showed a very low infection rate.[178] This difference is unlikely to be due to the testing procedures. As other studies have demonstrated high infection rates beef herds[151, 158] it is more likely that the difference between herds is a reflection of their stocking rate and environmental conditions.

[1] Calculated using a coefficient of inactivation calculated from different sampling times over 156 days

Calves generally become infected between 1 and 4 weeks of age and display clinical signs for 4-14 days. Animals of all ages can be infected but diarrhoea is mainly associated with calves pre-weaning.[151] Cryptosporidial infections are asymptomatic in bovines older than 4 months. *C. parvum* mainly infects the distal small intestine, but lesions are also found in the caecum and colon and occasionally the duodenum (reviewed in Graaf[179])[180]. The parasite invades the superficial cells of the mucosa in the intestine but remains extracytoplasmic, surrounded by an invagination of the host cell membrane. Sexual and asexual phases develop and recent research has indicated that extracellular developmental stages result in autoinfection, sporulating within the intestine and immediately infecting adjacent cells.[181] This can result in protracted clinical illness and relapses. The ability to autoinfect results in huge parasite burdens following very small infective doses.

Parasitic invasion of the mucosa results in epithelial destruction and mild to moderate villus atrophy, with microvillus shortening and destruction. This leads to impaired nutrient digestion and transport and a resulting malabsorption diarrhoea. Respiratory cryptosporidiosis has been reported in humans, and in one case in a calf but the significance of this is thought to be low.[179, 182, 183]

The faeces can vary in consistency from loose to watery and may contain undigested milk, blood, mucus and bile.[184] Affected calves often show no sign other than diarrhoea but can show depression, dehydration and anorexia.[176] Pyrexia and tenesmus has been noted.[185, 186] Variable levels of morbidity have been reported and mortality is generally low.[176, 185, 187] In one report of outbreaks on dairy farms in Australia the mortality was reported to be up to 50% but other agents were also implicated in this report.[187]

Giardia

Giardia duodenalis has been found in beef and dairy cattle worldwide and has been isolated from dairy calves in Western Australia.[143, 168, 188, 189] Affected calves are at least 2 weeks old, and often older than 1 month of age, with infection often becoming chronic and lasting for several months.[150, 168, 178, 190, 191] Giardia has a pre-patent period of 7-8 days[40] and the delayed interval between birth and infection is likely to relate to high levels of colostral protection against Giardia, but low protective levels in milk.[192] Many calves were shown to have a poor specific immune response to the infection, accounting for the chronicity of the infection.

The prevalence rates and dynamics of infection within a herd are affected by differences in management and climate. A prevalence of 100% has been demonstrated in dairy calves.[190] In a beef herd in Canada the percentage of calves infected peaked at 85% at 5 weeks of age and by 27 weeks 21% of the calves were infected. The geometric mean number of cysts shed in the faeces also peaked when the calves were 5 weeks of age at 2230 cysts g-1 of faeces and by 27 weeks had reduced to 2 cysts g-1 of faeces.[178] In a dairy herd in the Netherlands the peak infection rate occurred at 4-5 months of age with peak shedding occurring between 3 and 8 months of age.[150] Infection rates in adults are lower, but a peri-parturient increase in cyst excretion has been demonstrated.[143]

Giardia cysts are commonly found in watercourses with increased prevalence associated with access of cattle to the watershed.[169] Giardia cysts are infective after 18 weeks in mixed slurry, 11 weeks in water, 7 weeks in soil and 1 week in cattle faeces at 4-5 ^{o}C.[164, 193, 194] Temperature has a significant negative influence on survival with no infective cysts detected in any of these media after 1 week when the temperature was above 20 ^{o}C. Freezing at -4 ^{o}C also killed cysts in less than a week in soil, water and cattle faeces.[164] Consequently in Australia it is likely that calf-calf infection is more significant than environmental infections. This was recently confirmed by a study of 2 dairy properties in Western Australia with more than 80% of the calves

infected, where cysts were not isolated from any soil samples, including those taken from the calf pens.[168]

Giardia is often found in diarrhoeic calves in association with other pathogens, but it's relevance as a pathogen in its own right is unclear. Several authors have documented cases of diarrhoea where giardia infection has been implicated as the causative agent either by itself or in conjunction with *C. parvum and rotavirus.*[190, 195, 196] However infection rates are high in asymptomatic animals, and two studies have shown rates of infection in calves with diarrhoea to be similar to or lower than rates of infection in asymptomatic calves.[150, 151] whereas 1 study showed an increased prevalence of giardia cysts in diarrhoeic calves.[197] The causes of diarrhoea were not fully investigated in either of these studies

Treatment of affected calves with fenbendazole resulted in a decrease in the duration of episodes of diarrhoea, but no difference in the number of episodes.[196] Therefore it is likely that giardia have a secondary role in the pathogenesis of diarrhoea. Post mortem studies 7 days after treatment with fenbendazole demonstrated an increase in the intestinal brush border surface area and enzyme activity, however no changes were observed to the intestinal villus height or crypt depth.[198]

Giardia should only be considered to be the primary cause of NCD in the absence of any of the major enteric pathogens, or when histopathological lesions are demonstrated.

Coccidiosis

Eimeria spp. are found worldwide. Thirteen species have been reported in cattle, all localised to the intestine.[199] *E. bovis* and *E. zuuernii* are the most common pathogenic species in temperate climates although *E. alabamensis* is increasingly becoming recognised as a cause of diarrhoea in Europe.[200-203]] *Eimeria alabamensis* and *Eimeria brasiliensis* have also been reported as pathogenic species in Queensland.[204]

Transmission is faecal-oral. Infected animals pass unsporolated oocysts in their faeces that sporulate and become infective. The sporulated oocysts are protected from the environment by a double cyst wall (reviewed by Step.[205]). Sporulation is dependant on temperature and moisture, but in warm conditions can occur within a few days. The pre-patent period of the 2 main pathogenic species is 15-20 days, and the patent period around 11 days. *E. alabamensis* has a pre-patent period of only 8 days and a patent period of 5 days. Many millions of oocysts are present in a faecal specimen with *E. alabamensis* infection.[200]

Moist temperate cool conditions favour sporulation and oocysts can survive for several years.[40] Infection has been reported from hay made from pastures that calves infected with *E. alabamensis* had been grazed on 2 years previously.[206] Hay was shown to be infective 8 months after harvest. Sporulated oocysts can resist freezing to –8 ^{o}C for several months, but are destroyed by high temperatures and dry conditions within a few weeks.[207] Oocysts have been destroyed by ensiling, but only where temperatures reach 25 ^{o}C.[208] There are no published reports of transmission of via silage.

Calves start shedding at about one month of age and shed for 3-4 months. *E. bovis* and *E. zuernii* schizonts first reproduce in the lower small intestine and then produce second generation schizonts and gamonts in the caecum and colon, where they attack crypt cells.[199] These latter stages induce both local and more extensive lesions, and are capable of killing stem cells and impairing cellular repair.

Outbreaks of disease are related to overcrowded and confined conditions. Up to 95% of infections are sub-clinical causing decreased growth rates that are often unnoticed.[200] Clinical disease can be chronic or acute and is generally found in calves aged 3 weeks to 6 months, although animals of 2 years of age or older may be affected. In beef cattle the most common reports of clinical disease are associated with weaning stress.[40] The disease is usually self-limiting without reinfection.

Acute disease is classically associated with tenesmus and dysentery, but these signs are only observed in 50% of cases. Pyrexia, dehydration and anaemia may also be observed. Chronic disease is often under-diagnosed.[200] Calves appear weak and listless with pasty faeces, drooping eyes and a staring coat. Faecal oocyst count is low or negligible. Disease results from continual re-infection due to a heavily contaminated environment and a partial immune response barely holding the parasite in check.

Parasitic agents

Although parasitic agents can infect young beef calves these are not recognised as a cause of neonatal calf diarrhoea and are seldom a problem prior to weaning unless nutrition is poor.[40] There are both managemental and pharmaceutical methods of control, but the details of these are outside of the scope of this review.

Other aetiology's

Necrotising (or necrotic) enteritis was reported in suckler beef herds in Scotland in the mid 1990's.[52, 209] Affected calves were 2-3 months of age and presented with by severe diarrhoea and dysentery associated with oral and nasal lesions. Pneumonia and nephritis was observed in fatal cases and whilst the morbidity was low affected calves seldom recovered. Despite histopathological lesions being indicative of BVD no virus was isolated from the calves and the aetiology was not determined. No further reports to have been published on this condition.

Nutritional diarrhoea

Many producers report an increase in scours associated with lush feed. This presentation often has low morbidity and mortality.[2] However there is no documented research on this. Scours has not been reported as a problem in several studies where calves have been fed a large volume of milk (16 to 20% of body weight/day) or allowed ad libitum access to milk.[210, 211] However in studies where calves are also infected with enteric pathogens the diarrhoea and depression was exacerbated by feeding normal amounts of whole cows milk in the early stages. Moreover deliberate underfeeding of healthy calves has been shown to predispose to diarrhoea.[184]

Studies in Scotland have shown that poor clotting ability of milk is associated with diarrhoea and abdominal distension in calves aged 1-3 weeks of age in beef suckler herds.[212-214] Milk incubated with rennet should clot within 7 minutes , the milk from the affected cows took at least 1 hour to clot and in some cases more than 24 hours. Diarrhoea may be due to the rapid passage of undigested milk through the bowel, or to infection by enteric pathogens facilitated by the conditions created in the bowel. Rotavirus was isolated from 18% of samples and *E. coli* was cultured from all samples, although this is unlikely to be relevant. Calves responded to treatment with 30 mL of 1 molar solution of calcium chloridel administered three times daily p.o. and relapsed when this treatment was stopped.

Dams of calves that were not scouring had milk with normal clotting ability. Milks with poor clotting ability were shown to have low ultrafilterable calcium levels and low total magnesium

levels.[214] The majority of the milk samples clotted when 100 µL of 1 mol per L calcium chloride solution was added prior to the addition of rennet. The exact cause of the impaired clotting ability was not determined. The affected cows had normal serological mineral profiles although the diet of one group of affected cows was shown to be low in calcium[212, 213]. After a mineral mixture containing additional calcium was added to the diet of these cows the clotting time was reduced to ≤12 minutes, treatment of the calves was stopped and there was no recurrence of clinical symptoms.[212] On 10 of the affected farms hay was the principle fodder and there was a higher incidence of the problem in beef cows on farms where no minerals were fed.[213] Five outbreaks (9%) occurred when cows were at grass. A similar syndrome has also been reported from Poland.[215]

Risk factors associated with neonatal calf diarrhoea and calf mortality

Few studies have looked at risk factors associated with NCD or calf mortality in grazed beef cows in climates similar to Southern Australia. No research could be found relating to risk factors for diarrhoea in the Australian beef industry.

Enterprise level risks

The variation in incidence of diarrhoea between herds has been attributed to the genetic composition of the cattle, the environmental conditions on the property, variation in the degree of exposure to pathogens and individual herd management practices.[216]

Stocking Rates

High stocking rates at calving and use of one calving area are recognised as major risk factors in neonatal calf diarrhoea[217-219] Newborn calves that remain in the calving area further increase stocking rates, and this may increase stress and decrease the transfer of immunity[220] as well further assist in the transmission of infectious agents. For example high stocking rates have been associated with increased shedding of *C. parvum*[160] and larger herd size has been associated with an increased risk of *C. parvum* infection.[159, 175]

Calves born into a contaminated environment may become infected during or shortly after birth, but remain clinically normal and shed enteropathogens. This further increases the environmental load of infectious agents. Clinical cases of diarrhoea become a source of infection not just for other calves, but also adult animals and the environment.

Time of calving and length of calving season

Radostits[218] noted that in Canada the practice of autumn calving onto open pasture minimised the exposure of the calf to infectious agents. This may have been due to the decreased stocking intensity, or climatic effects on the pathogens, but this was not clarified. In California a study of faecal shedding of *C. parvum* in beef herds showed no difference in shedding pattern related to calving season.[160] Shedding of *C. parvum* oocysts was increased with longer calving periods. A longer calving season is likely to increase the environmental pathogen load unless calving paddocks and young calf paddocks are regularly changed, especially in moist cool climates.

The time of calving will also result in different weather patterns. Although no study has looked at the specific relationship between weather and NCD in pasture based cattle, there are several references to the provision of shelter, as part of minimising stress on the newborn calf.[1, 217, 219] Cold, wet, windy and hot weather causes cows to move to shelter and shade, concentrating cows and calves in small, contaminated areas. In hot weather cows also camp for longer in the shade. A study in dairy calves has shown that calves exposed to the prevailing winds and fed ad

lib for 2 -6 weeks after birth, had reduced weight gains compared to those provided with a dry, draught free fresh air environment. Exposed calves were also more susceptible to disease.[221]

Winds lead to dehydration, even in winter, and poor clearance of mucus from the airways. Combined with water they also lead to chilling. Solid structures provide little protection as eddies form in their lee, unless they are 4 sided, in which case they can lead to poor ventilation. The best shelter comes from semi-solid structures, such as open hedges, which provide shelter for 1 X height upwind and 7 X height downwind.

Longer calving seasons are logically likely to lead to a build-up of pathogens in the calving paddocks in a favourable environment. Increased mortality from diarrhoea has been associated with an increased duration of the calving season.[3] Another study in Quebec determined that herds with diarrhoea problems had a longer calving period than those without diarrhoea problems.[222]

Herd structure

Calves born to 2 and 3 year old cows have a higher death rate than those born to older cows.[3, 223] As a consequence the higher the percentage of heifers in the herd the greater the mortality rate from NCD. The risk of diarrhoea in calves born to heifers has been shown to be 3.9 times greater than that in calves born to cows.[216] Heifers have a poorer mothering ability, lower colostrum quality (see p 45) and an increased risk of dystocia. These factors are all likely to contribute to the increased mortality. It is also likely that heifers are kept at a higher stocking rate prior to calving to allow better observation and are consequently exposed to a greater environmental pathogen load. It has also been shown that where cows and calves are shedding rotavirus and coronavirus, calves from carrier heifers are more likely to develop clinical disease than calves born to carrier cows.[28]

Biosecurity

Mortality due to NCD has been shown to increase in farms purchasing replacement calves that were less than 4 weeks of age.[3] The stress of transport and arrival at a new location may well increase shedding or allow a calf to succumb to clinical disease, resulting in an increased environmental load of pathogens. Calves from a different environment may also introduce new pathogens onto a property, thus causing significant disease in a susceptible population.

Grazing practices

No difference in faecal shedding of *C. parvum* was observed in beef herds in California with a grazing rotation lengths < 1 week, 1- 4 weeks or greater than 4 weeks.[160] There is no other published research studying the effect of grazing practices on NCD.

Other Risks

An increase in the total number of other agricultural animals on the farm increased the risk of *C. parvum* infection.[159]

Mixed Infections

Many studies have detected mixed infections of pathogens in calves with NCD, and a range of major pathogens are likely to be endemic in many herds.[6, 9, 11, 12, 26, 224] Several studies have reported that infections with multiple enteropathogens are more commonly observed in diarrhoeic calves than healthy ones.[12, 57] Mixed infections are more common in younger

calves, with a significant age-associated decline in accordance with the age-dependent susceptibility of calves to all major enteropathogens except salmonella.[11] The most common pathogens found in mixed infections are rotavirus and cryptosporidium, probably reflecting a higher prevalence of these pathogens although this may be biased due to the ease of detection of these pathogens.[11, 26] It is likely that mixed infections may be a determining factor for clinical disease. Where no single pathogen predominates this may indicate a susceptibility to disease due to faulty husbandry conditions. Few studies have specifically looked at the pathophysiology, pathogen shedding, severity and duration of mixed pathogen infections, and those published have mainly studied the relationships between rotavirus and ETEC and between coronavirus and coccidia.[24, 225, 226]

Management of cows pre-calving

An increase in mortality from diarrhoea was noted when heifers and cows were run as one group prior to calving.[3] This may be because heifers were less able to compete for feed than the adult cows. However in this study herds that kept cows together prior to calving also tended to calve on the same ground, and it is likely that by the time calving commenced there was a build up of pathogens in the environment.

Nutrition of pre-parturient cows

There are no reports of a direct effect of pre-parturient nutrition on the subsequent incidence of NCD in the calf. High feed levels pre calving will increase calf birth weight but does not increase the risk of dystocia unless the animals become obese.[227-230] Poor nutrition resulting in weight loss is associated with prolonged labour, increased dystocia, increased perinatal mortality, reduced calf growth rates and has detrimental effects on the subsequent fertility of the cow.[228, 231] Where feed is limited it is important to run first-calf heifers separately from older cows to meet their higher energy requirements.

Management at birth

Make up of the calving group

No increase in mortality was observed when cows and heifers calved on the same ground despite an increase in mortality when heifers and cows were wintered in the same group.[3]

Time of calving

Calves born to animals that calve later in the calving season are more likely to develop diarrhoea.[216] As the calving season progresses it is also more likely that calves will develop diarrhoea at a younger age.

Management of calving paddocks

Increasing the drainage of the calving area has been related to a decrease in the mortality of calves due to NCD.[3] Shelter in the calving area had no relationship with mortality levels, however in these herds the cows were moved to a nursing areas after 24 hours and there was a significant relationship between shelter in the nursing area and mortality levels.

Dystocia

The 2 major causes of dystocia are disproportionately large calves and reduced maternal pelvic area. Of these 2 factors the former is the most significant.[232, 233] Heifers have an increased

rate of dystocia and dam pelvic diameter is an important determinant of dystocia.[234, 235] Studies have shown that nutrition of heifers during the period of 7 to 13 months when pelvic area is set has a profound effect on subsequent calving ability as a two-year-old.[236, 237] Pelvic measurements can be used to identify abnormally small or abnormally shaped pelvis's. Large frame size of the dam correlates with a reduced risk of dystocia, however continued selection for large frame size tends to select for larger birth weight and dimensions of calves.[238] The risk of dystocia in heifers is also increased by poor nutrition in the last trimester.[228] Age at first calving for heifers is not correlated with risk of dystocia as long as heifers are fed and managed to achieve appropriate growth and stature prior to calving.[239-241] Appropriate nutrition and management of replacement heifers to achieve appropriate size and stature at parturition reduces maternal and neonatal losses by reducing the incidence of dystocia.

Feto-pelvic incompatibility accounts for a lower proportion of dystocias in multiparous cows but weak labour secondary to hypocalcaemia, uterine torsion, and incomplete cervical dilation are more common.[242]

Management variables that influence the risk of dystocia and perinatal mortality include stocking density of pre parturient cows, timing of calving, and cow grouping. In a study of 123 beef herds the dystocia rate was highest for cows housed in a barn and decreased progressively through barn/yard, barn/pasture and pasture only calving location categories.[234] The most common cause of dystocia in penned heifers was vulval constriction, while dystocias in paddocked heifers were most commonly associated with mal-presentations.[243] Calving beef heifers 4-6 weeks prior to cows has been recommended to allow the heifers longer to recover and conceive after calving than cows.[244, 245] In a herd level comparative study this practice was associated with a higher incidence of dystocia and stillborn calves, however no assessment was made of heifer growth rate or weight and it is likely that earlier calving heifers were not as well grown.[234] Running heifers separately from cows prior to calving reduced dystocia, with the reduction proportional to the length of time the heifers were separate. This was presumably due to better nutritional management of the heifers.[234]

Foetal variables that influence the risk of mortality include sex, size, and number. Twins, and bull calves are more likely to die at birth.[223, 246] Low and high birth weight calves are at greater risk of mortality than average birth weight calves.[223] Small calves experience greatest mortality at parities greater than one and large calves at first parity.[239]

Dystocia is a risk factor for pre-weaning mortality with several studies showing that over 40% of pre-weaning deaths occurred in calves born to cows experiencing dystocia.[222, 223, 247] It has not been shown to be a risk factor for diarrhoea in pasture based beef systems,[216, 248] but has been associated with NCD in more intensive and dairy systems.[249, 250]

Calves that experience dystocia are likely to have oedema of the head and tongue, making suckling difficult. They are also weak and exhausted and likely to be recumbent for a longer period of time and expose themselves to more faecal pathogens.[250] Dystocia affects the uptake of immunoglobulins by the calf (see p 47) and calves that survive dystocia are between 2.4 times more likely to become sick in the first 45 days of life.[248]

The role of colostrum

Calves need to intake 100g of IgG within the first 12 hours.[251] Ensuring early intake of colostrum and sufficient absorption of IgG reduces neonatal morbidity and mortality.[252-254] In one study levels of IgG < 800 mg/dl at 24 hours after birth were associated with between a 3.2 and 9.5 times increase in morbidity and a 5.4 times increase in mortality. However good colostrum management will not be sufficient in poor environmental conditions as demonstrated

by a 10-year study of 3,479 Holstein replacement heifers. This showed that the relative risk of mortality associated with low serum protein concentration (< 5 g/dL) was not affected by the base mortality incidence for each farm allowing extrapolation to different farm environments with a different baseline risk.[255] The baseline mortality for calves with adequate passive transfer will depend on the pathogen types and strains, nutrition and hygiene on each farm, and where the challenge is high or the husbandry is poor, calf mortality can be unacceptable. The mortality of calves with a serum protein of 4.0 g/dL will be approximately double baseline mortality and for calves with a serum protein of 3.5 g/dL mortality approximately 4 times this level.

Despite the recognition of the role of colostrum and the factors that contribute to FPT in calves for over 80 years,[256] many studies show that it continues to be a problem in beef and dairy herds.[128, 220, 257-261] Calves are only able to absorb immunoglobulins for a limited time after birth and the subsequent serum Ig concentration is determined by the perinatal state of the calf, timing of colostrum ingestion, and the mass of Ig consumed.[262]

Factors affecting colostrum qualities of beef cattle

There is a large variation in colostrum immunoglobulin concentrations between individual cows and it has been shown that calves born to cows with lower colostrum immunoglobulin concentration have an increased risk of mortality.[263] However numerous studies[264-267] have shown that there is little association between the colostral Ig levels of the cow and the serum Ig of the calf indicating that there are a variety of influences; genetic, environmental and physical on the uptake of colostral antibodies by the calf. This could explain the lack of association found between colostrum management and the risk of diarrhoea,[250, 268] although it is also possible that few animals had low serum Ig in these studies.

At the herd level colostrum quality can be affected by breed, parity, nutrition, and climate. For individual cows the biggest determinant is volume is a dilution of immunoglobulin concentration as the volume of milk increases.[269] When the calf serum Ig level was considered a repeatable trait of the cow the IgG_1 and IgM concentration at 24 and 36 hours showed moderate repeatability (range .38 $\pm$ .13 to .52 $\pm$ .10).[266] It is likely that the deviation of a calf's serum Ig levels from the population average may be used as a predictor of future deviations in serum Ig for that dams calves.

Effect of breed

Many studies of colostrum and calf Ig levels in specific breeds have looked at minor breeds and crossbreds or used small numbers.[270, 271] Where Angus and Hereford breeds have been studied the results are often not significant or inconsistent.[266, 272-274] Variation in the volume of colostrum produced and the concentration of immunoglobulin in the colostrum between breeds of cows has been attributed to differences in the onset of lacto-genesis, and the resulting dilution of colostral immunoglobulins.[275] Whilst this study showed a significantly greater decline in serum IgG of pre-partum dairy cows compared with beef cows, the colostrum of dairy cows had lower concentrations of IgG due to the much larger volume. Some studies have shown very low volumes of colostrum in beef cows. In one survey 75% of 2-year-old Hereford heifers produced less than 750 mL of colostrum immediately after calving,[219] and in another study the mean volume of colostrum in fully fed Hereford Cross cows was 1655 mL.[276] It is likely that nutrition and the age of the dam have a greater effect on the volume of colostrum produced than breed.[277]

Inheritance studies have shown that the breed of the sire and the dam has an effect on the serum IgG_1 levels of the calf and that breed of sire also affects the IgM level of the calf.[266, 273, 274] Variation has also been shown within lines of the same breed.[273] A moderate heritability

was demonstrated in the serum IgG_1 levels of the calf at 24 and 36 hours after birth.[266] This result was not substantiated by a later experiment at the same institute, but when the heritability was considered as a dam trait it was estimated at .27 $\pm$ .17 indicating that greater progress would be achieved if selection pressure was put on the dam.[273] Recent studies have shown an increased incidence of FPT in specific genotypes with different haplotypes determining receptors for neonatal Ig absorption.[278, 279] This would substantiate the evidence that FPT is more prevalent in specific lines of cattle rather than breed per se.

Effect of parity

Several studies in beef[266, 280] and dairy cows[269, 281, 282] have shown that first and second calving cows have a lower immunoglobulin concentration than cows of third parity and above. Similarly it has also been shown that calves born to beef heifers have a significantly lower mean concentration of serum IgG compares with that of calves born to cows.[216, 272, 274, 280] Where calves from second parity cows were also evaluated this was also true.[273]

Effect of nutrition

There are many studies on the effect of nutrition on colostrum immunoglobulin levels. Several studies on beef cows and heifers have either shown no effect of poor nutrition, or non-significant trends towards higher IgG levels with pre-partum nutritional restriction.[283-286] In these trials animals were fed diets with restricted protein levels[283, 284] or restricted protein and energy[285, 286] for at least 100 days before calving, with dietary restrictions up to half of recommended levels (ARC or NRC). Other studies have shown an increase in IgG levels and a concurrent decrease in colostral volume with protein restriction.[272, 287] This is likely to be a direct volume response as shown by other studies of colostral IgG concentration.[269, 275] One study showed that low body condition of 2-year-old heifers at calving had a significant negative influence on calf serum immunoglobulin concentrations, but this finding was not repeated in older cattle of low body condition score.[272] This was the only study in which calves from heifers with normal births were allowed to suckle unassisted. It is likely that poorer condition animals had less colostral volume, and also the colostrum would be “thicker” and more difficult to suckle especially in heifers, resulting in decreased IgG levels in the calves. There was no significant effect of dietary restriction on IgM in any of the studies where it was measured.[272, 284, 286, 287]

There is some evidence that protein restriction in the last third of gestation may affect uptake of IgG by the calf.[284] Calves were fed 1 L of reconstituted colostrum previously collected from pluriparous dairy cows. However no relationship was found between concentrations of IgM in the calf sera and the daily crude protein intake of the dams. This finding was attributed to the selective absorption of IgM in newborn calves, which is highly efficient when colostrum intake is low. IgM is the primary immunoglobulin that provides protection to the neonatal calf during the first few days of life, and it has been suggested that the efficient absorption of IgM is an adaptive measure that provides immunity to calves even when they are hypogammaglobulinaemic.[288, 289]

It was proposed that the reduced uptake of IgG_1 and IgG_2 was due to underdevelopment of jejunal absorptive cells in calves from dams fed the low-protein rations. Reduced uptake of IgG by calves from protein restricted dams was not replicated when calves were fed 1 L of colostrum from a beef dam.[285] This study did show an increase in serum cortisol and decrease in T_3 concentrations in calves from dams with restricted nutrient uptake, suggesting that endocrine compensation occurs in calves in response to the nutritional stress in their dams. It was suggested that as cortisol and T_3 are necessary for the maturation of the intestinal epithelium and this may have been responsible for the results shown by the calves from beef heifers.[284]

None of the studies discussed above include large numbers of animals and it is likely that this may have compromised their ability to detect clinically relevant treatment effects (low statistical power). It can be concluded that dietary restriction of the dam prior to calving does not affect the immunoglobulin levels in calves' sera after absorption of colostrum. However it appears that there are compensatory mechanisms demonstrated where animals are nutritionally restricted with a trend towards increased levels of immunoglobulins and enhanced absorption of IgM by the calves.

Selenium supplementation has been shown to increase the IgG levels in the colostrum of selenium deficient cows.[290] Colostrum quality may also be affected by serum copper levels. IgG levels of calves were measured in 10 herds in the western states of the USA.[128] In one herd 44% of calves had serum IgG levels less than 1000 mg/dL (FPT) and 72% of calves had serum IgG levels less than 1500 mg/dL (partial FPT). This was notably worse than the other herds in the study. Serum copper was measured in 20% of the cows in each herd. The mean copper level of the cows in the herd with the high proportion of calves with FPT was 28 ppm with 95% of the cows having a blood serum level < 40 ppm and classified as deficient. It is not possible to determine whether the low copper was affecting colostrum quality, the uptake of colostrum from the gut lumen or whether the calves were too weak to suckle properly. However in areas of copper deficiency calf serum IgG should be monitored in the face of an outbreak of neonatal disease.

Other factors.

It has been shown that the shape of the dam has a significant effect on the time that the calf will take seeking the teats after standing.[291] Cows were classified into having a "good shape" where their udder and teats were on a similar or higher level than the xiphisternum, or having a "poor shape" where, due to the size of the abdomen or the udder, the xiphisternum was the highest part of the dam's underbelly. Calves born to dams with a "good shape" had an average teat seeking time prior to first suckling of 17 minutes whereas calves born to cows with a "poor shape" took 40 minutes to find the teats.

Studies have shown a seasonal variation in the Ig levels of calves after colostrum feeding, being lower in the winter in cold climates and lower in the summer in hot climates.[259, 265, 292] Colostrum immunoglobulin concentration is reduced in hot and cold weather,[293-295] and this is exacerbated by calves also being less willing to suckle in extremes of temperature. Heat stress results in smaller calves that may be less vigorous,[296] and one study has shown that calves subjected to a cold and wet environment had a slower rate of colostral absorption,[297] although the serum Ig at 24 hours was not significantly different from the control calves. In that study the calves exposed to cold temperatures were fed colostrum via an oesophageal feeder. The effect would be exacerbated in cold-stressed calves, as they are less likely to suckle voluntarily.

Factors affecting colostrum uptake by the calf

There is some variation in the published research as to the level of serum IgG that indicates adequate passive transfer, but most researchers use values of 10 g/L (1000 mg/dL).[1, 298-300] Some researchers use <1000 mg/dL as a predictor of FPT and >1500 mg/dL as a predictor of adequate passive transfer, classifying the intermediate group as partial FPT.[128] Several studies have used <800 mg/dL as a predictor of FPT and >1600 mg/dL as a predictor of adequate passive transfer,[253, 254] but 10 g/L is the cut-off used in most studies of FPT.

Amount of colostrum and time of intake

The age of the calf when it receives its first feed and the amount of immunoglobulins received will influence the time of closure of the intestinal permeability to colostral immunoglobulins and the final serum immunoglobulin levels of the calf.[301, 302] Cessation of absorption occurs by 24 hours in calves that receive a full feed of colostrum within the first 4 hours after birth. When the colostrum volume is less than 2 L, the gut will remain permeable for a longer time and the rate of absorption will increase in response to a subsequent feed. If the calf is older than 12 hours when it receives its first feed there is a significant increase in the possibility of the calf being agammaglobulinaemic. It is likely that the time of closure is related to the immunoglobulin concentration of the colostrum as well as the volume fed, but the immunoglobulin concentration of colostrum was not measured. Studies in dairy calves have shown that there is a significant increase in the absorption of IgG_1 when calves are fed 4 L of high Ig colostrum at birth rather than 2 L.[262] When 2 L or 4 L of low quality colostrum at birth were compared there was no significant difference in the rate of absorption, but there was an increase when an additional 2L of low quality colostrum was fed at 6 hours after birth.

Increased supervision of calving cows and early intervention to give colostrum to calves not suckling within 6 hours has been shown to reduce the number of calves with FPT.[220]

Effects of dystocia on colostrum absorption

Decreased levels of IgG in calves experiencing dystocia have been observed in several studies.[272, 273] This may be partially due to inadequate colostrum intake due to decreased vitality of the neonate and a slower time to stand and suck. Calves that have experienced severe dystocia may also have oedema of the head and tongue leading to a decreased ability to suck. Respiratory acidosis does not lead to a decrease in the efficiency of absorption of immunoglobulins.[298] Odde[272] showed a significant effect of calving difficulty on absorption even when all calves were supplemented with colostrum.

Other factors affecting Immunoglobulin levels in calves

Clinical mastitis in the dam at the time of calving has not been associated with FPT.[303]

Management to ensure adequate colostrum uptake

Administration of colostrum to dairy calves within 6 hours of birth has been shown to significantly reduce the incidence of FPT.[260] Routine administration of colostrum to the newborn beef calf is disruptive and delays the time until first sucking[304] but will decrease the incidence of FPT.[264, 305] This procedure has little benefit where FPT is minimal and the result is only an increase in Ig above 10 g/L. Where dairy colostrum was fed this was also shown to be a biosecurity risk.

It is likely that there is benefit in feeding colostrum to high-risk calves. Colostrum should be fresh and refrigerated or frozen. Storage of colostrum in the refrigerator for more than 24 hours will result in a decreased amount of IgG absorbed and levels may lead to FPT if stored for more than 48 hours.[306] There is no decrease in the IgG absorbed from colostrum frozen for a short period,[307] but long term storage in field conditions has not been evaluated.

Evaluation of colostrum

The quality of colostrum has traditionally been measured by colostrometers, which measure the specific gravity of the liquid. The reading needs to be adjusted for temperature. Field experience has shown that it is most accurate for diagnosing samples of moderate or inferior quality but may indicate erroneously high readings for samples in the superior range.[267] The specific gravity of

colostrum is more closely associated with colostral protein concentration than IgG1 concentration, differs between breeds, and is influenced by lactation number, month of calving, year of calving, and protein yield in the previous lactation.[293] It needs to be remembered that the markings on a colostrometer are calibrated for Holstein cows. However as a comparative field test it is a useful tool, where calves are force-fed colostrum. In a non-interventionist situation the dams colostral Ig is not a good predictor of the level of immunity that the calf will attain.[264, 265]

Biosecurity

Several diseases have been shown to be transmitted in colostrum including EBL and Johne's disease.[308, 309] It is also possible that faecal pathogens such as cryptosporidium, salmonella, or enteric viruses could be present in colostrum.[310, 311] Consequently the use of colostrum from cows from a different property should not be recommended. Pasteurisation of small batches of colostrum at 67 ^{o}C or less causes minimal decrease in the Ig concentration of colostrum and has little effect on the subsequent serum Ig in calves at 24 hours of age,[312, 313] but at this temperature *Mycobacterium paratuberculosis* may survive.[314, 315] Pasteurisation of large batches of colostrum or at higher temperatures (76 ^{o}C) does result in a significant decrease in the serum Ig levels of calves at 24 hours. There are no published studies on the subsequent disease levels in calves fed pasteurised colostrum.

Colostrum supplements and replacers

Colostrum supplements and replacers are derived from serum protein, milk, colostrum, and chicken eggs.[316] The majority of independent efficacy trials have not demonstrated adequate serum immunoglobulin levels.[262, 317-319] Moreover the addition of these products to colostrum has decreased the efficiency of absorption of colostral immunoglobulin.[262, 318] Serum based products appear to be absorbed more efficiently.[319]. Increasing the amount of serum-based product in an attempt to achieve the required serum immunoglobulin level resulted in decreased absorption efficiency and did not provide adequate protection.[320] Poor absorption was also shown when colostrum and cheese-whey derived products were fed at 3-4 times the recommended level.[317] Recently a colostrum replacer has been developed that results in blood levels greater than 10 g/L IgG in calf serum at 24 hours (Acquire, American Protein Corporation, Inc., Ames, Iowa).[316, 321, 322] This product contains 125 g of bovine immunoglobulin concentrated from processed bovine serum. The Federal Drug Administration in the USA have recently proposed a rule to ban the feeding of plasma and serum proteins to calves and this will limit the availability of these more efficacious products.[316]

There are few colostrum supplements and replacers available in Australia. Biocol (Intervet Australia Pty Ltd) is a colostrum supplement containing approximately 4 g of bovine IgG extracted from the blood of 3-6 day old calves. It also contains whey protein and dextrose. There are no efficacy studies available, but the dose of immunoglobulin is so low that it is unlikely to make a significant difference to calf serum IgG levels. It is recommended that it is fed in addition to sufficient colostrum in the first few hours of life.

Other risk factors at calving

Treatment of the navel at calving has no association with the risk of diarrhoea.[250] Cleaning calving facilities after each calving season has been shown to minimise the risk of diarrhoea.[250]

Management of calves post calving

Management of the nursing paddock

One study in Alberta showed a small but significant relationship between the provision of shelter for young calves and mortality due to NCD.[3] Shelter areas are important, as calves tend to lie down frequently in the first 2 weeks of life. Small shelters can become crowded in bad weather resulting in high concentrations of pathogens and increased opportunity for disease transmission. In Idaho protective wooden shelters have been used successfully to improve the survival and performance of spring born calves.[323]

One study has shown that an increase in the drainage of the area used for cows and young calves was related to a decrease in the mortality of calves due to NCD.[3]

Other factors

Other management risk factors are likely to include time of calf management procedures such as drenching and castrating as well as the stocking rates and group size. Radostits[217, 219] has recommendations for the latter in his control procedures, but there is little scientific evaluation of the benefits of different group sizes or stocking rates. Carrying out any procedures on beef calves in the first 2 days of life has associated with increased shedding of cryptosporidia.[324]

Nutrition of cows

It is likely that water source and availability, grazing rotation length and fertilisers use could have affect the incidence of NCD, but there is no published information evaluating these topics.

Mineral status of cows

Selenium supplementation has been shown to increase the IgG levels in deficient cows (see p 45). It has also been shown to reduce the incidence of NCD in one study.[325]

Water quality

C. parvum survives for at least 2 weeks in water sources and it has been shown that the risk of *C. parvum* infection in cattle is related to the distance of the barn water source from the septic system.[159]

Best practice diagnostic methods

Defining the problem

At the onset of an investigation it is important to define the problem both in a veterinary perspective and in the view of the producer. It is normal to assess the extent of the problem by comparing the mortality and morbidity with accepted industry standards. It is also important to define the time frame that the losses occur over. These are defined by Radostits[326] as follows

Perinatal mortality: stillbirths occurring at more than 270 days gestation, at full term and mortality up to 24 hours of age.

Neonatal mortality: Calves born alive but dying between 1 and 28 days of age

Older calf mortality: Calves born alive but dying between 29 and 84 days of age or 29 and 182 days of age.

In reality for most Australian operations the last 2 terms are grouped together as pre-weaning mortality, but when assessing the effect of NCD on an enterprise, neonatal morbidity and mortality are the most useful measures.

Outbreaks of calf scours in Australia have been shown to have a large variation in morbidity and mortality and there is no industry standard for an acceptable target.[2] Few standard definitions are available for the beef industry worldwide, but Heath has suggested a figure of 3% from 24 hours to 60 days for all calf mortality.[327] In the dairy industry the target for neonatal mortality is between 2%.[328] and 3%[326] and the target for older calf mortality is 1%.[326] The target for the prevalence of diarrhoea used by Brand[328] for dairy calves is 20% per pen.

The published literature does not always use these definitions, but would concur that a 3% neonatal mortality rate is an acceptable goal. In a study of 10 herds in Colorado the neonatal mortality (24 hours to 45 days) was 2.2% and incidence of NCD was 1.1%.[248] These herds were not selected randomly and consequently this may be a biased estimate. Four hundred and sixty seven cow-calf herds surveyed in Quebec had an average pre-weaning mortality of 5.6%.[222] In another Canadian study of 170 beef farms in Ontario the average pre-weaning mortality for calves from cows was 2.6% and 3.6% for heifers.[329]

It is important to check herd records to determine precise figures for mortality and morbidity as well as the age range of affected calves. However these figures are often not recorded by Australian beef farmers.[2]

The temporal relationship of the problem should also be defined.[330] The date of the first (index) case should be noted and then subsequent animals clinically affected or dying should be plotted on a calendar to assess if there is an epidemic curve or whether the problem can be related to climatic changes. If diarrhoea has not been a problem in the past it should be determined what has been changed. The herd structure, stocking rates and management of the stock should all be considered.

It should also be considered whether a sudden increase in cases is just chance. This is called the *scan statistic* (reviewed by Wikse[330]) and is based on the fact that biological events are not uniformly spaced but randomly distributed on a time line. If the number of events expected for any given year is known then the probability of a given number of events occurring in a shorter period of time (eg a 30 day period) can be calculated. This will allow the investigator to determine if the problem is possibly just a random cluster of affected animals. However in a contagious disease such as NCD it is likely that an initial random cluster may result in a significant problem due to the rapid increase in the environmental pathogen loading.

As well as defining the mortality and morbidity rates it may also be helpful to define the attack rate for different ages of dams (number of dams affected/number of dams at risk that age).[330] Should the major problem be in calves of first calf heifers it is important to investigate dystocia rates and FPT.

Spatial patterns (descriptive epidemiology) should also be defined by looking at the losses for each individual group or paddock on the property. If differences are shown between groups factors such as the nutrition or condition score of the cows, age of the cows, breed or sire of the calves and amount of shelter from severe weather should be considered when collecting the history.

Collecting the information required

Data Collection

Veterinarians and producers need to be aware that NCD at a herd level is an indication of underlying stress on the calf and the solutions are likely to be more managemental than pharmacological. Consequently obtaining a good history of the herd management and animals affected together with evaluating the property for risk factors are key aspects of minimising the problem.

Information useful for defining an outbreak of calf scours includes the type of operation (beef, dairy, veal), age range of affected calves (onset through to resolution), the demographics of the herd (number at risk, affected, and died), the duration of the problem and the historical progression of the problem including details of housing / stocking density, vaccination status, worming history, and prophylactic and therapeutic interventions for the affected group.

Information collected at a herd level should include in depth information on nutrition, paddock management reproduction, length of calving season, genetics, record keeping and labour.[330] Where possible the producer should answer a comprehensive questionnaire before the farm visit. This can be combined to also collect information on every possible risk factor for NCD as the third stage of the investigation.

Clinical investigation

Having collected a complete history, physical examination of affected and unaffected calves should occur. From a diagnostic point of view special attention should be paid to the animal's oral cavity, respiratory tract, evidence of any nasal discharge, evidence of fibrin clots in anterior chamber suggestive of bacteraemia and/or septicemia, whether it is pyrexic, the nature of faeces and any perineal staining. A high proportion of infected navels may be a reflection on hygiene but are not related to the cause of calf scours.[250] However septic physitis, arthritis and osteomyelitis may be secondary to salmonella.[331-333]

Calves should be classified using a simple decision tree as to the treatment that may be required and appropriate samples should be collected as discussed on p 55.

Examination of the environment

The property should be assessed to ensure that all risk factors have been correctly detailed in the history. This will also allow the investigator to better understand the dynamics of a property and understand difficulties that may be encountered either in the control of the current problem or in putting preventive measures in place.

Diagnostic strategies

Evaluation of FPT

There are many tests that have been used to evaluate passive transfer status in calves. Enzyme linked immunosorbent assay (ELISA) and radial immunodiffusion are the only tests that directly measure serum IgG concentration. All other tests estimate serum IgG concentration by measuring the concentration of immunoglobulins and other serum proteins. Indirect tests are generally cheaper and technically easier. Most tests can be used from 24 hours until 2 weeks after birth.

