MEAT RESEARCH CORPORATION

FINAL REPORT

ECOLOGICAL STUDIES OF VECTORS OF BLUETONGUE AND OTHER IMPORTANT ARBOVIRUSES IN SOUTHERN AUSTRALIA.

PROJECT DAN 72

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EXECUTIVE SUMMARY

Project Objectives

The objectives of this project were:

- to determine the ecological constraints for the survival, increase and spread of *Culicoides brevitarsis* in temperate Australia;
- to develop low cost sensitive systems for the definition of virus distribution;
- to refine a computer forecasting system for virus/vector distribution.

Methodology

Light traps, truck traps, sweep nets and larval rearing are recommended for sampling *Culicoides brevitarsis*. The vacuum sampling technique was also adapted for this purpose as part of the project. Each method was used selectively to sample *C. brevitarsis* during its different stages of development and behavioural activities. Light traps were used to record flight activity at night and to enable comparisons to be made in different geographical areas. Truck traps and sweep nets were used to record flight during daylight and at any time when the use of light traps was impractical. The immature stages were studied by using emergence chambers to rear larvae through to adults. A vacuum sampler was used to extract adult *C. brevitarsis* from vegetation and when they were associated with the breeding habitat (dung).

Monitoring sentinel cattle for antibodies to Simbu viruses which are efficiently transmitted by *Culicoides brevitarsis* has been shown to be a very economical method for determining the distribution of effective vector populations. However, tests less expensive than the cell culture virus neutralisation were needed and, preferably a single Simbu group test, in place of 5 individual VN tests. To achieve these objectives, two competitive ELISA (cELISA) tests were developed, one using monoclonal antibodies against antigens unique to Akabane virus, the other using a mixture of monoclonal antibodies against antigens which are shared by the Australian Simbu viruses. After developing prototype tests, an evaluation was carried out, firstly using a panel of sera with known antibody titres to individual Simbu viruses and,

secondly, in parallel with the VN tests during the monitoring of sentinel cattle. Finally, these cELISAs were used to test a large batch of sera both from southern arbovirus-free areas and a range of sera from cattle in the arbovirus endemic areas of WA, NT and Qld.

Routine monitoring of sentinel cattle was continued during the course of these studies, not only to assist with the evaluation of low cost tests, but also to compare with the results of vector monitoring. Serum samples were tested for antibodies to Bluetongue, Akabane and Bovine Ephemeral Fever viruses. Such a program of regular sampling allows the time of infection to be accurately determined and, if the sampling of sentinel herds across a region is synchronised, the geographical spread of infection can be determined. This allows the accurate definition of virus distribution on a regional and annual basis. From the sentinel monitoring, composite maps of virus distribution and hence the distribution of effective vector populations were prepared. These maps were then used to test and refine computerised forecasting systems based on the analysis of climatic data. Information derived from this project should allow both populations at risk of disease and virus/vector free areas to be accurately defined.

Results.

Vector ecology:

The entomological component of the project involved research to determine the constraints which affect the survival, multiplication and spread of the biting midge *Culicoides brevitarsis*, the main vector of several arboviruses infecting livestock in Australia. The study area for the species distribution was initially the Hunter-Manning region of NSW but was extended to include the southern coastal plain and the adjacent slopes. Preliminary work was also carried out on the mid-north coast in the final years.

The vector ecology research initially involved extensive field studies, where various midge habitats were monitored and vector population parameters examined and compared with environmental data. This research was supported by intensive laboratory based studies in which the effects of a broad range of temperatures on midge larval survival and maturation were examined. In order to obtain the necessary precision, these studies could only be conducted under controlled laboratory conditions. Field experiments were mainly conducted at the C.B. Alexander Agricultural College at Tocal. Laboratory experimentation was conducted at the Horticultural Research and Advisory Station, Gosford.

This study firstly established a greater understanding of the species behaviour in relation to on-farm habitats and activity throughout the day. The information was used to develop ecological principles and to improve sampling techniques. It was confirmed that peaks in flight activity mainly involved mating and feeding behaviour in the hour before sunset and for extended periods after sunset respectively. New information showed: smaller peaks in flight activity after dawn; peaks in activity by females near dung that was associated with egg laying; and distinct behaviour at resting sites where highest numbers of *C. brevitarsis* were found in grass and reed tussocks and along the margins of dams during dry periods. Larval activity was confined to bovine dung.

The effect of temperature on the emergence, development and survival of *C. brevitarsis* was quantified in the laboratory. The vector was active between 17°C and 36°C. It lived for periods outside of this range but its survival was ultimately dependent on the temperature eventually being raised above a 'critical' level of 17°C. The vector did not survive in dung for more than 50 days at any temperature.