Serum total protein

This test is relatively easy and cheap and although it is not a direct measure of IgG concentration, studies that have compared it to glutaraldehyde gelation, sodium sulphide and zinc sulphate turbidity and GGT activity have shown that refractometry is equivalent to or superior to these other available assay.[254, 255, 334, 335] It is also easier to standardise between laboratories and operators than many other semi-quantitative methods, and is cheaper and technically easier than most direct tests for IgG.[255] It can be carried out in most veterinary clinics and the equipment needed could be easily used by a large calf rearing unit after some training. Dehydrated calves will have a slightly higher serum protein and it is advisable to assess hydration status and adjust the cut-off point for sick calves. It is not a reliable estimate of IgG levels in calves that have received colostrum substitutes or replacers. [322]

Serum total protein was first used by McBeath in 1971.[336] In study of 185 calves from different sources (beef suckler calves, dairy calves and calves purchased from a market) a highly significant correlation was shown between the refractometer reading and the total immunoglobulin concentration (IgG and IgM) measured by radial immunodiffusion. A subsequent study compared the sensitivity, specificity, predictive values, and classification accuracy of the sodium sulfite turbidity test, the zinc sulfate turbidity test, and refractometry relative to serum IgG1 concentrations determined by radial immunodiffusion.[334] It was noted that there was a large variation in the sensitivity and specificity depending on the choice of endpoint but that refractometry correctly classified the largest proportion of calves with regard to their passive transfer status at test endpoints of 5.0 and 5.5 g/dL, being 83% and 82% respectively.

Another study over a ten-year period evaluated the relationship between serum protein concentration in the first week of life and survival to 16 weeks of age in 3,479 Holstein replacement heifers.[255] Optimal survival was observed in calves with serum protein concentrations greater than 5.5 g/dL and calves with a serum protein between 5.0-5.4 g/dL had only a slightly increased risk of mortality (RR=1.3). The highest relative risk of mortality was observed in calves with serum protein concentrations < 4 g/dL. A survival analysis comparing calves with serum protein < 5 g/dL and ≥ 5 g/dL indicated that the relative risk of mortality was significantly higher in the first six weeks of life for calves with a serum protein < 5 g/dL.

When clinically ill calves were studied there was a significant drop in sensitivity at the 5 g/dL cut-off and the proportion correctly classified was reduced to 70%.[251] At the 5.5 g/dL cut off 85% were correctly classified. The effect of hydration status on the result was not examined and it is likely that this change was due to dehydration of the calves causing an increase in serum protein. It was suggested that the accuracy of serum protein could be improved if a PCV was also measured as a proxy for hydration status.

Serum gamma-glutamyltransferase activity (GGT)

This test will indicate that a calf has ingested colostrum, but has a poor correlation with the actual IgG level. It also declines markedly in the first few days, so the age of the calf should also be known to interpret the test accurately. It is a relatively inexpensive test when used at a herd level, but has to be sent to a laboratory.

A high level of GGT activity in colostrum and in the serum of young calves was first documented in the 1980's.[337, 338] Both colostrum and milk contain GGT, but activities in milk are considerably lower than in colostrum.[337] GGT rises quickly after the ingestion of colostrum, and then falls rapidly over the subsequent 24 hours followed by a slow decline for the next two months. Calves that do not ingest colostrum have serum GGT activities similar to adult cattle.

In a study of 48 calves sampled 24 hours after birth Perino demonstrated that a cut off value of 200 IU of GGT/L gave a specificity and sensitivity of 80% and 97% for diagnosis of FPT.[254]

However there was a low degree of association between GGT levels and IgG levels indicating that although elevated GGT is an indicator that colostrum has been absorbed, it is not a reliable indicator of adequate IgG levels.

A study of Holstein calves by Parish et al in 1997 demonstrated that serum GGT activity drops rapidly from 200 IU/L 24 hours after birth to 75 IU/L by 1 week of life.[335] It was suggested that use of serum GGT should be restricted to calves less than 10 days of age, and that four-day old calves should have a serum GGT activity >100 IU/L and one week old calves should have a serum GGT activity >75 IU/L. Calves with serum GGT <50 IU/L should be classified as having FPT. A subsequent study in beef calves by the same authors measured IgG levels in calves at 24-72 hours of age, then measured GGT levels in these same calves at various ages between 3 and 18 days of age.[339] Minimum association was present between initial serum IgG concentration and serum GGT activity when all calves <18 days of age were considered, however when the study population was restricted to calves <8 days of age, there was an improved relationship, although the degree of association with initial IgG levels was again weak (r^2=.438). It was proposed that the poorer association was due to the smaller variation in the level of passive transfer observed in the beef calves compared with the dairy calves . It was concluded serum GGT activity has no apparent advantage relative to other assay procedures for predicting passive transfer status in beef calves. If serum GGT activity is to be used to assess passive transfer status in beef calves, application of this procedure should be restricted to calves <8 days of age.

GGT was a useful test when assessing the passive transfer status of clinically ill calves less than 21 days of age.[251] When compared with serum protein, zinc sulphate turbidity and sodium sulphite turbidity a GGT cut off of 50 IU/L classified 93% of the calves correctly (sensitivity 93% specificity 92%).

Turbidity tests

The sodium sulfite and zinc sulphate turbidity tests are based on the development of a precipitate after a measured amount of calf serum is added to a standard volume of solution and incubated at room temperature for up to 30 minutes. The test results are quantified according to the degree of cloudiness of the mixture caused by precipitation of the reactants. These tests are more complicated to perform than serum protein and less accurate than the whole blood immunoassay and consequently are seldom used.

Sodium sulfite turbidity test

This has traditionally been described as a 3-step semi-quantitative test using 14, 16 and 18% solutions. The sensitivity, specificity and degree of association with IgG are similar to serum protein, but the test is more complicated to perform. The test solutions cause selective precipitation of high molecular weight proteins including immunoglobulins resulting in turbidity, the measured endpoint. The results are recorded on a 0, 1+, 2+ and 3+ scale. 0 is equivalent to no turbidity in any tube, 1+ is observed turbidity in the 18% tube only, 2+ is observed turbidity in the 18% and 16% solutions and 3+ is observed turbidity in all solutions.[334]

Use of the 14% and 16% solutions have been associated with extremely high and clinically irrelevant serum IgG concentrations, and the 18% solution gives the best diagnostic ability.[334] The sensitivity has been estimated between 85 to 100% and the specificity from 53% to 87% using an 18% solution (1+ endpoint).[300, 334]

When used to test clinically ill calves less than 21 days of age 85% were correctly classified using the 18% solution. The specificity of the 16% and 14% solutions were 58% and 0 respectively.

Zinc sulphate turbidity test

This test can be influenced by haemolysis, making it difficult for routine use.[340] The standard test is carried out by adding 0.1 mL of calf serum to 6 mL of 208 mg/L zinc sulfate and incubating the mixture for 1 hour at 23 ^{o}C. The test has a sensitivity of 100% and a specificity of 55%, which is lower than that determined for serum protein of the sodium sulphite turbidity test.[334] In a study of serum samples from 242 calves it was shown that the endpoint of the test was higher than desired, with calves classified as having FPT having a mean IgG_1 concentration of 955 mg/dL. Consequently it was suggested that the test should be modified to use a stronger concentration of solution. Subsequent experiments showed that the test could be improved by increasing the strength of the solution to 300 or 350 mg/L.[341] These solutions resulted in an increase of the specificity to 65% and 76% respectively with little decrease in the sensitivity.

Whole blood glutaraldehyde coagulation test

Studies have shown this test to be inadequate for routine use with poor sensitivity and specificity. At the 5 minute end point as recommended by the manufacturers the sensitivity was 5% and the specificity 99%.[299]

Lateral flow immunoassay

A lateral flow immunoassay was developed for calves in the late 1990s and is performed on whole blood or serum [2]. This test gives a semi-quantitative immunoglobulin concentration. The kit consists of a 4-mm lateral flow membrane enclosed in a plastic test device, Tests kits are incubated for 20 minutes. If the sample concentration is greater than 10 mg/mL (1000 mg/dL) a single red line develops on the membrane strip indicating adequate passive transfer. If the sample is less than 10mg/mL double lines develop. Published results show the sensitivity ranges from 93-99% and specificity from 88-89%.[300, 342]

Radial immune diffusion

This is the gold standard for measurement of serum IgG. However its usefulness is compromised by the processing time.

The choice as to which test to use should depend on the purpose for which it is being used, the ease, speed and accuracy of the result required, the cost and availability of the test and on the degree to which results can be standardised. Where a group of calves is being monitored for FPT serum protein is probably the most cost effective test and as accurate as any of the alternatives. For a rapid calf-side monitoring test for small groups of animals the whole blood immunoassay may be the appropriate choice, and when sick calves need to be evaluated GGT should be considered.

[2] Quick test calf kit. Midland Bio-Products, Boone, Iowa

Diagnostic investigation of the affected group

Sample Collection

Appropriate selection of diagnostic specimens is required to achieve a meaningful diagnosis. Best results are obtained when fresh samples and specimens are collected from calves early in the course of disease. It is important to ensure that the calves exhibit a typical presentation for that outbreak. Numerous tests are available to detect the various enteric pathogens of calves in faeces. Six or more fresh faecal samples should be collected from calves early in the course of the disease to determine the proportion of calves shedding known pathogens. When possible fresh necropsies are particularly informative as it provides an opportunity to relate the presence of pathogens to a disease process. This is required to establish causality. The quality of the information gathered is to a large extent determined by the quality of the samples submitted to the diagnostic laboratory. Autolysis and bacterial invasion of gut mucosa begin within 5 minutes of death. Autolysis is a common cause of poor tissue sections for histopathology, this may reflect prolonged post mortem interval or poor tissue preparation, handling, or transport. To avoid autolysis formalin needs to distribute into the lumen of intestinal sections hence intestinal specimens should be no longer than an inch long and the tissue to formalin ratio should be no greater than 1 to 10. Necropsy diagnosis is based on the demonstration of the agent associated with compatible gross and histopathology. Tissues are also examined by high power light microscopy to look for the presence of bacteria adhering to the mucosa and cryptosporidia associated with the brush border of epithelial cells. Fluorescent antibodies techniques may be used for in situ detection of enterotoxigenic *E. coli,* rotavirus, and coronavirus. When multiple dead calves are available for necropsy it is worth taking the opportunity to check the consistency of findings across the affected group.

Calf Necropsy

The focus of this discussion is on observations and samples pertinent to the diagnosis of calf scours. It is not intended to reflect a protocol for conducting a complete necropsy. During the necropsy each of the following organs should be checked and the indicated samples collected. The possible pathogen associations are indicated in parentheses. While economics usually dictates the submission and ordering of tests there is only one opportunity to collect samples so it is prudent to collect everything.

- Mouth: Check for oral erosions (Pestivirus) or proliferative lesions (Bovine papular stomatitis), if observed collect and fix tissues.
- Oesophagus: Check for erosions, if observed collect and fix tissues. (Pestivirus)
- Abomasum: Check for inflammation and emphysema in rugal folds (Clostridia) collect tissue for histopathology and abomasal contents (on ice) for detection of clostridia and clostridial toxins.
- Small intestine: Examine for signs of inflammation and fluid distension. Collect a sample of duodenum, mid jejunum, and ileum for histopathology (Rotavirus, coronavirus, salmonella, cryptosporidia).
- Mesenteric Lymph Nodes: Note if enlarged and collect samples for culture (salmonella) and histopathology.
- Caecum: Check for evidence of inflammation (coronavirus, salmonella, coccidia) and collect contents on ice for culture and tissue for histopathology

- Spiral colon: Check for inflammation and collect tissue for histopathology (salmonella, coronavirus, coccidia)
- Rectum: Examine for inflammation and collect tissue for histopathology (coccidia, pestivirus)
- Brain should be examined for evidence of Neospora, that can contribute to weak and sickly calves after birth and for evidence of meningitis, encephalitis and polioencephalomalacia
- Comment should be made on the nutritional status of the calf by examining fat reserves around the kidneys and coronary band.

Diagnostic tests for neonatal enteric pathogens

This summary includes a review of diagnostic tests available for the diagnosis of enteric pathogens of calves.

Bacterial pathogens

Escherichia coli

E. coli is a normal inhabitant of the gastro-intestinal tract. Isolation of *E. coli* from faecal samples or gut contents is therefore of no significance unless the isolates are demonstrated to possess virulent attributes that are consistent with the clinical and or pathological presentation. Virulence attributes include adhesins, enterotoxins, and cytotoxins.

Enterotoxigenic E. coli

Enterotoxigenic *E. coli* possess two virulence factors: fimbriae (pili) and enterotoxins. F5 (K99) and or F41 fimbriae mediate adherence and heat stable enterotoxin a (STa) stimulates a secretory response into the gut lumen. Enterotoxigenic *E. coli* adhere to enterocytes in the jejunum and ileum.[343] On gross pathology, enterotoxigenic *E. coli* is associated with fluid-distended loops of bowel without enteritis.[344] Calves infected with enterotoxigenic *E. coli* have a mild inflammatory reaction in the small intestinal wall and some villous atrophy. In fresh specimens sheets of gram negative bacilli can be seen adhering to the small intestinal wall.[343] Susceptibility to Enterotoxigenic *E. coli* is age dependent according to the binding specificity of pilli antigens to immature enterocytes.[79] Disease is typically observed in calves less than 3 days of age, however concurrent infection with rotavirus may extend this window to 14 days of age.[24, 80] Intestinal cells of calves greater than two days of age acquire natural resistance to F5 adhesion.[79] Despite this F5 positive *E. coli* have been isolated from healthy 4–12 week old calves and F5 positive ETEC are shed in faeces for several weeks following experimental infection of newborn calves.[82]

Definitive diagnosis of enterotoxigenicity rests on demonstration of the ability of the *E. coli* to dilate intestinal loops.[345] Enterotoxigenic *E. coli* can also be identified by the presence of the F5 (K99) using antigen specific immunoassays including; latex agglutination,[346] ELISA,[347] fluorescent antibody,[348] slide agglutination[348] and rapid dipstick tests. A potential limitation of immunoassays is the specificity of the antibodies used, strains of enterotoxigenic *E. coli* utilising non-F5 fimbriae will not be detected by these tests.[85, 349]

Attaching and effacing E. coli (AEEC) and Shiga toxin-producing E. coli (STEC)

Attaching and effacing *E. coli* (AEEC) and Shiga toxin-producing *E. coli* (STEC) have also been identified as causes of diarrhoea and dysentery in calves.[83, 84] Disease is mediated by

cytotoxic damage to the intestinal mucosa. Lesions may be observed in the ileum, caecum and colon.[86] Lesions may include mucohaemorrhagic colitis, with petechial or ecchymotic haemorrhages in the wall of the colon and rectum.[85, 88] Histological demonstration of gram-negative bacilli adherent to the colonic mucosa in necropsy specimens is consistent with this diagnosis. Attaching and effacing lesion formation is mediated by products of the locus of enterocyte effacement (LEE).[350] Intimin, the product of the *eae* gene (located in, and sometimes used as a marker for, the LEE pathogenicity island), is required for adherence.[351, 352] Attaching and effacing *E. coli* which cause disease and does not produce enterotoxins or shiga toxin is referred to as enteropathogenic *E. coli* (EPEC). STEC produce two types of shiga toxins, those that are immunologically similar to the Shiga toxin produced by *Shigella dysenteriae* (Stx1) and those that are immunologically distinct from *Shigella dysenteriae* Shiga toxin (STx2).[93] Bovine STEC produce either STx1, STx2, or both.[94]

Diagnosis of *E. coli* infection may be achieved using phenotypic differentiation of pathogenic strains from non-pathogenic normal flora *E. coli* via bioassays or immunoassays for toxins and fimbriae. Immunoassays have been developed to identify the presence of STx1 and Stx2 in faeces as a presumptive test for the detection of STEC in cattle faeces.[353-355] An alternative approach is to identify and differentiate ETEC, AEEC, and STEC is to utilise PCR to identify virulence associated genes commonly found in these *E. coli* strains (F5, F41, enterotoxin, intimin, Stx1, and Stx2).[94] The significance of STEC, EPEC, and AEEC in bovine enteritis is probably underestimated due to a lack of appropriate assays for routine detection and because of the widespread presence of verotoxin producing *E. coli* strains in healthy cattle that complicate the interpretation of detecting faecal shedding in sick animals.[356-358] Demonstration of verotoxin in cultures from bovine enteritis is not sufficient to imply a causative association.

Clostridium spp.

Clostridium perfringens has been associated with enterotoxaemia and haemorrhagic abomasitis in calves.[127, 129, 130] *C. perfringens* are normal flora of the gastrointestinal tract hence isolation of *C. perfringens* from faeces is not in itself diagnostic. Pathogenic strains of *C. perfringens* produce exotoxins, five of these (α,β,ε,ι, and enterotoxin) are involved in the pathogenesis of disease.[127] The complete pathogenesis of enterotoxaemia and abomasitis has yet to be completely elucidated. Production of specific toxins can only be demonstrated in a proportion of cases.[359] Isolation of toxin positive *C. perfringens* from intestinal contents does not confirm a clinical diagnosis of bovine enterotoxaemia as almost as many *C. perfringens* isolates from normal calves produce toxin and toxin production cannot be demonstrated in as many as 40% of affected calves.[360]

A fresh necropsy is required to definitively diagnose clostridial enteritis. Observing many gram positive bacilli in the mucosa associated with haemorrhagic enteritis is suggestive of clostridial enterotoxaemia. Quantitative bacterial counts of intestinal contents at the site of the lesion have proven to be one of the most reliable methods for diagnosing enterotoxaemia.[129] A *C. perfringens* count greater than 10^6/mL of intestinal contents is consistent with a diagnosis of enterotoxaemia.[129] Demonstrating the presence of *C. perfringens* toxins or the capacity to produce toxins provides support for the diagnosis. Tests for detecting toxins or the bacteria's capacity to produce toxins include bioassays, immunoassays, western blot and PCR.[361] The basis of the bioassay is to demonstrate protection of mice using antitoxin. In this assay bacterial free filtrates are injected into mice which have also received different antitoxins. *C. perfringens* enterotoxin is produced during sporulation. In-vitro detection of enterotoxin production capacity by a *C. perfringens* isolate using western blot or immunoassays requires sporulation to occur. In vitro techniques to induce sporulation are not 100% efficient so detection of enterotoxin using these methods are less sensitive than PCR is at detecting the genes required to produce

enterotoxin.[362] Multiplex PCR assays have been developed for detection of *C. perfringens* toxin producing genes to genotype *C. perfringens* isolates.[360]

Salmonella spp.

Salmonellae are capable of causing disease in cattle of all ages. Neonatal infections are common. *Salmonella typhimurium* and *S. dublin* are the most common serovars associated with disease in cattle. The classic pathological lesion is fibrinous or fibrino-necrotic to ulcerative enteritis.[363] The severity of lesions is usually greatest in the distal small intestine and proximal large bowel. Hypertrophy of the mesenteric lymph nodes is a common finding.[364] Serosal haemorrhages may be observed in the small and large intestine. Septic infarcts in the kidneys and inflammation of the gall bladder are less common findings. Pneumonia is a common finding with *S. dublin* infections and gangrenous necrosis of distal extremities may also be observed.[365] Bacteraemia is a feature of neonatal salmonellosis and may manifest as osteomyelitis and or meningitis.

Isolation of salmonella from faeces of calves with diarrhoea is consistent with a diagnosis of salmonellosis but in itself does not necessarily establish causality as salmonella may be isolated from the faeces of apparently healthy calves.[366] Isolation of salmonella from tissues at necropsy is indicative of invasive salmonellosis. A definitive diagnosis of salmonellosis is based on the clinical presentation, pathological lesions, and isolation of salmonella from tissues at necropsy.

There are numerous methods for isolating and detecting the presence of salmonella. These include direct culture, enrichment cultures, PCR, immunoseparation, and immunoassays.

The process of directly inoculating tissues or other samples on to plating media, except in the case of acute infections, is usually non-productive. Typically, with subclinical infection the number of salmonellae is low relative to the high number of other bacteria. These samples should be inoculated into selective enrichment media for optimal recovery of salmonella. Selective-enrichment broths are formulated to selectively inhibit other bacteria while allowing salmonella to multiply to levels that may be detected after plating. There are three major types of selective enriching media: tetrathionate, selenite, and Rappaport-Vassiliadis (RV) with various formulations within each type. Generally as the number of enrichment media is increased, the level of detection increases. Several studies have shown that tetrathionate enrichment is better than selenite enrichment.[367, 368] Pre enrichment of the sample, regardless of the type or source, is advocated with RV broth. The inoculum ratio commonly used for tetrathionate and selenite enrichment broths is 1:10; with RV broth, however it is 1:100.[369] Enriched samples should be incubated for at least 32 hours.[370] The optimal growth temperature for salmonella is 37 ^{0}C. Generally, samples such as internal organs or tissues having low levels of background flora are incubated at 35–37 ^{0}C. A higher temperature is not necessary to suppress contaminants in these samples. Intestinal and environmental samples, which generally have higher levels of competing bacteria, may be incubated at higher temperatures (40–43 ^{0}C), because salmonella are more tolerant to higher temperature.[369]

Internal organs which are normally sterile do not need to be inoculated on selective media rather they should be inoculated on to non-selective (blood agar) or weakly selective (MacConkey agar) media.

There are numerous plating media available for the detection of salmonella. Plating media are incubated at 35–37 ^{0}C for 20–24 hours and observed for suspected salmonella. Some salmonella grow slowly so the plates should be re-examined at 48 hours. Several studies have compared the various plating media; however, no single study has compared all the media.

Generally there is a correlation between sensitivity and specificity. As the specificity increases the sensitivity also increases. Plating media that generally performs well includes: XLT4, Novobiocin brilliant green glycerol lactose, XLD novobiocin, brilliant green novobiocin, and Rambach agar. Further details regarding specific media and comparisons of culture techniques can be found in the review by Waltman.[369]

A number of rapid detection methods have been developed to expedite the detection of salmonella. These methods include electrical conductance and impedance, immunological techniques, nucleic acid based assays, and PCR. These methods generally take 24–52 hours to screen for or detect and identify salmonella. The majority of these tests, particularly the enzyme linked immunological techniques require 10^5 cells per mL for reliable results. Accordingly all these tests involve a pre-enrichment stage, and some also involve a selective enrichment culture.[371]

Numerous tests have been developed for detection of salmonella, many of these for detection of low numbers in food and environmental samples. When salmonella is causing disease, clinically affected calves may shed 10^9 salmonella per gram of faeces.[372] Detection of salmonella in clinical samples when it is the inciting cause of the disease process is not normally difficult when multiple samples are collected from a representative sample of the affected population.

Viral enteropathogens

Viruses are usually identified by direct examination of the faeces, immunoassays, or fluorescent antibody examination of gut tissues. Molecular techniques involving PCR and RT-PCR have been described for most pathogens but are not routinely available in Australian diagnostic laboratories. Electron microscopic examination of faeces is not a sensitive means of detecting virus particles but it has the advantage that many different types of viruses can be detected, including those such as parvovirus that are not recognised as common causes of diarrhoea. Viral isolation is not commonly used because the process is expensive, the quality of samples received at diagnostic laboratories is usually inappropriate for survival of the viruses, enteric viruses are difficult to grow, and the methods are not particularly sensitive. The recent development of relatively inexpensive immunoassay diagnostic test kits make these an attractive option, limited test specific data regarding test sensitivity and specificity limits the application of some of these tests.

Coronavirus

Diarrhoea associated with bovine coronavirus infection is most frequently observed between 1 and 2 weeks of age.[45] Virus replication occurs in the epithelial cells of the distal half of the villi of the lower small intestine and colon. Infected cells die, slough, and are replaced by immature cells. In the small intestine these changes result in stunting and fusion of adjacent villi, and in the large intestine they lead to atrophy of the colonic ridges. On histopathology the tall columnar epithelial cells are replaced by cuboidal and squamous epithelial cells and in severe infections there may be areas of complete desquamation.[45]

Bovine coronavirus is shed in respiratory secretions and faeces. There are a number of methods for detecting bovine coronavirus virus in faeces. These include isolation of the virus in cell culture,[373] electron microscopy,[374] immunoelectron microscopy,[42] immunoassays[30, 347, 375-378] and molecular techniques including dot blot hybridisation assays[379] and RT-PCR.[380, 381] Isolation of bovine coronavirus using cell culture techniques is not often performed in diagnostic laboratories as the technique is difficult and requires viable virus (fresh samples or shipped on dry ice).[382] Electron microscopy has been utilised as a standard diagnostic method for bovine coronavirus. Although the intact virion of bovine coronavirus is fairly

characteristic in appearance it is not uncommon for the identifying surface projections of the virus to be lost during sample preparation or storage, making it difficult to properly identify virus particles by EM. Electron microscopy is not however a very sensitive method of detecting viruses requiring approximately 1 million viral particles per millilitre.[374] In addition, coronavirus can be confused morphologically with non viral particles such as intestinal brush border epithelium and with other morphologically similar viruses, leading to false-positive results.[383] The sensitivity of electron microscopic examination can be increased using techniques that concentrate viral particles prior to examination or through the use of virus specific antibodies to facilitate detection of the virus.[384]

Numerous ELISA assays have been described for the detection of BCV antigen in faeces. A number of companies have developed commercial kits utilising this technology. The use of monoclonal antibodies rather than polyclonal antibodies is reported to increase the sensitivity and specificity of bovine coronavirus ELISAs.[378] The limit of detection for ELISA assays range from 10^4-10^5 virions/mL of faeces. Antigen capture ELISA techniques are not as effective for detection of coronavirus shedding by adult cattle as the virus is often complexed to host antibody.[377] Assessment of the host immune response to coronavirus provides an alternate method of establishing coronavirus exposure in adult cattle.[377]

A 1-step RT-PCR assay, targeting a 730 bp fragment of the nucleocapsid gene of bovine coronavirus, and a nested PCR assay, targeting a 407 bp fragment of the nucleocapsid gene have been developed to detect bovine coronavirus. Compared to an antigen capture ELISA the limit of detection for the RT-PCR and nested PCR was 10^3 and 10 virions/mL respectively compared to 10^5 virions/mL for the ELISA.[47]

Rotavirus

Bovine rotavirus infects enterocytes of the intestinal villus. Infected cells are predominantly in the distal third to half of the villus. Susceptibility to infection is age related with the newborn calf most susceptible.[20, 21, 24] The age at the time of infection influences the distribution of the virus in the gastrointestinal tract and the number of virions shed in faeces. In experimental challenge studies infection of day old calves resulted in a uniform distribution of virus throughout the small intestine.[21] Challenge of 10 day old calves led to a patchy distribution of the virus with maximal viral load observed in the mid small intestine.[21] The degree of villus stunting is also influenced by the age of the calf with less stunting observed in calves infected at an older age.

Methods for detection of rotavirus include cell culture, fluorescent antibody staining, electron microscopy, immuno-electron microscopy, immunoassays, electrophoretic procedures, and RT-PCR.[13-16, 17 , 346, 347, 376, 385-387] Bovine rotavirus is difficult to isolate in cell cultures because of the cytotoxic nature of faeces and faecal filtrates and because the virus is inconsistent in production of cytopathic effects.[386] The fluorescent antibody technique is simple, rapid and specific, however rotaviral antigen is usually difficult to detect within 24 to 72 hours after the onset of diarrhoea because rotavirus infected epithelial cells are rapidly shed from the tips of the villi.[388] Comparative studies evaluating methods of detecting rotavirus in faeces give good agreement between antigen capture assays (ELISA, latex agglutination) and electron microscopy.[346, 376, 386, 389] Direct immunoflorescence testing of faecal samples gives good agreement (90%) with electron microscopic examination for rotaviruses when samples are collected during the 24 hours following the onset of diarrhoea,[390] but poor agreement (33%) for field specimens submitted to a diagnostic laboratory.[386]

A potential limitation of commercial immunoassays is that they only detect group A rotaviruses. This problem does not occur with other more laborious methods such as electron microscopy, polyacrylamide gel electrophoresis, or RT-PCR.[14, 15, 391]

Bovine Pestivirus

Bovine pestivirus rarely causes diarrhoea in neonatal calves.[50] Sporadic disease may be observed in persistently infected calves. Pathological lesions include ulceration of the oral cavity, particularly on the hard and soft palate, and blunting of the buccal papillae.[392] Erosions may be observed in the oesophagus and necrosis of Peyer's patches may be observed in the ileum. In the United States and Belgium thrombocytopenia has been observed with BVD type II infections. Outbreaks of neonatal disease have been observed with this strain. Petechial and ecchymotic haemorrhages are a feature of this condition.[49, 393, 394] Haematological findings often include leucopenia and thrombocytopenia.

Several options are available for the detection of pestivirus, these include virus isolation,[395, 396] RT-PCR,[397] immuno-histochemistry,[398] and antigen capture ELISA.[399] Immuno-histochemistry and antigen capture ELISA assays are utilized in most commercial laboratories. Maternal antibodies reduce the sensitivity of the ELISA assay in young calves.[397]

Bovine Torovirus (Breda virus)

Bovine torovirus is an enveloped, single-stranded RNA virus in the Torovirus genus within the coronaviridae family, which produces cytolytic infections of villi and crypt enterocytes in the small and large intestine.[400] Bovine torovirus does not grow in tissue culture, cell culture, or in embryonated eggs.[401] Therefore, the large scale preparation of reference antisera and antigens for the development of diagnostic tests has been precluded. Torovirus is capable of causing diarrhoea in cattle with disease observed most frequently in calves less than 3 weeks of age.[56, 57, 60, 402-404] Like other enteric viruses bovine torovirus has been detected in faeces of normal calves therefore detection of the virus in faeces from diarrheic cattle cannot be interpreted as causal. The lack of diagnostic reagents has limited the study of BoTV, leaving questions about its epidemiology and relative importance in calf diarrhoea.[57] Diagnostic methods that have been used to detect bovine torovirus include electron microscopy, immunofluorescence, antigen capture ELISA and RT-PCR[56, 57] ELISA based seroepidemiological studies have been utilized to determine the prevalence of the virus in the United States.[56] There are however no commercial diagnostic assays currently available for bovine torovirus.

Miscellaneous Viruses

There are a number of other enteric viruses that have been identified in the faeces of calves with diarrhoea. The clinical significance of these viruses is unknown. Astrovirus and calicivirus were originally detected in the faeces of calves with diarrhoea in the late seventies using electron microscopy.[62] There has been renewed interest in bovine calicivirus in recent years because 2 of the genera within the caliciviridae family are commonly associated with enteritis in humans and the question has been raised that cattle may represent a reservoir of infection for these agents.[67, 70, 405, 406] Experimental infection of gnotobiotic calves with calicivirus is claimed to have induced disease in calves however the results of this study have not been published.[70] Commercial assays are not available for detection of calicivirus or astrovirus. Epidemiological investigation has been based on molecular detection and typing methods.[65, 68, 406]

Protozoa

Eimeria spp

Eimeria spp. are host specific. *Eimeria bovis* and *Eimeria zuernii* are the common cause of coccidiosis in cattle[407] although *Eimeria alabamensis* and *Eimeria brasiliensis* have emerged more recently as pathogenic species in Queensland.[204] Eimeria alabamensis has also been

reported to be a common cause of coccidiosis in Europe.[201, 203] Coccidiosis is generally not considered to cause diarrhoea in calves less than 30 days of age.[22] *E. bovis* affects primarily the mucosa of the caecum and the proximal part of the large intestine, whereas *E. zuernii* affects the mucosa of the caecum as well as the entire large intestine, including sometimes the rectum.[407] The clinical signs of bovine coccidiosis are associated with the final stages of the eimerian life-cycle and commence shortly prior to oocyst shedding. Clinical signs may include diarrhoea, ill thrift, increased susceptibility to pneumonia, tenesmus, increased mucus in faeces, and haematochezia. Depending on the severity of the infection, gross lesions in the caecum and large intestine range from semi-liquid contents with little or no blood and few areas of epithelial sloughing to extensive haemorrhage and large areas of epithelial sloughing and necrosis of the mucosa.[407] The serosal surface is often reddened opposite the affected mucosal area and the submucosa and external muscular layers thickened by oedema.

Oocysts usually can be recovered 2 to 4 days after the onset of diarrhoea.[408] Oocysts can be identified microscopically either by direct smear, flotation or centrifugation methods. The oocysts of *E. alabamensis* are smaller and less distinctive than oocysts of other coccidian but approximately 4 x larger than cryptosporidia.[204] Oocyst counts of 5,000 per gram of faeces or greater are considered significant in cattle.[205] The mere identification of oocysts in faeces is not diagnostic for clinical coccidiosis as the parasite is frequently detected in small numbers in the faeces of healthy cattle.[409] When investigating scour problems multiple samples should be collected for oocyst counts to provide an indication as to the level of infection within the group. The potential for discord between clinical signs and faecal shedding limits the diagnostic utility of a single sample from an individual animal.

Giardia

Giardia have been reported to cause disease in calves from 11 days to 5 months of age[191, 195, 410] and has been isolated from calves in Australia.[189] Trophozoites may be found in the lumen of all intestinal segments (duodenum, proximal jejunum, distal jejunum, and ileum). Infection is not associated with changes in intestinal villus height or crypt depth. However, transmission electron microscopy has been used to demonstrate a reduction in microvillus surface area.[198]

Diagnostic methods for detection of Giardia include direct microscopy, immunomagnetic separation, fluorescent antibody staining,[195, 411] ELISA,[412] and PCR.[413] When using direct microscopy faecal samples should be examined within 24 hours of collection. Cysts are concentrated by sucrose or zinc sulfate centrifugation.[414] Fresh faecal samples can be examined as a squash preparation on a glass slide. Stained and unstained preparations should be examined. Staining is achieved by adding a drop of Lugol's iodine solution to a preparation. Magnification of 100x is sufficient to observe mobile trophozoites. Trophozoites need to be differentiated from trichomonads, which are commonly seen in faecal samples and are not of pathogenic significance. Trophozoites are distinguished by the presence of two nuclei on each side of two recurrent flagella (axonemes).[414] Trichomonads have a long flagella that appears as an undulating membrane. Magnification of 400x is required to find dead trophozoites or cysts. Multiple samples are often required to identify the organism.

Concentration of trophozoites and cysts via density gradient centrifugation or filtration followed by fluorescent antibody staining is the diagnostic method utilised in most veterinary epidemiological studies of Giardia in calves.[150, 415, 416] The use of other immunoassays and PCR techniques are emerging in human diagnostic laboratories.[412, 413]

Cryptosporidia

Cryptosporidium infections are commonly associated with diarrhoea in calves 7 – 21 days of age.[149-152] *Cryptosporidium parvum*, infects the intestine of young calves producing acute enteritis.[177, 417] *Cryptosporidium parvum* infections are mainly concentrated in the distal small intestine but lesions may also be found in the caecum and colon, and occasionally in the duodenum.[180] The pathological findings associated with *Cryptosporidium* are a mild to moderate villous atrophy, villous fusion, and changes in the surface epithelium with infiltration of mononuclear cells and neutrophils in the lamina propria.[180] The parasite develops inside the epithelial cell of the digestive tract, although on the edge of the host cell cytoplasm and separated from it by a feeder organelle membrane. This intracellular extracytoplasmatic location is unique for the coccidia.[417] The other species of *Cryptosporidium* that infects cattle, *Cryptosporidium andersoni,* infects the abomasum of juvenile and mature cattle and is not associated with overt clinical disease.[144, 418]

Calves infected with *C. parvum* usually develop diarrhoea in 72-96 hours, diarrhoea is observed for 8 to 23 days,[177] during which time oocysts are excreted in faeces. Oocysts are stable in faeces for many days at room temperature.[162] Laboratory methods for the diagnosis of cryptosporidial infections include microscopic examination of faecal smears or faecal preparations, immunoassays, and PCR.

Cryptosporidia oocysts are small (4–6 µm in diameter) and are easily missed on a faecal smear. Because faecal smears do not concentrate the oocysts, this technique is less sensitive than faecal flotation. Concentration of the protozoa is achieved by salt[419] or sugar flotation: 1 g of faeces is mixed with 12 mL of water, strained through a tea strainer, and centrifuged. The sediment is mixed with 12 mL of sugar solution with a specific gravity of 1.27 and re-centrifuged with a cover slip on the top of the tube. The cover slip is removed and placed on a slide and observed at 400x.[408] Special stains may be used to facilitate detection of cryptosporidia during microscopic examination. Differential staining techniques are useful to distinguish cryptosporidium oocysts from other faecal components (especially some yeasts) of similar size and shape. Differential stains include safranine-methylene blue[420] Kinyoun[421] Ziehl-Neelsen,[422] DMSO-carbol fuchsin.[423] Differential staining techniques, however, are time consuming and vary in their sensitivity and specificity[423, 424] Negative staining techniques using nigrosine[423] light green, malachite green,[425] and merbromide,[426] which stain background yeasts and bacteria but not the oocysts, have also been developed. Negative staining methods are faster, however, some methods are less sensitive than conventional staining techniques.[419, 427] Malachite green has been reported to be both fast and sensitive.[425]

A number of immunoassays have been developed for the detection of cryptosporidia. The detection threshold of the different methods have been reported to be 3×10^5 oocysts/g for a monoclonal antibody based antigen capture ELISA, compared with 1×10^6 oocysts /g detected by examination of acid-fast stained faecal smears and 1×10^3 oocysts/g detected by indirect immunofluorescence.[428] The detection threshold may be further enhanced by using a combination of immunomagnetic separation coupled with immunofluorescent microscopy. With this combination it is possible to detect as few as 10 oocysts/g.[429] A number of dipstick immunoassays have also been developed. The detection threshold for this technology is reported to be 1×10^3 oocysts/g.[430] This technology offers the potential for rapid, cost effective detection of cryptosporidia in faecal specimens.

Molecular techniques have been described for detection and typing of cryptosporidia.[146, 418] The capacity to differentiate the different genotypes makes this approach useful for

epidemiological studies of cryptosporidia. Speciation is based on sequence analysis of the 18S ribosome amplified using PCR.[418]

Commercial Diagnostic Kits

1. Coris distributed by Dutec Diagnostics: (Rota,[387] Corona, *E. coli* K99, Cryptosporidium,[430] Dipstick Immunochromatographic test)
2. Bio X diagnostics distributed by Laboratory Diagnostics (Rota, Corona, *E. coli* K99, Cryptosporidium, Dipstick ELISA)
3. VMRD Excherichia Coli K99 Test Kit Distributed by Laboratory Diagnostics[346]
4. Institute Pourquier ELISA (Rota, Corona, E. col1 K99, 96 well break off plates) Distributed by Laboratory Diagnostics
5. Syracuse Bioanalytical Group A Rotavirus and Bovine Coronavirus Antigen Test Kit. 23 Corporate Circle, East Syracuse, NY 13057
6. Cellabs. PO Box 421, Brookvale, NSW, 2100, Australia Ph 02 9905 0133 Fluorescent antibody detection kits for cryptosporidium and Giardia.[150]
7. Direct immunofluorescence assay for cryptosporidia (Merifluor; Meridian Diagnostics, Inc., Cincinnati, Ohio)

Distributors

Laboratory Diagnostics

VMRD (*E. coli* K99 Slide Agglutination)

Institute Pourquier (Rota, Corona, Cryptosporidia, and *E. coli* ELISA)

Bio X (Rota, Corona, Cryptosporidia, *E. coli* K99 Dipstick).

Dutec Diagnostics

Coris (Rota, Corona, Cryptosporidia, *E. coli* K99, Giardia Dipstick)

Recognised methods of prevention of neonatal calf diarrhoea

Preventive management strategies

Management techniques are similar for both the prevention and the control of epidemics of neonatal calf diarrhoea. However preventive management allows integration of a variety of procedures into the herd management program, whereas control of an epidemic only allows shorter term and more “reactive” management procedures. The producer with good management skills will pre-empt a potentially hazardous situation and adjust their management procedures to minimise the risk. If a problem arises they are able to recognise and correct the cause of the problem.

Radostits[217, 219] recommended five basic management principles for the prevention and control of neonatal calf diarrhoea based on epidemiological survey of the disease in beef herds in Western Canada from 1973 to 1976. These are as follows:

1. Remove the source of the infection from the calf's environment

2. Remove the calf from contaminated environment
3. Increase the non-specific resistance of the calf
4. Increase the specific immunity of the calf
5. Reduce stress

These recommendations were expanded as follows[3]:

Remove the source of the infection from the calf's environment

Many pathogens are carried by the cow and transmitted to the calf during or shortly after calving.[28, 110-112, 157, 431] Some pathogens can survive for a long periods in the environment.[39, 163] Excess surface water and mud may be another source of contamination, and people can also become a major source after treating or handling infected calves. It is important to keep the level of environmental contamination low so that the calf's natural defence mechanisms are not overwhelmed, particularly before it ingests colostrum.

Minimising infection in the environment can be achieved by using the following procedures

I. Avoid confining the herd prior to calving. Rotate feeding and bedding areas so that animals are not forced to remain in a contaminated environment. This will help to reduce the number of infected cows that shed enteropathogens in their manure.

   It has been suggested that hay bales should be spread around the calving area at a different location every day, both in the calving area and in paddocks with cows and young calves.[1] Feed areas should be separated from watering points to encourage cow dispersal and minimise contamination. Where appropriate supplementary feed should be fed to dry cows to ensure that there is enough fresh pasture available for calving and nursing cows.

II. Put cows and heifers in a clean area 1-2 weeks prior to the start of calving.

III. Do not restrict calving animals to small areas, especially muddy paddocks. Even when they have a large area avoid physical or management procedures that encourage cows to congregate in small areas.

IV. If the ground is wet or muddy decrease the stocking rate in the calving paddock.

V. Rotate the calving paddocks from year to year, especially if neonatal calf diarrhoea has previously been a problem in the calving area.

VI. Locate the calving paddock to take advantage of natural shelter and drainage.

VII. Calving paddocks should be left vacant during the summer. If a calving pad is used all manure and bedding should be removed to expose the underlying soil.

A shorter calving period will also reduce the build-up of enteric pathogens in the environment.[1] This can be achieved by good nutrition and reproductive management.

[3] these recommendations have been abridged and adapted for Australian conditions

Remove the calf from contaminated environment

Leaving calves and their mothers in the calving area will increase contamination of this area and decreases the transmission distance between animals, hence increasing the rate of passage of infectious agents from animal to animal. Moreover the increased stocking rate may increase stress and impair the transfer of passive immunity from cow to calf. Often calving areas are small due to the perceived need to assist cows for dystocia.[1] Well-grown and appropriately fed heifers mated to suitable sires can minimise this need.

To prevent contamination in the calving area it was suggested that the calving herd should be divided into small sub groups and newborn calves and their dams should be moved away from the calving area soon after birth.[217, 219]

In his 1980 paper Radostits[217] presents recommendations adapted from Bradley,[432] as used at the Agriculture Canada Lacombe Research Station. Cows are moved into the calving area about two weeks before the start of calving at a stocking rate of 5 to 10 cows per acre with a maximum of 200 cows per calving paddock. Subsequently it was recommended that when the herd size is greater than 100 cows, the calving area is subdivided into areas containing 50-75 head. A minimum of 1000 square feet per cow (93 sq m) is necessary to minimise infection pressure and up to 2000 square feet (186 sq m) would be ideal.[218] This recommendation is equivalent to a stocking rate of 54-108 cows per hectare, and although not stated must apply to a feeding pad situation. Newborn calves and their dams are then moved within 24 hours of birth (as soon as the bond between cow and calf is established) into a nursing area that is 10-12 acres and holds between 35 and 40 cows and calves.

The system helps to overcome problems of crowding, mismothering, failure of calves to suck colostrum early enough as well as neonatal calf diarrhoea. The following recommendations were suggested for the calving and nursing areas:

i The calving area is sheltered by trees or a windbreak fence (2.5 m high, 20 % porocity). A shelter and handling facilities may be included for difficult calvings.

ii The nursing paddocks each contain 2 calf shelters with a windbreak fence at each end. The recommended dimensions of the shelters are 7.3 m long, 3.05 m deep and 2.44 m high

iii Shelters are movable and contain fresh clean bedding.

iv Clean water is available in a trough that is accessible to cows and calves.

v There is a separate isolation area for chronically sick animals, weak calves and cows with no milk.

vi Once a nursing area has a maximum of 40 cows with their calves, a new nursing area must be started.

vii Do not leave cows and calves in the nursing area longer than four weeks, move each group out when the youngest calves are three weeks old.

viii With herds larger than 200 cows the system can be duplicated, alternately older cows may be allowed to calve in a much more extensive area.

ix Calving area should be harrowed and left vacant until the grass is a suitable height for grazing

The advantages of the “Lacombe-type” system are:

a) It is easier to examine the pregnant cows and heifers as one group and the cows that have already calved as a separate group.

b) Cows and calves are together with their own kind and find one another more readily. They are not disturbed by cows close to calving claiming another cow’s calf. In large herds this will help to minimise the problem of mismothering, particularly in the first-calf heifers.

c) There is a more relaxed environment for the calf and less movement in the herd. The smaller group size minimises the risk of the calf getting injured or trampled.

d) Calves of similar ages are grouped together facilitating management procedures.

e) The herd is already divided if an outbreak of diarrhoea develops.

Disadvantages include the cost of setting up the system and the increased time and labour for managing multiple groups. Is suggested this would be compensated for by reduced expenditure on medication, reduced to calf losses and reduced time in treating the sick animals. Although there are no controlled studies of calf shelters reported in the literature it has been noted that calves in the shelters had decreased morbidity and severity of clinical disease.[267] Once producers had tried them was not possible to persuade them to place their calves in an experiment without access to man-made or natural protective shelter.

Increase the non-specific resistance of the calf

Calves need to ingest an adequate volume of colostrum within the first 12 to 24 hours of life which is dependent on three factors:

i The amount of colostrum available from the dam.

ii The maternal behaviour of the dam and whether or not she lets the calf suck.

Poor maternal instinct is more commonly a problem in heifers and it is suggested that the heifers should be confined with their calf in a small pen for a few days until they accept their calf.

iii The vigour of the calf and whether or not it can the suck the cow.

Calves may be weak at birth because of congenital defects, infection, or a prolonged and difficult birth. Prolonged difficult dystocia may cause intrapartum hypoxia, oedema of the soft tissues of the head including the tongue, and inability of the calf to suck early enough. Calves born in inclement weather may also become hypoxic. Any calf that is weak and unable to stand or suck within one to two hours should be supplemented with colostrum. Calving areas should also be designed to facilitate regular checking of the cows and to allow easy movement of animals requiring assistance into a yard.[1]

Increase the specific immunity of the calf

Research in the 1970s indicated that under natural conditions the colostrum of less than 10% of beef cows contains antibodies against enterotoxigenic *E. coli* (K99+)[433] and 1980 Radostits[217] recommended that vaccination program against these *E. coli* should be used routinely.

In 1983 Radostits[219] qualified his recommendation for any vaccination program by noting that the decision to vaccinate pregnant dams would depend on consideration of the risk factors in the herd. These were as follows

i Has the enteropathogen being isolated for diarrhoeic calves in previous years?

ii Is the disease considered to be economically important in the herd?

iii What are the characteristics of the calving grounds? If there sufficient area per calving animal; is the ground surface well-drained; is there adequate protection from cold winds, and is it easy to move animals from one place to another?

iv What is nutritional status of the pregnant animals? Will they have sufficient colostrum? A major factor in the efficacy of vaccine is the amount of colostrum ingested by the calf.

v What is the level of management? Vaccination is not a replacement for inadequate management.

With rotavirus vaccination he noted that although 73% of cows in 95 percent of herds had colostral antibodies to rotavirus, levels decline rapidly after calving and calves can become susceptible to infection at one week of age.[32] This decline in antibody levels was thought to be responsible for outbreaks of rotavirus diarrhoea year after year, despite the presence of colostral antibodies in most cows at calving. In 1983 Radostits stated that there was not enough evidence to evaluate vaccines available against rotavirus and coronavirus. In 1991 he stated that he was unaware of the availability of any multiple component vaccines (*E. coli*, rotavirus, coronavirus) in Canada that were effective based on field trials where the vaccines had been tested, with concomitant unvaccinated controls, against naturally occurring diarrhoea in calves from birth to 30 days of age.[218]

Radostits also noted that apparent failure of vaccination programs can occur when other pathogens are responsible for an outbreak of neonatal calf diarrhoea, or when the protective level of the colostrum is overwhelmed by infection pressure.

Reduce stress

The ability of newborn animals to adapt to changes in the environment is limited and conditions that appear to have no effect on mature animals may be detrimental to the newborn calf. The results from a questionnaire sent to producers in Alberta and Saskatchewan identified inclement weather, poor ground surface conditions and crowding as risk factors leading to outbreaks of neonatal calf diarrhoea. During the first 2 weeks of life most calves spend most of their time sleeping or suckling and under crowded conditions their resting and feeding patterns may be altered. Radostits is recommended every effort should be made to ensure that calves have a clean, dry, sheltered area in which nursing and resting our not disturbed.

More recently Larson[1] noted that many calving areas are nearly devoid of natural cover or windbreaks. This encourages cows to gravitate into protected valleys or gullies or concentrate near any buildings or trees that provide shelter. Cows and their calves then concentrate in small, wet and heavily contaminated areas, increasing the environmental pathogen load. He recommended that a calving area should be free of mud, sunny and protected from the wind. Windbreaks should be large enough to avoid cows concentrating in a small area, and feed and water sources should be located away from the windbreak to encourage dispersal of the cows.

The time of calving should be chosen to avoid extremes of weather. When the calving season does fall in periods of low ambient temperatures and inclement weather facilities should be provided that allows warming and drying of newborn calves.[1] Calving heifers earlier has been associated with increased dystocia and it is important to ensure heifers reach target weight at start of mating.[234]

Management of heifers

Planning for heifers to calve 2 or more weeks earlier than mature cows and having a short calving period will reduce the exposure of the most vulnerable calves to the build up of environmental pathogens that is likely to occur later in the calving season.[1]

Efficacy of current vaccines available in Australia and overseas

There are only two vaccines available in Australia directed at preventing calf scours. The first is an *E. coli* bacterin (BOvac, Intervet Australia Pty Ltd) to prevent enterotoxigenic *E. coli* and the second is a salmonella bacterin (Bovilis, Intervet Australia Pty Ltd) to prevent salmonellosis. In the U.S.A. and Europe a number of viral vaccines are available these include killed and attenuated rotavirus and coronavirus vaccines.

Enterotoxigenic E. coli

The protective efficacy of enterotoxigenic *E. coli* bacterins is well documented.[434-437] Because ETEC scours occurs during the first 3 days of life the neonate does not have time to mount a protective immune response to vaccination. Protection is afforded by vaccinating cows in late gestation so as to ensure high concentrations of anti-K99 colostral antibodies. Anti-pilus antibodies block the adhesion of the pathogen to enterocytes and subsequently prevent disease. Good maternal management is required to ensure that the calf receives the maternal antibodies. Vaccination of un-vaccinated cows in the face of an outbreak is likely to be beneficial as some beneficial immunity may develop within three weeks of the first injection, and cows that calve within 45 to 60 days of the second injection will have a protective antibody concentration.

The decision to vaccinate on a particular farm will be influenced by recognition of risk factors:

1. Prior history of ETEC scours (based on a definitive diagnosis or history of scours in calves less than 3 days of age)
2. High stocking density or use of a common calving area
3. Projected calving during the wet season
4. Large numbers of heifers projected to calve.

Salmonella

The successful reduction of salmonella prevalence in livestock on a national level via implementation of a salmonella control program emphasising immunoprophylaxis with modified live and killed salmonella vaccines indicates the potential benefits that can be derived from the application of effective salmonella vaccines.[438] Salmonella vaccine studies in cattle have focused on salmonella bacterins and attenuated modified live salmonella.

The salmonella vaccine licensed for use in Australia is a bacterin. There is conflicting reports regarding the efficacy of salmonella bacterins. The reported efficacy of salmonella bacterins ranges from good to ineffective.[439-447] The overall consensus of these reports is that vaccination of cattle with salmonella bacterins provides partial protection against salmonella challenge. In the only reported controlled field trial an autogenous salmonella bacterin was not found to have any effect.[447] Adverse reactions in the form of anaphylactic reactions are occasionally reported in cattle vaccinated with salmonella bacterins. The cause of these reactions is unknown but has been suggested to be associated with LPS content of these products. Similar allergic type reactions in humans caused by salmonella bacterin vaccination during typhoid outbreaks are well documented.[448]

There are a number of naturally occurring and genetically manipulated attenuated salmonella strains that have been used to immunise cattle against salmonellosis. The most widely tested genetically altered salmonella mutant vaccines in cattle are the auxotrophic strains. Aromatic amino acid (aro) and purine (pur) auxotrophs of salmonella are attenuated and have decreased virulence.[449-455] Auxotrophic salmonella penetrate cells and survive for a limited time in the liver and spleen stimulating an immune response. Their capacity to stimulate protective immunity to virulent salmonella infection is well documented.[449-455] Comparative vaccine trials indicate modified live attenuated salmonella vaccines provide greater protection against virulent salmonella challenge than salmonella bacterins.[441, 445, 455] Vaccination with modified live salmonella vaccines attenuates the severity of clinical signs and pathological lesions and reduces salmonella shedding and mortality.[438, 451, 456] Killed vaccines also reduce salmonella shedding, severity of clinical signs, and mortality however lower challenge doses overwhelm immunity induced by bacterins.[445, 457]

Calves immunised with modified live salmonella vaccines are protected from homologous and heterologous salmonella serotypes when challenged within 3 weeks of vaccination.[451, 458, 459] Live salmonella vaccines induce transitory T-cell independent non-specific protection which disappears about 1 month after immunisation following clearance of the organisms from the reticuloendothelial system. Thereafter, protection to oral challenge is species and serotype specific with recall of immunity presumably involving specific antigen recognition.[460, 461]

The level of passive protection of calves achieved via feeding colostrum from vaccinated cows is questionable. A number of reports suggest immune colostrum provides passive protection and others report no protective effect. The results of the different trials may partly be explained by the study designs employed. Immunisation of pregnant cows with formalin-killed *S. typhimurium* 7 and 2 weeks prior to parturition protected their calves against experimental *S. typhimurium* challenge in the first week of life.[462] Mortola also reported reduced severity of clinical disease in calves fed colostrum from salmonella bacterin vaccinated cows.[463] In contrast, Smith reported a lack of protection associated with passive transfer from maternal vaccination.[444] Calves in this trial were challenged at 3 weeks of age in contrast to 1 week of age where protection was observed suggesting that the duration of passive immunity associated with colostral transfer is relatively short. Even though the duration of immunity associated with colostral transfer is short considering that many calves are exposed to salmonella in the first week of life colostral protection may be useful. The impact of colostral transfer on the development of acquired immunity to salmonella has not been evaluated.

Rotavirus and Coronavirus Vaccines (Not Available in Australia)

Faecal shedding of rotavirus and coronavirus by adult cows is common,[30, 48, 464] providing a source of infection for newborn calves. Currently there is one type of coronavirus known to cause disease in calves. Conversely there are 7 serogroups of rotavirus with group A accounting for the majority of pathogenic isolates. Members of the group A rotaviruses are further classified

according to antigenic and genetic differences in their outer capsid proteins, G and P. Both of these proteins are involved in neutralisation of infectivity in vitro and in vivo.[465] In the United states 8 G serotypes/genotypes and four P serotypes/genotypes have been identified in cattle isolates.[18] The genome of rotavirus is composed of 11 gene segments that can be exchanged among isolates when animals are infected by more than one virus at the same time.[466] Genetic re-assortment can generate new progeny viruses that can evade what was once a protective immune response, thus allowing persistence of rotavirus in susceptible populations.[465]

Two approaches have been taken with immunoprophylaxis against rotavirus and coronavirus infections in calves. The first approach involves oral vaccination of neonatal calves with a modified live vaccine. Calves begin producing detectable levels of local secretory IgM within 4–6 days of vaccination.[467] Calves are resistant to challenge from the initial appearance of local IgM antibodies.[467] In order to consistently elicit an effective immune response, the vaccine must be administered orally, immediately after birth, and before the calf has nursed because the colostrum of most cows contains virus neutralising antibodies that interfere with the vaccine.[464] There are conflicting reports of efficacy with these type of vaccines. In double blind field studies that include vaccinated and non-vaccinated calves the vaccine was not shown to be effective.[468] Conversely when all calves were either vaccinated or not vaccinated in sequential comparisons, morbidity and mortality were significantly reduced.[468]

The second approach involves intramuscular vaccination of pregnant cows with either modified live vaccine or inactivated viral vaccines to stimulate high levels of specific viral neutralising antibodies in colostrum and milk during the first several days of the calf's life. Infectious viral particles are neutralised within the gut lumen preventing infection of intestinal villus enterocytes. One advantage of passive immunisation is the fact that cross-protection between serotypes becomes much less of a problem. This is due to the fact that vaccination of a mature cow that has had natural rotavirus exposure leads to cross-serotype stimulation of heterotypic antibodies.[469] Single serotype vaccination therefore stimulates antibody production to a wide range of rotavirus serotypes, negating the need for multivalent rotavirus vaccines. Passively absorbed anti bovine rotavirus IgG1 antibodies are transferred to the small intestinal lumen, where (in suitable concentrations) they protect against experimental challenge.[470] Antigen sensitised maternal lymphocytes also confer partial protection against challenge with virulent bovine rotavirus.[471] Colostrum and milk with a high virus-neutralising antibody titre is highly protective while it is being consumed by the calf. For example administering 400 mL of immune colostrum daily to calves from day 2 to 12 reduced the incidence of diarrhoea from 41 to 3% in one study.[472] The concentration of rotavirus and coronavirus neutralising antibodies in milk of vaccinated cows fall below protective levels by 3 to 7 days following parturition.[473-475] In lieu of complete protection, the manifestations of passive immunity to bovine rotavirus that are often noted are (1) a delay of a few days in the onset of clinical signs and or (2) a reduced severity of clinical signs, and or (3) a reduction in the length of the period of viral shedding associated with infection.[476] Although there are reports of successful field trials involving bovine rotavirus/bovine rotavirus-coronavirus – vaccinated cows,[436, 477, 478] negative results have also been reported.[479] A common problem with commercial vaccines on the market in the U.S.A. and Europe is a lack of vaccine specific data supporting efficacy claims. Protection correlates with serum titres, independent studies have sometimes failed to demonstrate effective seroconversion with some products.[480]

Control of an outbreak

Generic management strategies

Management of stock

In the face of an outbreak stock should be dispersed as widely as possible.[5] Affected and unaffected cow calf pairs should be separated. Radostits[217] suggests that the Lacombe style system detailed on page 66 smay also be applied during an outbreak. Cows not yet calved should be removed from contaminated calving area to a clean location and the population density should be reduced. Larson[1] also suggests that removing cows not yet calved to a distant clean paddock is one of the best intervention strategies in the face of an outbreak and has few obvious drawbacks in herds with low dystocia rates. Where a herd has moderate dystocia, the increased potential costs associated with calf deaths from NCD must be weighed against the potential cow calf losses due to dystocia.

Minimising Pathogen Spread

A fence line is generally sufficient to control spread.[217] Calves should not be handled during the first 24 hours to minimise the risk of enterotoxigenic *E. coli* diarrhoea. Stockpersons should take special precautions to avoid contaminating their hands, clothing and boots to break the cycle of infection. In some cases it may be beneficial to wear disposable rubber gloves and clean overalls when handling newborn calves, and to use footbaths for workers when moving from one paddock to another. Specific individuals, equipment, clothing and facilities should be dedicated to the treatment of affected calves and where possible these people and their equipment should not come into contact with healthy cow/calf pairs.[5]

Calf flow is an important control measure in an outbreak. Paddocks should be set up to ensure that unaffected calves and affected calves are kept separately especially when moved around the farm.[481]

Management of pathogen build-up

The strategies to achieve this can be based of first principles from the knowledge of the epizootiology of the common pathogens. They are summarised in Holliman as methods for control of coccidiosis but apply to all faecal pathogens.[200]

- Isolate and treat all affected cases.
- Reduce stocking density.
- Use troughs when feeding out to avoid faecal contamination.
- Avoid faecal contamination of water supplies.
- Minimise stressful management procedures during outbreak. e.g. dehorning, castration, dietary changes including weaning, transport.
- Where appropriate food or water can be mass medicated of for 28+ days in the face of disease or in anticipation of stress.
- At pasture, move water troughs and feeders regularly.

- Ensure thorough cleaning and disinfection of treatment areas.
- Minimise the exposure of calves and cows to wet and muddy areas.

The importance of moving calves out of the calving paddock into a separate area soon after birth has already been discussed as a preventive measure. This minimises exposure of the newborn calf to potential pathogens and should be rigorously applied in the face of an outbreak. It is also important to recognise the role of older calves as a potential source of a large numbers of pathogens and apply management strategies to minimise the exposure of young calves to older calves.[1]

Isolation of affected animals

Radostits[219] suggests that once an outbreak has begun it is difficult to remove the source of contamination from the calving area. Even if diarrhoeic calves are isolated, infectious agents may survive for weeks or months in the environment. Where possible the person treating the calves should not have any direct contact with newborn healthy calves. In the 1991 paper Radostits[218] does suggest that diarrhoeic calves should be removed and this solution is also suggested by Larson[1] who also suggests that treated cows should not be returned to the group until all the calves are at low risk of disease (> 30 days of age).

Treatment of affected calves

A simple program should be put in place to identify sick calves with a decision tree for the treatment options.[5] Protocols for the isolation and treatment of sick calves should be designed, as well as for the disinfection of equipment and treatment areas

Disinfection

Most enteric pathogens are transmitted by the faecal-oral route, that is, from the faeces of infected animals to the mouths of susceptible animals. Transmission may occur directly or indirectly. Mixing of infected and susceptible calves provides opportunity for direct contact. Indirect transmission requires that the infectious agent survive in the environment. Most enteric pathogens survive in the environment for weeks to years.[482, 483] Transmission occurs when susceptible calves come in contact with a contaminated environment or fomites such as equipment or vectors such as flies. Key variables involved in pathogen transmission include the number of organisms shed, survival characteristics in the environment, and the dose required to establish an infection in susceptible hosts. Variables that influence the survival of pathogenic micro-organisms in the environment include the physical characteristics of the substrate material (e.g., faeces, water, milk, manure slurry, dust), temperature, pH, water activity, and competing micro-organisms.

The first step in disinfection is cleaning to remove organic material (faeces, milk film).[484] Physical removal of organic contamination through scrubbing is preferred to application of high pressure sprays which can produce aerosols containing organisms allowing dissemination. Physical cleaning cannot be replaced by applying disinfectants in larger quantities as organic material neutralises most disinfectants. Disinfectant solutions are applied following cleaning and pathogen elimination is time dependent.[485] Other important variables that influence the effectiveness of disinfectants and rate of pathogen reduction include concentration, temperature, pH, and water hardness. The relationships between these factors are not straightforward.[486] For example, halving the concentration of formaldehyde requires a 2-fold increase in contact time to obtain similar microbial destruction, where as halving the concentration of phenolics requires a 64-fold increase in contact time. A 10 ^{o}C rise in temperature increases the activity of alcohols 30-

fold, yet only increases the activity of formaldehyde 1.5-fold. Iodophors are highly active at low pH but are inactive at an alkaline pH.[310]

The characteristics of environmental surfaces also influences the effectiveness of disinfection.[487] Unfinished plywood retains 15-fold more micro-organisms than varnished plywood, which supports 15-fold more micro-organisms than plastic surfaces. On smooth ideal surfaces, physical removal of visible contamination by thorough washing with soap and water removes 99% of the microbial load (2 logs). However, on typical housing surfaces washing only removes 90% (1 log). Application of disinfectant following washing is important to eliminate remaining pathogens and to prevent bacterial pathogens from proliferating. Within any given facility will be areas that are difficult to disinfect. Implementation of disinfection protocols in a farm environment usually translates into pathogen reduction not pathogen elimination.

Sodium hypochlorite (bleach) is effective against the bacterial and viral agents of neonatal enteric disease, but at practical levels not *Cryptosporidium* oocysts.[488] It is rapidly inactivated by the presence of any appreciable organic material and increasing concentration or contact time does not recover this loss. The recommended concentration for bleach is 1750 ppm sodium hypochlorite solution. For effective microbial killing this concentration of bleach requires a 10 minute contact time when applied at room temperature and a pH 6 to 7.[489]

Virkon is a trade name of a newer disinfectant/cleaner containing potassium monopersulfate as the active ingredient. Normally, a 1% solution is used and is prepared by mixing 10 g of powder to 1 L of water. It is virucidal and bactericidal and has a detergent like action. It is inhibitory to but not effective at killing cryptosporidia.[490] It is not corrosive, and has a low toxicity. Contact of the powder to skin or eyes or inhalation of the powder must be avoided.

A number of microbial characteristics should be considered when disinfecting equipment that contacts calves. Rotavirus is susceptible to sodium hypochlorite and povodine iodine with 1% available iodine [491] but is relatively resistant to many common disinfectants, such as chlorhexidine, under the same exposure conditions. Because as a non-enveloped virus it is not affected by soaps, washing with soap alone may actually spread the virus around on the washed surface.[492] Coronavirus is an enveloped single-stranded RNA virus and is not as stable in the environment as rotavirus. Because of their envelope, these viruses retain infectiousness better at lower rather than higher relative humidity[483] and are considerably more sensitive to soaps and common disinfectants than are non-enveloped viruses. Because *Cryptosporidium* can auto-infect the original host, the infectious dose can be exceedingly small. In the environment, cryptosporidia are extremely resistant to most veterinary disinfectants except 5% ammonia, 6% hydrogen peroxide or 10% formalin.[166, 493, 494] They survive very well in water, requiring 4 to 11 weeks to decline by one log.[495] On the other hand cryptosporidia are susceptible to drying with oocyst infectivity declining in one to 4 days.[167]

Treatment

Management of sick calves –

Criteria for treatment

Many calves with diarrhoea can be bright, alert and well hydrated. It is possible that these calves may have drunk excess milk, but there are no pathological changes in the enteric system. Calves can drink up to 26% of their body weight a day without causing diarrhoea.[210] Ingestion of large volumes of milk increases faecal fat content and changes faecal appearance from thick brown to a green-white gelatinous paste.

A full clinical examination should be carried out on all scouring calves, paying special attention to any evidence of a septicaemia or bacteraemia and the calf's hydration status. The degree of dehydration can be estimated from skin tent time, suckle response, degree of enopthalmus, the degree of peripheral perfusion and the activity of the calf (see Table 2).[496, 497]} The age of the calf, its demeanour and the estimated dehydration can be used to determine the likelihood of acidosis, with acidosis being much more likely in calves aged more than 8 days of age.[497, 498] The degree of acidosis can also be estimated from urine pH.[499] The faeces should be examined for melaena or dysentery and to estimate the daily fluid loss. Calves that are less than 5% dehydrated, not depressed, have no evidence of either a bacterial infection or coccidiosis and have a good suckle reflex do not require treatment, but should continue to be observed.

Role of electrolytes

Oral electrolyte solutions will ensure the survival of more than 95% of diarrhoeic calves if given early enough in the course of the disease and the treatment is continued for long enough with sufficient quantity.[500] However oral treatment is ineffective in neonates with severe or rapidly progressing dehydration and intravenous therapy is required.[497]

Calves that are more than 5% dehydrated, depressed or have a reduced suckle reflex should be given electrolytes. The total fluid required is the amount to correct the deficit, plus the estimated losses through diarrhoea (1-4 L/day) and a maintenance rate of 50-100 mL/kg.[184, 501] Only 60-80% of oral fluids are absorbed and consequently calves given oral solutions have more liquid faeces than those supplemented intravenously.[184, 497]

The objective of fluid therapy is to restore a normal systemic state by replacing fluid lost in the diarrhoea and reverse acidosis. In some calves it may also be necessary to correct hypoglycaemia.[184] Metabolic acidosis occurs due to fermentation of nutrient in the intestines and forestomachs, bicarbonate loss in the faeces as well as production of lactic acid by dehydrated tissues. Acidosis is an important cause of mortality in scouring calves.[502]

Table 2: Determining the degree of dehydration

% Dehydration	**Degree of enopthalmus**	**Skin Tent time (seconds)**	**Mucous Membranes**	**Estimated base deficit** of blood (mmol/L **)**	
				≤ 8 days old	> 8 days old
1-5	None / slight	1-4	Moist	0	5
6-8	Slight separation between eyeball and orbit	5-10	Tacky	5	10
9-10	Up to 0.5 cm between eyeball and orbit	11-15	Tacky	10	15
11+	Gap between eyeball and orbit is 0.5 to 1 cm	> 15	Dry	10	20

Oral electrolyte solutions

Any calf that is 5-8% dehydrated or is depressed should be treated with oral electrolyte solutions. These calves show mild skin tenting over the eyes and neck when pinched, minimal enophthalmos, moist to tacky mucous membranes, warm extremities, will stand when aroused and will suckle, although the suckle reflex may be reduced.[496, 497] These calves may be difficult to catch in a paddock.

Any oral solution used in the treatment of diarrhoea has four aims:[503]

1) supply sufficient sodium to facilitate normalisation of extracellular fluid deficits

2) provide agents that facilitate absorption of sodium and water from the intestine

3) provide an alkalising agent to treat metabolic acidosis

4) provide sufficient energy, as these electrolyte solutions may be administered instead of milk or milk replacer for short periods of time.

The first two requirements depend on the coupled active transport of glucose and sodium ions across the brush border membranes of enterocytes, which results in passive absorption of water and other electrolytes (reviewed by Bhan et al.[504]). This function remains largely intact in calves with enterotoxigenic *E. coli* diarrhoea but where there is endothelial damage it may be impaired.[184] Certain amino acids (glycine, L-alanine, L-glutamine) enhance the absorption of sodium and water,[504] as do acetate and propionate.[502] Glucose and the disaccharide (maltose), trisaccharide (maltotriose), oligosaccharide mixtures (maltodextrins of various grades), and polysaccharides (starches from rice or other cereals) from which glucose is derived may also be used to promote the absorption of sodium and water.[504]

In order to effectively combat acidosis oral electrolyte solutions need to contain 50 to 80 mmol/L of alkalising agent.[184] Acetate, lactate, citrate, gluconate, or bicarbonate are all used as alkalising agents. Bicarbonate combines with hydrogen ions directly whereas the other agents remove hydrogen ions during their metabolism within cells.[505] Electrolyte solutions that contain > 40 mmol/L of bicarbonate or citrate have marked adverse effects on milk clotting.[506] Bicarbonate raises abomasal pH, while citrate binds calcium, and so the presence of either interferes with the normal clotting of milk in the abomasum. Breakdown of abomasal milk clots results in the gradual release of some nutrients into the small intestine. Bicarbonate also reduces milk digestibility. A reduced growth rate was recorded when electrolyte solutions with bicarbonate were fed to milk fed calves.[500] Solutions containing bicarbonate may also alkalinise the gastrointestinal tract of milk-fed calves and promote bacterial overgrowth in the small intestine as well as ETEC attachment and toxin production.[507] Acetate and propionate are the preferred alkalising agents for treating calves that are still receiving milk as they do not interfere with milk clotting in the abomasum as long as the final pH of the solution is acidic and the citrate concentrations are low.[506, 508] Acid phosphate salts have also been shown to enhance clotting.[506]

There are a range of electrolyte solutions promoted with differing osmolalities and energy sources. Sodium is required to rapidly correct extracellular electrolyte and fluid losses that typically develop in calves with diarrhoea and dehydration. Recommended levels vary between 60 to 133 mM/L.[508] Potassium is also depleted in diarrhoeic calves.[497, 501] Most oral electrolyte solutions contain between 10-30 mmol of potassium/L.[184] There are no clinical trials reported on the efficacy of different concentrations of potassium.

Oral solutions are compared to the osmolarity of plasma which is 306 mOsm/L. Solutions can be iso-osmolar (300-312 mOsm/L), hyperosmolar (>312 mOsm/L), and hypo-osmolar (<300 mOsm/L)[508] Hyperosmolar solutions are also referred to as hyperosmotic solutions and iso-osmolar solutions are often referred to as isotonic. Hyperosmolar solutions are preferred for initial therapy by several researchers as they provide better nutritional support and have been shown to minimise the body weight loss that that occurs when healthy calves are deprived of milk.[496, 509] In a trial comparing a hyperosmolar solution containing glucose, acetate and propionate in diarrhoeic calves with an iso-osmolar solution and milk, Constable et al[503] showed that the hyperosmolar solution produced a similar resuscitative response to the iso-osmolar solution, but maintained higher blood glucose concentrations and lower ß-OH butyrate. Constable

recommends the use of hyperosmolar solutions if the calf is not being fed milk and requires additional energy, and the use of isotonic solutions if milk feeding is continuing. However hyperosmolar solutions will induce hypernatraemia if they are the only source of fluids, and whilst superior for initial rehydration should only be used repeatedly when the calf is consuming milk or water from other sources.[496] Naylor states that the differences between hyperosmolar and isotonic solutions are too small to be clinically significant.[184]

Hypo-osmolar solutions are recommended for children with acute non-cholera diarrhoea and have been shown to reduce faecal output and the need for intravenous fluids.[510] In calves hypo-osmolar solutions with an osmolarity < 250 mOsm/L promote rapid absorption of the water from the gastro-intestinal tract decreasing the plasma osmolarity, and causing haemolysis of the red blood cells. Consequently Constable discourages the oral administration of hypo-osmolar solutions or water to calves.[508]

Some oral rehydration solutions contain bulking agents. This is often psyllium (ispagula husk), which is a mucopolysaccharide, but rice flour is also used. One study has shown a benefit of including mucopolysaccharides in electrolyte formulations to treat calves infected with *E. coli*.[511] The treated calves had increased growth rates and fewer days with diarrhoea. Two subsequent controlled studies using solutions containing psyllium in naturally occurring undifferentiated diarrhoea did not show a clinical improvement in outcome compared with a standard oral electrolyte solution.[512, 513] It was postulated that psyllium would increase glucose absorption from the intestine, however there was no difference in blood glucose between calves receiving a standard electrolyte solution and those receiving the standard solution with added psyllium.[512] Studies on the use of rice-based products in treating scours in calves are limited. One study fed 6 calves a rice-based rehydration solution instead of milk for 3 days and it induced diarrhoea in 5 of the 6 calves.[514] This effect was reversed when calves were returned to the milk replacer diet. It was concluded that calves are unable to properly digest the rice-derived carbohydrate, and this type of formula is not recommended for oral rehydration of calves.

Clinical trials have studied the addition of glutamine to rehydration solutions. Glutamine promotes mucosal repair and stimulate sodium and chloride absorption.[515-517] Studies in piglets and calves demonstrated no difference in the percentage reduction in villous area, mucosal protein content or lactase specific activity when a glucose-based electrolyte was compared with a glutamine-based electrolyte.[518, 519] Similarly no significant difference was shown when treating diarrhoeic calves with solutions containing glutamine as the main amino acid compared with glycine.[520]

Commercial oral rehydration solutions do not contain enough energy, protein, minerals and vitamins to meet the maintenance and growth requirement of the calf. Consequently there is debate about the withdrawal of milk and for how long. Continued feeding of milk has been shown to maintain weight gain, but in one study continuing full rations initially led to greater inappetence.[500, 521] Withdrawal of milk without replacement with a high energy alternative can rapidly result in cachexia and malnourishment. However lactose digestion and xylose absorption are impaired in the diarrhoeic calf[522] and it is argued that continual feeding of milk leads to increased dehydration due to the osmotic effect of unabsorbed nutrients drawing water into the gut.[502] The undigested nutrients also promote bacterial overgrowth and possibly malfermentation with production of organic acids.[184] Naylor[184] recommends feeding limited amounts of milk for less severely affected calves and the withdrawal of milk when the calf is depressed and not interested in sucking. Heath[327] also recommends that affected calves should drink milk voluntarily, and be allowed as much milk as they would if they were healthy. Where calves are depressed and not sucking voluntarily 1-2 days of electrolyte therapy should restore vigour and sucking drive and milk can be gradually reintroduced in 1 L amounts given 2 to 4 times daily.[184] Where calves are not interested in drinking after several days or get

depressed when re-introduced to milk, a high energy oral electrolyte preparation can be used instead.

Oral electrolytes products available in Australia

Oral electrolytes products available in Australia have recently been comprehensively reviewed and it was concluded that there is no product with the “ideal” composition and only four products have adequate alkalising ability (see Table 3).[523, 524] Furthermore none of the label doses have the appropriate volumes recommended to meet the requirements of a calf with diarrhoea. Three of these products use citrate as an alkalising agent and two of these also use bicarbonate. Only one product uses acetate and propionate and is suitable for feeding to calves that are receiving milk ie suckling calves left on their mothers. However this product has lactose rather than glucose as the energy source. Lactose is the main carbohydrate in milk and the only carbohydrate that the preruminant calf can digest in the first 3 weeks of life.[525] However lactose digestion and xylose absorption are impaired in the diarrhoeic calf.[522] Lactose is hydrolysed at the intestinal mucosa by lactase to form glucose and galactose, which are then absorbed by the sodium co-transport mechanism. There is little information available on the use of lactose in electrolyte solutions in diarrhoeic calves. One study has shown that diarrhoeic calves appear to digest and absorb lactose when fed in small amounts.[522] Another study showed that lactase activity remained normal in calves with ETEC infections but rotavirus or combined rotavirus/ETEC infection led to more severe mucosal damage and a decrease in lactase activity.[81] This is likely to apply to diarrhoea caused by other agents that destroy the mucosal epithelium such as coronavirus and cryptosporidium. Calves that are unable to turn lactose into glucose and galactose may not have a sufficient glucose drive for sodium and water absorption from oral electrolyte solutions.

Table 3: Composition[§] of oral rehydration solutions available in Australia from Cannon[524]

Product *Company*	Sodium 60-130 mmol/L^	Chloride 40-80 mmol/L^	Potassium 10-20 mmol/L^	Alkalinising agent 40-80 mmol/L^	Glucose 110-140 mmol/L^	Glucose/glycine –sodium ratio (moles) 1:1-3:1^	Osmolarity 300-700 mOsm/L^	*kcal/L**	Cost per treatment~	Other ingredients
Bovelyte Plus *Provico Australia*	112.0	46.4	17.6	75 Bicarbonate	189	1.7:1	365.0	137	$1.71 (2kg) $1.63 (4kg) $1.27 (10kg) $1.21 (20kg)	Vitamins A, D3, E, B1, B2, B3, B5, B6, B9, B12, C, H Flavouring
Dexolyte *Pharm Tech Pty Ltd*	43.85	58.22	14.32	—	183.77	4:1	300.16	131	—	Vit A 800 IU/L
Diarrest *Virbac (Australia) Pty Ltd*	*147.60*	*102.72*	*30.85*	52.21 Acetate, citrate	111.01	*0.8:1*	536.53	320	$3.58 (9s) $3.29 (18s)	Lactose: 73.04 mmol/L[a] $H_2P_2O_7^{2-}$: 7.89 mmol/L Alanine: 11.22 mmol/L Rice flour Pre-gelatinised starch
Hydrate Liquid Pharm Tech Pty Ltd	75.82	73.58	14.95	*2.24* Propionate	136.18	2.3:1	388.90	98	$2.26 (2L)	Glycine: 41.29 mmol/L $H_2PO_4^-$: 14.95 mmol/L[†]
Lactolyte Virbac (Australia) Pty Ltd	79.32	55.15	*37.33*	47.32 Acetate, propionate	—	—	333.48	144	$2.44 (12s) $1.31 (5kg)	PO_4^{3-}: 6.41 mmol/L[†] Mg^{2+}: 2.52 mmol/L Lactose 105.43 mmol/L[a]
Lectade / Vy'Trate (sachets) *Jurox Pty Ltd*	73.41	73.41	15.58	*1.45* Citrate	123.81	2.2:1	377.53	89	$3.24 (12s)	Glycine: 41.16 mmol/L $H_2PO_4^-$: 14.99 mmol/L[†]
Megalyte *Sykes Vet International Pty Ltd*	88.20	59.03	—	29.16 Bicarbonate	222.28	2.5:1	398.68	184	$1.01 (1kg) 92c (2kg) 78c (4kg) 64c (10kg)	—
Megalyte Plus *Sykes Vet International Pty Ltd*	81.73	46.05	14.22	40.76 Bicarbonate, citrate	*90.14*	1.1:1	*291.41*	65	$2.10 (1kg) $1.53 (2kg) $1.19 (4 kg) $1.06 (10kg)	$Mg(OH)_2$: 6.17 mmol/L Mucopolysaccharide
Pronto/Resus Dasco Pty Ltd	84.11	57.32	—	*26.78* Bicarbonate	*214.46*	2.5:1	382.67	170	$1.51 (1.75kg) $1.46 (4kg) $1.09 (8kg) $1.06 (16kg)	—
Res-Q DeLaval Pty Ltd	88.27	58.86	—	*29.40* Bicarbonate	*222.48*	2.5:1	399.02	176	$1.82 (2kg) $1.50 (10kg) $1.44 (20kg)	—
Scourlyte *Novartis Animal Health Australasia*	89.13	75.72	18.91	*10.77* Citrate	*161.52*	1.8:1	356.06	176	$2.70 (1kg) $1.62 (5kg)	Pre-gelatinised maize starch microcrystalline cellulose
Scourproof Bayer Australia Ltd	81.73	46.05	14.22	40.76 Bicarbonate, citrate	*90.14*	1.1:1	*291.41*	65	$3.81 (1.15kg)	$Mg(OH)_2$: 6.17 mmol/L Mucopolysaccharide
Vy'Trate Liquid Concentrate *Jurox Pty Ltd*	73.16	73.16	*29.45*	*1.56* Citrate	123.78	2.3:1	355.92	89	$2.85 (1L)	Glycine: 41.09 mmol/L PO_4^{3-}: 9.62 mmol/L[†]

[§] Osmolarities and molarities assume that all compounds dissociate completely in solution
^ Highest and lowest recommended values from review of published recommendations
* 1g of carbohydrate (glucose, lactose and rice flour) = 4 kcal
~ Treatment cost is per 1.5 or 2L dose and includes a mark-up and GST

When the three solutions containing citrate or bicarbonate are considered for use in calves that are not receiving milk, two are mildly hypo-osmolar and have low glucose levels. These solutions also both contain ispaghula husk. The third solution is the most hyperosmolar solution available with above recommended levels of sodium, chloride and potassium. It also contains rice flour.

Therefore the Australian industry has one solution that can be used in milk fed calves and two mildly hypo-osmolar solutions and one hyper-osmolar solution that should be fed separately from milk. It can be concluded that there is no obviously best solution or solutions that veterinarians can recommend, especially for beef calves in a cow-calf operation.

Intravenous Fluid Therapy

If a calf is depressed and unwilling to suckle, intravenous fluids are the preferred and often only method of reviving them. Administration of fluids and electrolytes orally corrects deficits more slowly than can be achieved by IV fluid administration due to the limited capacity of the gastrointestinal tract.[526] The sodium concentration of oral electrolyte solutions is also limited because of requirements for optimal osmolality for abomasal and intestinal absorption, plus maintaining the optimal sodium-glucose ratio for sodium transport.[506, 527] IV fluids should always be given if the calf is more than 8% dehydrated.[528]

Ideally dehydration and acidosis should be corrected over 24 hours, but few problems are seen with correction over 2-8hours.[508]

Isotonic fluids

Saline based fluids may be used but an alkalising agent is preferable and clinical trials show that bicarbonate is the most effective, although lactate, acetate and gluconate have been used.[529] Bicarbonate is generally administered as an isotonic (1.3%) solution [508, 529]. Sodium bicarbonate can be added to saline (12.5 g to 1 L of 0.9% saline), but not to Lactated Ringers as calcium precipitates can form.[184] It is also possible to make up crystalloid solutions using clean or preferably sterile water. Directions for mixing solutions required for different levels of acidosis are reviewed by Jubb.[530] There is little requirement for the addition of glucose or potassium as most calves are not hypoglycaemic and any hypokalaemia will respond to hydration.[184]

Bicarbonate requirements can be calculated from base deficit values determined by blood gas measurement but blood gas analysers are seldom available in clinical veterinary practice in Australia. Therefore it is necessary to estimate the calf's deficit using the physical findings.

The maximum rate recommended for administration of iso-osmotic crystalloid solutions to a calf is 80 mL/kg body weight/ hour, which can be used for a severely dehydrated calf.[508] When fluid is administered rapidly calves should be monitored for any signs of fluid overload. This includes pulmonary oedema, which will manifest as tachypnoea, increased depth of respiration, flared nostrils, chemosis (oedema of the bulbar conjunctiva forming a swelling around the iris of the eye) and occasionally a moist cough.

Hypertonic fluids

Hypertonic saline dextran (7.2% saline containing 6% dextran 70) administered at 4 mL/kg BW during a 4 minute period concurrently with an isotonic alkalising oral electrolyte solution is effective in resuscitating dehydrated calves with diarrhoea.[526, 531] This treatment results in an immediate and sustained increase in plasma volume, cardiac output and stroke volume, together with an immediate and sustained decrease in HCT, serum albumin and protein, and plasma calcium. The treatment was comparable with administration of Lactated Ringer's solution at 80 mL/kg/hr for 1 hour (aggressive shock resuscitation) and continued at 4 mL/kg/hr for 7 hours, and with 32 mL/kg Lactated Ringer's solution. All groups of calves were maintained on appropriate oral electrolyte solutions for the 24 hours after intravenous administration of fluids. Unfortunately

the study of clinical cases, as opposed to experimentally induced cases of diarrhoea, did not measure the base deficit, so the efficacy of this treatment with severely acidotic calves is not known.

Calves with diarrhoea have a larger decrease in the extracellular fluids compared to the intracellular fluid.[532] Moreover the intestinal loss of sodium, chloride, potassium and bicarbonate results in the plasma and extracellular fluid becoming hypotonic. Consequently fluid moves from the extracellular fluid into the cells, exacerbating the hypovolaemic shock.[526] Treatment with hypertonic saline dextran reverses the flow from the extracellular fluid into the cells, and by using an isotonic oral electrolyte solution, the osmotic gradient also favours movement from the gastro-intestinal tract to the plasma. The predominant cation in the extracellular fluid is sodium and administration of hypertonic saline dextran achieves a more rapid correction of this deficit, together with expansion of the plasma volume, than achieved by administration of an isotonic intravenous solution.[526] The hypertonic saline results in a volume expansion of approximately 3 mL for every 1 mL administered.[533] The addition of dextran to the solution sustains the plasma volume expansion as 1 mL of dextran 70 expands the plasma by 0.8 to 1.2 mL. Because 50% of the dextran remains after 24 hours, a more sustained treatment is provided than by using hypertonic saline alone.[508]

Hypertonic saline dextran should always be given concurrently with an isotonic oral electrolyte solution. The oral fluid should ideally be given before the hypertonic saline dextran, due to the rapid expansion of the plasma, however administration of the hypertonic saline dextran first may temporarily resuscitate the calf and allow it to suckle.[526] When treating animals with hypertonic solutions care should be taken to remain in the vein as tissue damage will result from perivascular administration.[526]

This treatment would be an effective field treatment for severely dehydrated calves and a practical alternative to isotonic fluids. There are no published trials evaluating the response with hypertonic saline solutions that do not contain dextran, however these solutions are commonly used in the USA and the response is very similar to that with hypertonic saline dextran.[534, 535] Hypertonic saline dextran is not readily available in Australia, but isotonic saline dextran (0.9% saline containing 6% dextran 70) is requiring additional sodium chloride to produce a hypertonic solution. Hypertonic saline ± dextran should be administered at 1 mL/kg body weight/min. For most calves a 16 gu 1½" needle can be placed in the jugular vein, however a catheter should be placed if the calf is particularly mobile.