The laboratory data strongly supported the second phase of the study which used field data to define the ability of *C. brevitarsis* to overwinter at the southern end of its distribution. Low minimum and low maximum temperature thresholds of 8.1°C and 17.5°C were calculated from regression equations describing the decrease in *C. brevitarsis* with temperature through autumn into winter. Below these thresholds, *C. brevitarsis* did not emerge and could not survive in dung for more than 1 to 2 months.

However, in both the laboratory and field experiments, emergence of adults after a month below the threshold occurred almost immediately the temperature was raised. This enabled the species to respond quickly to the short periods of high temperatures that are sometimes experienced in the study area. The data were used to estimate probabilities for survival during winter at several locations. Probabilities may be estimated for any site where temperature records have been kept. These were mapped and indicated a low chance of survival in the Hunter/Manning region as was recorded. Absolute probability for survival increased significantly in coastal areas as far south as Moruya, when the temperatures were raised by an arbitrary 2°C to simulate the possible consequences of global warming.

Efficient tests for virus distribution:

During the course of the project, two new ELISA tests were successfully developed and evaluated. One was a serogroup test for detecting antibodies to any of the Australian Simbu viruses. The other test was specific for antibodies to Akabane virus. Both tests were shown to have a high level of sensitivity and specificity. As both tests are monoclonal antibody-based competitive ELISAs (cELISAs), serum samples

from any animal species can be tested in the one test. These tests are also very economical and rapid. They cost less than 25% of the cost of a conventional virus neutralisation (VN) test and can be completed in a few hours compared to 5 days for a VN test. Further, the Simbu group test can detect antibodies to any one of the 5 Simbu viruses. Previously, 5 individual VN tests were required.

Arbovirus epidemiology:

During the course of this project, a trend which had been observed previously was again observed. In any one year, one or two viruses appear to be "dominant". This is probably the outcome of a complex set of interactions between virus, host, vector and environmental variables.

Each year, even under relatively adverse environmental conditions, there was one or more of the Simbu viruses transmitted at a high level. These viruses appear to be transmitted more efficiently and consistently than any of the other viruses. The drought conditions did however, limit the extent of transmission of the Simbu viruses. In 1992/93 the southern and western limits of Akabane transmission were restricted, while in 1993/94, infection occurred later in the season and at a lower incidence than usual. In 1995, the pattern of spread of Akabane virus was relatively normal. In each of these years, there was evidence of infection with one or more of the other Simbu viruses. In 1992/93 there was a very high incidence of Douglas virus infection. In 1993/94, there was an unusual pattern in that there was a low level of infection with each of the Simbu viruses, compared to the usual dominance of one or two viruses. In 1994/95 sentinel animals were infected with both Douglas and Peaton viruses. The complex transmission patterns of the 5 Simbu viruses were efficiently monitored with the new Simbu group cELISA.

Bluetongue virus transmission was observed in 2 of the 3 seasons covered by this project. It is unusual to observe a significant level of transmission of this virus in NSW in 2 successive seasons, and perhaps also unusual to have a high incidence of infection only 3 seasons apart from the previous high level transmission. In 1992/93 there was a moderate level of infection on the North Coast and a separate independent focus in the Manning region. in 1993/94 infection was widespread throughout the endemic area and was found as far south as Camden. In 1994/95 no Bluetongue virus infections were observed.

The trend for ephemeral fever virus transmission is one of alternate seasons of epidemic transmission followed by an absence or low level of infection. During the course of this project, this pattern was again observed, with no infection in 1992/93/93, a significant epizootic in 1993/94, with infections as far west as Dubbo,

and finally only a small focus in the north west of NSW in 1994/95.

Forecasting vector/virus distribution:

As a result of the vector ecology studies it was possible to define the key parameters which influence the distribution and abundance of *Culicoides brevitarsis*. It was shown that the most critical determinants affecting vector distribution and abundance in the study area are its ability to survive through winter, factors affecting its migration into otherwise vector-free areas, population development after survival or re-establishment and its association with viruses. Temperature was vital to each of these determinants. Models were then developed which predict the probability of survival throughout NSW, the first occurrence and subsequent distribution of *C. brevitarsis* and the probable month that *C. brevitarsis* should last occur before its activity is stopped by the onset of winter. From the vector ecology data, it has also been possible to determine the number of months that the midge can survive in different regions throughout NSW and also the last month that is conducive for midge survival in that location. This information can be used to provide advice for disease control on the probability of a disease outbreak in a given area and, if disease occurs, the length of time that transmission could continue.

The results of monitoring sentinel cattle for infection with each of the *Culicoides brevitarsis*-borne arboviruses were also successfully combined to produce composite maps of vector distribution for each of the 3 years studied. These maps were then compared with the distribution of *C. brevitarsis* predicted by the models. There was a high level of agreement, confirming that these techniques are efficient ways to define virus and vector-free populations. Associations were established between the distributions of the vector and some viruses for the first time in Australia.