Hyperosmotic sodium bicarbonate solutions have also been used in acidotic diarrhoeic calves, especially in Europe.[536] Calves with experimentally induced respiratory and strong ion (metabolic) acidosis have been safely treated with 5 mL/kg of 8.4% sodium bicarbonate solution at a rate of 1 mL/min/kg and with 1.9 mL/kg of a 7% sodium bicarbonate solution administered over 15 minutes.[537, 538] Treatment resulted in an immediate and sustained (>60 minutes) increase in arterial pH, base excess, and [HCO3−], and a small transient (<15 minutes) increase in mean PCO_2 with no change in respiratory rate or minute volume.[537] This indicated that hyperosmotic sodium bicarbonate rapidly corrected the strong ion acidosis and created a strong ion alkalosis, while mildly and transiently increasing the severity of the respiratory acidosis. An immediate and sustained plasma volume expansion, increase in plasma sodium concentration, and decrease in plasma potassium concentration, haematocrit, haemoglobin, and plasma protein concentration were also measured. Whilst hyperosmotic sodium bicarbonate has the potential to be used in conjunction with an isotonic oral electrolyte solution to produce rapid reversal of acidosis together with increased plasma volume and cardiac parameters, there is no published data on such a study.

Intraosseous administration

If the calf is comatose and the blood pressure is so low that it is impossible to raise a vein, fluids may be administered intraosseously until perfusion is sufficient for placement of an intravenous

catheter. Intraosseous administration provides rapid access to the central circulatory system through the capillary-rich bone marrow.[539] In terms of the rate of fluid uptake, the intraosseous route is second only to a central venous route with the tip of the catheter resting within the large thoracic vena cava.[540] Placement of an intra-osseous needle can usually be achieved within 3 minutes. An area 2.5 cm^2 over the proximal humerus or femur is shaved and aseptically prepared prior to insertion of a 14-gauge 1 ½" needle into the centre of the bone, longitudinal to the length of the bone. The bone is soft and the needle can be "drilled" in. The needle will contain a core of bone so it should be removed carefully observing the position and a second 14-gauge 1 ½" needle placed in the same hole. A syringe containing 50 mL of saline is attached to the needle and injected to establish flow, then a litre bag of isotonic fluids is attached and run in as fast as possible. The preferred solution would be 1.3% sodium bicarbonate, although saline or lactated Ringers could be used if bicarbonate solution is not available. After 1 L has been administered it is usually possible to find the jugular, but if not a second litre may be administered. Once the calf regains consciousness this technique is difficult to maintain as the movement makes it difficult to keep the needle clean and in place. It is important to maintain asepsis when administering intraosseous fluids to minimise the risk of inducing osteomyelitis. Calves should also be covered with systemic antimicrobial therapy.

Subcutaneous fluids

A moderately dehydrated calf may be treated with subcutaneous fluids but this method should not be used in calves > 8% dehydrated as severe peripheral vasoconstriction will mean that fluids are not absorbed.[496] No more than 500 mL should be given at any one site up to a total of 2 L. Fluids should be isotonic, warm and should not contain glucose as this may lead to abscessation. They should be administered high on neck or thorax with a fast drip rate to allow more even distribution into subcutaneous space.

Intraperitoneal fluids

The effectiveness of the administration of intraperitoneal fluids has not been evaluated, concerns have been raised that there is altered absorption from the peritoneal cavity in dehydrated calves and that there is a risk of peritonitis, but these claims have not been substantiated.[508]

Calculation of fluid requirements

The total fluid required is the amount to correct the deficit, plus to replace ongoing losses through diarrhoea and provide daily maintenance. The hydration status of the calf can be estimated from the degree of enopthalmus, the degree of skin tent on the neck and evaluating the mucous membranes (see Table 2).[184] The volume (L) required to replace the deficit is % dehydration x calf body weight (kg). The ongoing losses through diarrhoea should be estimated from the nature and volume of the diarrhoea. Studies have shown that faecal loss can account for 1-6 L in diarrhoeic calves.[505]. Maintenance requirements have been estimated at 50-100 mL/kg/day.[501, 505]. The degree of hydration and the volume of faeces passed should be reassessed daily and the treatment adjusted accordingly. Only 60-80% of oral fluids are absorbed and this needs to be accounted for in the calculation.[184, 497] It should also be noted that calves given oral solutions have more liquid faeces than those supplemented intravenously.

Administration of fluids

Large volumes of oral electrolyte solutions can safely be administered to neonatal calves.[526] Calves have been shown to drink up to 19% of bodyweight at one feed, and the abomasum expands to accommodate this volume of fluid.[210] The fastest and most efficient way to administer oral fluids is using an oesophageal feeder. When fluid is given by oesophageal feeder it is initially deposited in the rumen and reticulum, but overflows into the abomasum after the administration of only 400 mL in calves aged less than 18 days of age and after 2 L in older calves.[541] Despite this some calves will become bloated and uncomfortable[508], and in these

cases administration of smaller amounts is preferable, but in many situations not practical when aiming to correct a deficit and compensate for further losses within the first 12 hours. Calves should be monitored for any evidence of reflux, or excessive distress and if this occurs the treatment should be stopped.

Ongoing fluid therapy

Calves should be reassessed after the initial volume of fluid is administered and therapy adjusted accordingly. The calf should improve over 24 hours and persistent depression is most likely to be a sign of uncorrected acidosis or toxaemia. ([184] However, hypoglycaemia, hypernatraemia and hyponatraemia should also be ruled out.

Hypoglycaemia is a common sequelae to withdrawal of milk for more than 48 hours, especially in cold weather. Affected calves are weak or recumbent, but appear to be normally hydrated, or minimally dehydrated.[501] They are often emaciated, and can occasionally have neurological signs including facial twitches, convulsions, opisthotonus and coma. They will respond to infusion of 5% glucose, but often this response is temporary, especially if suffering from severe malabsorptive disease. It is important to rapidly restore adequate energy intake to ensure resolution of these cases.

Hyponatraemia and hypernatraemia are less common findings, but may be a result of improper mixing of oral electrolyte solutions.[542] Hypernatraemia may also result from the use of high sodium content milk replacer or limited access to fresh water, consequently it is often farm specific.

Hyponatraemia occurs when a massive loss of isotonic fluid through the gastro-intestinal tract is replaced by free water or hypotonic solutions. The latter often occurs when oral electrolyte solutions are made too dilute. Hyponatraemia may also occur with isotonic solutions when the ability to absorb sodium is compromised. This may be due to severe pathological changes or an inadequate level of agents that facilitate sodium co-transport within the oral electrolyte solution. Hyponatraemia results in a fluid shift from the extracellular space to intracellular compartment along the osmotic gradient and the resultant swelling of the cells can result in neurological disturbances; depression, disorientation and even convulsions.[542] Hyponatraemia should be considered in calves with a serum sodium < 132 mmol/L and calves with a serum sodium < 120 mmol/L have severe hyponatraemia.

The goal of therapy is to restore serum sodium levels to > 125 mmol/L over the first 6 hours and then to restore to normal levels over 24 hours.[542] In hypovolaemic calves normal saline should be administered, and in normovolaemic calves hypotonic saline should be used initially because the administration of large fluid volumes will exacerbate the oedema. If the calves are suspected to be acidotic this should also be corrected with bicarbonate solutions of appropriate tonicity.

The amount of sodium required in the first 6 hours to raise the sodium level to 125 mmol/L can be calculated as follows:[542]

$$\text{Sodium (mmol)} = [125 - \text{measured serum sodium (mmol/L)}] \times [0.6 \times \text{Bodyweight (kg)}]$$

Calves should then be maintained on sodium containing isotonic fluid, such as normal saline or lactated ringers and treated with oral electrolyte solution as appropriate. The sodium level should be monitored frequently in the first 24 hours due to unknown losses through the gastro-intestinal tract and unknown kidney function in a severely dehydrated patient.

Hypernatraemia is defined as a serum sodium concentration over 152 mmol/L, but only levels greater than 170 mmol/L have been associated with nervous dysfunction.[543] Hypernatraemia can occur due to the loss of hypotonic fluid in faeces or when oral electrolyte solutions are improperly diluted. This will be exacerbated if calves have no access to water or have stopped

suckling.[542] Rapid development of hypernatraemia results in fluid moving from cells into the extracellular fluid and produces cellular dehydration. Neurological signs include lethargy, weakness, depression coma and death.

Treatment for severe hypernatraemia should only occur when serum sodium levels are greater than 170 mmol/L. Gradual treatment is preferred as rapid treatment may lead to cerebral oedema.[542] Treatment protocols recommended in the mid-1990s involved administration of isotonic fluids to produce volume expansion if required, followed by 5% dextrose solution too supply free water. With this protocol calves some died of cerebral oedema and treatment with 0.45% saline in 5% dextrose was recommended to reduce this risk.[542]

More recently the use of intravenous fluids manipulated to contain concentrations of sodium approximately equal to that of the plasma has been recommended.[544] The goal is to reduce plasma sodium by less than 5 meq/L/day over the first 48 hours by slow excretion through the kidneys. The volume given should provide rehydration and cover maintenance and ongoing losses similar to the treatment of any other diarrhoeic calf. The solution may require bicarbonate if the calf is suspected to be acidotic. Until plasma sodium levels are approaching normal, sodium should also be added to any oral fluids (ie milk replacer) so that the concentration is approximately equal to the intravenous fluids. Cerebral oedema will present as coma or seizures and may be treated with 25% solution of mannitol at 1 g/kg IV over 30 minutes or an oral solution of glycerin given at 1 g/kg diluted 1:1 with water.

Role of antibiotics

There is some controversy regarding the use of antimicrobials for the treatment of calf scours. Reports questioning the use of antimicrobial therapy are derived from lack of efficacy, potential for adverse effects, the potential for violative residues, and selection for antimicrobial resistance. Conversely there are reports describing attenuation of clinical disease, reduced pathogen shedding and lower mortality following the use of antimicrobials to treat scouring calves. The following review of antimicrobial use in calves is restricted to drugs available for use in Australia. The results of studies using chloramphenicol, fluroquinolones, and nitrofurazone have been omitted as these drugs are not recommended for use in food producing animals in Australia.

Therapeutic targeting

Bacterial pathogens associated with neonatal calf diarrhoea include salmonella and *E. coli*. During disease outbreaks caused by these pathogens antimicrobial use may be targeted at the specific pathogen. Beneficial responses to antimicrobial therapy have also been reported in field trials involving undifferentiated pathogens.[545, 546] Calves with diarrhoea often have small intestinal overgrowth with *E. coli*, regardless of the inciting cause. [547-549] and this colonisation is associated with altered small intestinal function, morphologic damage, and increased susceptibility to bacteraemia.[549] Faecal bacterial culture and antimicrobial susceptibility testing is not recommended in calves with diarrhoea because faecal bacterial populations do not accurately reflect small intestinal or blood bacterial populations.

Calves with diarrhoea are more likely to have failure or partial failure of passive transfer, and this group of calves, in turn, is more likely to be bacteraemic.[550, 551] Two studies of diarrhoeic calves that presented with depressed mentation detected bacteremia in a significantly ($P < .01$) greater proportion of calves with failure of passive transfer (44/129 = 34% and 47/103 = 46%) than in calves with adequate passive transfer (3/40 = 8% and 21/116 = 18%).[550, 551] The median and mean age of bacteraemic calves in these studies were 8[550] and 9[551] days respectively. Blood cultures indicate gram negative bacteria account for approximately 80% of bacterial isolates, *E. coli* is the most common bacteria isolated.[550, 552, 553] In a study of 190 recumbent calves on a large calf raising facility 31% were determined to be bacteraemic, *E coli* accounted for 51% of the isolates, other gram negatives 25%, gram negative anaerobes 5.9%, gram positive cocci 11.8%, and gram positive rods 5.9%.[550]

Antimicrobial therapy may therefore be targeted at a specific bacterial enteric pathogen isolated from sick calves or in severely ill calves (as manifested by reduced suckle reflex, >5% dehydration, weakness, inability to stand, or clinical depression) used prophylactically to manage the risk of bacteraemia, for this application emphasis should be directed toward gram negative organisms particularly *E. coli*.

Antimicrobial susceptibility

Antimicrobial susceptibility testing of faecal isolates has not proven to be a good predictor of clinical outcome. Two reports concluded that a "good correlation" existed between in vitro antimicrobial susceptibility of faecal *E. coli* isolates and clinical response to antimicrobial treatment.[554, 555] Three other studies reported no correlation between in vitro antimicrobial susceptibility of faecal *E. coli* and *Salmonella* spp isolates and clinical response to antimicrobial treatment.[556-558] The only study to statistically test the predictive ability of fecal antimicrobial susceptibility results found that the rectal swab was an inaccurate method of predicting clinical outcome.[558] Antimicrobial efficacy is best evaluated by the clinical response of a number of calves to treatment, with calves randomly assigned to treatment groups, rather than the results of in vitro antimicrobial susceptibility testing performed on faecal *E. coli* isolates.[559]

Antimicrobial susceptibility testing has more clinical relevance for predicting the clinical response to antimicrobial treatment when applied to bacteria isolated from blood or tissues of bacteremic calves because the minimal inhibitory concentration break points are based on achievable antimicrobial concentrations in human plasma and MIC_{90} values for human *E. coli* isolates, which provide a reasonable approximation to achievable MIC values in calf plasma and MIC_{90} values for bovine *E. coli* isolates.[559] Even within a given herd there will be a diversity of bacteria isolated from bacteraemic calves so the collection of blood cultures and assessment of antimicrobial susceptibility does not necessarily provide information applicable to the next case. In the US, studies conducted on calf rearing operations have provided indications of the relative frequency of resistance to different antimicrobials present in those facilities,[550] knowledge that could be applied as a general guide for antimicrobial selection in that facility. There are no such reports originating from Australian isolates and given the different management practices and antimicrobial use patterns it is likely that the patterns of antimicrobial resistance will differ.

Antimicrobial safety

A number of antimicrobials have been demonstrated to produce deleterious effects when administered orally to healthy milk-fed dairy calves. The addition to milk replacer powder of procaine penicillin (2–60 mg/kg of milk replacer) increased the incidence and duration of diarrhea and decreased growth rate compared with untreated controls in a total of 36 milk-fed calves.[560] Penicillin is not labelled for treatment of calf scours and has an inappropriate antimicrobial spectrum to prevent or treat calf scours. Administration of neomycin sulfate (300 mg PO q24 h for the 1st 4 days of life) tended (P = .060) to increase the proportion of calves developing diarrhea (99/233 = 43%) compared with the proportion in an untreated control group (58/174 = 33%).[561] Administration of neomycin sulfate (25 mg/kg PO q6 h, n = 10), ampicillin trihydrate (12 mg/kg PO q8 h, n = 6), or tetracycline hydrochloride (11 mg/kg PO q12 h, n = 6) for 5 days increased the occurrence of diarrhea and decreased glucose absorption through unknown mechanisms compared with untreated controls (n = 6),[562] The doses used in this study were higher than the recommended doses in oral anti-diarrhoeal formulations sold in Australia. Two other studies did not observe adverse side affects in calves administered tetracycline hydrochloride (40 mg PO q12 h; 11 mg/kg PO q12 h).[563, 564]

In another report that questions the use of antimicrobial therapy for treatment of calf scours a survey was conducted of dairy farms and it was found that calf mortality was higher on farms that treated calves with antimicrobials.[565] This was subsequently interpreted to indicate that mortality was greater when antimicrobials were used to treat calf scours.[562] This conclusion appears to overstate the findings of Oxender et al as the incidence of calf scours on antimicrobial

use and no use farms was not defined so it is impossible to determine if antimicrobial use was in response to calf scours.[565]

Efficacy of Oral Antimicrobial Therapy

Sulfadimidine, sulfadiazine, streptomycin sulfate, dihydrostreptomycin sulfate, neomycin sulfate, amoxycillin trihydrate clavulanic acid, oxytetracycline, and apramycin are labeled for oral administration for the treatment and prevention of calf scours calves in Australia. Orally administered apramycin has proven to be efficacious in field studies.[546] The results of field and experimental trials with the other antimicrobials available in Australia have been equivocal.[559]

In a study involving 347 dairy calves with diarrhoea[546] apramycin significantly decreased mortality rate in calves treated at 20 mg/kg PO q24 h for 5 days (mortality 10/118 = 9%, $P < .001$) or 40 mg/kg PO q24 h (mortality 6/108 = 6%, $P < .001$) when compared with untreated controls (mortality 36/121 = 30%). Apramycin administration PO also increased growth rate in survivors.

In a field study evaluating the efficacy of neomycin (dose not stated) administered orally twice a day for 2 days mortality rate for treated calves (6/21 = 28%) was similar to non-treated calves (6/21 = 28%).[566] The mean duration of diarrhea tended to be shorter in treated calves (6.5 days) compared with untreated calves (9.7 days). In an experimental *S. dublin* challenge trial no significant difference in mortality (P=0.29) was observed in 1 – 2 week old calves treated with 500 mg of neomycin sulfate PO q12 h (3/6 = 50% died, $P = .29$) compared to non-treated control calves (16/20 = 80% died).[567] Treatment began when calves had profuse diarrhoea and fever.

No statistical difference ($P = 0.83$) in mortality was observed between calves treated with orally administered ampicillin (12 mg/kg PO q12 h for 3–5 days) (mortality 26/83 = 31%) and non-medicated control calves (mortality 27/82 = 33%).[568] In this study antimicrobials were not administered until diarrhea had been present for a number of days. In another field study 48 of 80 (60%) calves held off milk for 24 hours that did not receive antibiotics survived verses 45 of 62 (73%) that were held off milk and treated orally with ampicillin (dose not defined).[569] Thirty four of 45 (76%) calves that were not held off milk and were not treated with antibiotics survived verses 38 of 60 (63%) of calves not held off milk and treated with ampicillin.[569] None of the outcomes were statistically significantly different. In this study the cause of the diarrhoea was not defined. Salmonella was isolated from some calves at necropsy but the antimicrobial sensitivity of the isolates and its relation to the antimicrobial therapy implemented was not defined.[569]

In a field study evaluating the efficacy of trimethoprim sulpha and a sulfamethazine neomycin combinationadministration of trimethoprim (5 mg/kg PO q24 h) and sulfadiazine (25 mg/kg PO q24 h) for 3–5 days had no effect ($P = 0.17$) on the proportion of calves returning to normal fecal consistency (recovery rate 88/101 = 87%) when compared with a combined treatment of 87 mg/kg PO q12 h sulfamethazine and 11 mg/kg PO q12 h neomycin sulfate (recovery rate 62/78 = 80%) or with an untreated control group (recovery rate 23/31 = 74%, $P = 0.097$). [570] Conversely in a *S. dublin* experimental challenge study involving 2–3 week old calves, daily administration of trimethoprim, sulfadiazine, or both (in 1:5 ratio) was started 24 hours after challenge, at which time the calves were slightly subdued but otherwise clinically normal, and continued for 5 days.[571] Compared with an untreated control group (5/7 = 71% died), the mortality rate tended to be lower in calves treated with trimethoprim/sulfadiazine boluses (5 mg/kg trimethoprim and 25 mg/kg sulfadiazine; 1/7 = 14% died, $P = 0.10$). Similar mortality rates were observed in control calves and calves treated with a lower dose of trimethoprim/sulfadiazine (2.5 mg/kg trimethoprim and 12.5 mg/kg sulfadiazine; 4/7 = 57% died, $P = 1.00$), trimethoprim (10 mg/kg; 4/7 = 57% died, $P = 1.00$), or sulfadiazine (50 mg/kg; 6/7 = 86% died, $P = 1.00$).

The efficacy of amoxycillin trihydrate has been evaluated in two experimental enterotoxigenic *E. coli* challenge studies. In the first study diarrhoea was experimentally induced in forty 5 – 10 day old calves and treatment was administered immediately after diarrhoea was detected.[572] Mortality rate was significantly ($P < 0.05$) lower in calves administered amoxycillin trihydrate in

milk replacer (at 10 mg/kg PO q12 h for 4 days; 1/20 = 5%) than in non-medicated control calves (6/20 = 30%). The duration of diarrhoea was significantly (P < 0.01) shorter in calves administered amoxycillin (3.9 ± 0.1 days) than in non-medicated control calves (5.7 ± 0.2 days). In the second 82 calves were orally challenged with enterotoxigenic *E. coli.*[573] Treatment was administered immediately after the onset of diarrhoea. The mortality rate tended to be lower in calves administered amoxycillin (as amoxycillin trihydrate, 10 mg/kg PO q12 h for 2 days; 1/21 = 5%), oral electrolyte solution (1/20 = 5%), or oral electrolyte solution and amoxycillin (0/20 = 0%) than in untreated control calves (4/21 = 19%). The duration of diarrhoea was significantly (P < .05) shorter in calves administered amoxycillin (3.1 ± 1.9 days), oral electrolyte solution (3.1 ± 1.1 days), or oral electrolyte solution and amoxycillin (2.3 ± 1.5 days) than in untreated control calves (4.6 ± 2.3 days). Rotavirus was also isolated from calves in this study.

In an epidemiological study of salmonella in dairy calves conducted in the United States, feeding medicated milk replacer and hay to calves from 24 hrs of age to weaning was associated with a reduced risk of salmonella shedding.[574] This observation contradicts an experimental study in which feeding chlortetracycline in milk replacer increased the severity of disease and the rate and duration of salmonella shedding.[575] Similarly in another experimental trial daily drenching of calves with 50 mg or 100 mg of chlortetracycline failed to alter the excretion pattern or the number of organisms excreted by calves infected orally with 10^6 *S. typhimurium*.[576] In another study that examined sub-therapeutic and therapeutic antimicrobial therapy two of four groups of seven calves were maintained on a sub-therapeutic amount of chlortetracycline. All calves were then challenged with *S. typhimurium*, and with the onset of clinical symptoms one group with and one group without sub therapeutic chlortetracycline were given a therapeutic dose of oxytetracycline. The two groups receiving a therapeutic dosage of oxytetracycline had the quickest decline in body temperature and the highest average body weights after challenge. Two calves died in the group receiving no antibiotic treatment, and one calf died in the group receiving only the sub-therapeutic treatment.[577]

Efficacy of Parenteral Antimicrobial Therapy

There are no parenteral antimicrobial formulations specifically labelled for the treatment of calf scours in Australia. Antimicrobial drugs with an appropriate gram negative spectrum of activity include third generation cephalosporins (ceftiofur), potentiated penicillins (amoxycillin), trimethoprim sulphonamide combinations (TMS), aminoglycosides, sulfonamides, florphenicol, and tetracyclines. There is a paucity of efficacy data to support the use of aminoglycosides, tetracycline, non potentiated sulfonamides, and florphenicol.

Ceftiofur has an appropriate antimicrobial spectrum and therapeutic drug concentrations can be maintained with once daily dosing. In a *S. typhimurium* challenge experiment intramuscular administration of ceftiofur hydrochloride (5 mg/kg q 24hrs for 5 days) reduced the severity of clinical signs and reduced faecal shedding of salmonella. The MIC of the challenge strain in this experiment was 1 ug/mL and the therapeutic protocol maintained plasma concentrations above this concentration for the duration of therapy.[372]

Potentiated sulphonamides have been evaluated in enterotoxigenic *E. coli* and salmonella challenge experiments. Mortality in 2–3 week old calves medicated with trimethoprim sulfadiazine (in a 1:5 ratio) for 5 days 24 hours following *S. dublin* oral challenge was reduced.[571] Compared with untreated controls (19/22 = 86% died), the mortality rate was significantly lower in calves treated with trimethoprim/sulfadiazine (20 mg/kg sulfadiazine and 4 mg/kg trimethoprim IV; 2/14 = 14% died, P < 0.0001), trimethoprim/sulfadiazine (20 mg/kg sulfadiazine and 4 mg/kg trimethoprim IM; 1/14 = 7% died, P < 0.0001), or a lower dose of trimethoprim/sulfadiazine (10 mg/kg sulfadiazine and 2 mg/kg trimethoprim IV; 1/7 = 14% died, P = 0.0011). Administration of either sulfadiazine or trimethoprim alone did not reduce mortality.[571] TMS may be used to treat sepsis in neonatal calves, but its half life rapidly declines as rumen function develops. In ruminating (6-8 wk old) calves, subcutaneous or oral

administration of trimethoprim sulfa leads to high serum levels of sulfadiazine but little or no serum trimethoprim.[578]

Intramuscular administration of amoxycillin reduced mortality in *S. dublin* challenged calves.[579] Six calves medicated with amoxycillin (20 mg/kg on the first day followed by 10 mg/kg daily for 4 days) survived following oral challenge with 7.6 x 10^8 S. *dublin*. The three non-medicated calves administered the same dose died. In a comparative trial of amoxycillin and trimethoprim sulphadiazine both drugs were found to have equal efficacy in reducing adverse clinical signs of disease when dosage regimens were based on the MIC of the pathogen.[580]

The frequency of bacteraemia is sufficiently high that treatment regimes for severely ill calves with diarrhoea (as manifested by reduced suckle reflex, >5% dehydration, weakness, inability to stand, or clinical depression) should include routine antibacterial treatment, with emphasis on treating potential *E coli* bacteremia.[559] Parenteral administration of a broad-spectrum beta-lactam antimicrobial - ceftiofur (5 mg/kg IM q24 h), amoxycillin (10 mg/kg IM q12 h), or Trimethoprim Sulfadiazine (20 mg/kg sulfadiazine with 5 mg/kg trimethoprim IV or IM, q24 h for 5 days) is recommended for treating calves with diarrhoea and systemic illness (Note these are off label doses and require an extended meat withholding period). Antimicrobial therapy is not recommended for calves with diarrhoea and no systemic illness (normal appetite for milk or milk replacer, no fever). [559]

Antiprotozoal Drugs

There are currently no effective therapeutic options for treatment of cryptosporidiosis in Australia. Drugs reported to have some efficacy against cryptosporidia in calves include, halfuginon,[581-587] paromymicin,[588, 589] decoquionate,[590, 591] and β_-cyclodextrin.[592] Halfuginon is licensed for treatment of calves in Europe and appears to be the most efficacious. The efficacy of decoquionate is questionable with the only controlled clinical study failing to demonstrate a beneficial therapeutic effect with daily treatment at (2 mg/kg per day).[591] Lasalocid has been trialed for treatment of cryptosporidia. Using a toxic dose of 8 mg/kg it was found to reduce the shedding of cryptosporidia however the calves suffered adverse side effects. At a dose of 0.8 mg per kg lasalocid was not effective.[593] The registered dose for preventing coccidiosis in calves is 1 mg/kg per head per day.

Coccidiosis is uncommon in calves less than 6 weeks of age. In hand reared calves coccidiostats (lasalocid, amprolium, or decoquionate) may be added to milk replacer. Prophylactic options for beef calves are restricted to coccidiostat medicated pellets (monensin, lasalocid, amprolium, or decoquionate) or water (amprolium or sulfonamides). Therapeutic options include amprolium or sulfonamides such as sulfadimidine.

Both fenbendazole (5 mg/kg once daily for 3 days PO.) or albendazole (20 mg/kg once daily for 3 days PO.) have been shown as effective treatments for Giardia.[196, 198, 594] Due to the high level of subclinically affected animals all cows and their dams need to be treated and reinfection is likely to occur unless calves are removed from environmental sources of infection.

Other Treatments

A recent study looked at the benefits of a single or double injection of flunixin meglumine in scouring calves.[595] Although a trend towards decreased morbidity was shown this was not statistically significant.

Probiotics

Probiotics are a food or drug containing live microbes that, when ingested, is expected to confer beneficial physiological effects to the host animal through microbial actions. A number of probiotic products are licensed for the prevention and treatment of calf scours in Australia.

Bacterial and fungal species included in these products include *Enterococcus faecium*, *Lactobacillus acidophilus, Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Streptococcus salivarius* subsp. *thermophilus*, *Aspergillus oryzae*, and *Candida pintolepesii*. General mechanisms of action that have been prescribed to probiotics include competition for receptor sites on the intestinal surface, immune system stimulation, excretion of anti-microbial substances, and competition with pathogens for intraluminal nutrients.[596]

The number of controlled clinical trials evaluating probiotic formulations in calves is limited. In one report feeding antimicrobial resistant *Streptococcus faecalis* to calves reared on an antibiotic-containing diet reduced salmonella intestinal colonisation of calves.[597] An improvement in weight gain and a reduction in diarrhoea has been reported when calves were fed either 3 x 10^9 *Bifidobacterium pseudolongum* or *Lactobacillus acidophilus* daily from 1 to 56 days of age or a cell mixture containing 10^{10} colony forming units (cfu) of *Bacillus thermophilum*, 10^{10} cfu of *Enterococcus faecium*, and 10^9 cfu of *Lactobacillus acidophilus* for 28 days.[598] In another study body weight gain and severity of scours was similar in calves fed milk replacer containing antimicrobials or probiotics (undefined microbes), this study did not include a non-medicated control group.[599]

A probiotic available in Germany that is not available in Australia is a non-pathogenic *E. coli* strain Nissle 1917. In a controlled blind study *E. coli* Strain Nissle 1917 significantly reduced the incidence of diarrhoea in calves administered 10^8 organisms daily for the first 10–12 days of life.[600]

The number of viable organisms present in commercial formulations available in Australia ranges from 10^5 to 1.8 x 10^8, the number of species ranges from 2 to 9.

Intestinal Protectants

A number of products that include intestinal protectants are marketed for treatment of calves with scours. Intestinal protectants include bismuth subsalicylate, kaolin or pectin, and activated charcoal. There is no efficacy data available regarding the use of kaolin in scouring calves. Experiments using a rat diarrhoea model found that administration of kaolin-pectin increased sodium and potassium losses and reduced fat losses suggesting that increased caution is required to maintain electrolyte status when kaolin-pectin adsorbents are used for symptomatic relief of neonatal diarrhoea.[601] Suggested advantages of bismuth subsalicylate are its neutralisation of bacterial toxins and antisecretory effect through its local antiprostaglandin activity.[602, 603]

Ancillary Therapies

Catechu an extract of the plant *Acacia catechu* is included as an ingredient in at least one anti-diarrhoeal product marketed in Australia. Catechu is used as a traditional medicine in India to treat enteric diseases. In vitro studies have demonstrated that catechu is inhibitory to salmonella and *E. coli* O157:H7. There are no published controlled studies evaluating the efficacy of this compound as an aid for the prevention or treatment of calf scours.[604, 605]

Mucopolysaccharides are included in a number of anti-diarrhoeal products and trials using oral electrolyte solutions containing psyllium are discussed in the section on oral electrolyte solutions

Ascorbic acid has been used as a preventive measure in calf scours and shown to have a beneficial effect.[606] However the frequent administration required is unlikely to be practical in a cow calf operation.

Biocol (Intervet Australia Pty Ltd) is a colostrum supplement containing approximately 4 g of bovine IgG extracted from the blood of 3-6 day old calves. It also contains whey protein and

dextrose. The label claims a protective effect against *E. coli* and rotavirus, however there are no published trials to support this claim.

Hyperimmune Products

A number of pathogen-specific hyperimmune products have been evaluated as adjunct therapies for the prevention of calf scours. Sources of antibodies include serum, colostrum, milk and eggs.[607-610] This approach to prophylaxis has been most effective for enterotoxigenic *E. coli*.[611-613] The success of the ETEC directed products in part reflects the limited duration of susceptibility to enterotoxigenic *E. coli* infection providing a defined opportunity for immunoprophylaxis at birth.[614] While hyper-immune products have been developed and are generally effective at preventing or attenuating the severity of other enteric pathogens specifically cryptosporidia,[615, 616] rotavirus[608, 617-619] salmonella,[620] and coronavirus[619, 621] the application of these products is more difficult on a practical level in beef calves as they need to be administered daily for the first 7 to 14 days of life.

For bucket or bottle fed calves addition of hyperimmune colostrum to calf milk or milk replacer at 1% of the total volume at each feeding or the inclusion of 2 to 8 grams of hyperimmune egg powder for the first 7 to 14 days of life is effective at providing protection against the viral pathogens.[608, 622, 623]

Practical aspects

Calves should be encouraged to suck milk voluntarily. Calves that do not want to feed should be encouraged to stand and rubbed vigorously along the back and over the chest and neck. This simulates maternal caring, and stimulates the calves appetites.[327] Where calves do not feed voluntarily an oesophageal feeder should be used. Milk should not be fed in conjunction with alkalising agents that affect the formation of a clot in the stomach (see p 75). The success of therapy should be based on based on the calf's clinical signs and restoration of urination

If intravenous fluids are required and it proves difficult to catheterise the calf it can be suspended by its hind legs so that blood will pool and distend the jugular veins.[184] . The calf's neck should be clipped and prepared prior to inversion and the calf laid flat as soon as the catheter is placed.

Benefits of separating from mothers

There appears to be no scientific research published on this. Presumably if the calf is able to stand and walk it is less stressful for the cow and calf to keep them together. If the calf is collapsed and dehydrated then it will need separation for administration of IV fluids. Leaving the calf with its mother will allow it to suckle voluntarily as recommended.[184, 327] The main concern if left with the mother is ensuring the correct electrolyte solution is used.

Supportive care

Calves should be evaluated for secondary problems such as hypoglycaemia and hypothermia. Hypoglycaemia is likely to occur if the calf is malnourished or endotoxaemic.[327] Hypothermia occurs due to poor hydration, poor nutrition or poor adaptation and can occur in all climates. The calf should be placed in a warm sheltered environment and warm air is one of the most effective ways of treating hypothermia.

Conclusions

This literature review has demonstrated that whilst significant research is occurring in some areas relating to neonatal calf diarrhoea, there are still many areas that are poorly understood, or there is conflicting information. Moreover there is virtually no published Australian research pertaining to the prevention and control of calf scours in suckler beef herds.

For instance there is a great deal of research on the contribution of individual aetiological agents, but few studies that consider all aspects of this complex disease and attempt to quantify the significance of the different predisposing factors. Molecular biology is resulting in significant advances in laboratory techniques to diagnose pathogens, yet there are enteropathogenic viruses for which there is no commercially affordable diagnostic test and consequently their contribution to the aetiology of neonatal calf diarrhoea is unknown. Although there is a reasonable understanding of the epidemiology of the respective pathogens there is little knowledge of the significance of environment reservoirs of pathogens relative to the role of subclinically infected animals within a herd.

An active area of research is the development of oral electrolyte solutions, and best practice methods of intravenous administration of fluids, and this review will result in improved recommendations for treatment of scouring calves. There are many studies into factors that influence colostrum quality, however the trials are often small and the information contradictory. The significance of FPT in outbreaks of neonatal calf diarrhoea in beef herds in Southern Australia is unknown.

Significantly there is a paucity of information on the prevention of calf scours in pasture based herds. Published research has taken place in much colder climates such as Canada. Recommendations can be made by adapting these techniques and applying knowledge of the epidemiology of the aetiological agents, however it is difficult to rank the impact of these suggestions.

Many producers view vaccines as the best prevention for a calf scour problem and many vaccines have been developed and trialed overseas. However vaccination is not a panacea for a specific pathogen and should only be considered when the property owner is prepared to address other significant risk factors, especially nutritional and environmental. It is also not a replacement for poor management. The protective efficacy of enterotoxigenic *E. coli* bacterins is well documented, but the efficacy of vaccines against salmonella, rotavirus and coronavirus is variable, depending on the type of vaccine and the study design.

In summary this literature review has demonstrated new developments in the diagnosis and treatment of neonatal calf diarrhoea that can be extended into the Australian industry. More significantly it has identified areas of research from overseas that need clarification here in Australia – for example the significance of milk clotting times could explain the regional and seasonal variation in the incidence of neonatal calf diarrhoea. It has clearly demonstrated that ongoing research is required into the prevention of neonatal calf diarrhoea in pasture based suckler beef enterprises.

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8.2 Appendix 2: Documents for Veterinarians

1. Prevention of calf scours

The objective of this paper is to provide a series of preventive strategies that can be applied to properties with a history of neonatal calf diarrhoea. Many of these are good management practices that will also promote general calf health and reduce the risk of transmitting diseases such as Johne's. Although practices can be applied generically without a diagnosis, it is advisable to have a thorough knowledge of the property and the calf scour problems they have experienced. Producer compliance is often better when preventive efforts are directed at a tangible pathogen rather than an abstract "bug". Identification of specific pathogens also recognises the need for pathogen specific interventions such as vaccination. Conducting a risk assessment of current management practices strategies highlights weaknesses in the system. For each area where action is required a means of assessing the outcome of the intervention should be identified to determine the effectiveness not only of the intervention but also the effectiveness of the implementation of the intervention.

This paper focuses on achievable management changes for the majority of beef enterprises, but most effective ways of achieving these goals will vary between management systems. Some preventive strategies will require a major change in how cows are managed and may be more time consuming. Producers are most likely to be interested in preventive strategies if they have experienced a calf scour outbreak.

Key information

- Calves are often subclinically affected by the major enteric pathogens and act as biological amplifiers
- All major enteric pathogens are commonly carried by asymptomatic adult cows and shedding is likely to increase at the time of calving
- All major enteric pathogens will survive in the environment and water sources for weeks to months (and in most cases over a year) in cool damp conditions
- Most other animal species, many birds and flies are potential vectors of cryptosporidium and salmonella.
- Feral animals and domestic pets are a potential reservoir of rotavirus

Principles of prevention

The susceptibility of a herd to calf scours can be reduced by:

1. minimising stress (nutritional and environmental) on both cows and calves both pre and post partum
2. minimising exposure to enteric pathogens
3. increasing the non-specific resistance of the calf
4. increasing pathogen specific immunity

1. Minimise stress

During the first 2 weeks of life most calves spend most of their time sleeping or suckling, and under crowded conditions their resting and feeding patterns may be altered. Crowding can be

due to a high stocking rate or environmental factors causing calves to concentrate in a small area.

Calving paddocks are often crowded because producers bring cows due to calve into close paddocks for observation. They also traditionally used the same paddocks throughout the calving season and year after year.

The following guidelines should be applied to minimise stress for the neonatal calf:

Key Points

- ✓ Ensure cows calve in good condition (CS 3.0-3.5 out of 5)
- ✓ Ensure adequate feed after calving (Minimum post grazing cover of 1500 kg DM/ha)
- ✓ Cows and calves should be drifted off with minimum stress 24 hours after calving into a nursing group tht where practical should be no more than 40 cows and calves.
- ✓ Calves should be provided with adequate shelter and shade.
- ✓ In windy areas paddocks should have shelter belts planted to protect from the prevailing winds. Windbreak fences (2.5 m high 20 % porosity) could be used as an interim measure. Alternatively calf coats could be used on particularly susceptible calves; Small, premature, difficult calving etc.
- ✓ In areas where heat stress appears to precipitate calf scours large areas of shade should be provided
- ✓ Cows and calves should have adequate access to fresh water within 300 metres at all times
- ✓ The time of calving should be chosen to avoid extremes of weather

Other suggestions that may be applicable in some herds

- ✓ Use breeding and management strategies to optimise calving ease particularly in heifers, and minimise the need for close supervision by reducing dystocia rates (acceptable dystocia rates are 2% of cows and 10% of heifers)
- ✓ Where inclement weather is a problem, producers could consider trialing calf shelters. These have been used successfully in the United States. The recommended dimensions of the shelters are 7.3 m long, 3.05 m deep and 2.44 m high and they are designed so that only the calves can enter. Shelters should be movable and contain fresh clean bedding

2. Minimise exposure to enteric pathogens

In order to minimise exposure to enteric pathogens in a moderate to intensively farmed situation it is necessary to run the stock in small groups and change paddocks frequently. This will require careful planning to determine the best way to utilise paddocks and will often require the creation of temporary paddocks with electric fences. In a more extensive farming situation many of these suggestions may not be practical. The benefit of these measures will also depend on the pathogen(s) causing problems on each property (see “Diagnosis of Calf Scours”)

Key Points

Management of cows

- ✓ Start calving heifers at least 2 weeks before the cows to reduce the exposure of the most vulnerable calves to the build up of environmental pathogens
- ✓ Have short calving periods to minimise the exposure of calves from late calving cows (6 weeks for heifers and 9 weeks for older cows)
- ✓ Move cows and heifers into calving paddock no more than 2 weeks before calving
- ✓ Calve cows and heifers in separate groups
- ✓ Do not bring in/purchase replacement calves from other properties

Calving paddocks

- ✓ Have a minimum of two and preferably 3 calving paddocks. It may be wise not to use the best-drained paddock first in the winter/spring as this will mean the other paddocks will be very wet and muddy. Instead save it for when it gets wet
- ✓ Use one calving paddock at a time and rotate every three weeks or more frequently if there is an outbreak of calf scours
- ✓ Change calving paddocks if there is more than 2 pats of manure per square metre
- ✓ Calving paddocks should be well-drained. If water is visible on the surface or in boot prints /hoof prints it is not dry enough and should be changed
- ✓ Use different paddocks for calving in from year to year. Do not use paddocks in which calf diarrhoea has been a problem in the past 12 months.
- ✓ Where it is not possible to provide a well-drained paddock, decrease the stocking rate
- ✓ Leave the calving paddocks vacant over the summer (cut for hay or silage)

Feeding cows

- ✓ Change feeding areas so that cows are not forced to remain in a contaminated environment. Feed bales should be spread around the calving area at a different location every day
- ✓ Where it is not possible to feed out in clean areas use bale feeders to avoid contamination of feed
- ✓ Separate feed areas from watering points to encourage cow dispersal and minimise contamination
- ✓ Provide clean fresh water. Dams and watercourses that cows and older calves have access to and can dung in may be contaminated with cryptosporidium or rotavirus, so it is important to minimise access of calves to these areas where practical

Nursing paddocks

- ✓ Move calves and cows from the calving paddocks 24 hours after calving and run nursing group's with no more than 40 cows with calves, and no more than four weeks between the oldest and young calves in the group

- ✓ Consider using temporary fencing to restrict access to suspected heavily contaminated areas, especially favourite cow camps

- ✓ Move cows and calves out from a nursing area when the youngest calf is three-week old

Other suggestions that may be applicable in some herds

- ✓ A 1/16th to 1/8th infusion of *Bos indicus* into British breeds of cattle in hotter climates (NSW) will result in cattle that are more inclined to walk and graze out

- ✓ Use multiple watering points to spread cattle out more in extensive country

- ✓ Use troughs for any supplementary/creep feeding to avoid faecal-oral transmission

- ✓ Encourage producers to fix chronically leaking water troughs

3. Increase the non-specific resistance of the calf

This is achieved by ingestion of an adequate quantity and quality of colostrum within the first 12 to 24 hours of life, which is dependent on four factors:

- ✓ The amount of colostrum available from the dam

- ✓ The maternal behaviour of the dam and whether or not she lets the calf suck

- ✓ The conformation of the cows udder

- ✓ The vigour of the calf and whether or not it can the suck the cow

The level of passive transfer on a property should always be evaluated when setting up a preventive program for producers with a calf scour problem. Whilst calf scours can still be a problem when there is adequate passive transfer it, is important to rule out a high proportion of failure of passive transfer (FPT) as a predisposing factor. Research has shown a large increase in the risk of mortality for calves from heifers and also those from assisted calvings. This is mainly because of an increased risk of FPT in these calves and producers should pay special attention to ensure these calves receive adequate colostrum early enough.

The following steps should be taken to optimise the chance of calves receiving adequate colostrum

Key Points

- ✓ Use management techniques to optimise calving ease

- ✓ Design calving areas to facilitate regular checking of the cows and to allow easy movement of animals requiring assistance into a yard

- ✓ Monitor calves from heifers and assisted calvings closely and ensure they are sucking well from the cows

- ✓ Where FPT appears to be a problem in a herd a thorough investigation of the underlying causes should be carried out (see “Diagnosis of calf scours)

- ✓ Calves that are not standing and suckling well within two hours of birth should be supplemented with colostrum

- ✓ Put cows with poor conformation on a preferential culling list. These cows have pendulous abdomens or udders so that the xiphoid-axillary region is the highest part of the cow's underbelly. Consequently calves will teat search around the forelegs and this will cause a delay in time from standing to suckling. Cows with large or "bottle" teats may also be difficult for newborn calves to suck from.

- ✓ Whilst careful monitoring of calving cows is necessary, it is also important not to disturb calving cows too much, especially in extensively managed herds. Therefore good stock handling techniques with judicious use of working dogs is important to minimise mismothering. It is possible for producers to interfere too much and cause problems. This may be a staff training issue.

Other suggestions that may be applicable in some herds

- ✓ Provide adequate feed in the last third of gestation to ensure cows calve in condition score 3 to 3.5

- ✓ Ensure cows are replete in selenium and copper

- ✓ Provide shelter to calves with assisted calvings born in cold wet weather

- ✓ When a heifer has poor maternal instinct she should be confined with her calf in a small pen for a few days until she has accepted it

- ✓ Heifers with poor maternal instinct or temperament should be culled

- ✓ Any cows with excessively large and swollen udders after calving should be checked for mastitis

- ✓ Predator control programs should be implemented to minimise disturbance of calving cows

Travelling stock

Cows calving whilst travelling can result in high relative stocking rates and increased risk of mismothering, especially when cattle are put in a break yard every night. There are also significantly increased risks of transit, nutritional and handling stress.

Drovers should be encouraged to:

- ⇨ set up a nursery and try to keep calving cows separate if possible,

- ⇨ tube feed all new born calves with 2–4 L of stored colostrum (or colostrum supplement),

- ⇨ evacuate calved cows to a fixed paddock if possible

4. Increase the specific immunity of the calf

Before recommending a vaccination program, the relevant pathogen must be shown to be a significant problem on a property. The cost of the vaccination should be weighed up against the likely costs of a calf scour outbreak due to that pathogen. These should include cost of treatment, time taken to treat and manage a calf scour outbreak, likely mortality and cost of culling a cow because she is dry.

Vaccination is not a panacea for a specific pathogen and it is only worth using when the property owner is prepared to address other significant risk factors, especially nutritional and environmental. It is also not a replacement for poor management.

Currently there are only 2 vaccines available in Australia directed at preventing calf scours. The first is an *E. coli* bacterin (Bovac, Intervet Australia Pty Ltd) to prevent enterotoxigenic *E. coli* and the second is a salmonella bacterin (Bovilis, Intervet Australia Pty Ltd) to prevent salmonellosis. In the U.S.A., Europe and New Zealand a number of viral vaccines are available these include killed and attenuated rotavirus and coronavirus vaccines.

E. coli vaccination

Research has shown that under natural conditions the colostrum of less than 10% of beef cows contains antibodies against enterotoxigenic *E. coli* (K99+). Because ETEC scours occurs during the first 3 days of life the neonate does not have time to mount a protective immune response to vaccination. Protection is afforded by vaccinating cows in late gestation so as to ensure high concentrations of anti-K99 colostral antibodies. Anti-pilus antibodies block the adhesion of the pathogen to enterocytes and subsequently prevent disease. The protective efficacy of enterotoxigenic *E. coli* bacterins is well documented. Good maternal management is required to ensure that the calf receives the maternal antibodies.

The decision to vaccinate will be based on a cost/benefit basis on a particular farm and will be influenced by recognition of risk factors:

- Prior history of ETEC scours (based on a definitive diagnosis or history of scours in calves less than 3 days of age)
- High stocking density or use of a common calving area
- Projected calving during the wet season
- Large numbers of heifers projected to calve

Pregnant cows should be vaccinated 3 and 8 weeks prior to calving and followed up with annual boosters.

Salmonella vaccination

The salmonella vaccine available in Australia is a bacterin (chemically inactivated salmonella) and there is a paucity of data available regarding its efficacy. Vaccination of cattle with salmonella bacterin provides partial protection against salmonella challenge. Moreover the level of passive protection of calves achieved via feeding colostrum from vaccinated cows is questionable and it is likely that the duration of passive immunity associated with colostral transfer is relatively short. However because many calves are exposed to salmonella in the first week of life colostral protection may be useful in an endemically infected herd.

Where salmonella is a problem in a herd it is particularly important to eliminate environmental and nutritional stresses, and focus on hygiene and increased colostral intake. Where vaccination is likely to be justified, producers should be advised that vaccination may not result in a complete cessation of the problem. Vaccination should be considered as a long-term strategy to reduce shedding by affected animals and administered to cows during late gestation to promote passive immunity. During the summer cattle should be vaccinated during the cooler times of the day to minimise the risk of adverse vaccination reactions.

Rotavirus and Coronavirus Vaccines (Not Available in Australia)

Currently there is one type of coronavirus known to cause disease in calves. Conversely there are 7 serogroups of rotavirus with group A accounting for the majority of pathogenic isolates. The

genome of rotavirus is composed of 11 gene segments that can be exchanged among isolates when animals are infected by more than one virus at the same time. Genetic re-assortment can generate new progeny viruses that can evade what was once a protective immune response, thus allowing persistence of rotavirus in susceptible populations.

Two approaches have been taken with immunoprophylaxis against rotavirus and coronavirus infections in calves. The first approach involves oral vaccination of neonatal calves with a modified live vaccine. In order to consistently elicit an effective immune response, the vaccine must be administered orally, immediately after birth, and before the calf has nursed because the colostrum of most cows contains virus neutralising antibodies that interfere with the vaccine. There are conflicting reports of efficacy with these type of vaccines.

The second approach involves intramuscular vaccination of pregnant cows with either modified live vaccine or inactivated viral vaccines prior to calving. This stimulates high levels of specific viral neutralising antibodies in colostrum and milk during the first several days of the calf's life. Colostrum and milk with a high virus-neutralising antibody titre is highly protective. However concentration of rotavirus and coronavirus neutralising antibodies in milk of vaccinated cows fall below protective levels by 3 to 7 days following parturition. One advantage of passive immunisation is the fact that cross-protection between serotypes becomes much less of a problem. This is due to the fact that vaccination of a mature cow that has had natural rotavirus exposure leads to cross-serotype stimulation of heterotypic antibodies. Single serotype vaccination therefore stimulates antibody production to a wide range of rotavirus serotypes, negating the need for multivalent rotavirus vaccines

In lieu of complete protection, the manifestations of passive immunity to bovine rotavirus that are often noted are (1) a delay of a few days in the onset of clinical signs and or (2) a reduced severity of clinical signs, and or (3) a reduction in the length of the period of viral shedding associated with infection. Although there are reports of a positive response in field trials involving bovine rotavirus/bovine rotavirus-coronavirus – vaccinated cows, no beneficial response has been observed in other trials. A common problem with commercial vaccines on the market in the U.S.A. and Europe is a lack of vaccine specific data supporting efficacy claims. Protection correlates with serum titres, independent studies have sometimes failed to demonstrate effective seroconversion with some products.

Management strategies specific to aetiology

Rotavirus and coronavirus

Rotavirus is transmitted faecal–orally and coronavirus is transmitted both faecal–orally and through the respiratory tract. Both rotavirus and coronavirus are shed intermittently by adult cows, with an increase in the amount of shedding around the time of calving. Calves from carrier cows have a significantly higher risk of clinical disease and the birth of calves from known carrier cows have been associated with the beginning of an outbreak. Many calves will be subclinically infected with no difference in the duration of infection or the levels of antigen in the faeces. Recovered calves can become reinfected and shed virus.

Bovine rotavirus has also been shown to be transmitted by cats and dogs and feral animals (deer, pigs, foxes and rabbits). Cross transmission is known to occur between dogs and between cats. Transmission from cats to dogs has also been demonstrated. Low amounts of virus are sufficient to produce infection, viral multiplication and excretion in dogs.

The environment may be an important source of infection for rotaviruses whereas coronaviruses are more fragile. Rotaviruses can survive in fresh water for more than 2 weeks at 23^{o}C and for months in water or soil < 5^{o}C. They are also stable in faeces and effluent for up to 9 months and therefore are likely to remain in calving areas from year to year.

Incubation time is short with infection occurring 1-3 days after transmission.

The emphasis for control for both should be on minimising the exposure of young calves to both calving cows and older calves that may be sub-clinically infected.

With rotavirus infection exposure to pet and feral animals should be minimised and calves should be provided with a clean easily accessible water source.

Protozoal infections

Protozoa are transmitted faecal–orally. Adult cows are a potential source of infection but emphasis must be on providing good quality water, a clean environment and minimising access to other species, including wildlife. Outbreaks of coccidiosis are associated with stress, especially post weaning and in dry conditions when cattle are being supplementary fed off the ground and are congregating around watering points. Chronically leaking troughs can create a great environment for coccidia to survive. Encouraging dispersal and improving hygiene will minimise spread.

Coccidial and Cryptosporidial oocysts will survive for months or years in water and in soil in cool conditions. Drying of oocysts has been shown to dramatically reduce their viability and infectivity. Giardial cysts are more sensitive to environmental conditions but can still survive for 5 months in cool water and several months in soil in cool wet conditions.

The prepatent period for cryptosporidia is 3-6 days and the infective dose required to produce clinical disease is low, due to the ability of the parasite to sporulate within the intestine and immediately infect adjacent cells, although oocyst excretion has been documented at 2 days of age. Giardia has a prepatent period of 7-8 days and coccidia 15-20 days. Calves 1 to 4 months of age are most likely to be actively shedding significant numbers of cryptosporidial oocysts with peak shedding occurring between 1 and 3 weeks of age. Shedding of giardial cysts is common in calves up to 8 months of age and peak shedding has been documented between 5 weeks and 8 months. The levels of oocysts/cysts in the water/environment can be minimised by preventing this age group from accessing watercourses. Viable oocysts/cysts can also be found in run-off irrigation water with subsequent contamination of watercourses. Management strategies to minimise infection levels in young calves will decrease the potential for high numbers of infective oocysts on a property.

Cryptosporidia infections are more common when there are other species on the property. Outbreaks of cryptosporidial diarrhoea in beef suckler herds have been associated with the introduction of dairy calves to beef herds as replacement calves.

Salmonella

Salmonella may be introduced onto a property by contaminated feed or water, or by infected livestock or other animals and birds. The bacteria can survive in the environment for several years. There is also an increased shedding and risk of clinical disease in periparturient cows. This disease is often associated with recent introductions of stock or stresses such as drought, droving and rapid dietary change or stress.

Preventive measures should include addressing the precipitating stressors, isolating young calves from calving cows, providing clean water and minimising stock access to likely sites of contamination such as effluent contaminated water. If an outbreak is associated with a change in the diet the introduced feed should be cultured for salmonella, preferably utilising samples that cattle have not had access to. Replacement neonatal calves, especially those purchased from saleyards, represent a high risk for introduction of salmonella and other enteric pathogens. Vaccination should be considered when salmonella is a problem at a herd level (see p 131)

E. coli

E. coli is a faecal bacteria that can survive in soil for more than 6 months and in water for at least 3 months. Calves are only susceptible to ETEC for 14 days and mainly for the first 3 days of life, therefore the emphasis should be on minimising the exposure to faeces in this time. Adult cows shed the bacteria, but there is no documented evidence of an increase associated with calving. It has been observed that specific properties tend to have recurrent *E. coli* problems and vaccination should be considered on these properties and where ETEC has been shown to cause significant losses in a herd.

Colostrum management

In a beef herd supplementation of colostrum can make a difference at an individual calf level. In particular, when a stressed calf is delivered after assistance, then it is advisable to strip colostrum from the mother while still restrained and supplement the calf via stomach tube immediately. However routine administration of colostrum to the newborn beef calf at a herd level is disruptive and difficult and will delay the time until first sucking. If a herd has a problem with FPT the cause should be determined and addressed. Only where there is no short-term solution to this problem and the percentage of calves with FPT is greater than 25% should routine supplementation be considered.

Where multiple calves are likely to require colostrum it is advisable to develop a colostrum bank. In this situation oxytocin may be used to allow for rapid collection of colostrum. The safest way to milk beef cows is to do it from directly behind the cow as they are less likely to kick, if they do then a tail jack can be used to reduce the risk and force of a kick. Enough colostrum should be collected for a second feed by tube if necessary.

Colostrum should be fresh and refrigerated or frozen in 1.5-2 L containers, or in zip-lock plastic bags laid flat, so they are thin and easy to defrost. Colostrum should not be refrigerated for more than 48 hours, as there will be a significant decline in IgG levels. There is no decrease in the IgG absorbed from colostrum frozen under laboratory conditions but long term storage in field conditions has not been evaluated. Colostrum should be defrosted in warm water or at low power in a microwave. Thawing at full power in a microwave or in boiling water will result in a decrease in the immunoglobulin levels. When defrosting colostrum in the microwave it is advisable to decant the liquid phase regularly and keep the solid portion in the microwave.

In an ideal world calves should be supplemented with 1-2 L of colostrum (5% of bodyweight) within 6 hours of birth and this should be repeated 6 hours later. Practical experience in the dairy industry has shown few side effects by giving up to 4 L (10% of body weight for smaller calves) as a single amount within the first 6 hours using an oesophageal feeder. This allows for rapid administration and minimal time input from the producer, plus is less likely to result in mismothering. However beef calves are often smaller than dairy calves, and become more distressed when large volumes of fluid are administered using an oesophageal feeder. Therefore it is important not to give more than 10% of bodyweight, and administration should stop if the calf become distressed or regurgitates.

The sourcing of colostrum is a problem on beef farms. Sourcing of colostrum from cows on a different property, especially dairy cows, is a high biosecurity risk. Colostrum can be a source of Johne's Disease (*Mycobacterium paratuberculosis),* EBL and also of the major enteric pathogens that cause neonatal calf diarrhoea. Facilities for pasteurisation of colostrum are not readily available in Australia, and pasteurisation at temperatures sufficient to kill *M. paratuberculosis* is likely to result in a significant decrease of IgG levels. Dairy cows are also likely to have a lower concentration of immunoglobulin in their colostrum.

To minimise these risks it is best that colostrum is sourced on the home property. The best source of colostrum is from cows that have had at least 3 calves and have been on the property

for at least 1 year. There is some evidence of a decline in colostrum levels in very old cows, so cows that are older than 10 years of age should not be used. Obtaining sufficient milk from beef cows can be an occupational health and safety risk. Where calf scours is a problem or there are high losses from dystocia farms could consider “taming” several cows for a colostrum supply. However more benefit is likely to be achieved by addressing the underlying causes of FPT or dystocia.

Colostrum supplements are available, but research has not found subsequent serum IgG levels to be sufficient in where independent trials have been carried out on commercial products. They are also expensive to use when a large proportion of calves require treatment.

Evaluation of colostrum

Colostrum may be evaluated with a colostrometer after collection. Whilst in many situations “any colostrum is good colostrum”, evaluation should be considered in a herd with a significant FPT problem where many calves are supplemented, or when a colostrum bank in being established. Colostrometers are most accurate for diagnosing samples of moderate or inferior quality but may indicate erroneously high readings for samples in the superior range. The reading needs to be adjusted for temperature. The specific gravity of colostrum is more closely associated with colostral protein concentration than IgG1 concentration, differs between breeds, and is influenced by lactation number, month of calving, volume of colostrum produced, year of calving, and protein yield in the previous lactation. The scale on a colostrometer is calibrated for Holstein cows, but is useful as a comparative field tool.

2. Investigation of calf scours

Calf scours needs to be approached as an enterprise level problem as there are risk factors common across all pathogens. It is important to identify environmental, management and nutritional factors that are contributing to the problem, failure to do so will limit the effectiveness of disease prevention efforts. Implementation of pathogen specific interventions such as antimicrobial therapy and immunoprophylaxis should be guided by further diagnostic investigation.

Pinpointing the aetiological agent(s) may be more difficult, as is discussed in this paper, however submission of a moderate number of samples for an appropriate diagnostic protocol should allow determination of the more common aetiological agents on a property. This knowledge is important to establish the appropriate treatment for affected calves, but greater benefit will be gained from recommending appropriate management changes to control the current outbreak and prevent recurrence in subsequent calving seasons.

Defining the problem

At the onset of an investigation it is important to define the problem. The duration, progression, morbidity, mortality, age affected, and response to treatment. This information is sometimes difficult to obtain depending on the availability of producer records and may require prospective investigation. It should be remembered that producers will call animals “calves” up to one year of age and that there are 2 separate disease syndromes “post weaning calf scours” and “neonatal calf scours”. This document primarily applies to neonatal calf diarrhoea (NCD)

The veterinarian should be concerned about a calf scour outbreak if any of the following apply to the outbreak: In larger more extensive operations, the producers may be comfortable with higher mortality rates, and the trigger level should be discussed with individual property owners.

Percentage of calves requiring treatment over the past month > 4% (1% per week)

Calves require treatment when they

- are more than 5% dehydrated
- are unwilling/unable to walk
- have a reduced suckle reflex
- are passing large volumes of extremely watery faeces

For more information see the document on treatment

Neonatal mortality (Calves born alive but dying between 1 and 28 days of age) > 3%

Older calf mortality (Calves born alive but dying between 1 and 6 months of age) > 2%

or if these figures are not clearly defined

Pre-weaning mortality (Calves born alive but dying between 1 day of age and weaning) > 5%

Producers often approach veterinarians for over the counter advice regarding calf scours. Long term effective management and prevention is more likely to be achieved through on farm consultation with a review of management procedures. If any of the above trigger points are exceeded a farm visit is likely to be beneficial to identify risk factors responsible for the outbreak, to determine the aetiological agent and most appropriate therapeutic interventions, and to plan for the following season to avoid recurrent problems.

Collecting a good history

Where available it is best to evaluate the producer's records. It is important to define:

- Maternal distribution of cases (Incidence in calves born to heifers, first calvers, mature cows)?
- What type of country are they on (pasture type, quality, toxic plants)
- Recent introductions of stock.
- Are the cattle on agistment, with a drover or have they been recently?
- General health and condition of cows.
- Any management changes that have coincided with the onset of the problem.
- What is the expected calving span – are they year round joined or not.
- The date calving started
- The date of the first (index) case
- The date of subsequent cases: is this an epidemic curve or sporadic clusters that may be related to climatic change or management factors
- The number and date of any deaths that have occurred (Confirm that the deaths were not related to calving problems)
- The age groups of the affected calves. Commonly the age of calves affected reduces as the outbreak progresses.
- The areas of the farm that the cows and affected calves have had access to
- The number and location of cows still to calve
- Has this been a problem in previous years and how frequently
- Any management changes that have occurred on the property in the past 12 months (particularly relating to risk factors)
- The vaccination history
- Other prophylactic and therapeutic interventions

Identifying Risk factors

Field observation, deduction and compilation of a complete history of the mob of cows from before calving is required to identify predisposing risk factors.

Enterprise level risks

Some properties are at higher risk of scour problems due to the genetic composition of herd, environmental conditions, farm management practices and a variation in the degree of exposure to pathogens and that these need to be considered when understanding how all the risk factors interact together

Herd structure

Heifers have a poorer mothering ability, lower colostrum quality and an increased risk of dystocia and consequently have been shown to have an increased incidence of NCD in their calves. Therefore the percentage of heifers in the herd will affect the risk of mortality from NCD. It is also likely that heifers are kept at a higher stocking rate prior to calving to allow better observation and are consequently exposed to a greater environmental pathogen load. It has also been shown that where cows and calves are shedding rotavirus and coronavirus, calves from carrier heifers are more likely to develop clinical disease than calves born to carrier cows.

Nutrition

Calves from heifers are at increased risk and rearing management should ensure well-grown heifers and sire selection for calving ease to minimise the risk of dystocia.

There are no reports of a direct effect of pre-parturient nutrition on the subsequent incidence of NCD in the calf. High feed levels pre calving will increase calf birth weight but does not increase the risk of dystocia unless the animals become obese. Poor nutrition resulting in weight loss is associated with prolonged labour, increased dystocia, increased perinatal mortality, reduced calf growth rates and has detrimental effects on the subsequent fertility of the cow. Poor colostral uptake due to periparturient problems may result in an increased susceptibility to NCD Where feed is limited it is important to run first-calf heifers separately from older cows to meet their higher energy requirements.

Whilst nutrition post calving may have little direct impact on the incidence of NCD there are many management factors resulting from a shortage of feed that will increase stress and environmental pathogen load.

These include:

- ✓ The necessity to feed out leading to a concentration of stock in specific areas of the paddock
- ✓ Closer grazing to dung pats in the paddock
- ✓ the herd being put on the road with a drover, or sent on agistment, resulting in an increased likelihood of exposure to pathogens, increased stress on calves and increased possibility of mismothering
- ✓ Where troughs are not used drought may also lead to fewer sources of fresh water and increased contamination of remaining water sources with faecal pathogens

When cows are receiving supplementary feed the following procedures will result in an increased risk of exposure to enteric pathogens:

- ✓ Feeding grain on the ground
- ✓ Feeding hay or silage in the same area of the paddock every day
- ✓ Feeding out close to watering points
- ✓ If there are limited areas to feed out bale feeders should be used to minimise faecal contamination of the hay or silage

Mineral status of cows

Selenium supplementation has been shown to increase IgG levels in deficient cows. Trace mineral deficiency may compromise innate and acquired immune mechanisms.

Management before calving

Running heifers and cows as one group prior to calving has been shown to increase the risk of mortality from diarrhoea. This is possibly because heifers are unable to compete with cows for feed. Animals should be moved into the calving paddock no more than 2 weeks before calving.

Management at calving

Risk factors for NCD are factors that compromise host immunity and increase pathogen exposure.

Major risk factors are:

- High stocking rates (> 10 cows/ha) resulting in high levels of faecal contamination in the calving paddock
- Poorly drained calving paddocks
- The use of a single calving area
- Use of the same calving paddock from year to year/ calving season to calving season
- Newborn calves and their dams remaining in the paddock with the calving cows for longer than 24 hours
- Extremes of temperature
- Limited access to shelter and shade
- Situations that result in disturbance of the dam and newborn calf, such as predators, droving or excessive monitoring.

Time of calving

A longer calving season is likely to increase the environmental pathogen load, especially in moist cool climates. Calves born to animals that calve later in the calving season are more likely to develop diarrhoea. As the calving season progresses it is also more likely that calves will develop diarrhoea at a younger age. Calves from heifers are more susceptible to NCD and will be more at risk if heifers calve later in the season

Dystocia

Calves that experience dystocia are likely to have oedema of the head and tongue, hypoxic injuries and acid base imbalances making suckling difficult. They are also weak and exhausted and likely to be recumbent for a longer period of time and expose themselves to more faecal pathogens. Dystocia affects the uptake of immunoglobulins by the calf (see p 142) and calves that survive dystocia are between 2.4 times more likely to become sick in the first 45 days of life

Other risk factors at calving

Cleaning calving facilities after each calving season has been shown to minimise the risk of diarrhoea. Whilst this is not possible in a paddock situation, cleaning of the crush area, calving equipment and equipment used to treat sick calves will minimise cross contamination. It is particularly important that equipment used to treat sick calves is not used to administer colostrum to newborn calves.

Treatment of the navel after calving has no association with the risk of diarrhoea.

Management of calves post calving

Young calves should not be run with calving cows or calves > 1 month of age.

Shelter areas and shade are important, as calves tend to lie down frequently in the first 2 weeks of life. Small shelters can become crowded in bad weather resulting in high concentrations of pathogens and increased opportunity for disease transmission. In Idaho protective wooden shelters have been used successfully to improve the survival and performance of spring born calves.

Paddocks should be well drained and stocking rate should be less than 10 cows/ha

C. parvum survives for at least 2 weeks in water sources and it has been shown that the risk of *C. parvum* infection in cattle is related to the distance of the water source from septic systems. Calves require easy access to fresh water and at risk groups should have water provided in clean troughs, and water should be accessible within 300 metres.

Biosecurity

Mortality due to NCD has been shown to increase in farms purchasing replacement calves that were less than 4 weeks of age.

Weather

Wet windy weather and hot weather are both likely to increase the risk of calf scours. Wet cold windy weather increases energy requirements of calves, and will cause calves to huddle in sheltered areas, effectively increasing the stocking rate and environmental pathogen load. Wet weather may also result in rotavirus and cryptosporidial oocysts that are bound to soil, leaching to the surface, increasing concentrations in surface water or water courses. Hot weather will cause cows and calves to concentrate in the shade, again increasing environmental pathogen load.

Failure of passive transfer (FPT)

Factors affecting colostrum qualities of beef cattle

There is a large variation in the colostrum immunoglobulin concentration between individual cows but numerous studies have shown that there is little association between the colostral Ig levels of the cow and the serum Ig of the calf on an individual level. However factors resulting in poor colostrum quality of a group of cows will increase the incidence of FPT in their calves. At herd level colostrum quality can be affected by breed, parity, nutrition and their subsequent effect on volume, as well as climate. At an individual level colostrum quality has been shown to have poor heritability but when the calf serum Ig level was considered as a repeatable trait of the cow, the IgG_1 and IgM at 24 and 36 hours show moderate repeatability. It is likely that the deviation of a calf's serum Ig levels from the population average may be used as a predictor of future deviations in serum Ig for that dam's calves, and consequently this could be used as a selection criteria when breeding.

Risk factors for FPT

Factors affecting colostrum quality of the cow

Effect of breed

There is no clear evidence of a consistent variation in colostral Ig levels between breeds, although individual sire and dam effects have been shown. Recent studies have shown an increased incidence of FPT in specific genotypes and it is likely that FPT is more prevalent in

specific lines of cattle rather than breed per se. Cows producing large volumes of colostrum (>12 L) will have a lower concentration of IgG in their colostrum and this may be a problem with dairy cross cows.

When cows have a “poor shape” where, due to the size of the abdomen or the udder, the xiphisternum was the highest part of the dam’s underbelly, calves will take significantly longer to find the teats and suckle. Cows with bottle teats can also be a problem for calves to suck. Conformational problems are more likely to make a difference at an individual cow level than a herd level.

Effect of parity

First and second calving cows have a lower immunoglobulin concentration than cows of third parity and above. Calves born to these dams have a significantly lower mean concentration of serum IgG compared with that of calves born to older cows.

Effect of nutrition

Dietary restriction of the dam prior to calving does not affect the immunoglobulin levels in calves’ sera after absorption of colostrum. Nutritionally restricted cows are likely to have a lower volume of colostrum, but compensatory mechanisms have been demonstrated with a trend towards increased levels of immunoglobulins and enhanced absorption of IgM by the calves.

Selenium supplementation has been shown to increase the IgG levels in the colostrum of selenium deficient cows. Calf serum Ig levels may also be decreased when cows are severely deficient in copper.

Climatic factors

There is a seasonal variation in the Ig levels of calves after colostrum feeding, being lower in the winter in cold climates and lower in the summer in hot climates. Colostrum immunoglobulin concentration is reduced in hot and cold weather, and this is exacerbated by calves also being less willing to suckle in extremes of temperature. Heat stress results in smaller calves that may be less vigorous and calves subjected to an extremely cold and wet environment also have a slower rate of colostral absorption.

Factors affecting colostrum uptake by the calf

Amount of colostrum and time of intake

The age of the calf when it receives its first feed and the amount of immunoglobulins received will influence the time of closure of intestinal permeability to colostral immunoglobulins, and the final serum immunoglobulin levels of the calf. Cessation of absorption occurs by 24 hours in calves that receive a full feed of colostrum within the first 4 hours after birth. When the colostrum volume is less than 2 L, the gut will remain permeable for a longer time and the rate of absorption will increase in response to a subsequent feed. If the calf is older than 12 hours when it receives its first feed there is a significant increase in the possibility of the calf being agammaglobulinaemic. Studies in dairy calves have shown that there is a significant increase in the absorption of IgG_1 when calves are fed 4 L of high Ig colostrum at birth rather than 2 L. When 2 L or 4 L of low quality colostrum at birth were compared there was no significant difference in the rate of absorption.

Increased supervision of calving cows and early intervention to give colostrum to calves not suckling within 6 hours has been shown to reduce the number of calves with FPT.

Effects of dystocia on colostrum absorption

Decreased levels of IgG in calves experiencing dystocia have been observed in several studies. This may be partially due to inadequate colostrum intake due to decreased vitality of the neonate and a slower time to stand and suck. Calves that have experienced severe dystocia may also have oedema of the head and tongue leading to a decreased ability to suck, also the dam is less likely to be interested in the calf, and may have delayed milk let down. Calving difficulty has been shown to have a significant affect on absorption even when all calves were supplemented with colostrum.

Other factors affecting Immunoglobulin levels in calves

Clinical mastitis in the dam at the time of calving has not been associated with FPT .

FPT should be minimised in the current calving group, by effective observation of recently calved cows and supplementation with colostrum where practical and appropriate. In the longer term it is important to address mineral deficiencies

Other Risks

An increase in the total number of other agricultural animals on the farm increases the risk of *C. parvum* infection

Possible risk factors

The following are possible risk factors for diarrhoea but there appears to be no reports evaluating them in the scientific literature.

- time of calf management procedures such as drenching and castrating
- stocking rates and group size under an Australian pasture based system
- Effluent contamination of water sources. Salmonella, cryptosporidium and rotavirus have all been isolated from water. Logically creeks and dams are likely to be more contaminated as calves are more likely to defecate in them. However there has been no comparative study published and in an outbreak it may be beneficial to test samples from various water sources on the property.
- grazing rotation length
- fertiliser use
- Purchased feeds

Examination of the herd and individual calf

Calf scour problems usually relate to management practices, hence it is important to take time to view the big picture prior to focusing on the individual. Assess the body condition of the calves and the dams, evaluate environmental conditions and determine the age and proportion of calves currently affected. This information is useful for identifying predisposing causes and for prioritising the diagnostic work up.

Assess the proportion of calves in the following 4 groups:

- Unaffected
- Scouring, but still suckling, bright and alert (Can’t catch)

- Scouring and ≤ 8% dehydrated (Can catch with some exertion)

- Scouring and > 8% dehydrated (Easy to catch/collapsed)

The following presentations may be helpful in pin-pointing some differential diagnoses.

- the majority of animals affected are less than 3 days of age and all animals are less than 14 days there is a strong possibility of an enterotoxigenic (K99+) *E. coli*

- there is a wide range of age groups infected, possibly including yearling or adults cows then salmonella or yersinia should be considered – both these agents may be associated with the presence of blood and mucous in the scour

- the majority of animals affected are over five weeks of age and all over three weeks of age, and a higher proportion of animals have perineal faecal staining, rectal straining and excessive tail swishing, or an increased incidence of rectal prolapse, then coccidiosis should be considered a strong diagnostic possibility

- calves are dying suddenly with no signs of dehydration then consider a toxicity or toxaemia or septicemia (salmonella, clostridial disease, or *E. coli*)

Apart from these four syndromes there are few classic clinical or age-related signs that can be used as pointers for the other major enteropathogens.

A minimum of four to six calves representing the range of presentations should be given a full clinical examination. Neonates are prone to sepsis which may be reflected by scleral injection, hypopyon (fibrin in the anterior chamber), omphalitis, and septic arthritis. A high proportion of infected navels may reflect failure of passive transfer and or a poor calving environment. Septic arthritis is indicative of a prior bacteraemia that may occur secondary to calf scours. Fever is an unreliable indicator of sepsis in neonates and an absence of a fever should not be interpreted to indicate absence of sepsis. Depressed mentation is often observed in scouring neonates and may reflect a metabolic acidosis, hypothermia, electrolyte derangements (hypo or hypernatremia, hypo or hyperkalemia), hypoglycaemia, or septicemia.

On an individual level mentation, suckle reflex, ability to stand, and hydration should be assessed to determine the appropriate treatment (see information sheet on treatment).

The udder of the dam of the affected calf should be observed to determine that she has an adequate milk supply. If practical the dam should be stripped to give a better estimation of milk supply.

Sample collection

Most farmers are unlikely to want repeat visits, therefore it is important to collect as many samples as needed to do a preliminary diagnostic work-up at the initial visit. Samples should be collected from calves that are exhibiting a “typical” presentation for that outbreak. It is helpful to sample calves at different stages of the disease but preferably half of the samples should be taken from calves early in the course of the disease prior to the initiation of treatment. These calves will often have watery diarrhoea and little faecal staining around the perineal region and tail, and therefore can be hard to pick unless they are depressed or not suckling. A minimum of 6 samples is recommended and more are desirable when there are larger numbers of affected calves with representative signs of disease available to sample. If there are only 2-3 calves affected collecting samples from non-affected calves may also be beneficial to build up a picture of pathogen prevalence on the property, as cohorts may be sub-clinically infected. Routine collection of samples from unaffected animals for comparison is unlikely to add additional information to an investigation because a proportion of unaffected animals will shed aetiological

agents. A single faecal sample from an individual animal has limited diagnostic utility due to the potential for discord between clinical signs and faecal shedding.

Where possible blood samples should be collected from 10 calves less than a week of age to assess the effectiveness of passive transfer. If there are inadequate calves of this age group calves up to 2 weeks of age may be evaluated. If this can only be achieved by yarding the cattle and increasing the risk of spread across the farm (see document on "Control of an outbreak") then affected calves less than 21 days of age should be evaluated. The most reliable diagnostic tests in sick calves are the whole blood immunoassay[4] or GGT levels. If more than 20% of sick calves have inadequate levels then the problem should be confirmed by establishing the degree of FPT in the healthy newborn calves.

In many cases it will be necessary to run the mob into a yard to facilitate catching calves. Moving the mob will increase the pathogen load around the farm, and it is important to minimise any contact between the affected mob and other calves less than 6 weeks of age. Because of this it is important to get enough appropriate samples in one visit to have the best chance of a diagnosis. If the affected mob is put through a crush that is also used for calving cows, this area must be thoroughly cleaned after use. If the floor of the crush and yards are concrete the area should be scraped and cleaned with bleach. If the floor of the crush is soil and the top 10 cm should be removed and replaced. In an outbreak of NCD of bacterial origin lime should be put in the base of a crush with a soil floor.

Calves should be rectally stimulated to get a good sample using a new disposable glove for each calf. The glove should be lubricated with obstetrical lubricant that does not contain antiseptic. For a full diagnostic work-up it is necessary to collect at least 5-10 g into leak-proof container: Where possible provide a yellow top container that is at least ½ full. Sample should be pre-labelled (1-5) with indelible pen, and all information, including the ID of the cow and calf should be put on an accompanying sheet of paper. This will minimise the faecal contamination of the packaging.

The most valuable information as to the aetiology of a calf scour outbreak will come from the post mortem of freshly dead calves. However it should be remembered that autolysis within the gut occurs within 5 minutes and it is likely that the samples will be non-diagnostic within a few hours of death. A gross evaluation of these calves may be nonetheless rewarding in confirming or challenging findings in other calves necropsied. Where there is a mortality rate > 2% or previous post mortem results have not been rewarding, consider euthanasia of a calf early in the disease course. Producers will often present a cachectic calf that has had a protracted illness. These calves are unlikely to have high diagnostic yield due to debilitation and secondary infections. Consequently the producer and the veterinarian become disillusioned with the diagnostic process and are unwilling to sacrifice a calf with early clinical signs. As a single necropsy could be an unrelated isolated case, it is important to backup any results with faecal samples collected from other animals in the group, or at least one more necropsy.

A necropsy is not a sample collecting exercise. It should be approached in a systematic manner in the same way as the clinical examination of live animal. It is important to describe and interpret what is found even if the tissues appear normal. Start by describing the body condition. To ensure the correct diagnosis it is important to examine all cavities and organs of the body in a systematic fashion. Where enteric disease is suspected the whole gastrointestinal tract should be examined from mouth to anus. Special note should be taken of:

- Erosions, proliferative lesions, ecchymosis, petechial haemorrhages
- Congestion, oedema, Inflammation, emphysema

[4] Midland Bioproducts Quick test kit

- Fluid distension of gastrointestinal tract
- Enlarged lymph nodes
- The nature of intestinal and colonic contents

Samples for histology

Calves that have died within 48 hours of birth should be examined for signs of calving difficulties: meconium staining inside the back legs, swollen head or tongue and scleral haemorrhages.

During the necropsy each of the following organs should be checked and the indicated samples collected. The possible pathogen associations are indicated in parentheses. **This is not intended to reflect a protocol for conducting a complete necropsy.** While economics usually dictates the submission and ordering of tests there is only one opportunity to collect samples so it is prudent to collect everything.

Mouth: Check for oral erosions (Pestivirus) or proliferative lesions (Bovine papular stomatitis), if observed collect and fix tissues.

Oesophagus: Check for erosions, if observed collect and fix tissues. (Pestivirus)

Abomasum: Check for inflammation and emphysema in rugal folds (Clostridia) collect tissue for histopathology and abomasal contents (on ice) for detection of clostridia and clostridial toxins.

Small intestine: Examine for signs of inflammation and fluid distension. Collect a sample of duodenum, mid jejunum, and ileum for histopathology (Rotavirus, coronavirus, salmonella, cryptosporidia).

Mesenteric Lymph Nodes: Note if enlarged and collect samples for culture (salmonella) and histopathology.

Caecum: Check for evidence of inflammation (coronavirus, salmonella, coccidia) and collect contents on ice for culture and tissue for histopathology

Spiral colon: Check for inflammation and collect tissue for histopathology (salmonella, coronavirus, coccidia)

Rectum: Examine for inflammation and collect tissue for histopathology (coccidia, pestivirus)

Brain should be examined for evidence of Neospora, that can contribute to weak and sickly calves after birth and for evidence of meningitis, encephalitis and polioencephalomalacia

Comment should be made on the nutritional status of the calf by examining fat reserves around the kidneys and coronary band.

Always sample upper & lower SI, spiral colon and abomasum even when there are no obvious lesions as some changes are not visible macroscopically. Mesenteric lymph nodes should be included as autolysis is always faster in gut, therefore lymph nodes can be used to detect an inflammatory response. Other sections of the intestine should be collected if there are obvious gross changes. Samples should also be collected from all the major organs, even if they are not processed initially.

Intestinal samples should be 3-4 cm long, if too short a section the samples will curl at the end. Make sure that the gut lumen is open, syringe formalin through or dunk sample in formalin with the lumen open to ensure formalin distributes the whole way through. It is important not to

traumatise the gut in any way. Do not cut intestine lengthwise as this will smear off the intestinal lumen cells. Do not take large samples. Tissue samples collected from other organs should be approx 1cm x 2cm to allow adequate fixation. It is better to take several small samples than one large one. Tissue: formalin ratio must be 1:10

Formalin should be added to the sample pots in the field. This is important to minimise post mortem change. Avoid contaminating the formalin with blood and gut contents and change the formalin in the pots before the samples are sent to the laboratory. If samples are not sent until the next day the formalin can be drained after fixing overnight, to minimise the risk of leakage of formalin in transit. (Except for large specimens).

If the client is paying by for each sample separately then put different sites in different pots when sections are to be processed sequentially.

Fresh samples

Samples of the rectal contents should be sent for testing for enteric pathogens. Fresh samples of the mesenteric lymph nodes, spleen and liver are useful to culture for salmonella.

If there are sections of haemorrhagic intestine and the presentation of the case is suggestive of enterotoxaemia sections of the affected bowel should be submitted for histopathology. Intestinal contents may also be tested for the presence of toxin. Demonstrating the presence of *C. perfringens* toxins or the capacity to produce toxins provides support for the diagnosis, but does not confirm a clinical diagnosis as almost as many *C. perfringens* isolates from normal calves produce toxin. Diagnostic laboratories in Australia do not routinely perform quantitative Clostridial counts. A diagnosis of clostridial enteritis is therefore based on the cumulative picture created by the case history, clinical presentation, gross pathology, histopathology, and ancillary testing (toxin production).

Sending samples to the laboratory

Calf scour samples are potentially zoonotic and should be packaged to IATA 650 standards (e.g. the outside of the packaging should be clean) to ensure that there is no risk to personnel transporting or unpacking the samples. Any containers with liquid should be sealed inside a second leak-proof container, especially if they contain formalin. Where possible sample should be fixed in formalin overnight at a 1:10 ratio and then placed in a fresh container with some formalin-soaked gauze to send to the laboratory.

Containers with faecal samples should be placed in a Zip-lock bag and sent to the laboratory in an esky with an iceblock.

Submission forms should be placed in a plastic envelope separate from the samples so that they remain clean if a sample should leak.

Epidemiological details to include on a laboratory submission form

The following details should be included on the laboratory submission forms

- Location
- Age range of affected calves
- Breed
- Sex
- History including
    - ✓ Type of operation (beef, dairy, veal)

- ✓ Number of animals at risk, affected and dead
- ✓ Presenting syndrome
- ✓ Duration of disease
- ✓ Severity of disease
- ✓ Historical progression of the problem
- ✓ Stocking density
- ✓ Diet (where appropriate)
- ✓ Worming history
- ✓ Vaccination history
- ✓ Prophylactic and therapeutic interventions
- ✓ Relevant management changes/risk factors
- ✓ Environmental factors

- Differential diagnoses
- Reason for test

Providing laboratories with adequate information will enable them to correlate the histopathology with the clinical presentation to provide a more meaningful assessment of significance. Quality data recording also facilitates national disease monitoring by government and industry groups. This can allow targeted prioritisation of resources and research.

Laboratory tests

Ideally all faecal samples should be tested for rotavirus, cryptosporidia, coronavirus, and salmonella. Enterotoxigenic *E. coli* should be included when calves less than 3 days of age are affected. This strategy is most likely to result in a diagnosis, and will also determine if there are multiple pathogens involved, which allows for the correct emphasis to be placed on preventive strategies. Moreover, with the recent introduction of ELISA tests for rotavirus and coronavirus it is more likely that a diagnosis will be established.

Should funds be limited, it may be possible to target tests for ETEC, salmonella, yersiniosis, or coccidiosis from the presenting syndrome (see page 142), but a limited panel of tests is more likely to result in a misdiagnosis. Moreover with more ELISA and subsequently PCR technologies becoming available there has been a reduction in the cost of laboratory diagnosis.

Should five samples test negative for all major pathogens, ancillary tests should be considered depending on the clinical signs. Alternately a necropsy may be useful in providing the answer.