Conclusions.

During this project, knowledge of the ecology of *C. brevitarsis* in coastal NSW has been greatly expanded. Factors limiting the distribution and abundance of the midge have been identified. Models have been developed which predict the survival of *C. brevitarsis* during winter, its first occurrence and distribution. It is now also possible to determine the length of time that this vector will survive in a location and the probability for it to survive through the winter. The data that is now available for use in these new vector forecasting systems will allow refinement of insect monitoring in the future.

As an outcome of the development of new ELISA tests for the detection of antibodies to Akabane and the other Simbu viruses, sensitive low cost tools are available for determining the distribution of infection of livestock with these important viruses.

With a combination of the vector models and low cost ELISA tests, virus and vector free areas can be more readily and efficiently defined. The accurate description of these free areas should facilitate livestock exports. Further, changes in vector and virus distribution may be predicted and populations of livestock at risk of arbovirus induced disease identified, allowing the implementation of control measures.

However, care should be taken during the promotion of virus and vector free areas not to compromise the status of this area by making any remarks about Bovine Ephemeral Fever virus. With a probable mosquito vector, Bovine Ephemeral Fever virus is occasionally found well beyond the limits of the *C. brevitarsis* endemic area, in the "vector-free" area and should always be considered in a separate category to the *Culicoides*-borne viruses.

Recommendations and Application of Outcome.

Predictive models which describe potential vector distribution and probabilities of survival in a remote location have been developed. These can be used to provide advice on the potential for disease risks, duration of a possible disease outbreak, the likelihood of resurgence in the next season and to define the limits to vector free areas.

These models will optimise vector monitoring by both reducing the number of locations required for vector collections in the longer term and by identifying key sites of probable vector occurrence for more intensive sampling. A consequence will be more precise descriptions of vector distribution which are generated at lower cost.

The key to using a strategy to predict and monitor the distribution of *C. brevitarsis* in NSW and in the southern Australian states is to be able to designate a single point where monitoring for the vector should initially be started (i.e. the basis for use of the distribution models entails monitoring at a key site), to confirm predictions and refine estimates of arrival at more southerly locations. Taree would appear to be suitable for this purpose at this time. However, preliminary research shows that the most appropriate site could be even further north. The further north the site, the greater the length of time for control/disease prevention strategies to be implemented.

It is recommended that further research be conducted to establish the best site for monitoring. It is considered that this site should have the following characteristics:

- Be outside the endemic area (i.e. where vectors are found continuously);
- Be in the path of any migrating *C. brevitarsis*;
- Be supported by a limited number of check sites to ensure that any abnormal movements (e.g. rapid and relatively long distance movements described by Murray and Kirkland 1995) or the survival of populations in southern foci do not go undetected.

Although Taree appears to be a suitable location for entomological surveillance to support a model, further research should be carried out on the mid-north and north coasts to help establish the northerly monitoring site and to define alternative movements (e.g. up coastal valleys to the northern tablelands) by the vector and spread of the viruses. This should include the following objectives:

- Defining the endemic area (where vectors are continuously present) and further defining events within this area over a range of seasonal conditions;
- Establishing and validating the pattern of dispersal down the mid north coast and up the coastal valleys to the northern tablelands. This is necessary to ensure that the vector cannot bypass any site designated as 'the' monitoring site
- Considering the feasibility of being able to block movements of *C. brevitarsis* (or other *Culicoides* vector species not currently present in NSW) or new or particularly virulent strains of virus down the narrow coastal plain between Coffs Harbour and Taree.

As some viruses appear to move southwards from locations on the Far North Coast or Southern Queensland, there is a need for detailed studies of the ecology of C. brevitarsis and its relationships with key viruses such as Bluetongue at the NSW/ Queensland interface. In particular, there is a need to determine if the current models are suitable for predicting the south-western inland movement of vectors in favourable seasons, bypassing the coastal plains and providing rapid access to susceptible sheep populations in North Western NSW.

There is a need for an extension of research into other states, particularly Queensland and the areas of interface between Queensland and NSW because:

- while *C. brevitarsis* is endemic in S.E. Coastal Queensland, another vector, *Culicoides wadai,* is also present. This species could alter the pattern of virus transmission should it reinvade NSW;

- the most likely movement of *C. brevitarsis* into NSW west of the dividing range would be from Queensland and not the NSW coastal plain;
- models of survival and last occurrence should apply to other states and need to be evaluated;
- the model of dispersal of *C. brevitarsis* could be tested in other states by evaluating prevailing weather conditions in concert with the monitoring of vector and viruses;

As an extension of the work undertaken in this project, and to utilise the new tools available, meteorological data from the past 20 years should be analysed to determine the likely annual distribution of *C. brevitarsis*. These annual estimates of vector distribution should be compared with available virological data and new *"brevitarsis"* lines created, as it is anticipated that these will be less expansive than the currently accepted limits. By comparing the predicted limits with actual distributions of viruses from virus monitoring, the new vector limits should be more readily adopted by trading partners.