When indicated by the clinical history, further tests should include faecal float for coccidiosis, intestinal worms or giardia and antigen capture of BVD from spleen. If ante-mortem bloating is a presenting feature clostridial abomasitis should be considered

Isolation of *E. coli* from the gastrointestinal tract does not constitute a diagnosis unless the isolate is demonstrated to possess virulence attributes that correlate with the clinical presentation and histopathology. Most laboratories utilise immunoassays to demonstrate the presence of fimbrial antigens to identify Enterotoxigenic *E. coli*. Enteropathogenic *E. coli* may be identified utilising PCR assays to demonstrate the presence of genes involved in producing proteins involved in adhesion and cytotoxicity. A diagnosis of enteropathogenic *E. coli* should be supported by histopathology as healthy calves may shed *E. coli* which posses the same virulence attributes.

Evaluation of passive transfer

Calves with adequate passive transfer, have a serum IgG concentration greater than 10g/L (1000 mg/dL). There are many tests that have been used to evaluate passive transfer status in calves. Enzyme linked immunosorbent assay (ELISA) and radial immunodiffusion are the only tests that directly measure serum IgG concentration. All other tests estimate serum IgG concentration from the total globulin level of other proteins whose transfer is associated with the absorption of IgG. Indirect tests are generally cheaper and technically easier. The most appropriate test depends on the purpose of the testing and the health of the calf.

Serum protein is probably the most cost-effective test and is as accurate as any of the alternatives when assessing or monitoring healthy calves for FPT. Values < 5.0 g/L indicate FPT, but 5.5 g/L should be used as the end point in sick calves as serum protein will be affected by dehydration. For a rapid calf-side monitoring test for small groups of calves the whole blood immunoassay[5] may be the appropriate choice. When sick calves need to be evaluated GGT gives the most accurate indication with levels < 50 IU indicative of FPT in calves aged less than 21 days of age. Serum GGT may also be used to assess IgG levels in normal calves, but there is a rapid decrease with age. Consequently it is necessary to know the age of the calves tested and the use of this test should be restricted to calves less than 10 days of age. The levels indicating FPT are as follows:

Calf age	**Serum GGT level**
≤ 4 days	< 100 IU/L
5-7 days	< 75 IU/L
8-10 days	< 50 IU/L

Where FPT is a problem in a herd the risk factors contributing to this need to be assessed

Putting all the information together

Interpretation of laboratory results

The major enteric pathogens may be shed by apparently normal calves, and the relevance of these pathogens on a faecal sample may be questioned. A causal relationship may be inferred by a high prevalence of a known or multiple known enteric pathogens. Correlating the presence of a pathogen with compatible clinical signs, response to treatment, gross pathology, and histopathology makes for a more definitive diagnosis. Multiple pathogens are frequently incriminated in calf scour problems. Identification of specific pathogens can facilitate prevention, treatment, and control efforts. In the case of bacterial pathogens determining the pathogens antimicrobial sensitivity allows for targeted therapeutic intervention. An effective maternal vaccine is available for enterotoxigenic *E. coli* and it is likely that antiprotozoal drugs will be released in Australia for the treatment of Cryptosporidia. Coccidiostats may be included in creep feed for calves infected with coccidia. There are currently no vaccines available for rotavirus and corona virus in Australia. However if it is determined that the cause of a scour problem is primarily viral in origin it is useful to indicate to the producer why antimicrobial therapy is not effective and to redirect efforts to provision of appropriate supportive fluid therapy and preventive management interventions.

Reporting to the farmer

Information gathered from examination of calves, the laboratory diagnosis and assessment of risk factors should be consolidated to determine the major factors that have resulted in the

[5] Midland Bioproducts Quick test kit

disease outbreak. The farmers should be provided with advice on further treatment and a prioritised list of management changes to control the outbreak, and prevent problem in subsequent years.

This is best presented as a single page containing the most important action items. Further information can be attached to this page.

3. Control of a calf scours outbreak

In order to target specific control measures it is useful to have an established aetiology. However in many cases initial control measures will have to be taken in the absence of this. Many of the management strategies outlined for the prevention of calf scours can also be applied in the face of an outbreak.

Management of stock

The most important control measure is to change the calving paddock and isolate any new calves from the existing group(s) of neonatal calves. Where cows and calves are running with cows yet to calve this results in 3-4 groups of cattle:

1. Cows with calves currently at foot (infected group)

2. Cow yet to calve

3. A new group of cows and calves that will be created by drifting off cows and their calves as they calve down

4. If there is a large age range in the affected group, it may be beneficial to separate calves more than 6 weeks of age from the younger calves

For most infections an adjacent paddock is sufficient, but if a further one were available then this would be the preferred choice for separating the infected group from newborn calves. Both salmonella and coronavirus are spread in nasal secretions so if these diseases are suspected or diagnosed increased separation is necessary. If there are only a few paddocks on the farm hot wires may be used to divide paddocks.

If affected calves are in a paddock where treatment is very difficult or time consuming it may be necessary to move the affected mob to allow for treatment of sick calves. Apart from this situation mobs of affected calves should only be moved when they will not come into contact with other calves less than 6 weeks of age. The paddock that they are moved out of must be isolated from calves less than 6 weeks of age, but can be used for older classes of stock such as yearlings, cows with calves > 4 months old etc. Because many enteric pathogens can survive for months to years in the environment it is possible for a paddock to remain contaminated from one season to the next. The risk of pathogen carry over will depend on the environmental conditions with cool wet conditions favouring pathogen survival. Working a paddock to turn over 4 inches of dirt is one way to reduce the pathogen load between seasons, but in most situations forward planning to allow for multiple calving patterns may be more practical.

Where possible the stocking rate in the affected group should be decreased to minimise the rate of spread. This may be achieved by separating calves greater than 6 weeks of age from the younger calves. The older calves are likely to shed large numbers of pathogens and increase the level of environmental contamination, but are unlikely to suffer from severe clinical disease. These calves can be put with older calves but should not be mixed with other calves less than 6 weeks of age at any stage.

When there are unaffected mobs of cattle on the farm with calves less than 1 month, maximum effort should be applied to minimise the risk of infection spreading to these animals. Not only are they the most susceptible but they will also shed the highest numbers of pathogens.

Management of affected animals

The management of mobs of cattle in a calf scour outbreak depends on the severity of infection, the percentage of calves affected and the ease with which the cattle can be handled. Where only

5-10% of the calves require treatment the affected calves and their dams should be isolated into a confined area to ensure monitoring and ease of repeat treatments. Where a larger proportion is affected it is likely that a high number of the remaining animals will develop clinical disease or are already subclinically affected and there would be little benefit in separating the clinical cases.

Isolating cows and sick calves may be achieved by restraining the calves and drifting unaffected cow-calf pairs into a different paddock, or by enticing the dams to follow a vehicle transporting the sick calves. In most pasture based systems separating cows and sick calves from the rest of the mob is difficult without yarding the whole mob. Yarding of the mob has 4 drawbacks:

1. It will bring adult cows and affected and unaffected calves into close proximity and increase the chance of disease spread

2. It is likely that more calves will be incubating the disease, resulting in the requirement for frequent (daily) yarding of the mob

3. Yarding the mob is likely to increase the stress on young calves and increase their susceptibility to clinical disease

4. Moving and yarding the mob increased the chance of mismothering

Unless a producer has cooperative cattle or extremely good handling facilities it would be easier to set up a temporary treatment shelter in the corner or at the edge of the paddock for dehydrated calves requiring treatment. The shelter should be designed to allow affected calves to be confined and provide shelter and warmth, but also allow contact (nose to nose) with the dam to minimise separation stress. It could be simply constructed with 3 gates, movable yard sections or large square bales of hay, with tin or tarpaulin to provide shade and shelter. Severely dehydrated or depressed calves can be confined in the shelter to allow for repeat administration of fluids or IV therapy. Where calves do require separation the emphasis should be on 12-24 hours intensive therapy before returning them to their mothers. In outbreaks of scours where calves do require treatment for longer periods of time it will be necessary to set up an isolation area that allows calves to be with their mothers, but are still easy to catch and treat. Unless calves are severely dehydrated and require repeated administration of fluids they should be kept with their dam to minimise stress and ensure adequate energy intake.

The treatment shelter should be moved weekly to an adjacent area to prevent pathogen build up. The ground where the shelter has been moved from should be isolated eg fenced off with an electric fence, such that the isolated area gets slightly bigger each week.

A simple program should be put in place to identify sick calves. Raddle, spray paint or a band around the calves' neck could be used to identify treated calves, if they have no ear tags.

Minimising pathogen spread

The following guidelines should be followed to minimise the spread of pathogens between mobs:

Ensure Good Hygiene

- ✓ Wear clean overalls and disposable gloves when handling newborn calves.

- ✓ Minimise the transmission of faeces between mobs of animals.

- ✓ Keep a set of overalls and gloves specifically for treating sick calves

- ✓ Where possible the sick calves should be treated by a stockperson that is not coming in contact with unaffected calves

- ✓ Management of cattle to minimise spread. Where possible affected groups should be separated from unaffected calves for as long as possible and preferably the youngest calves are 4 months old. The minimum time that two groups can be merged is when both groups are more than 6 weeks of age

- ✓ Where affected calves and their dams are isolated from healthy calves they should be kept isolated until they and the rest of the mob are more than 6 weeks of age.

- ✓ Ensure that affected mobs (and preferably all mobs with calves less than 4 months of age) have no access to watercourses or surface water

- ✓ If the outbreak occurs close to branding or weaning, delay this procedure for the affected calves for as long as practical to allow the outbreak to run its course. Where procedures requiring yarding are being carried out on affected and unaffected mobs, run the affected mob through the yards last, so that there is minimal contact between them and healthy mobs

The following steps should be used to minimise pathogen build-up and spread in the affected mobs

- ✓ minimise stressful management procedures during outbreak. e.g. dehorning, castration, dietary changes including weaning, transport

- ✓ newborn calves should not be handled unnecessarily in the first 24 hours in herds where enterotoxigenic *E. coli* has been diagnosed

- ✓ reduce stocking density

- ✓ Put supplementary feed in troughs, feeders or racks to avoid faecal contamination

- ✓ avoid faecal contamination of water supplies

- ✓ move feeders regularly

- ✓ Top dress around water troughs with soil or wood chips mixed with lime

- ✓ minimise the exposure of calves and cows to wet and muddy areas

- ✓ Fence off existing known calf camps

Disinfection

Treatment areas and equipment should be made of plastic or metal as opposed to wood to facilitate cleaning. Surfaces should be regularly cleaned of faeces by scrubbing, as most disinfectants are inactivated by organic material, even when an increased concentration is applied. Scrubbing is also preferred to application of high-pressure sprays that can aerosolise organisms allowing dissemination.

Separate equipment should be used to administer oral electrolytes and colostrum. Salmonella and coronavirus are shed in saliva and can contaminate equipment used for oral medication. This equipment should be washed with warm soapy water to remove the fat residue left by milk and colostrum. Detergents should be rinsed prior to application of disinfectant solutions.

It is important to appreciate that the physical cleaning and removal of organic debris removes 90% of the bacterial load. Disinfectants are not effective in the presence of gross contamination and cannot compensate for inadequate cleaning.

Pathogen elimination is time dependent, therefore it is important to allow sufficient contact time prior to rinsing. The effectiveness of disinfectants and the rate of pathogen reduction are also affected by concentration, temperature, pH, and water hardness. The impact of these variables is product dependent. Manufacturer's directions should be observed.

Bacterial and viral agents of neonatal enteric disease are susceptible to:

- Sodium hypochlorite (bleach) (1750 ppm solution) with a 10 minutes contact time when applied at room temperature and a pH 6 to 7
- Povodine iodine (1% available iodine)
- Potassium monopersulfate (Virkon™) (1% solution).

Virkon™ has a detergent action that facilitates cleaning.

Rotavirus is relatively resistant to many common disinfectants, such as chlorhexidine. It is not affected by soaps and washing with soap alone may actually spread the virus around on the washed surface.

Cryptosporidia are extremely resistant to most veterinary disinfectants and it is therefore important to physically remove the debris. They survive for months in water but are susceptible to drying. Potassium monopersulfate (Virkon™) is inhibitory to but not effective at killing cryptosporidia.

Cryptosporidia are extremely resistant to most veterinary disinfectants except 5% ammonia, 6% hydrogen peroxide or 10% formalin, they survive for months in water but are susceptible to drying. There is effectively no practical disinfection protocol for eliminating cryptosporidia from the environment emphasising the need for thorough physical cleaning and subsequent drying to reduce the challenge exposure.

Increasing resistance to disease

The degree of failure of passive transfer should be evaluated as part of any calf scour outbreak, as it can be a common risk factor especially where heifer's calves are involved. (See "Diagnosis of calf scours"). Where FPT is occurring the underlying causes should be assessed and animals at increased risk of FPT should be supplemented with colostrum (see more details under "Prevention of calf scours")

It is unlikely that it is possible to address the causes of FPT in the current calving season, but it is possible to put in place management strategies to minimise the problem in the future. These should be based on assessment of the risk factors (See Diagnosis of calf scours) and may include:

- Ensuring adequate levels of copper and selenium
- Breeding and nutritional strategies to minimise dystocia
- Management of heifers to ensure adequate nutrition prior to calving

Vaccinating in the face of an outbreak

There are only 2 vaccines available in Australia directed at preventing calf scours, and both are bacterins (killed vaccines). The first is an *E. coli* bacterin (Bovac, Intervet Australia Pty Ltd) to prevent enterotoxigenic *E. coli* and the second is a salmonella bacterin (Bovilis, Intervet Australia Pty Ltd) to prevent salmonellosis. Vaccination of unvaccinated pregnant cows in the face of an outbreak of enterotoxigenic *E. coli* is likely to be beneficial when the outbreak occurs towards the beginning of the calving season. Some beneficial immunity may develop within three weeks of the first injection, and cows that calve within 45 to 60 days of the second injection will have a protective antibody concentration. It is important to ensure that all calves get sufficient colostrum by feeding colostrum to calves that may not have suckled within 6 hours of birth (See document on prevention of calf scours)

There is a paucity of data available regarding the efficacy of the salmonella bacterin available in Australia. Trials with salmonella bacterins have had variable results either having no effect or providing partial protection manifest by reduction in faecal shedding, severity of clinical signs, and mortality however the immunity can be overwhelmed by high challenge doses. Passive protection from colostral transfer has been observed when calves are challenged during the first week of life. Because many calves are exposed to salmonella during this period this protection is may be useful in an endemically infected herd. Vaccinating pregnant unvaccinated cows in the face of a salmonella outbreak is warranted, however it should be appreciated that the protection afforded by salmonella bacterins is generally limited and the importance of other management interventions that reduce pathogen exposure and increase host immunity (nutrition and environmental management) be emphasised. Because of the time required for stimulation of an immune response vaccination of neonatal calves is unlikely to be effective at preventing neonatal scours. During the summer cattle should be vaccinated during the cooler times of the day to minimise the risk of adverse vaccination reactions.

4. Treatment of the Scouring beef Calf

Evaluating the calf and determining the treatment options

Dehydration, metabolic derangements, and sepsis are common problems experienced by scouring calves. Medical management is directed at correcting either measured or estimated fluid, electrolyte, metabolic, and acid base derangements and at preventing or treatment of sepsis.

Depressed mentation is commonly observed in diarrhoeic calves and may be secondary to hypovolemia, acidosis, hypoglycaemia, hypothermia, hyperthermia, sepsis, and electrolyte disturbances. Hydration is estimated by measuring skin tent, evaluating mucous membranes, and observing eyeball position (See Table 4). Acutely affected calves may present with dehydration prior to the onset of diarrhoea when fluid is sequestered in the lumen of the gastrointestinal tract. Acidosis is particularly common and in the absence of laboratory support should be anticipated as a contributing factor to depressed mentation in obtunded, dehydrated, scouring calves. The best way to assess acid base status is measure arterial blood gas to determine the calf's blood pH and base deficit. This is usually not an option in the field. Alternatively the calf's base deficit may be estimated by measuring serum bicarbonate or TCO_2. When this is not possible urine pH can be measured to provide an indication of the calf's acid base status. A urine pH values less than 6.0 indicates moderate to severe acidosis. PH values between 6.0 and 6.4 indicate that the calf is developing acidosis.

Without laboratory support it is not possible to completely assess acid base, electrolyte, and metabolic status. Field evaluation and therapeutic interventions are therefore directed at the most common derangements, dehydration, acidosis, and hypoglycaemia. Poor response to treatment on a farm level should prompt further diagnostic investigation as it may reflect a specific management induced problem such as hypernatraemia with the use of high sodium containing milk replacer.

Scouring calves that are bright and alert, suckling, and not dehydrated often do not require treatment. A decision tree such as in Figure 1 can then be used to direct the implementation of therapeutic protocols. Early detection of cases is important to correct deficits and prevent calves from losing their suckle reflex and becoming recumbent. Administration of alkalising electrolyte solutions before calves become profoundly depressed aids recovery. Special attention should be paid to detect calves with very watery faeces. These calves often do not have staining around their rectum and may be easily missed. Treatment of calves with fluids at this stage will maximise their chances of survival.

Calves ≤ 5% dehydrated, and that still have some suckle reflex should be given a single treatment or oral electrolytes, marked and left with their mother (See figure 1). Calves that are more seriously affected should be isolated for repeat treatment.

Calves that are isolated should be provided with warmth and shelter. On farm the easiest option may be to construct a temporary shelter in the corner of the paddock with gates or straw bales, using tin or tarps to provide shade and wind protection. Shelters should be designed to allow cows to see and smell their calf. In wet cold conditions the use of "space blankets" or calf coats may help sick cold calves. The treatment area should be moved to an adjacent area weekly to minimise build up of pathogens and the "Treatment corner" should be fenced off with electric tape to prevent other calves accessing this area.

Table 4: Determining the degree of dehydration

% Dehydration	Clinical signs	Eyeball Sunkenness	Skin Tent time (seconds)	Mucous Membranes	Estimated base deficit of blood (mmol/L)		Fluid therapy required
					≤ 8 days old	> 8 days old	
1-5	Bright and alert	None / slight	1-4	Moist	0	5	None
6-8	Standing or sitting quietly – will move if disturbed	Slight separation between eyeball and orbit	5-10	Tacky	5	10	Likely to respond to oral electrolyte therapy
9-10	Depressed, unwilling/ unable to stand	up to 0.5 cm between eyeball and orbit	11-15	Tacky	10	15	Requires intravenous fluids
11+	Collapsed, death imminent	Gap between eyeball and orbit is 0.5 to 1 cm	> 15	Dry	10	20	Requires intravenous fluids & repeated monitoring

Fluid Therapy

Re-hydration, and correction of electrolyte and acid base disorders are the founding principles behind the treatment of calf scours as more calves die from dehydration and acidosis than from sepsis. Oral rehydration solutions are very effective when given early in the course of the disease and when the composition of the electrolyte solution is appropriate to meet calves needs and the volume of fluid administered is sufficient to correct dehydration. Oral administration of electrolyte solutions is the preferred route of administration for calves that are between 5 and 8% dehydrated, can still stand and have a weak suck reflex, or chew at the examiners fingers. If treatment is initiated too late, or the disease is particularly acute then intravenous fluids should be used. It is important to impress on the producer that it is dehydration that kills the calf not infection, and consequently fluids are the preferred treatment not antibiotics.

The total daily fluid volume required is the amount to required to correct the deficit (% dehydration x body weight (L)) plus the estimated losses through diarrhoea (1-4 L/day) and maintenance requirements (3 mL/kg/hour). As only 60-80% of oral fluids are absorbed this needs to be accounted for when calculating fluid requirements. It should be remembered that because 20-40% of fluid is not absorbed in the intestine the faeces of calves treated with oral electrolytes may get more watery and increase in volume even when the calf is improving clinically.

Treating with oral electrolyte solutions

Selecting the correct oral electrolyte solution

Oral solutions should:

1) Supply sufficient sodium to facilitate normalisation of extracellular fluid deficits. Recommended concentrations between 60-133 mmol/L

2) Provide agents that facilitate absorption of sodium and water from the intestine. This can be glucose, specific amino acids (eg. Glycine), acetate or propionate.

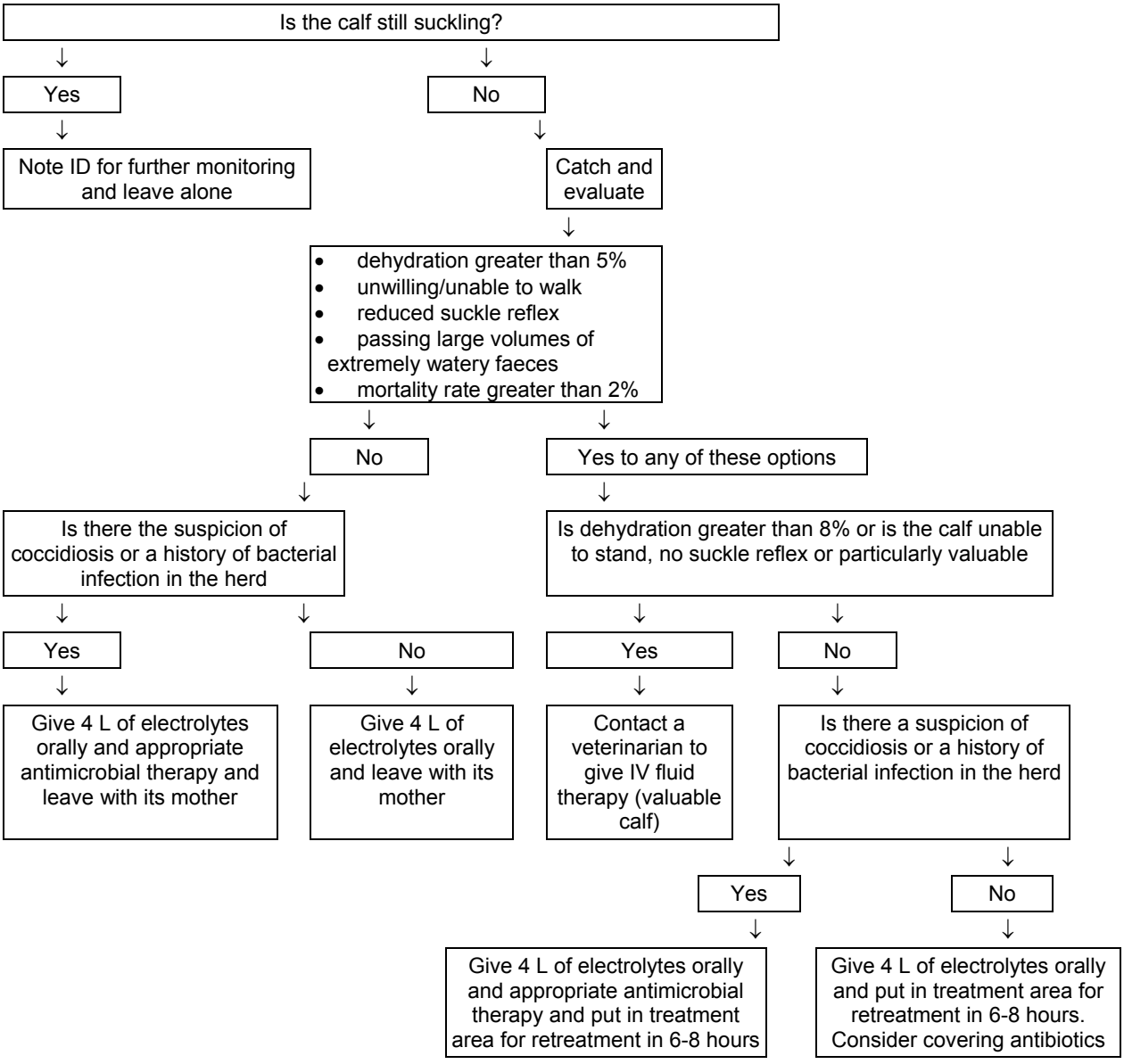
3) Provide an alkalising agent to treat metabolic acidosis. Sodium bicarbonate, citrate, acetate and propionate are commonly included in electrolyte formulations for this purpose.

4) Provide energy, as these electrolyte solutions may be administered instead of milk or milk replacer for short periods of time.

5) Provide potassium, which is depleted in a diarrhoeic calf due to faecal loss. There are no clinical trials reported on the efficacy of different concentrations of potassium but most oral electrolyte solutions contain between 10-30 mmol of potassium/L.

Electrolyte solutions that contain > 40 mmol/L of bicarbonate or citrate have marked adverse effects on milk clotting. Solutions containing bicarbonate may also alkalise the gastrointestinal tract of milk-fed calves and promote bacterial overgrowth in the small intestine as well as ETEC attachment and toxin production. Therefore these solutions should not be fed to beef calves if they are to be left with their mother and are bright enough to suckle within 2 hours. If a calf is unwilling to stand or more than 5% dehydrated it is likely to be acidotic. In this situation bicarbonate containing solutions will be the most effective to reverse the acidosis.

Figure 1: Decision tree used to determine the appropriate treatment for a scouring calf



if the calf is unable to suckle then fluids need to be administered with a tube feeder

Oral electrolyte solutions are compared to the osmolarity of plasma which is 306 mOsm/L.. Solutions can be iso-osmolar (300-312 mOsm/L), hyperosmolar (>312 mOsm/L), and hypo-osmolar (<300 mOsm/L). Solutions with a osmolarity < 250 mOsm/L can cause haemolysis and should be avoided. For short term administration in a calf that is still standing the osmolarity is unlikely to be clinically relevant, and the sodium and glucose levels together with the alkalising

ability are more important. Hyperosmolar solutions probably result in a better outcome in a severely dehydrated calf or when repeated administration is required, however they will induce hypernatraemia if they are the only source of fluids, and should only be used repeatedly when the calf is consuming milk or water from other sources.

There is no perfect rehydration solution currently available in Australia that can be used for all situations on a beef property. As many calves are likely to be acidotic the product should be primarily chosen on the type and concentration of its alkalising agent. Consequently the producer should be advised to use at least 2 products.

1. A solution containing acetate or propionate should be selected for use in calves that are still standing and can be left with their dams

2. A solution containing bicarbonate should be selected for calves that are severely affected and require repeat therapy and isolation.

Where calves are slow to respond to therapy, or there is a high mortality, it may also be necessary to have a third solution that contains additional glucose.

Administration of oral electrolyte solutions

Because of the difficulty of catching and treating beef calves farmers should be advised to give 4 L (or 10% of bodyweight if the calf weighs less than 40 kg) at the initial treatment, and where required a second 4 L (10% of body weight) 6 to 12 hours later. For calves weighing more than 70 kg 5 L can be given at a time. An oesophageal feeder is the quickest and most effective method for giving this much fluid and producers should be instructed on their use.

Large volumes of oral electrolyte solutions can safely be administered to neonatal calves. Calves have been shown to drink up to 19% of bodyweight at one feed, and the abomasum expands to accommodate this volume of fluid. When fluid is given by oesophageal feeder it is initially deposited in the rumen and reticulum, but overflows into the abomasum after the administration of only 400 mL in calves less than 18 days of age and after 2 L in older calves. Despite this some calves will become bloated and uncomfortable, and in these cases administration of smaller amounts more frequently is preferable. However it is important to correct the deficit and compensate for further losses within the first 12 hours and this may require large volumes of fluids to be administered as efficiently as possible over this time (see Table 5). Calves should be monitored for any evidence of reflux, or excessive distress and if this occurs the treatment should be stopped.

In outbreaks where longer treatment is required the amount should be tailored to the weight and estimated requirements of the affected calves and reassessed on a daily basis. Table 5 shows the volume of oral electrolyte solution required to cover maintenance and losses through scouring for different weight ranges. If calves are dehydrated the volume of fluids required to correct this should be added to the maintenance amount. Therefore a 40 kg calf that is 5% dehydrated and has an estimated loss of 2 L per day through scouring will require 9 L of oral electrolyte solution on the first day to correct the deficit and compensate for ongoing losses assuming that 70% of the fluids are absorbed.

Table 5: Calculation of daily fluid requirements of scouring calves

Weight of calf (kg)	**30**	**40**	**50**	**60**	**70**	**80**	**100**
Maintenance requirements (L)	1.5	2	2.5	3	3.5	4	5
	Total requirement (L)						

Loss through scours (L)	**1**	4.0	4.5	5.0	6.0	6.5	7.5	9.0
	2	5.0	6.0	6.5	7.5	8.0	9.0	10.0
	4	8.0	9.0	9.5	10.0	11.0	11.5	13.0

Intravenous fluid therapy

If a calf is depressed and unwilling to suckle intravenous fluids are indicated and often the only way of reviving them. Ideally dehydration and acidosis should be corrected over 24 hours, but few problems are seen if this occurs over 4 hours. These two goals may be achieved by administration of large volume isotonic solutions or low volume hypertonic solutions. Both methods are effective as long as any acidosis is corrected in addition to replacement of extracellular fluid and sodium.

Treatment of acidosis

Calves requiring IV fluids should initially be treated for acidosis, especially if they are over 8 days of age. Clinical trials show that bicarbonate is the most effective alkalising agent. Bicarbonate requirements can be calculated from base deficit values determined from blood gas measurements but the equipment for this is seldom available in clinical veterinary practice in Australia. Therefore it is necessary to estimate this from physical findings (see Table 4).

The base deficit is used to calculate the bicarbonate required using the following equation:

mmol bicarbonate = Body Weight (kg) x Base Deficit (mmol/L) x 0.5

Bicarbonate solutions can be made up by the three methods shown below. Solutions 1 or 2 should be used to correct the acidosis, and then a mildly alkalising solution should be used to correct the rest of the fluid deficit. Solution 1 is the preferred option as it is isotonic, but solution 2 may be easier to make up because most veterinary clinics will have intravenous infusion packs of sterile saline on hand to which bicarbonate can be added.

Solution 3 contains less bicarbonate per litre and will not correct the acidosis as rapidly. However volume replacement will improve perfusion reducing production of lactate in tissues and facilitating further correction by improving renal function. This approach may be easier to set up on farm and leave running, without the farmer having to change solutions. The balance of the fluid deficit could then be corrected with oral electrolytes.

- Solution 1: Addition of 13 g of sodium bicarbonate to 1 L of distilled water. This is an isotonic solution containing 155 mmol bicarbonate/L. = 1.3% bicarbonate solution. Sterile water is available in 1 L fluid infusion packs from veterinary wholesalers.

- Solution 2: Addition of 12.5 g of sodium bicarbonate to 1 L of 0.9% saline solution. This is a hypertonic solution containing 300 mmol/L sodium and 150 mmol/L of both bicarbonate and chloride.

- Solution 3: Mixing the solution for severe acidosis as shown in Table 7. This is an isotonic solution containing 161 mmol/L sodium and 79 mmol/L of bicarbonate and 82 mmol/L chloride.

It is important that the bicarbonate and other ingredients in all mixtures are fully dissolved and mixed through the solution and this will be aided by gentle warming. Sodium bicarbonate should not be added to Ringer's solution as the calcium in Ringer's solution precipitates as calcium carbonate.

Practical tip: 13 g Sodium bicarbonate = 16 mL volume in a syringe barrel and 12.5 g = 15.5 mL volume

The volumes required of isotonic 1.3% bicarbonate solution (solution 1) for calves of different weight are shown in Table 6. The volume of the hypertonic saline/bicarbonate solution (solution 2) will be marginally higher as the mmol bicarbonate/L are slightly less. The volume of isotonic solution for severe acidosis (solution 3) required will be approximately double that of the 1.3% bicarbonate solution. Whilst it is important to make a reasonable attempt at estimating the base deficit it should be remembered that volume expansion will increase renal perfusion allowing the kidneys to perform their function of establishing an appropriate sodium balance.

Table 6: Bicarbonate requirements of calves depending on estimated base deficit

Weight of calf (kg)	Base deficit (mmol/L)	Bicarbonate requirements (mmol/L)	Volume of 1.3% NaHCO3 solution (L)
	10	150	1.0
30	15	225	1.5
	20	300	1.9
	10	200	1.3
40	15	300	1.9
	20	400	2.6
	10	250	1.6
50	15	375	2.4
	20	500	3.2
	10	300	1.9
60	15	450	2.9
	20	600	3.9
	10	350	2.3
70	15	525	3.4
	20	700	4.5
	10	400	2.6
80	15	600	3.9
	20	800	5.2
	10	450	2.9
90	15	675	4.4
	20	900	5.8
	10	500	3.2
100	15	750	4.8
	20	1000	6.5

Correcting the dehydration and ongoing fluids for maintenance

The volume of fluids administered to correct metabolic acidosis may be insufficient to fully correct the calf's dehydration. Lactated Ringers (Hartmann's) or isotonic saline may be used to correct the remaining fluid deficit. Hartmann's is a balanced, alkalising, iso-osmotic solution containing physiological concentrations of Na^+ , K^+, Ca^+, Cl^- and lactate. Alkalisation is produced by the metabolism of lactate to glucose. Calves with diarrhoea often have increased lactate concentrations and the rate of conversion of lactate to glucose is decreased by 50% in severely dehydrated animals. Therefore Hartmann's is not the appropriate first choice in severely dehydrated calves, without prior administration of a solution containing bicarbonate. It is

recommended that 100 mL of 50% dextrose is added to each litre of isotonic solution (creating a 5% dextrose solution) to prevent hypoglycaemia in cachectic hypothermic calves.

It is possible to mix your own intravenous solutions using clean or preferably sterile water. Table 7 gives some recipes for making up isotonic solutions. Salt and bicarbonate can be weighed or measured by volume into a syringe. Solutions for mild or moderate acidosis could be used for ongoing treatment of a scouring calf that has been treated for acidosis or for a calf that is not so seriously affected. The recipe for severe acidosis should only be used for initial therapy of the collapsed calf. These solutions do not contain potassium or glucose, and where calves are cold, wet or cachectic 500 mL of 50% dextrose should be added to the sterile water to make up a total of 5 L, in which the salts are dissolved.

Hypovolaemic calves are normally tachycardic reflecting an attempt to maintain blood pressure. Paradoxical bradycardia is likely to reflect atrial block secondary to hyperkalaemic due to a shift of hydrogen ions into cells in exchange for potassium ions secondary to acidosis. Rapid correction of the hyperkalaemia is indicated and can be achieved via intravenous administration of bicarbonate and glucose containing fluids. Following correction of the acidosis it is not uncommon for calves to be hypokalaemia reflecting a total body deficit of potassium secondary to faecal loss. Profound hypokalaemia may contribute to weakness. In a field situation it is important that the oral electrolytes given as a follow up contain potassium. In a clinic situation potassium chloride (40 mEq/L) may be added to the follow up fluids and administered at a rate of less than 1 mEq/kg/hr.

In most cases the initial IV therapy can be followed up by ongoing oral electrolytes, once the dehydration and acidosis has been corrected.

Table 7: Methods for making up isotonic solutions in 5 L of sterile water

Degree of acidosis	Salt		Sodium Bicarbonate		Electrolyte concentrations (mmol/l)		
	Weight (g)	Volume (mL)	Weight (g)	Volume (mL)	Na+	Cl-	HCO_3^-
Mild	38	30	13	16	161	130	31
Moderate	29	23	26	32	159	97	62
Severe	24	20	33	40	161	82	79

Administration of intravenous fluids

Recommended fluid rates vary from 40-80 mL/kg/hour. Where higher rates are used the patient should be monitored for fast and/or laboured respiration that may indicate pulmonary oedema. Once the fluid deficit has been corrected the fluid rate can be adjusted to provide for maintenance (3 mL/kg/hr) and to compensate for ongoing losses due to diarrhoea. The additional fluid to compensate for ongoing losses will vary from an additional 40 mL/hr (1 L/day) to 250 mL/hr (6 L/day).

A catheter can be placed in either the jugular or auricular vein. If the calf is collapsed and comatose intraosseous administration may be required.

Jugular vein

This can be catheterised with a 14 - 18gu, 2.5 - 7.5cm catheter. The catheter can be stitched in place or positioned with cyanoacrylate (“superglue” or tissue adhesive), and once the IV line is connected it can be secured with more tape and cyanoacrylate, together with a bandage around

the neck. A wide bore extension tube will allow for rapid flow rates, and where necessary this can be connected to the catheter with an extension tube. If the calf is collapsed and the vein hard to localise the calf can be suspended upside down so that blood will pool and distend the jugular veins. The calf's neck should be clipped and prepared prior to inversion and the calf laid flat as soon as the catheter is placed. If this method doesn't work it may be necessary to cut down on the vein

Intraosseous administration

If the calf is comatose and the blood pressure is so low that it is impossible to raise a vein, fluids may be administered intraosseously until perfusion is sufficient for placement of an intravenous catheter. Shave an area 2.5 cm^2 over the proximal humerus or femur. Prepare the site and insert a 14-gauge 1 ½" needle into the centre of the bone longitudinal to the length of the bone. The bone is soft and the needle can be "drilled" in. The needle will contain a core of bone so it should be removed carefully observing the position and a second 14-gauge 1 ½" needle placed in the same hole. A syringe containing 50 mL of saline is attached to the needle and injected to create a space with the trabeculae, then a litre bag of isotonic fluids is attached and run in as fast as possible. The preferred solution would be 1.3% sodium bicarbonate, although saline or lactated Ringers could be used if bicarbonate solution is not available. After 1 L has been administered it is usually possible to find the jugular, but if not a second litre may be administered. Once the calf regains consciousness this technique is difficult to maintain as the movement makes it difficult to keep the needle clean and in place.

Auricular

This vein runs on the outer surface of the pinna and can be located by placing a rubber band or similar tourniquet around the base of the ear. Warming the ear will also facilitate localisation. A 22gu 1" catheter can be secured with a suture or cyanoacrylate, attached to a giving set and the ear is then bandaged to the head. It is useful to place a wad of bandage or the inner cardboard tube from the centre of a bandage on the inside of the head when bandaging. The auricular vein is more difficult to maintain over the longer term compared to the jugular vein.

Subcutaneous and intraperitoneal administration

The administration of fluids subcutaneously is only appropriate for maintenance or mild dehydration because severe peripheral vasoconstriction in calves > 8% dehydrated will mean that fluids are not absorbed. Consequently there is no advantage over oral administration. Where this method is used no more than 500 mL should be given at any one site. Fluids must be sterile and should be isotonic, warm and not contain glucose as this may lead to abscessation. They should be administered high on neck or thorax with a fast drip rate to allow more even distribution into subcutaneous space

Fluids are absorbed more rapidly from the intraperitoneal route compared to subcutaneous administration, but this is not recommended due to the potential for peritonitis. If it has to be used the fluid must be isotonic and balanced to prevent fluid and electrolyte imbalances.

Hypertonic fluids

Hypertonic saline dextran (7.2% saline containing 6% dextran 70) or hypertonic saline administered at 4 mL/kg BW during a 4 minute period concurrently with an isotonic alkalising oral electrolyte solution is effective in resuscitating dehydrated calves with diarrhoea. Calves with diarrhoea have a larger decrease in the extracellular fluids compared to the intracellular fluid and the intestinal loss of sodium, chloride, potassium and bicarbonate results in the plasma and extracellular fluid becoming hypotonic. Consequently fluid moves from the extracellular fluid into the cells, exacerbating the hypovolaemic shock. Treatment with hypertonic saline reverses the flow from the extracellular fluid into the cells, and by using an isotonic oral electrolyte solution,

the osmotic gradient also favours movement from the gastro-intestinal tract to the plasma. The predominant cation in the extracellular fluid is sodium and administration of hypertonic saline achieves a more rapid correction of this deficit, together with expansion of the plasma volume, than can be achieved by administration of an isotonic intravenous solution. Hypertonic saline dextran will give prolonged vascular support, but in most calves the hypertonic saline alone is sufficient to resuscitate the calf.

Hypertonic saline should always be given concurrently with an isotonic alkalising oral electrolyte solution. The oral fluid should ideally be given before the hypertonic saline, due to the rapid expansion of the plasma, however administration of the hypertonic saline first may temporarily resuscitate the calf and allow it to suckle. When treating animals with hypertonic solutions care should be taken to remain in the vein, as tissue damage will result from perivascular administration.

This treatment is likely to be an effective field treatment for severely dehydrated calves and a practical alternative to isotonic fluids. However it has not been evaluated in severely acidotic calves and collapsed calves, especially those over 8 days of age, should initially be treated with a bicarbonate solution as discussed above. Hypertonic sodium bicarbonate (8.4%) is reported to have a similar effect, and could be used in acidotic calves at 4-5 mL/kg in a similar fashion. This would contain enough bicarbonate to correct a base deficit of 10 mmol/L. However there are no published clinical trials.

Hypertonic saline dextran is not readily available in Australia, but isotonic saline dextran (0.9% saline containing 6% dextran 70) is and additional sodium chloride could be added to produce a hypertonic solution. (31.5 g of sodium chloride (25.5 mL volume) should be added to a 500 mL bag of isotonic saline dextran). Hypertonic saline should be administered at 1 mL/kg body weight/min. For most calves a 16 gu 1½" needle can be placed in the jugular vein, however a catheter should be placed if the calf is particularly mobile.

What to do if the calf is not responding to fluid therapy

Calves should be reassessed after the initial volume of fluid is administered and therapy adjusted accordingly. The calf should improve over 24 hours and if it hasn't the following steps should be taken to address the underlying problem:

1. Perform a thorough physical examination: Look for congenital anomalies such as ventral septal defects or atresia ani, check for umbilical or joint infections, scleral injection or hypopyon that may indicate septicaemia and look for strabismus, anisocoria and facial twitching that may indicate meningitis or other neurological derangement, abnormal sodium levels or hypoglycaemia.

2. Assess the current degree of dehydration and recalculate the fluid required. If the calf is still dehydrated it is likely that the losses are higher than has been estimated and the daily requirement should be adjusted accordingly. If it has only been treated with oral electrolytes, intravenous therapy may be required

3. Assume that the calf has a bacteraemia and initiate appropriate antibiotic therapy if the calf is not already being treated (see p 166)

4. Revisit the possibility of acidosis: Unless the calf is fully rehydrated and has already been treated for severe acidosis, this is likely to still be a problem. Calves that are > 5% dehydrated should be treated for severe acidosis (Base deficit 10 - 20 mmol/L depending on the age of the calf (see Table 4 and Table 6). If the calf is ≤ 5% dehydrated, but has not been treated for severe acidosis treat for a 10 mmol/L base deficit (see Table 6)

5. If the calf is hydrated and has been adequately treated for acidosis take a blood and check serum glucose and sodium levels. It would also be useful to check serum protein, albumin, fibrinogen and a complete blood count for any indications of a bacteraemia. Blood tests should always be considered where there is a poor response to therapy at a herd level to identify areas for improving treatment regimes and rule out other potential diagnoses. In this case a complete biochemistry panel should be run.

Hypoglycaemia is a common sequelae to withdrawal of milk for more than 48 hours, especially in cold weather. Affected calves are weak or recumbent, but appear to be normally hydrated, or minimally dehydrated. They are often emaciated, and can occasionally have neurological signs including petit mal or grand mal seizures, opisthotonus and coma. Hypoglycaemia is particularly common in scouring calves that experience weather stress. Moribund hypothermic calves should be administered IV glucose prior to warming as warming will increase glucose demands and may lead to seizures and death. Addition of glucose to rehydration fluids to a concentration of 5% is usually sufficient to maintain blood glucose. Calves that have experienced chronic scours are also prone to hypoglycaemia, these calves can be identified by their cachexia. Energy malnutrition contributes to their debilitated state and it is important to rapidly restore adequate energy intake to ensure resolution.

Hyponatraemia and hypernatraemia are less common findings, but may be a result of improper mixing of oral electrolyte solutions. Hypernatraemia may also result from the use of high sodium content milk replacer or limited access to fresh water, consequently it is often farm specific.

Hyponatraemia occurs when a massive loss of isotonic fluid through the gastro-intestinal tract is replaced by free water or hypotonic solutions. The latter often occurs when too much water is added when making up an oral electrolyte solution. Hyponatraemia may also occur with isotonic solutions when the ability to absorb sodium is compromised, This may be due to severe pathological changes or an inadequate level of agents that facilitate sodium co-transport within the oral electrolyte solution. Hyponatraemia results in a fluid shift from the extracellular space to intracellular compartment along the osmotic gradient and the resultant swelling of the cells can result in neurological disturbances; depression, disorientation and even convulsions. Hyponatraemia should be considered in calves with a serum sodium < 132 mmol/L and calves with a serum sodium < 120 mmol/L have severe hyponatraemia.

The goal of therapy is to restore serum sodium levels to > 125 mmol/L over the first 6 hours and then to restore to normal levels over 24 hours. In hypovolaemic calves the initial treatment should be achieved using normal saline, and in normovolaemic calves hypotonic saline should be used for the initial treatment as the administration of large fluid volumes will exacerbate the oedema. If the calves are also suspected to be acidotic this should also be corrected with bicarbonate solutions of appropriate tonicity.

The amount of sodium required in the first 6 hours to raise the sodium level to 125 mmol/L can be calculated as follows:

$$\text{Sodium (mmol)} = [125 - \text{measured serum sodium (mmol/L)}] \times [0.6 \times \text{Bodyweight (kg)}]$$

Calves should then be maintained on sodium containing isotonic fluid, such as normal saline or lactated ringers and treated with oral electrolyte solution as appropriate. The sodium level should be monitored frequently in the first 24 hours due to unknown losses through the gastro-intestinal tract, as well as unknown kidney function in a severely dehydrated patient.

Hypernatraemia is defined as a serum sodium concentration over 152 mmol/L, but only levels greater than 170 mmol/L have been associated with nervous dysfunction. Hypernatraemia can occur due to the loss of hypotonic fluid in faeces or when oral electrolyte solutions are improperly diluted. This will be exacerbated if calves have no access to water or have stopped suckling. Rapid development of hypernatraemia results in fluid moving from cells into the extracellular fluid

and produces cellular dehydration. Neurological signs include lethargy, weakness, depression coma and death.

Treatment for severe hypernatraemia should only occur when serum sodium levels are greater than 170 mmol/L. When hypernatraemia occurs over 4-7 days the CSF sodium concentration will increase to parallel sodium serum concentration and the brain intracellular osmolality will also increase. Gradual treatment over 4-5 days is required as rapid treatment may lead to cerebral oedema. Calves should be treated with intravenous fluids that have been manipulated to contain concentrations of sodium approximately equal to that of the plasma with the goal of reducing plasma sodium by less than 5 meq/L/day over the first 48 hours. The volume given should be that to provide rehydration, cover maintenance and ongoing losses similar to the treatment of any other diarrhoeic calf. The solution may require bicarbonate if the calf is suspected to be acidotic. Until plasma sodium levels are approaching normal sodium should also be added to any oral fluids (ie milk replacer) so that the concentration is approximately equal to the intravenous fluids. Cerebral oedema will present as coma or seizures and may be treated with 25% solution of mannitol at 1 g/kg IV over 30 minutes or an oral solution of glycerin given at 1 g/kg diluted 1:1 with water.

Providing ongoing energy requirements

As a general rule calves that are willing and able to suckle should be encouraged to do so and electrolyte therapy should be adjusted accordingly.

Calves that have not received milk for over 24 hours should be encouraged to suckle. They should be helped to stand and rubbed vigorously along the back and over the chest and neck. This simulates maternal caring, and stimulates the calf's appetites. Unless they are still collapsed beef calves should not be kept from their mothers for more than 36 hours. Calves that are too weak to suckle from their dam, but have a suckle reflex and are willing to take a bottle should be fed 1-2 L of milk twice a day between electrolyte feeds. (total daily milk intake = 10% of bodyweight).

If calves are unwilling to suckle after 36 hours the treatment protocol should always be reassessed (see 163). Calves that have not been provided with enough energy for maintenance will be cachectic and hypoglycaemic.

Administering milk by oesophageal feeder to calves that are unwilling to suckle may make them uncomfortable if they have an abomasal ulcer. If cachexia demands an increase in energy intake is required, and milk needs to be fed to anorexic calves with a tube feeder it is best to start with small volumes (≤ 1 L). Alternatively if the calf is in better condition it may be worth administering a high-energy oral electrolyte solution. Energy malnutrition compromises host immunity and may lead to the demise of chronically affected calves so calves should be rubbed and encouraged to stand every 12 hours and their suckle reflex reassessed. Milk should be fed as soon as they are willing to suck.

In the case of valuable calves additional nutritional support may be provided through administration of glucose, amino acid, and lipid solutions utilising a procedure referred to as total parenteral nutrition.

Antimicrobials

Use of antimicrobial therapy in calves with diarrhoea should be risk based. The underlying aetiology in the majority of scour outbreaks in suckled beef calves is likely to be viral or protozoal. Therefore there is no place for the indiscriminate use of antibiotics in every slightly dehydrated scouring calf, especially those willing to suckle, unless a bacterial origin is known.

There are 2 indications for antibiotic use in scouring calves:

- When diagnostic investigation indicates the diagnosis of scours is linked to a bacterial pathogen(salmonella or Enterotoxigenic (K99+) *E. coli*)

- Calves at high risk of bacteraemia and sepsis (calves requiring intravenous fluid therapy)

Calves at high risk of bacteraemia and sepsis

Calves with diarrhoea often have small intestinal overgrowth with *E. coli*, regardless of the inciting cause, and approximately 30% of systemically ill calves that have failure of passive transfer are bacteraemic as are 8 % of systemically ill calves that do not have failure of passive transfer. *E. coli* is the most common bacterial isolate in these cases. Rapid recognition and treatment of sepsis improves the likelihood of a successful outcome. Therefore prophylactic antibiotics should be considered

- ✓ where calves are considered sick enough to require repeat fluid therapy (see Figure 1), especially in herds where there is a history of FPT

- ✓ where calves are pyrexic or have fibrin clots in anterior chamber indicating septicaemia

- ✓ when there is blood in the faeces

- ✓ when calves are treated with intravenous fluid therapy even when there is no documentation of an enteric bacterial pathogen,

Antibiotics should always be given when non-sterile fluids are used.

Selection of the appropriate antibiotic

Prophylactic antimicrobial treatment of calves at high risk of bacteraemia and sepsis should include a gram negative and gram positive spectrum and be focused against *E. coli* in the small intestine and blood, the 2 sites of infection. Faecal bacterial culture and antimicrobial susceptibility testing is not recommended as a basis for selecting the appropriate antibiotics in these cases because faecal bacterial populations do not accurately reflect small intestinal or blood bacterial populations. Antimicrobial efficacy is therefore best evaluated by the clinical response of a number of calves to treatment.

Parenteral administration of a broad-spectrum beta -lactam antimicrobial - ceftiofur (5 mg/kg IM q24 h) or amoxicillin (10 mg/kg IM q12 h), trimethoprim sulphur (25 mg/kg IV or IM q24 h), or florfenicol (20 mg/kg IM q 48 h) is recommended for treating neonatal calves with diarrhoea and systemic illness. (Note all of these except for florfenicol are off label doses and require an extended meat withholding period). In a beef suckler situation florfenicol may be the most appropriate due to the longevity of its action. The bacteriostatic action and frequency of antimicrobial resistance to tetracyclines and non-potentiated sulphonamides limits their effectiveness in septic neonates. Trimethoprim sulphur may be used to treat sepsis in neonatal calves, but its half life rapidly declines as rumen function develops, and cannot be recommended in calves older than 8 weeks of age.

Sulfadimidine, sulfadiazine, streptomycin sulfate, dihydrostreptomycin sulfate, neomycin sulfate, and apramycin are labelled for oral administration for the treatment and prevention of calf scours calves. Orally administered apramycin has proven to be efficacious in field studies. These trials involved daily medication of calves at risk of diarrhoea, or in the early stages of the disease. When calves are severely dehydrated the absorption of oral antimicrobials is likely to be compromised consequently when sepsis is suspected parenteral administration is advisable. The results of field and experimental trials with the other antimicrobials available in Australia have been equivocal

Treatment of a confirmed bacterial pathogen should be based on the sensitivity information provided by the laboratory. Parenteral treatment is recommended for salmonella and ceftiofur, amoxicillin, florfenicol or trimethoprim sulphur (at the doses shown above) have been shown to be efficacious against sensitive strains. ETEC is non-invasive and hence oral therapy is preferred, with apramycin being the drug of choice in sensitive isolates. This may be administered in oral electrolyte solutions. However if the calf is sick enough to require fluid therapy it should be treated with intravenous antibiotics

Treatment for protozoal infections

There are currently no effective therapeutic options for treatment of cryptosporidiosis in Australia. Drugs reported to have some efficacy against cryptosporidia in calves include, halfuginon, paromymicin, decoquionate, and β_-cyclodextrin. Lasalocid has some efficacy, but only at a dose that is toxic to calves. Halfuginon is licensed for treatment of calves in Europe and appears to be the most efficacious. The efficacy of decoquionate is questionable with the only controlled clinical study failing to demonstrate a beneficial therapeutic affect with daily treatment at 2 mg/kg per day.

Coccidiosis is uncommon in calves less than 6 weeks of age. In hand reared calves coccidiostats (lasalocid, amprolium, or decoquionate) may be added to milk replacer. Prophylactic options for beef calves are restricted to coccidiostat medicated pellets (monensin, lasalocid, amprolium, or decoquionate) or water (amprolium or sulfonamides). Therapeutic options include amprolium or sulfonamides such as sulfadimidine

both fenbendazole (5 mg/kg once daily for 3 days p.o.) and albendazole (20 mg/kg once daily for 3 days p.o.) have been shown as effective treatments for Giardia. {xiao 1996; O'Handley 2000, 2001} Both of these treatment are above label dose and will require an extended withhold period. Due to the high level of subclinically affected animals all cows and their dams need to be treated and reinfection is likely to occur unless calves are removed from environmental sources of infection.

Other treatments

A recent study of the benefits of a single or double injection of flunixin meglumine in scouring calves was equivocal. The use of non-steroidal antiinflammatories (NSAIDs) in severely dehydrated and shocked animals has also been questioned in small animal medicine, as their use may result in renal necrosis

Ascorbic acid has been used as a preventive measure in calf scours and shown to have a beneficial effect. However the frequent administration required is unlikely to be practical in a cow calf operation. Vitamin B injections may be given to ensure adequate levels, whilst nutritional intake is low, and in selenium deficient areas selenium injections should be given to calves with diarrhoea, if the dams have not been supplemented.

Probiotics

Probiotics are a food or drug containing live microbes that, when ingested, is expected to confer beneficial physiological effects to the host animal through microbial actions. A number of probiotic products are licensed for the prevention and treatment of calf scours in Australia. Bacterial and fungal species included in these products include *Enterococcus faecium*, *Lactobacillus acidophilus, Lactobacillus delbrueckii* subspecies bulgaricus, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Streptococcus salivarius* subspecies thermophilus, *Aspergillus oryzae*, and *Candida pintolepesii*. General mechanisms of action that have been prescribed to probiotics include competition for receptor sites on the intestinal surface, immune system stimulation, excretion of anti-microbial substances, and competition with pathogens for intraluminal nutrients

The number of controlled clinical trials evaluating probiotic formulations in calves is limited, and where a significant difference has been shown, the probiotics have been fed prophylactically to hand reared calves, as opposed to being used as part of a treatment regime for calves already affected with diarrhoea.

Supportive and ongoing care

Calves that are unable to suckle should be placed in a warm sheltered or cool and shaded environment depending on the climate. They should be evaluated for secondary problems such as hypoglycaemia and hypothermia. Hypoglycaemia is likely to occur if the calf is malnourished or endotoxaemic. Hypothermia occurs due to poor hydration, poor nutrition or poor adaptation and can occur in all climates. Calf jackets should be considered for severely debilitated calves or in cold weather. Warm air is one of the most effective ways of treating hypothermia. Warmth may also be provided by filling 2 x 20 L containers with hot water and covering with a tarpaulin or hay and placing near to affected calves. These calves will require ongoing treatment with electrolytes for 24 hours or possibly several days. Calves should be rubbed and encouraged to stand every 12 hours and their suckle reflex reassessed, and milk should be fed as soon as they are willing to suck.

Calves that are being returned to their mother should be given 2-4 L of electrolytes immediately before they are let go, so that they don't suddenly suckle a large volume of milk. Where calves are left with or returned to their mothers it is important that they also have easy access to water and appropriate shelter.

Record keeping

This is needed to monitor the outbreak and ascertain the effectiveness of treatment protocols as well as mandatory to determine withhold periods. The use of individual ear tags should be encouraged but where this is not done raddle, spray paint or a band around the calves' neck could be used to identify treated calves.

8.3 Appendix 3: Documents for Producers

1. Why do calves get scours?

Calf scours "neonatal calf diarrhoea" is most commonly seen in calves less than two months of age and often less than 4 weeks of age. Scouring calves can have yellow-green, white or brown manure and it may be pasty or very liquid. Sometimes there is blood in it. Often you will see the faeces stuck around their tail and down their legs. When the manure is very watery you may not notice it or there may be hair loss around the top of the tail, anus, and back of the hind legs. There may also be a lot more lot more flies than usual around the back of the calf. It is impossible to tell what is causing scours from the colour or type of the scour. Calves can have pasty whitish manure when they consume a lot of milk. **If "diarrhoea" is not associated with a depression in the calves attitude and appetite no action may be required.**

Sick scouring calves will be lethargic and spend a lot of time lying down. If they are standing up you may see them straining to pass manure. Their eyes may be sunken and sometimes they will have crusty scabs around their nose. Often you can see the diarrhoea around where they have been lying.

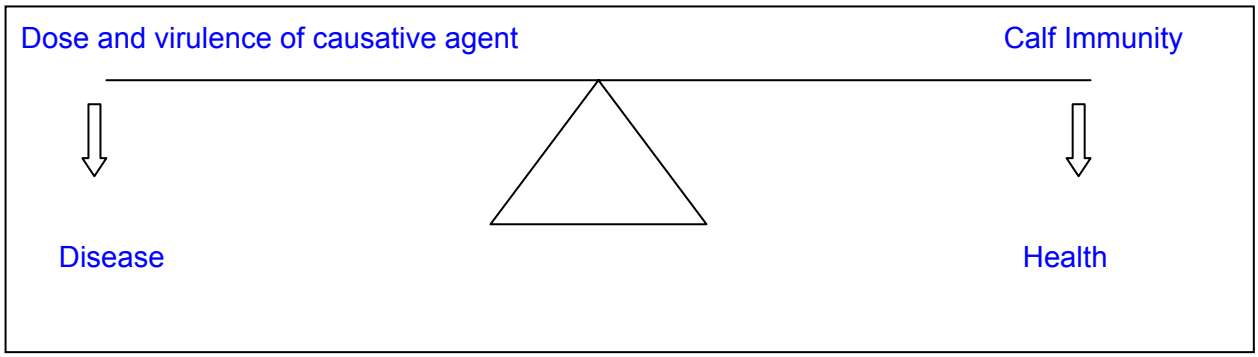
Bacteria, viruses and protozoa can all be involved in calf scours (protozoa are single celled organisms that are bigger than bacteria). These causative agents are often present in the manure of adult cows in many herds, but seldom cause sickness in adult animals. Cows are more likely to pass these agents in their manure around the time of calving. All causative agents most involved with scours will survive in the environment for many weeks and often for over a year in cool wet conditions. Consequently calving paddocks may remain infective from one season to the next.

Newborn calves are susceptible to disease, and receive colostrum from their mother to provide temporary protection and limit the degree of damage that an infective agent can do. Over the first few months of life they will become infected with a variety of diseases and then develop their own antibodies to them.

Exposure to these agents is an important part of the calf's early life and stimulates the development of immunity. For most calves the protection that they have received from their mother will limit the disease so they don't become ill. When calves are exposed to a *high dose of causative agent* or a particularly nasty (virulent) causative agent or have a *reduced ability to fight the infection* they become sick.

The result is like a weigh scale: If the dose and virulence of the causative agent is greater than the calves immunity the calf will become sick, but if the calf's immunity is able to overcome the causative agent then the calf will stay healthy (See Figure 2)

Figure 2: The balance scale of infection and immunity



Calves become infected with diarrhoea agents by ingesting them when suckling or nibbling at surfaces contaminated with manure. This can be their mother's teats, the ground, pasture or hay, or they may drink contaminated water. Causative agents either damage cells in the gut lining or alter the movement of fluid across the gut wall. **When calves ingest a small number of the causative agent if their immune system is strong they may control the infection. Conversely when calves receive a high dose of causative agent, or have a reduced ability to fight the infection the antibodies from colostrum do not control the infection and the damage to the gut is more widespread, resulting in diarrhoea and in some cases death.**

Sick calves have large numbers of causative agents in their scour, markedly increasing environmental contamination and the exposure of their herd-mates to the causative agent. Once they recover they can still shed causative agent in their manure and act as a source of infection to other calves.

Calves with good immunity against disease may not show signs of disease but may develop transient low-grade infections and also shed the causative agents in manure. Calves up to 4 months of age will shed infective particles in very large numbers (10,000 – 1 million particles per gram of manure) even when they do not have diarrhoea. These calves are therefore a major source of infection for younger calves in the herd, and when the age range of calves within a group is more than 1 month, a high proportion of the older calves may be shedding enormous numbers of infective particles, hence creating a very contaminated environment for the more susceptible younger calves. The build-up of causative agents tends to increase throughout the calving season, so calves from late calving cows are exposed to much greater levels of infection than earlier calving cows. Areas of high stocking density for example where calves camp are especially high-risk for infection of younger calves.

Therefore the key to prevention of calf scours to minimise contamination with causative agents and maximise the calf's ability to fight infection.

Stocking density, calving pattern and the management of paddocks, pastures and soil are key variables that may either minimise or promote environmental contamination with the causative agents. High stocking density and management procedures that bring cows and young calves together result in an increase risk of other calves becoming infected with scours. Calves brought in from another property to replace calves that have died may be carrying causative agents that are new to the herd, so there is no protective immunity in the colostrum or milk. Long calving intervals allow for build up of the causative agents during the calving period with calves born early in the calving period increasing the level of environmental contamination and increasing the challenge dose of causative agents that calves born later in the season will be exposed to.

Weather conditions play a role as the environmental conditions will influence the survival of causative agents. Moist conditions generally favour survival of the causative agents and rain may release some viral and protozoal particles that are bound in the soil. Hot weather will result in calves congregating in the shade, and they will get dehydrated more rapidly, so a mild case of scours becomes much more serious. Maternal management during pregnancy, colostral transfer, and nutrition influences the calf's defences against disease. **Nutrition of the cow before and after calving and good environments can improve calf viability, growth, and defence against infection.**

There are 5 causative agents of calf scours frequently diagnosed in Australia. It is not possible to determine the cause of the problem from the nature of the scour. All agents can produce severe watery diarrhoea or yellow or white pasty scour, and a variable percentage of affected and dead calves. Cryptosporidia and salmonella may also cause disease in people. Young children and persons that are immunosuppressed should not be exposed to scouring calves.

Cryptosporidia

Cryptosporidia is a protozoa that is commonly found in watercourses, and is especially associated with irrigation run off and access of cattle to the watercourse. It is one of the most common causes of diarrhoea in beef calves. The cryptosporidia that infect cattle also infects many other species including domestic animals, wildlife species and humans and these can act as a source of infection to the calf. Most calves are affected between 1 and 4 weeks of age and have diarrhoea for 4-14 days. Animals of all ages can be infected but cryptosporidia do not cause diarrhoea in cattle older than 4 months.

Rotavirus

This virus also affects other animals including deer, dogs, cats and feral animals and these can act as a source of infection to cattle as well as environmental sources. It is another common cause of diarrhoea in beef calves. In infected herds many cows will have antibodies in their milk but these decline rapidly after calving and calves can become susceptible to infection at one week of age. Affected calves are generally 5 days to 2 weeks of age, although disease can occur at 24 hours, particularly when calves have not suckled colostrum. Disease has also been reported at two to three months of age when calves are first exposed to this virus later in life.

Bovine coronavirus

This virus only affects cows and can be transmitted through the respiratory tract, so animals can become infected by direct contact with each other as well as ingesting contaminated manure. It is not so hardy in the environment and persistently infected cows and older calves are most likely to be the greatest source of infection in a herd. Calves are most commonly affected between 5 days and 1 month of age, but diarrhoea can occur at 24 hours when calves do not receive colostrum. Disease may be more severe and last longer than rotavirus infections. Some animals may have respiratory signs: nasal discharge, coughing and sneezing. Although coronavirus has been commonly reported in beef calves in other countries, it has not been found so commonly in Australia. However there is now a better diagnostic test available and it is likely to have a significant contribution to calf scours in suckler beef operations.

E. coli

E. coli are part of the normal bacteria of the bovine gastrointestinal tract and are found in all bovine manure. Most strains are harmless even to baby calves but some strains can cause sickness and diarrhoea. The most common *E. coli* to cause diarrhoea is Enterotoxigenic *E. coli* (ETEC) that produces disease in calves aged from 1-14 days of age, and mainly less than 4 days of age. Cows do not have good antibodies to this disease in their colostrum unless they have been vaccinated

Salmonella

Salmonella can be spread by contaminated feed and water, insects, birds, reptiles and other mammals. Infections in calves are often linked to shedding of the bacteria in the manure and milk of adult cattle. Salmonella shedding by adult cattle increases around calving and in cattle that experience nutritional (including rapid dietary change) or heat stress. Calves may show signs of disease from 2 days of age onwards, although disease is more frequently observed in calves 5 – 28 days of age. There are numerous types of salmonella. One type, *S.* dublin also causes respiratory disease. Occasionally salmonella causes very severe infections and calves die before diarrhoea is seen. Salmonella is also capable of causing disease in humans.

Other causes of diarrhoea

There are other causes of diarrhoea, but for calves aged less than 6 weeks of age it is important to eliminate the main 5 causative agents first. One common cause in slightly older calves in

coccidiosis. This may be found in calves as young as 3 weeks of age, but generally will affect calves that are 6 weeks of age or older. Calves will often strain, switch their tails and have blood in their manure. It is associated with high stocking densities and mud around water troughs. It is also associated with weaning stress.

Human health considerations

Farm employees should be advised that the agents that cause scours in calves may also cause disease in people. Recommendations for people working with calves include:

1. Wear disposable gloves when working with sick calves.

2. Always wash your hands after working with calves.

3. Do not eat, drink, or smoke while working with calves.

4. Do not work with sick calves when you are on antibiotics, immunocompromised, infected with HIV, or taking immunosuppressive medication.

5. Be particularly careful not to inadvertently infect your children by exposing them to sick calves and clothing and equipment used around sick calves. Young children can end up in hospital and sometimes require intensive care.

2. Prevention of calf scours

Calves develop scours when they ingest a large dose of infective agent and/or have a lowered resistance to disease. In order to prevent a calf scours problem it is necessary to manage your cows to prevent a build-up of causative agent on the farm and to optimise the health of pregnant dam and subsequently the newborn calf. There are many ways to manage a farm and there are also many ways to minimise the risk of calf scours. It is not possible to make generic recommendations that will suit all farms and all management systems. The purpose of this document is to highlight best management strategies. The implementation of these strategies will depend on the resources and restraints of each producer.

Common reasons for calves to be exposed to a high infective dose are:

- ✓ Their mother is shedding diarrhoea agents in her faeces
- ✓ There is a high stocking density in the paddock they are in (> 5 cows /ha), or the paddock has been used for a long time and there is more than 2 pats of manure per square metre
- ✓ They are running with calving cows that are more likely to have diarrhoea agents in their faeces
- ✓ They are running with older calves that are likely to be shedding large amounts of diarrhoea agents in their faeces
- ✓ They are calves of late calving cows so their has been a lot of cows and calves already in the calving paddock
- ✓ There is a lot of mud and manure around troughs, feed out areas and cow camps
- ✓ The only source of water is a dam or a stream which cows and calves also stand in
- ✓ Infectious agents have been introduced by bobby calves purchased to replace lost calves

Common reasons for calves to have a lowered resistance to disease are:

- ✓ They did not suckle enough colostrum from their dam, or their dam had poor quality colostrum (see p 177)
- ✓ They are cold and wet, and there is inadequate shelter.
- ✓ They are hot and dehydrated and there is inadequate shade
- ✓ They are recovering from a difficult calving
- ✓ Calves are not able to lie and sleep undisturbed because there is a high stocking density
- ✓ They have poor access to fresh water. Calves will often drink water from a few days of age. When they start to get diarrhoea they will drink more to prevent themselves getting dehydrated. If there is no fresh water within 300 m or they are unable to access the water due to high troughs or steep banks etc they will become dehydrated
- ✓ Cows have inadequate nutrition and are unable to produce sufficient milk

A farm plan to minimise calf scours

Prevention of calf scours requires a whole farm management plan that will allow you to prepare your farm for calving, both nutritionally and to minimise disease. Many of the management recommendations are those promoted by other MLA programs such as “More Beef from Pasture”. In order to protect baby calves from exposure to a high infective dose of causative agents it is necessary to isolate them both from calving cows and from older calves. It is also important that cows calve in a clean paddock that was not used for calving animals in the previous year. Recurrent use of a single calving paddock is likely to result in a build-up of contamination with carry over from one season to the next.

This requires identification of paddocks for cows before calving that are different from the paddocks that they will calve down in. There is also a requirement for a minimum of 2 and preferably at least 3 calving areas, and then paddocks that you can drift cows and calves into once they have calved. This may be achieved by using electric tape in a set stocking system, and in a rotational grazing system having different areas of the farm for different ages of animals. It may be easier in a rotational system to drift out the cows yet to calve and leave the cow and newborn calf behind. While the logistics of implementing paddock management systems to minimise pathogen exposure will vary from farm to farm the concept carries more benefit than just preventing calf scours. For example, the same principle is equally applicable to control and prevention of Johne’s disease. The following principles should be followed to minimise calf scours on your farm

Herd management strategies

- ✓ The time of calving should be chosen to avoid extremes of weather and to match stocking density to carrying capacity. If extreme weather or reduced feed availability is likely to be having a serious negative impact on the occurrence of calf scours in your herd, then it may be beneficial to change your joining dates. This needs to be a planned process and should be discussed with your veterinarian.

- ✓ Aim for a short tight calving season - mate cows for no longer than 9 weeks and heifers for 6 weeks. This will minimise the build up of infection in the calving paddocks

- ✓ Cull heifers that are not interested in their calves, or have calves that seem continually hungry

- ✓ Cull cows with low abdomens or udders that prevent calves from finding the teats easily

- ✓ If you want to introduce calves to replace calves that die at calving, or as additional calves for dairy cross dams, you need to consider the disease status of the herd of origin. Calves from other herds may introduce a causative agent that your herd has no protection against. Young calves should never be purchased through a market as they may not have received colostrum, and also will have been exposed to a variety of different causative agents, and are therefore a much bigger disease risk to your cattle.

- ✓ Ensure cows receive adequate trace minerals especially selenium and copper

- ✓ Run programs to control feral animals

- ✓ Minimise exposure of cows with young calves to domestic pets, farm dogs and wildlife

- ✓ If salmonella or enterotoxigenic *E. coli* has previously been diagnosed on your farm discuss the benefits of a vaccination program with your veterinarian (see p 178)

Management of heifers

Calves born to heifers carry a greater risk of calf scours, due to the increased risk of calving difficulty, mismothering and poor quality colostrum. Calving difficulty and low colostrum volumes are more likely to be a problem in poorly grown, poorly fed heifers. To minimise these risks it is important that heifers

- ✓ reach target weight at mating, (a minimum of 300 kg for British breed heifers aged 15 months old).(See Tool 6.1 in the More Beef from Pastures manual for other breeds)
- ✓ are mated to a high calving ease bull
- ✓ are fed well from weaning to calving (average gain 0.5-0.75 kg/day)

  Strategic supplementary feeding may be necessary to achieve this weight gain and will not increase the risk of calving problems unless the heifers become fat close to calving (greater than condition score 4).

- ✓ Mate heifers for six weeks and start mating 2 weeks before the cows.

MLA effective breeding workshops include cover information on achieving well grown, early calving heifers in much more detail.

Before calving

- ✓ Run cows and heifers separately to allow for preferential treatment of heifers
- ✓ Ensure cows have adequate feed (>1500 kg/DM residual after grazing) and are condition score 3.0-3.5
- ✓ Don't put cows or heifers into the calving paddock(s) more than 2 weeks before the planned start of calving

Management of cows at calving

- ✓ Have a minimum of two and preferably at least 3 calving areas, to prevent build up of contamination.
- ✓ Use one calving areas at a time and rotate every three weeks or more frequently if
  - there is an outbreak of calf scours
  - there is more than 2 pats of manure per square metre
  - the weather is excessively wet and the paddock becomes wet and boggy
- ✓ Where possible use different calving areas from year to year – (it is possible to use the same calving areas every 2nd year)
- ✓ Calving areas should
  - be sheltered from the prevailing wind and have plenty of shade
  - be well-drained. If water is visible on the surface or in boot prints /hoof prints it is not dry enough (If this is not possible decrease the stocking density)
  - have easy access to a crush to facilitate animals that require assistance

- should have water troughs not dams or creeks as a water source

✓ When feeding out, feed cows in a different area of the paddock each day

✓ Separate feed areas from watering points to encourage cow dispersal and minimise contamination

✓ Leave the calving paddocks vacant over the summer (cut for hay or silage)

Management of newborn calves

✓ Provide a sheltered dry area for calves that require an assisted calving in wet cold weather. This can become a site of infection, and should have a removable floor (straw or sand) so that it can be cleaned out

✓ All calves that have not obviously fed within 6 hours of birth should be supplemented with colostrum (see p 177). Special attention should be paid to heifer's calves and calves that have required assistance at birth

✓ Drift cows and calves into a separate paddock 24 hours after calving

Management of cows and calves after calving

✓ Drift off cows and calves into a nursing group

✓ Start a new nursing group every 3 weeks, or earlier if paddock size dictates

✓ Do not merge groups until the youngest calf in the group is 6 weeks old

✓ The nursing paddocks should

- be sheltered from the prevailing wind and have plenty of shady sheltered areas for cow camps
- be well-drained. If water is visible on the surface or in boot prints /hoof prints it is not dry enough (If this is not possible decrease the stocking density)
- have a stocking density appropriate to the district but no greater than 5 cows/ha
- should have water troughs not dams or creeks as a water source

✓ In windy areas paddocks should have shelter belts planted to protect from the prevailing winds

✓ If wind and cold is thought to be a contributing problem and there is insufficient shelter the following measures should be considered as an interim measure:

- calf coats for more susceptible calves eg those from heifers and assisted calvings
- windbreak fences (2.5 m high 20 % porosity)
- moveable calf shelters. These are constructed of wood and have straw or sand on the floor. The recommended dimensions are 7.3 m long, 3.05 m deep and 2.44 m high and are designed so that calves can enter but cows can't. They should be moved regularly to avoid build-up of the causative agents

- ✓ When feeding out, feed cows in a different area of the paddock each day
- ✓ Troughs should be spaced such that cows and calves should have easy access to fresh water within 300 metres at all times
- ✓ Separate feed areas from watering points to encourage cow dispersal and minimise contamination

Colostrum management

Newborn calves are susceptible to disease. Colostrum increases their resistance to disease. The protective effect of colostrum is in part derived from antibodies that are absorbed across the gut wall into the blood stream. The calf's gut loses its ability to absorb antibodies over time following birth. After 12 hours the gut wall will start to close so less antibodies can cross, and by 24-36 hours no antibodies will be able to get into the blood stream. To maximise the amount of antibodies in the blood stream calves should receive 2-4 L of colostrum in the first 12 hours and preferably at least half of this in the first 6 hours after birth (Table 8).

Routine administration of colostrum to the newborn beef calf is disruptive and difficult and will delay the time until first sucking. However with calves that have a difficult calving or are not sucking well in the first 4-6 hours due to adverse weather conditions or poor mothering, it is important to make sure that they get enough colostrum. Initially it may be easier to get the calf up, rub it down and encourage it to suck from its mother whilst she is restrained in a crush. However it is important that it has a really good long suck, otherwise it will not receive the minimum volume that it requires (see Table 8). It is important that the calf gets colostrum, not powdered milk or "home-made colostrum" as other sources will not supply any protective antibodies, but will cause the gut wall to close so that if the calf suckles later the antibodies will not be able to cross into the blood stream.

Colostrum should be sourced from cows on the home property. Sourcing colostrum from cows on other properties runs the risk of bringing new diseases onto your property, especially when it comes from dairy cows. Colostrum can be a source of Johne's Disease, Enzootic Bovine Leucosis *(*EBL) and also some of the infectious agents that cause calf scours. The best source of colostrum is from cows that have had between 3 and 6 calves and have been on the property for at least 1 year.

Obtaining sufficient milk from beef cows can be an occupational health and safety risk. The best method is to restrain the cow in a crush, jack up the cows tail and milk the colostrum from behind the cow between the back legs. Always try and get enough for a second feed and if the calf is sucking well you can freeze it for another calf. Colostrum can be stored in a refrigerator for 72 hours or frozen for 2 months. Longer storage than this will affect the antibody levels. Store colostrum in zip-lock bags laid flat to facilitate defrosting when you need to use it. Colostrum should be defrosted in warm water or at half power in a microwave. Defrosting at full power in a microwave or in boiling water will damage antibodies reducing the effectiveness of the colostrum.

Colostrum substitutes are available but are expensive and generally do not result in adequate antibody levels.

Calves should be given colostrum with a bottle and teat or with an oesophageal (tube) feeder. Direction on how to use an oesophageal feeder can be found in "Approach to a calf scours outbreak" The volume given depends on the weight of the calf and the source of colostrum. Colostrum sourced from beef cows is often more concentrated, so it is generally sufficient to milk out the mother and give this to the calf. If the mother has a lot of colostrum or if the milk is sourced from dairy cows the volume to administer is given Table 8.

Table 8: Volume of colostrum to be given at first feeding

Calf weight (kg)	Volume of colostrum required (Litres)
20	2
25	2.5
30	3
35	3.5
40 +	4

Vaccination programs to prevent calf scours

Before starting a vaccination program, the relevant causative agents should be a significant problem on a property. The cost of vaccination should be weighed up against the likely costs of a calf scour outbreak due to that causative agent. These should include cost of treatment, time taken to treat and manage a calf scour outbreak, likely death rate and cost of culling a cow because she is dry.

Vaccination is not a replacement for poor management and is only worth using when other predisposing factors are addressed, especially nutritional and environmental.

Currently there are only 2 vaccines available in Australia directed at preventing calf scours. The first is an *E. coli* vaccine (Bovac, Intervet Australia Pty Ltd) to prevent enterotoxigenic *E. coli* and the second is a salmonella vaccine (Bovilis, Intervet Australia Pty Ltd) to prevent salmonellosis. In the U.S.A., Europe and New Zealand a number of viral vaccines are available. These include vaccines for rotavirus and coronavirus.

E. coli vaccination

Research has shown that under natural conditions the colostrum of less than 10% of beef cows contains protection against enterotoxigenic *E. coli* (K99+). Calves are protected by vaccinating cows in late pregnancy. Many studies have shown this to be an effective vaccination but it is important that all calves get adequate colostrum.

The decision to vaccinate will be based on a cost/benefit basis on a particular farm and should be considered when there is a history of enterotoxigenic *E. coli* scours on the farm and especially if there is:

- High stocking density or use of a common calving area
- Projected calving during the wet season
- Large numbers of heifers projected to calve

Pregnant cows should be vaccinated 3 and 8 weeks prior to calving and followed up with annual boosters.

Salmonella vaccination

The results of trials with similar vaccines to the vaccine available in Australia have been variable. Some have demonstrated a beneficial response others have not. The consensus of the research

to date suggests that this type of vaccine provides partial protection. The protection associated with colostrum is short lived, but as many calves are exposed to salmonella in the first week of life vaccination may help to control disease on infected properties.

Where salmonella is a problem in a herd it is particularly important to eliminate environmental and nutritional stresses, and focus on hygiene and increased colostral intake.

The decision to vaccinate will be based on a cost/benefit basis on a particular farm but may not result in a complete cessation of the problem. In an infected herd there will be some animals that are chronically infected and will intermittently shed salmonella in their faeces. Vaccination of cows during late pregnancy will increase stimulate production of anti salmonella antibodies raising the level of these antibodies in colostrum. During summer cattle should be vaccinated during the cooler times of the day to minimise the risk of adverse vaccination reactions.

Rotavirus and Coronavirus Vaccines (Not Available in Australia)

There are many different types of vaccine available on the market for both of these causative agents. Results from independent field trials have shown variable results. Where vaccines have been shown to have benefit there has been 1) a delay of a few days in the onset of diarrhoea and or (2) reduced severity of diarrhoea, and or (3) a reduction in the length of the time that of the virus is shed by infected animals.

3. Approach to a calf scours outbreak

When do I need to worry about scouring calves

Exposure to the agents that cause calf scours is a normal part of "growing up" for a calf and almost every property will have a couple of calves that have sticky white or yellow diarrhoea around their tail. One or two calves that are scouring but remain bright and continue suckling are not a problem, although it is advisable to observe them daily to ensure rapid treatment if they do become sick.

Beyond this outbreaks should be classified as follows

Mild disease

- ✓ Variable number affected (2-100%)
- ✓ All affected calves are bright and suckling
- ✓ No deaths

Moderate outbreak

- ✓ Variable number affected (2-100%)
- ✓ Less than 4% of calves are sick and have required treatment[6] over the past month (< 1% per week)
- ✓ Less than 2% of calves aged less than 1 month have died over the past month
- ✓ Less than 1% of calves aged more than 1 month have died over the past month

Severe outbreak

- ✓ Variable number affected (5-100%)
- ✓ More than 4% of calves are sick and have required treatment[6] over the past month (< 1% per week)

or

- ✓ More than 2% of calves aged less than 1 month have died over the past month

or

- ✓ More than 1% of calves aged more than 1 month have died over the past month

When do I contact my veterinarian?

Your veterinarian should always be contacted if:

- ✓ You have a severe outbreak

[6] See Figure 3 for definition of needing treatment

- ✓ You have a moderate outbreak and
    - calves are dying
    - calves have blood in their faeces
    - you are unsure as to the correct treatment protocol

Contacting your veterinarian can help to diagnose factors causing of the problem. The most important reason for a diagnosis is to allow you to put specific preventive strategies in place. It will also allow you to establish a correct treatment protocol. Diagnostic tests can take several days before you get results, so once animals start getting sick it is important to consider the benefits of establishing a diagnosis

Management strategies to control the outbreak

Many of the management strategies used to control calf scours are similar to those used to prevent calf scours. They revolve around minimising contamination with causative agent and increasing the resistance to disease. It is most important to apply these strategies early in an outbreak to try and reduce the number of calves affected, especially if you have calves requiring treatment or dying.

Mild disease

A mild case of scours often starts in the older calves and can lead to more serious disease as it affects younger calves. If you regularly get scouring calves on your property, but very few that are sick enough to require treatment, it may be sufficient just to observe and monitor. However on most properties it is wise to set in place some preventive strategies to minimise build up of infection and the prevent infection of newborn calves. The most important preventive measures are:

- ✓ Change the calving paddock
- ✓ ensure stocking density is less than 5 cows/ha.
- ✓ Separate cows yet to calve from cows with calves
- ✓ Drift off newborn calves into a new nursing group in a fresh paddock (an adjacent paddock is usually sufficient). Do not put any new calves with affected group
- ✓ Leave the affected calves where they are as long as feed allows to avoid spreading contamination. In a rotational grazing system try to restrict to one area of the farm
- ✓ Do not move the affected calves anywhere where they or their manure will come into contact with other calves aged less than 6 weeks old
- ✓ Ensure affected calves have no access to water courses or surface water
- ✓ Decrease stocking density by drifting off calves older than 6 weeks of age from the affected group or spreading out group of calves over several paddocks
- ✓ Minimise exposure of cows with young calves to domestic pets and farm dogs– these can spread causative agents between groups

Moderate outbreak

- ✓ Carry out all management procedures used in mild outbreak
- ✓ Fence off heavily used calf camps in the paddock where the affected calves are with an electric fence
- ✓ Ensure all calves have access to a water trough within 300m
- ✓ If the area around water troughs is wet and muddy apply soil or woodchips and treat the area with lime
- ✓ When feeding out, feed cows in a different area of the paddock each day
- ✓ Separate feed areas from watering points to encourage cow dispersal and minimise contamination
- ✓ Continue to change the calving paddock every three weeks or more frequently if
    - there is more than 2 pats of manure per square metre
    - the weather is excessively wet and the paddock becomes wet and boggy
- ✓ Keep a set of overalls and gloves specifically for treating sick calves
- ✓ Wear clean overalls and disposable gloves when handling newborn calves.
- ✓ When possible the sick calves should be treated by a stock person that is not coming in contact with unaffected calves, especially newborn calves
- ✓ Minimise stressful management procedures during outbreak. e.g. dehorning, castration, dietary changes including weaning, transport
- ✓ Ensure all calves are getting sufficient colostrum and supplement where necessary (See section on colostrum management in prevention document)
- ✓ Calves with scours are more susceptible to cold wet, windy, conditions. Where possible provide access to shelter from the wind
- ✓ If salmonella or enterotoxigenic *E. coli* (K99) is diagnosed on laboratory samples discuss the benefits of vaccination with your veterinarian

Severe outbreak

Carry out all recommendations for moderate outbreaks plus

- ✓ If less than 25% of the affected group have had scours consider separating the affected and unaffected calves to minimise spread and make treatment of the affected calves easier

Diagnosis of calf scours

Knowing the causative agents present on your farm allows you to determine the treatment that is most likely to be cost effective, but more importantly will allow your veterinarian to suggest specific control measures.

This may be achieved in one of 2 ways:

- ✓ Taking at least 6 diarrhoea samples yourself from the most recently affected calves and delivering them to your local veterinary clinic for them to send away for laboratory testing.

- ✓ Having your veterinarian come to the farm, examine the sick calves and take appropriate samples

The second option will allow your veterinarian to build up a picture of what is happening on your farm, detect any predisposing factors and get a good clinical picture of the sick calves. They will then be much better equipped to provide specific advice on both control and treatment.

If you are going to take your own samples, they should be from calves that have been recently affected that have not been treated with antibiotics. Each calf should be rectally stimulated using a clean glove, do not collect samples from the ground. One simple trick is to cover you finger with an inside-out zip-lock bag, whist stimulating the calf and as the calf passes the sample turn the bag in the right way to collect the manure. Use a separate bag or glove for each calf so there is no cross-contamination of the samples. Each bag should be clearly labelled with the calves id or sample number, and information on the calves age and symptoms provided on a separate sheet. Samples should be refrigerated and taken to the vet the same day in an esky with an iceblock.

Results will take at least 48 hours and often up to one week, depending on the tests requested.

Your veterinarian will want details on the calves that have been affected in an outbreak. It is good practice to record details of sick calves daily in a diary from the beginning of calving.

Important information to record will include:

- ✓ Age of affected calves

- ✓ Age of affected mothers

- ✓ Date that affected calves were treated

- ✓ Effectiveness of treatment

- ✓ Number of calves died

- ✓ Age of dead calves

- ✓ Date that calvers died

Your veterinarian will then examine some of the affected calves, set up treatment protocols for them and take samples for diagnosis

There are 3 tests that are most commonly used by your veterinarian to determine the cause of a calf scour problem:

1. Blood tests of recently born calves to evaluate whether calves are receiving sufficient colostrum

2. Tests of scour samples to determine the causative agent

   The agents that cause calf scours are not found in the faeces all of the time and consequently it is necessary to take several samples to get a diagnosis. Samples that are taken from recently affected calves are more likely to give a diagnosis, but these calves may be harder to find as they will often have liquid faeces not tacky yellow or white faeces that sticks to the tail. The availability and quality of diagnostic tests has improved over the last 5

years. Appropriate sample collection and submission, and testing should result in isolation of the causative agents in 80 – 90% of calf scour investigations. If inadequate or inappropriate samples are collected or not all agents are tested for, then it will be more difficult to extablish the causative agents.

3. Post mortem of any dead or dying calves.

   Post mortems are often the best way to reach a precise diagnosis, but they must be carried out on freshly dead calves. You are much more likely to get a diagnosis if the calf is one that has been recently affected. Calves that have been treated for a few days will have chronic changes in the gut and the underlying cause may no longer be evident. Damage to the cells of the gut wall will occur within 5 minutes of death, and soon makes it difficult to tell whether the damage was caused by a causative agent before the calf died, or bacteria after death. Therefore whilst a post mortem examination of a calf that has been dead for several hours may allow your veterinarian to narrow down on the likely causes, laboratory testing often becomes an expensive exercise with no result. The best results are obtained when a dying calf is euthanased and samples are taken immediately.

If there is a possibility of mineral deficiency in your herd, your veterinarian may also take samples from the cows to assess this.

Although diagnostic tests can be expensive, the results can be used to set up preventive strategies for future years as well as allow the development of control and treatment protocols for the current outbreak.

Treatment of scouring calves

Assessing the sick calf

It is important to check the sick calf for it attitude, degree of dehydration, ability to suckle and volume of faeces passed. It is also important to note whether the dam appears to have an adequate milk supply.

Calves with scours pass many L of fluid a day as diarrhoea. Death is usually due to dehydration, not infection.

The most important treatment for a scouring calf is electrolyte solutions

Most calves with diarrhoea do not have a bacterial infection. Therefore antibiotics are not indicated in all cases and use at the herd level should be directed by diagnostic testing to identify a bacterial cause of calf scours. Targeted antibiotic therapy can be effective at reducing disease and calf death. Indiscriminate use of antibiotics may compromise the effectiveness of antibiotics on a farm by selecting for resistant bugs.

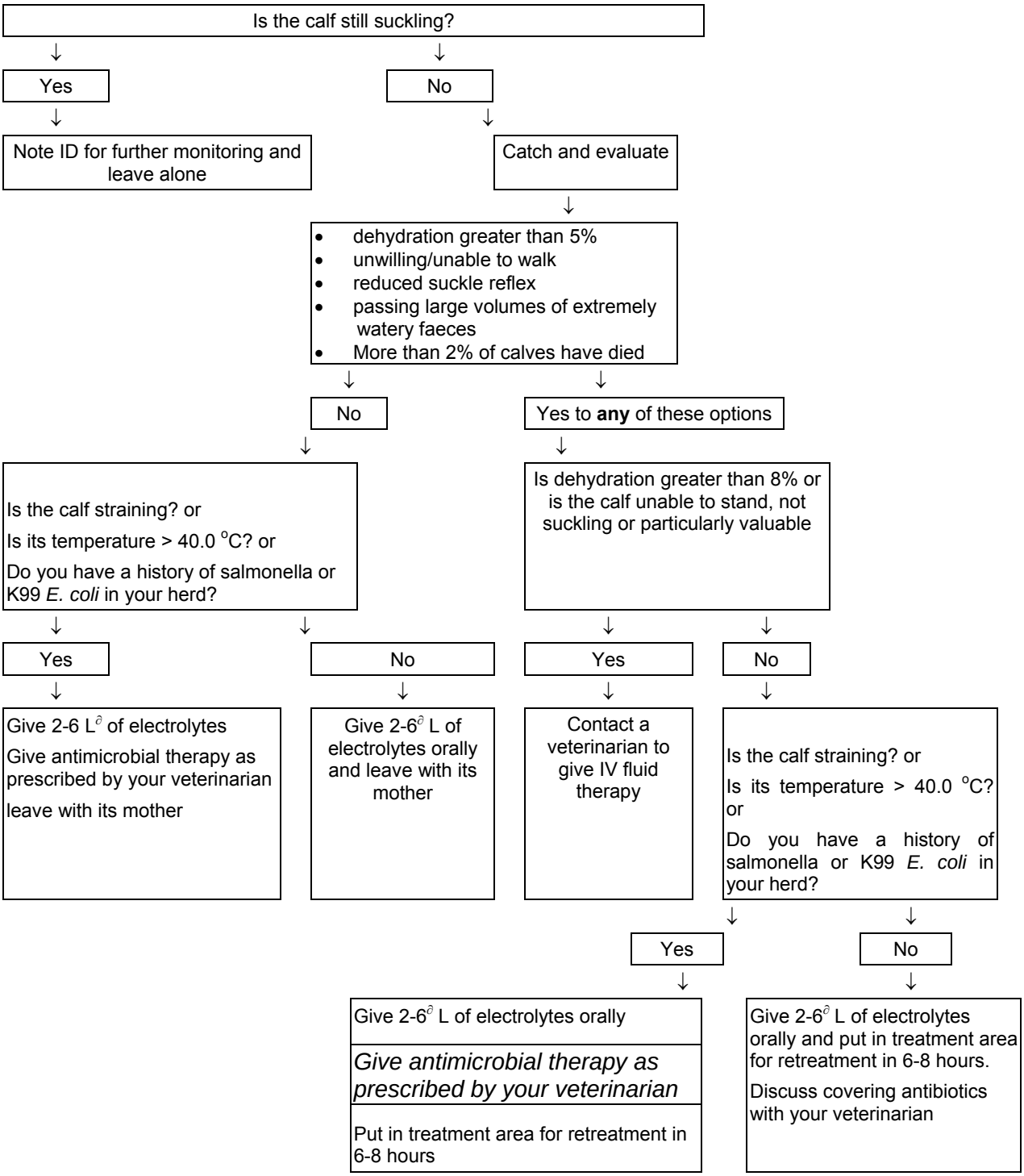
Table 9: How to estimate how dehydrated a calf is

% Dehydration	Attitude	Suckling	Eyeball Sunkenness	Skin Tent time (seconds)	Gums and nose
1-5	Bright and running, head up	Yes	None / slight	1-4	Moist
6-8	Standing or sitting, unwilling to move, head down	Maybe slowly	Slight separation between eyeball and orbit	5-10	Sticky
9+	Sitting or lying, head down	No	More than 0.5 cm between eyeball and orbit	11+	Sticky -Dry

Because dehydration will kill a calf quickly it is important to check cows and calves at least daily once a calf scour outbreak has begun. As a rule of thumb, if calves are suckling and run too fast to be caught they do not need treatment. If they can be caught they should be, and having been caught they should be categorised as to whether a single treatment is effective or whether they will need follow up treatment. This can be done by assessing the level of dehydration and other signs as shown in Figure 3: How to decide the appropriate treatment for a calf.

The degree of dehydration can be assessed as shown in Table 9. The skin tent time is measured by pinching up the skin on the neck and seeing how long it takes for it to return back to flat.

Figure 3: How to decide the appropriate treatment for a calf



∂ See Table 10

Treatment protocols

If calves are sick enough to catch it is important to give them enough electrolyte solution at the first treatment to aid a rapid recovery. The easiest way to administer oral electrolyte solutions is by using an oesophageal feeder, which is sometimes called a tube feeder. Information on the use of an oesophageal feeder can be found on p 188. A guide to the amount to administer is shown in Table 10. Calves often require large volumes of fluids to treat dehydration and to cover the losses from diarrhoea, therefore it is important to give a fairly large dose of fluids initially, as long as they are not too uncomfortable. If calves are to be left with their mother the oral electrolyte solution should not contain bicarbonate or citrate as this will prevent milk clotting and make the scours worse.

Table 10: Volume of electrolyte solution per feeding

Calf weight (kg)	Volume of electrolyte solution to give per feeding (L)
20	2
25	2.5
30	3
35	3.5
40	4
60	5
80	6

If calves are sick enough to require ongoing treatment the easiest option may be to construct a temporary shelter in the corner of the paddock with gates or straw bales, using tin or tarps to provide shade and wind protection. These should be designed to allow cows to see and smell their calf. In wet cold conditions the use of “space blankets” or calf coats may help sick cold calves. The treatment area should be moved to an adjacent area weekly to minimise build up of pathogens and the “Treatment corner” should be fenced off with electric tape to prevent other calves accessing this area.

Once calves are caught they can be put in here for 24–36 hours to allow repeat administration of fluids, or antibiotics if indicated. This will also be a good place for your veterinarian to administer intravenous fluids. It is important to give calves enough electrolyte solution each day, as they will continue to scour and lose body fluid. For the daily requirements see Table 11.

Calves that are too sick to be left with their dam should initially be given an oral electrolyte solution containing bicarbonate, as this is most effective for treating collapsed calves. Your veterinarian should be able to advise you of the appropriate brand.

Calves should be assessed daily according to the decision tree in Figure 3. Electrolyte solutions may make the scour runnier, so calves should be assessed on their attitude, willingness to suckle and degree of dehydration, not the consistency of the scour.

Calves should not be kept from their mothers for more than 36 hours and after 24 hours should be encouraged to suckle by encouraging to stand and rubbing vigorously along the back and over the chest and neck. Calves that are not suckling voluntarily after 36 hours should be left with the dam and given a high energy electrolyte solution to avoid excessive loss of condition. Calves that are too weak to suckle from their dam, but are able to suck on your fingers and willing to

take a bottle should be fed 2 L of milk twice a day between electrolyte feeds. Do not tube feed calves that are unwilling to suckle milk unless on the advise of your veterinarian.

Calves that have not responded to treatment within 36 hours should be reassessed and their treatment reassessed. If they are still dehydrated they will need an increased amount of oral electrolyte solution or an intravenous drip. They may also have an underlying infection and require treatment with antibiotics. If poor response to therapy within 36 hours is a recurrent problem or individual calves are taking longer than 3 days to respond it is advisable to consult your veterinarian to reassess the treatment protocol. Differences in response to treatment are observed with different pathogens. Establishing a diagnosis is helpful for assessing the response to treatment.

In a severe outbreak where calves are slow to respond to therapy it will be necessary to set up an isolation paddock for these calves and their mothers. Where possible isolated cows and calves should not be put back with other calves until all calves are 6 weeks of age.

Table 11: Daily fluid requirement for ongoing treatment of scouring calves[Ω]

Weight of calf (kg)	**30**	**40**	**50**	**60**	**70**	**80**	**100**
	Daily requirement (L)						
Sticky scours	4.0	4.5	5.0	6.0	6.5	7.5	9.0
Liquid scours	5.0	6.0	6.5	7.5	8.0	9.0	10.0
Profuse liquid scours	8.0	9.0	9.5	10.0	11.0	11.5	13.0

Ω Calves that are > 5% dehydrated (Table 9) will need another 2-4 L additional to this amount and more if they are collapsed

Antibiotic therapy

Antibiotics should only be given on the advice of your veterinarian. Most calf scour boluses, tablets and liquids contain antibiotics. While an antibiotic targeted at a specific disease that has been diagnosed on a property is beneficial, indiscriminate use of these products is unlikely to help. The causative agents of calf scours include viruses and protozoa that do not respond to antibiotics. Indiscriminate use of antibiotics as injections, boluses or calf scour liquid will result in an increased level of resistant bacteria. This may subsequently compromise the effectiveness of antibiotics used to treat other diseases that would normally respond favourably to treatment.

What do I do if calves are still dying?

In some frustrating cases producers will carry out the recommendations above and calves still die. This is frustrating both to the veterinarian and the producer. In most outbreaks where this occurs calves are either still being exposed to a high infective dose, have a lowered resistance to disease, or the therapeutic interventions have been abbreviated sufficiently by management constraints to become ineffectual.

Where deaths are still occurring it is important to review the situation with your veterinarian and especially consider the following points:

- ✓ What environmental and nutritional stresses could be increasing the calves' susceptibility to disease? Are there management changes that could be implemented to minimise these stresses?

- ✓ Are there more than 2 cowpats per m^2 in the areas where the calves spend most of their time? Can you fence off highly contaminated areas, or further decrease the stocking density of the mob?

- ✓ What are the possible sources of the disease? Can anything else be done to minimise exposure to high levels of causative pathogens

- ✓ Are you recognising sick calves quick enough? If calves are dying rapidly it may be necessary to check the affected group 2 or 3 times a day for a few days.

- ✓ Are you giving them enough fluids straight away?

- ✓ Are you confining sick calves and treating them with fluids at least twice daily?

- ✓ Have you established a diagnosis and correct treatment protocol?

- ✓ Does the ongoing pattern of deaths fit the patten expected by the causative agent that has been diagnosed? If not is the diagnosis correct or are their several causative agents involved in your problem

  Some cases can be hard to diagnose, they may be caused by a causative agent that is less commonly found requiring additional tests. It is possible any post mortem material that you have sent was too decomposed and not diagnostic. If deaths are continuing and calves are not responding to the treatment protocols suggested by your veterinarian, you should discuss with your veterinarian as to whether more tests are required.

- ✓ Have you prevented calves from accessing all obvious areas where there is likely to be high levels of contamination?

- ✓ Are you satisfied that calves from heifers and assisted calvings are receiving adequate colostrum

- ✓ Do you have a calving problem with your heifers that you need to address?

Use of the oesophageal feeder

Oesophageal feeders are a fast and efficient method of administering large volumes of fluids to calves by mouth. Oesophageal feeders have a rounded end on the tube to prevent the tube from passing into the lungs. It is important that this ball is not removed, and that the tube is replaced if the ball or tube is frayed, broken or has sharp edges.

To administer fluids to a calf the container should be filled with electrolytes and allowed to hang down so there is no fluid in the tube. Make sure that the calf is standing, or if it is unable to stand it should be sitting upright. With standing calves it is best to back them into a corner and use minimal restraint. Straddling the calf and holding the head often causes it to twist and sometimes flip upside down and kick you between the legs. Most calves will stand quietly with the tube in place when backed into a corner and blocked from moving forward.

The end of the tube is moistened with the electrolyte solution and the tube slowly passed into the calf's mouth and down its throat. Make sure no fluid is passing down the tube as it is inserted as it may flow into the lungs. The calf should be swallowing and not coughing. It is possible to see and feel the ball passing down the left-hand side of the neck. Extra care should be taken when passing the tube in very small calves (Wagyu, Dexter etc) to make sure not to damage the throat. Most tubes can be inserted for their full length, but it is important not to force the tube. Once the tube is in place, tip up the container and the contents will run down the tube. Stop feeding fluid if the calf is coughing excessively, if the fluid does not flow or if fluid comes out of the calf's mouth.

You should also stop if the calf becomes uncomfortable and has difficulty breathing. While the fluid is running through the tube make sure that the tube is not coming out of the mouth. If you don't watch this, the tube may come out to the point that the end is in the back of the calf's throat causing fluid to run into the lungs. Prior to removing the tube kink the soft portion so that there is no residual flow.

It is important to clean and disinfect the tube between each calf. Cleaning involves a three-phase cycle. First the feeder is cleaned with warm soapy water (dish washing detergent is effective) it is then rinsed to remove the soap and placed in a solution of bleach, 1% iodine or Virkon. The bleach solution made by mixing 300 mL of household bleach in 7.5 L of water. The feeder should be left to soak for at least 20 minutes in the bleach solution.

8.4 Appendix 4: Outcomes from the meeting with key laboratory personnel 04/11/04

Minutes from Meeting

Participants

Joan Lloyd	MLA	Keith Walker	EMAI
Alison Gunn	Project coordinator	Jeff Browning	Gippsland Pathology
John House	University of Sydney	Philippa McLaren	Gribbles
Cleve Main	Agriculture WA	Christine Trezise.	Gribbles
Steve Driesen	DPI Bendigo	Barry Richards	Idexx
Aileen Vanderfeen	DPI Bendigo	Ian Jerrett	Idexx
Stephen Pyecroft	DPIWE Tasmania		

Agenda

10.00am	Introductions and program outline. John House
	Calf Scours in Southern Beef Enterprises, the origin of the program and where we are up to. Alison Gunn
	Development of a practitioner calf scours disease investigation guide. Discussion / Workshop. led by John House
	SCAHLS and how it relates to investigation of diagnostic investigation of Calf Scours. Stephen Pyecroft
	Collecting information from laboratory databases. Alison Gunn
	Laboratory diagnostic testing for enteric pathogens of calves. Discussion / Workshop. led by John House
	Specific tests for each pathogen Testing modalities / Strengths / Weaknesses / Current best practice / Interpretation and reporting Limitations / required information (further research). led by John House

Calf Scours in Southern Beef Enterprises, the origin of the program and where we are up to:

Presentation of info from 1st phase of project. All laboratories should have copy of this document. PowerPoint sent as separate document FYI.

Jeff Browning then presented info from recent samples processed at Gippsland Pathology. Samples routinely tested for rotavirus, cryptosporidia and C&S for salmonella. If no result then tested for coronavirus and K99.

Positive result from 91% of submissions. About 20% of samples are from beef properties. Results included 29% crypto and 21% K99 +ve.

Development of a practitioner calf scours disease investigation guide

Clinical history

Need to differentiate between baby calf scours and post-weaning calf scours, producers call both "calf scours"

Suggested that information provided to lab should include:

Type of operation (beef, dairy, veal)

• *Age range of affected calves*

• *Demographics*

– *Stocking density*

– *Diet*

– *Worming history*

– *Vaccination history*

– *Number at risk*

– *# affected*

– *# dead*

• *Duration of the problem*

• *Historical progression of the problem*

• *Prophylactic and therapeutic interventions*

Comments that worming history and diet not important for NCD in suckling calves

Also need to add

Clinical description needed

Treatment type and response

Stocking density

Sample collection:

Collection of faeces from calves:

Faeces should be collected from a representative sample of calves:- need to rectally stimulate the calf. This will involve bringing cattle into the yard, but if worth taking samples then they should be good samples.

Look for calves with liquid faeces/clean bottom as white pasty faeces often late stage of disease. Consensus was off the ground not Ok as won't get fluid faeces, only late stage disease. Should be encouraging goo procedures.

Collect 5-10 g into leak-proof container: ½ full yellow tops, the more the better

Prelabel samples no 1 to 10 and put info on accompanying paper: minimises faecal contamination of packaging

Keep outside of the packaging clean to IATA 650 standards, need supply of good ready made IATA packages for veterinarians sending samples to labs that don't supply packaging.

Necropsy

Need to approach as a PM exam (similar to clinical examination), not a sample collecting exercise. Should describe and interpret what is there, description important even if normal. Checklist too complicated but remind of lesions they are looking for: Congestion, oedema, ecchymosis etc.

Include nutritional condition. Need to described what is nature of intestinal contents (fluid filled etc) especially rectal.

Samples required

Need multiple representative samples. Some areas are not macro obvious need to get samples regardless.

Samples in formalin

Must sample upper & lower SI, spiral colon and abomasum. Mesenteric lymph nodes should be included as autolysis always faster in gut:- will show inflammatory response.

Intestinal samples should be 3-4 cm long, if too short will curl. Need to make sure the lumen is open, syringe formalin through or dunk to ensure formalin the whole way through. Don't cut lengthwise as will smear off intestinal lumen cells; do not traumatise in any way.

Put formalin in sample pots in field. Minimise blood and gut contents in formalin, change formalin at practice.

Do not take large samples. Samples apart from GIT should be approx 1cm x 2cm to allow adequate fixation. Better to take several small samples than one large one. Tissue: formalin ratio must be 1:10. If samples are not sent until the next day the formalin can be drained. (Except for large specimens).

If paying by site put different sites in different pots when only want certain sections processed.

Primary container containing formalin should be packed with a secondary container outside.

Fresh samples

- Rectal contents
- Mesenteric LN or liver LN for culture for salmonella
- Lower SI contents *(There was some debate over this and I did not record why we need to take this – can someone please enlighten me!)*

Colon contents & MLN most important

Incorporating the diagnosis of calf scours under SCAHLS umbrella

Need to fit diagnosis into ANZ standard diagnostic protocols (available on web)

Appropriate category is "Testing for endemic disease of national significance". Other endemic disease: JD, salmonella etc on website, however none of these are multifactorial. Mastitis is included but is not up-to-date.

Process is very comprehensive, need to provide and prove specificity and sensitivity for every test. Need pathogen problem of the winner of the problem of the problem of present in x samples and estimate of reliability of test. May not be possible or practical and many tests used by laboratories are not covered by this process. If it is to be covered there will be a long-term goal.

Rigour needs to be applied to testing process if any chance of incorporating as an ANZ standard diagnostic protocols. However an agreed diagnostic protocol would be achievable but not necessarily recognised under SCAHLS.

Stephen Pyecroft to prepare a short agenda paper for next SCAHLS meeting discuss further

Epidemiological details to include on a laboratory submission form

The following details are included on all laboratory submission forms

- Location
- Age
- Breed
- Sex

The following details are included on some laboratory submission forms

- Number of animals at risk affected dead
- Duration of disease
- Presenting syndrome
- Differential diagnoses
- Severity of disease
- Duration of disease
- Reason for test

At present there is a large variation in the level of data stored in laboratory databases, and the information requested on submission forms.

If there is a requirement to collect data needs to be industry funded and target the private labs as well as government labs. There is no incentive for these laboratories to request or record

epidemiological data. Inherent problem with vets filling in forms, and also some private labs using medical systems where submission forms are scanned in and little data recorded in searchable fields. Data entry is expensive.

Specific tests for each pathogen

(Need to insert info from JH Slides)

E coli

DPI Bendigo have world reference strains and do own tests, some other labs sending samples to them.

Need to look for virulence factors in *E. coli*, if not attached then is it virulent? Can it produce a toxin? PCR more costly but does whole screen for fimbriae and virulence factors.

Only 10-15%b of calf samples cultured at Bendigo are K99, sometimes get sent one colony from lab, but prefer to receive original sample in case mistake in selecting appropriate colony

With PCR can take sweep across plate and check all colonies.

Bendigo do a faecal PCR, don't need to culture: detects F4 (K88), F5 (K99), F6, F18, F41 fimbriae and LT1, ST1, ST2, STXse, East1 and Eaea virulence genes.

DPI Bendigo prefers that samples come from a clinician as opposed to farmer, don't do interpretation, rely on vets to interpret results.

Ability to test for toxins has not been readily available in Australia

Ian Jerrett: not seen enteroeffacive and enteroinvasive colitis commonly, doesn't think that EAEC is a problem. Thinks that poor diagnosis is poor sampling technique. Possible problem in 2-3 months old

Keith Walker: EMAI also has PCR. 36% of 191 samples: genetic STEC and 15 % EPEC. Indications that these *E. coli* are possibly a problem in older calves

John House. No commercial process looking at interpretive diagnosis of STEC and EPEC. K99 is clear cut.

Best assay – no feelings

***Interpretation of significance of* E. coli**

Jeff Browning, doesn't culture calves faeces for *E. coli*, K99 can be determined without culture and will show if significant *E. coli*. ST toxin has 95% correlation with K99, used in stick tests

Steve Driesen thinks pure/predominant growths are significant, poss *E. coli* more relevant for dairy calves. Need more work to determine if this is the case.

Some disagreement on relevance of pure or profuse growth alone - pure growth may only be there due to non-selective media. Needs supporting histo evidence

Medical laboratories or USA do not report *E coli*. Should *E. coli* be reported at all by veterinary laboratories. Down side of not reporting is that if other forms of *E. coli* are not reported then unlikely to determine if there are non-K99 *E. coli* causing a problem in older calves.

Suggestion that *E. coli* only to be reported internally and if comes up consistently then to investigate further. Other option is qualifying statement on report to veterinarian that *E. coli* cultured not known to be a pathogen and more samples together with supporting histo evidence required to investigate if possible pathogen.

Noted that there is a huge range of serotypes that potentially produce shiga toxin – the relevance of this is unknown in calves. Consensus that much more research need to be carried out on the significance of non-K99 *E*. coli in calves

Other Bacteria

Clostridia: No laboratories routinely looking for toxins, toxin +ve animals are often not clinical disease, diff to interpret clinical relevance.

Clostridial abomasitis rarely seen not an issue

Campylobacter not an issue

Salmonella: most labs using culture, enrichments: rappaports or selenite for selective media. With clinical cases easy to detect.

Viral pathogens

Coronavirus: EM difficult to diagnose.

Quick dipstick tests available but need validation, coronavirus a problem as no gold standard. EM requires good samples. Crypt infection but crypt lesions not necessarily found on histology. Infection can be seen on FAT.

Barry Richards: IFAT correlates pathogen with lesion, need to get early in disease but affected areas show up longer with coronavirus. Requires GIT sample not faeces. Could be used as a gold standard

Fluorescent agents hard to find worldwide and being superseded by PCR. UC Davis website may have info on IFAT.

Bendigo DPI. Have used coronavirus/rotavirus ELISA on 80 samples, 20% shown to be rota and corona. Other labs not seeing it so much.

Bendigo didn't find latex agglutination for rotavirus as good as ELISA, latex misses samples shown on EM. ELISA is better test.

Corus (supplier of dipsticks) have published results on rota and crypto only. Tests need validating.

BVD need spleen or other high cell load tissues for antigen capture in calves. Can't use blood samples on less than 6 months for carrier status. Blood should be heparinised whole blood not clotted.

Protozoa

Cocci, will pick up in crypto smear.

Giardia, can cause lesions in the absence of another diagnosis

Smears for coccidia not as sensitive to some other tests, but correlate well with pathology (IJ). Intermittent shedding may mean place for sensitive test.

Coris tests trialed by Bendigo (22 samples): Appear to be more sensitive than latex agglutination for rotavirus (confirmed by EM), and more sensitive than faecal floats for crypto, Only a preliminary look and numbers are insignificant.

Discussion on future techniques

PCR may be best way to go in future, but only if better than current techniques

Virus vs bacteria PCR = RNA vs DNA, have place where used for research. Most labs likely to stick with ELISAs where tests are equivalent due to cost of running tests. PCR more difficult for viruses.

Future value in quantitative PCR, but diagnosis not just the answer, need clin path studies. Tests need to be properly validated, ease of doing this depends on how good current gold standard is.

ELISA's are often sensitive enough for most clinical syndromes. Should use for viruses and culture for salmonella. PCR may be good for E coli, but need PMs etc to go with them to determine relevance.

SCAHLS looking at standard protocols for PCR techniques. Standardisation also provided by the Australian National Quality Assurance Program (ANQAP) but only looking at standardisation of serological techniques (Mainly those used for Export). Need to consider similar QA program for PCR. At present no good standards for doing the testing.

Cost of PCR btwn $35 & $80 per test. PCR will allow for pooling of tests to do initial screening. Pooling will need further validation. Jeff Browning suggests tests may be cheaper: $60 for 1st sample and then $15 per test.

Phillipa McLaren: Need cost saving: time saving or decreased labour for PCR to be worthwhile. Takes 3 months to develop test and cost possible $10-$20 (Gribbles) All tests need evaluating and NATA will want proficiency testing on a lab basis. Depends on ISO system in your lab, lab determines which tests get accredited. NATA only require proficiency testing if proficiency testing exists for a test.

Validation of dipstick ELISAs:

Sample sticks cost approx $7 per agent per test. Takes 10 minutes

Discussion on need to validate kit tests in Australia or is there enough info abroad. Is info from companies good enough, are the viruses the same antigenically as the overseas pathogens that the tests have been developed from. Problem with validation is difficulty in finding reference samples. Work would have to be commissioned.

Discussion of a possibility of a few tests being trialed by all laboratories to share the costs of validation. May run into problems with sharing information between labs. Suggestion that a confidential database could be set up and all lab submit results. Best way to ensure cooperation between laboratories, test against known positive. However debate over confounding that is likely to occur with multi-site tests, maybe best to contract one laboratory to validate tests.

Also debate as to who should pay for the test to be validated: industry, laboratories or the supplier of the test. MLA concerned re funding such test as several suppliers of these tests (and likely to be more out of SE Asia) - want any funding to have long term benefit: need to determine how long test sticks are likely to remain useful and also the appropriate brand.

Suggestion that the providing company should be carefully evaluated: Will they share the information, what is their viability in the market, are they willing to share (or pay for) costs of evaluation.

Government labs likely cooperate, to contact labs and find out who is interested in cooperating.

Reporting of test results

Keith Walker: up to clinician to interpret result from clinical exam and lab result.

Where multiple pathogens are found then need more specimens to establish major pathogen(s). Also histopathology will be helpful in establishing the aetiology.

Summary

General acceptance for the need for nationally accepted guidelines/protocol for diagnosis of neonatal calf scours.

Requirement for education of veterinarians on sample taking and interpreting diagnosis

Question of test validation indicates the requirement for a National Veterinary Laboratory Funding body to look at standardisation and validation of new diagnostic tests as they become available. Need protocols developed for evaluating new tests.

Need to establish the benefits of pooled samples and how they relate to the clinical picture and clinical pathology

Indications that the Rotavirus latex agglutination has been superseded by the ELISA test.

Paper circulated to laboratory personnel

The following document was produced and circulated to laboratory personnel after the meeting with them on 4th November 2004

Diagnostic Investigation of Calf Scours

Practitioner Guidelines

Establishing an aetiologic diagnosis facilitates targeted vaccination programs, identifies zoonotic risk (salmonella, cryptosporidia, and giardia), and facilitates targeted prophylactic and therapeutic interventions. Prior to pursuing an aetiologic diagnosis a risk analysis of management strategies should be conducted as many of the management procedures that promote calf health and reduce the risk of calf scours are universal for all enteric pathogens.

Achieving a definitive diagnosis is more complex than simply isolating or identifying the presence of a pathogen since most enteropathogens in calves may be found in a percentage of normal calves and disease outbreaks often involve more than one pathogen. An etiological diagnosis is established by building a body of supportive evidence that includes a compatible clinical presentation, detection of the pathogen, compatible pathology, demonstrating an absence of other pathogens, and observing a compatible response to treatment. Diagnostic yield is maximised by a systematic approach to data and sample collection and via appropriate sample handling, packaging, and shipping. Simply speaking providing the diagnostic laboratory with a good history, physical exam findings, necropsy description, and appropriate well preserved samples gives them the opportunity to provide meaningful feedback.

The objective of this summary is to provide an outline of a systematic approach to investigating calf scours that will optimise diagnostic yield. Diagnostic yield following these procedures should approach 90%.

Data Collection

Clinical history

- *Type of operation (beef, dairy, veal)*
- *Age range of affected calves, onset of clinical signs and age at peak mortality*
- *Demographics*
    - *Stocking density*
    - *Distribution of affected calves in the herd, note if the problem is associated with calves born to heifers.*
    - *Maternal diet and if calves are hand reared record volume, frequency, and composition of milk fed*
    - *Coccidiostat +/-*
    - *Worming history*
    - *Vaccination history (Maternal and calf)*
    - *Number at risk*
    - *Number affected*
    - *Number dead*
- *Duration of the problem*
- *Historical progression of the problem*
- *Prophylactic and therapeutic interventions*
- *Response to treatment*

Physical Findings

- *Body condition score of cows*
- *Body condition score of calves*
- *General description of abnormal physical findings (Note prevalence of infected umbilical structures and joints)*

Gross Pathology

Provide a description of the gross pathology, remark on the condition of the whole gastrointestinal tract commenting on the normal as well as the abnormal.

Sample collection

Collection of faecal samples

Where possible a minimum of 6 faecal samples should be collected. It is helpful to samples calves at different stages of the disease but preferably half of the samples should be taken from calves early in the course of the disease prior to the initiation of treatment. Samples should be collected per rectum using a disposable glove. Digital stimulation of the rectum may be required

to stimulate defecation. Early in the disease process the perineum of calves may appear clean due to the liquid nature of the faeces.

Consideration should be given to the sample recipient at the diagnostic laboratory and the potential for zoonotic infections. Pre-labelling sample tubes promotes legibility and helps to minimise the amount of manure that ends up on the outside of the container. Five to ten grams of manure collected into a leak proof screw cap sample jar will provide adequate sample to test for all pathogens. Samples should be placed in a sealed secondary container and placed on ice to keep cool during transport.

For shipping packaging should conform to IATA 650 standards. Diagnostic laboratories can supply or direct you to suppliers of IATA approved shipping containers.

Necropsy

Necropsies should be performed systematically in a similar fashion to a physical examination. Descriptions of the gross pathology are useful for the pathologist examining histopathology sections. Comments regarding congestion, oedema, petechial and ecchymotic haemorrhages, erosions, ulcerations, fibrin casts, and the nature of intestinal contents (fluid filled etc) are particularly useful. The absence of gross pathology is also significant and supportive of should be noted.

Sample Collection

Fixed tissues - In addition to taking multiple samples from representative lesions sections should be collected from the abomasum, duodenum, jejunum, ileum, colon, and rectum for histopathology to detect microscopic lesions that may not be visible grossly. Autolysis is a common cause of diagnostic failure for intestinal samples and can be avoided by conducting a necropsy on an animal that has just died or has been euthanised and by avoiding placing large samples of intestine in formalin. Intestinal samples 3–4 cm in length should be placed in formalin with a tissue to formalin ratio of 1:10. Running formalin through the lumen of the intestinal section or dunking the section as it is placed in the formalin facilitates distribution of formalin into the intestinal lumen. It is unnecessary to cut sections lengthwise and it is important to avoid scraping the surface of mucosa as it will disrupt the tips of the intestinal villi.

Sections of mesenteric lymph nodes should also be collected for histopathology. Histopathology of the lymph nodes is useful for detecting inflammation and can be particularly useful if the intestinal samples are compromised by autolysis. Samples of tissues other than the gastrointestinal tract (liver, kidney, spleen, and lung) should be approx 1cm x 2cm to allow adequate fixation, it is better to take several small samples than one large sample. If samples are not sent until the next day and the tissue sections are small the formalin can be drained to reduce the volume of formalin shipped. Dividing tissue sections into multiple appropriately labelled sample containers facilitates identification of samples by laboratory personnel and the potential for stepwise sample processing to limit cost.

Fresh samples – Gut contents and tissue sections are collected for bacterial culture and pathogen detection. Appropriate samples include:

- *Mesenteric lymph node (Salmonella)*
- *Liver (Salmonella)*
- *Ileum contents. (Rotavirus, Coronavirus, Cryptosporidia, Salmonella, E. coli, Coccidia)*

Laboratory submission form

In addition to the history, physical findings, and gross pathology description the laboratory submission should include a list of differential diagnoses. Different laboratories have different protocols regarding testing for enteric pathogens. Providing an indication as to the pathogens suspected ensures that the testing is appropriately directed. This is particularly important when seeking to detect the less common pathogens.

Pathogen Specific Tests

Bacterial Pathogens

E. coli: Isolation of *E. coli* from the gastrointestinal tract does not constitute a diagnosis unless the isolate is demonstrated to possess virulence attributes that correlate with the clinical presentation and histopathology. Most laboratories utilise immunoassays to demonstrate the presence of fimbrial antigens to identify Enterotoxigenic *E. coli*. Enteropathogenic *E. coli* may be identified utilising PCR assays to demonstrate the presence of genes involved in adhesion and cytotoxicity. A diagnosis of enteropathogenic *E. coli* should be supported by histopathology as healthy calves may shed *E. coli* which posses the same virulence attributes.

Salmonella: Salmonella may be shed by apparently healthy calves. While isolation of salmonella from faeces supports a diagnosis of salmonellosis isolation of salmonella from tissues at necropsy provides evidence of a stronger causal relationship. Laboratories generally utilise enrichment cultures and selective plating media. With clinical salmonellosis a large number of organisms are shed in faeces and isolation is generally not difficult. The sensitivity of faecal culture may be increased by increasing the volume of sample cultured and through the use of multiple enrichment and selective plating media utilised.

Clostridia: Clostridia are rarely incriminated in calf scours and bacterial isolation and toxin detection are not routinely performed. If enterotoxaemia is suspected the laboratory submission form should indicate as such. Confirming a diagnosis of enterotoxaemia is difficult as clostridia are part of the normal intestinal flora. Fresh necropsy samples are particularly helpful to demonstrate histopathology that supports the clinical presentation. The presence of toxins may be determined using immunoassays. None of the veterinary diagnostic laboratories routinely conduct quantitative anaerobic bacterial cultures of gut contents.

Campylobacter: The significance of campylobacter in calf scours is questionable and testing is not routinely performed.

Viral pathogens

Coronavirus: Diagnostic tests available to diagnose coronavirus in Australian veterinary diagnostic laboratories include electron microscopy and immunoassays. Not all laboratories routinely look for Coronavirus. Sample handling is particularly important when using electron microscopy as the virus is fragile and the sensitivity of EM is compromised by freezing leading to viral particle degradation. A number of immunoassays have recently come onto the market, while this technology has the potential to provide a relatively sensitive diagnostic modality independent test validation is lacking for some of these test kits.

Rotavirus: Electron microscopy and immunoassays are also used to diagnose rotavirus infections. Enzyme linked immunoassays are more sensitive than latex agglutination and electron microscopy.

Bovine Pestivirus: Bovine pestivirus is rarely associated with calf scours in Australia. Sporadic cases may be observed in persistently infected calves. Detection of virus in blood is compromised by maternal antibody in calves less than 6 months of age. Spleen or lymphoid tissue that has a high viral load is the best sample for antigen detection in calves.

Protozoa

Cryptosporidia: Faecal flotation, faecal smears and immunoassays may be utilised to detect cryptosporidia. Because faecal flotation concentrates the protozoa it is a more sensitive technique than faecal smears. Special stains are utilised to facilitate pathogen detection. A number of dipstick immunoassays have been developed for detection of cryptosporidia. In-house testing by different laboratories reports that these tests are at least as sensitive as faecal smears and flotation techniques however there is a lack of published data for all tests.

Giardia: Giardia is detected by faecal flotation.

Coccidia: Faecal flotation.

Best Laboratory Practices for Investigating Calf Scours

Diagnostic yield for calf scour investigations approach 90% when testing for all enteric pathogens is conducted. While it is at the discretion of producers to dictate the amount of money they wish to direct toward pursuing a diagnosis the following recommendations are directed at promoting consistency of diagnostic recommendations and services offered across Australia. These recommendations are intended to reflect the consensus of the meeting of veterinary laboratory diagnosticians in Melbourne on November 4th 2004.

Specific recommendations include:

1. Faecal testing should include detection methods for all common enteric pathogens including rotavirus, coronavirus, cryptosporidia, salmonella, and coccidia.

2. Field necropsies should be promoted as a definitive diagnostic modality with distribution of guidelines to practitioners regarding appropriate sample and data collection and submission.

3. In regard to specific pathogens there are numerous diagnostic options. From the literature it is possible to make generic comments as to the relative sensitivity of different diagnostic modalities but there are little or no data comparing specific kits. Comments regarding the relative sensitivity and specificity of different diagnostic methods are therefore generic. It is also acknowledged that operator experience may significantly influence the performance of specific tests and influence the relative sensitivity of the diagnostic methods employed.

    a. *Rotavirus* – Enzyme linked immunoassays are generally more sensitive than agar gel immunodiffusion or electron microscopy. One limitation of enzyme immunoassays is that they will only detect type A rotavirus.

    b. *Coronavirus* – Enzyme linked immunoassays are generally more sensitive than electron microscopy. Virion degradation limits the application of electron microscopy to fresh samples.

    c. *Cryptosporidia* – Faecal flotation techniques used with selective strains are more sensitive than faecal smears. Enzyme immunoassays (dipstick) have emerged and preliminary reports suggest sensitivity at least equivalent to faecal flotation.

    d. *Salmonella* – Calves with salmonellosis typically shed large numbers of salmonella that can be detected via enrichment culture and selective plating media. Sensitivity is enhanced by utilising more than one enrichment and selective plating media.

e. *E. coli* – Diagnosis of *E. coli* should be based on the demonstration of virulence attributes associated with a compatible clinical presentation, gross pathology and histopathology. While the role of enterotoxigenic *E. coli* is clear and there are numerous immunoassays available for detection, the diagnosis of enteropathogenic *E. coli* infections on the basis of faecal culture is more difficult as normal calves frequently shed *E. coli* that possess virulence genes. Further research is required to establish the significance of faecal isolation of enteropathogenic strains of *E. coli* in scouring calves.

f. *Clostridia* - Clostridia are rarely incriminated in calf scours and bacterial isolation and toxin detection are not routinely performed. Practitioners submitting samples pursuing a diagnosis of enterotoxaemia should be encouraged to send fixed tissues from a fresh post-mortem along with intestinal contents for detection of toxin using immunoassays.

g. Pestivirus – Pestivirus sporadically causes diarrhoea in calves. Outbreaks of neonatal diarrhoea caused by pestivirus have not been reported in Australia. The optimum sample for antigen detection in calves less than 6 months of age is liver or spleen. Maternal antibodies may interfere with antigen detection in blood.

8.5 Appendix 5: Report from meeting with producers 15/12/04

Objectives of meeting

1. To receive feedback on the 3 documents prepared for farmers

2. To discuss options for further research

Participants

Joan Lloyd	MLA	**Producer Reference Group**
Gerald Martin	MLA	Carole Burden
Alison Gunn	Project coordinator	Darryl Croser
John House	University of Sydney	Rob England
Kevin McGrath	Millicent Veterinary Clinic	Nick Hunt
Colin Trengove	Pro-Ag Consulting	Nic Kentish
John Weaver	PIRSA	Hamish and Krista MacDonald
Peter Nosworthy	PIRSA	Donald McLennan
		Pip Rasenberg

Three members of the farmer reference group had responded to the farmer surveys in phase 1. The others were local farmers with an interest in the problem.

Meeting summary

After a round of introductions the participants were presented with the results from phase 1 of this project and the objectives and progress to date for phase 2.

The 3 documents were then discussed, together with much information about the scour problems that some of the participants were experiencing. The documents were reasonably well accepted, although all require modification and additional information. All documents need an introductory section detailing the key points so producers can quickly tell if the document is any value to them. It was suggested that a double-sided laminated sheet with a decision tree for the control (side one) and treatment (side two) of calf scours should be created.

When asked about their overall perspective on the documents, producers considered them to be useful for farmers with little experience of calf scours, or those experiencing an outbreak of significantly increased magnitude. However some farmers in the room felt that they had already applied many of these principles and had calf scours under control, and others had a significant problem, despite applying many of the management suggestions.

During the day there were many comments on the role of management and nutrition in the prevention of calf scours, and how control can require a paradigm shift in the whole farm management, addressing nutrition, grazing, and calving pattern/breeding management. However it is difficult to be prescriptive with farm management and there is no Australian (or similar climate and grazing system) research to determine exactly which management changes are important.

Areas identified for further research were

- ✓ The relationship of calf scours in Australia to paddock management, soil management, climate / meteorological conditions.
- ✓ The difference in incidence between different breeds / genetics differences.
- ✓ The role of probiotics and vaccines in the prevention of calf scours

It was suggested that a case control study across multiple areas would be extremely beneficial to elucidate the influence many of the managemental and climatic variables.