Finally, low cost, sensitive rapid diagnostic tests to detect antibodies to key viruses have been developed. These can be used to confirm predictions of vector distribution and to delineate effective vector populations. Steps should be taken to introduce the newly developed Simbu and Akabane cELISA tests into laboratories in other states. To facilitate this objective, reagents should be supplied for evaluation in the NAMP. We expect to provide trial batches of reagents for evaluation towards the end of the 1995/96 season, with a view to more widespread use of these tests in 1996/97.

Having generated valuable data for both virus and vector distribution, the remaining need is for a mechanism for the on-going promotion and supply of current data to key trading partners, animal health authorities (both domestic and overseas), AQIS personnel, exporters and livestock agents. This will require a consistent regular promotional program to raise awareness of the value of this data, whether for the negotiation of new, relaxed export protocols or to assist with the purchase of arbovirus-free stock from populations within the endemic areas. Promotional activities in this direction have been initiated through the NAMP management group. It is encouraging to be able to report that, in response to this awareness, AMLC staff have regularly brought overseas delegations (of scientists, administrators and livestock buyers) to EMAI for briefing on this project and NAMP results. However, outcomes of this project and of NAMP need to be aggressively "marketed" at every opportunity to ensure maximum benefit to industry.

DETAILED RESEARCH REPORT

PROJECT BACKGROUND

Viruses spread by biting insects (Arboviruses) have become of increasing importance to the livestock industries of Australia over the last 15 years. The viruses which have been of greatest significance to industry over this time have been Akabane, Bluetongue and Ephemeral Fever. These agents are a cause of current and potential economic loss in 3 main areas:

1. Direct production losses as a result of disease;
2. Indirect losses due to restricted access to valuable export markets as a consequence of the agents being present in Australia;
3. Future losses caused by agents which are currently exotic (or confined to remote areas of Australia) but pose a significant threat.

Previous research, including that funded by MRC under Project DAN 46, has shown that monitoring of sentinel cattle is an efficient system for defining the distribution of important arboviruses. In fact, this Australian system has gained international acceptance and has been recommended through OIE as the preferred method for epidemiological studies of arboviruses. In Australia, however, until relatively recently, sentinel herd monitoring in key areas of New South Wales, Queensland and the Northern Territory depended on the availability of diminishing state resources and a mix of funds from industry research councils. Clearly, such monitoring had moved beyond the province of the research funding agencies. Similarly, the States with endemic arbovirus populations are already disadvantaged by loss of export and other trade opportunities. They receive little direct benefit from virus and vector monitoring but are providing a "comfort zone" for the southern arbovirus-free States. Consequently, there was a need for the establishment of an integrated national program which has a sure financial footing. Soon after the commencement of this project, a mechanism was established for the establishment and funding of a co-ordinated National Arbovirus Monitoring Program (NAMP). This program, jointly funded by the States, Commonwealth and Industry includes both virus and vector monitoring elements. However, as financial resources continue to become further limited, there is a need to utilise the most economical and efficient tools for both

vector and virus monitoring.

The vector ecology studies which were commenced in the Hunter Valley under DAN 46 offered promise of a simple model to reduce the need for routine entomological surveillance. Similarly, the generation of composite maps of arbovirus activity allowed the definition of arbovirus distribution on a geographical and temporal basis but depended on testing of sera in several cell culture based neutralisation tests. These are relatively tedious and expensive. There was clearly a need for the development of low-cost tools to facilitate this approach. The purpose of this project was to address the need for models to assist with the prediction of vector distribution and to develop rapid economical diagnostic tests for key arboviruses.

OBJECTIVES

The objectives of this project were:

- to determine the ecological constraints for the survival, increase and spread of *Culicoides brevitarsis* in temperate Australia;
- to develop low cost sensitive systems for the definition of virus distribution;
- to refine a computer forecasting system for virus/vector distribution.

METHODOLOGY.

The objectives of this project involve both entomology and virology studies. Each objective is largely addressed by different methods, which in most cases are unique to one of the two disciplines involved. Therefore, for simplicity, the body of this report will contain 3 sub-sections, each describing initially the relevant methods together with the results of the research which was undertaken to achieve the 3 objectives of this project.

RESULTS.

Objective 1: Vector Ecology Studies.

Many aspects of the biology of *C. brevitarsis* had been described by other researchers prior to the commencement of this study. While these provided a sound base for the proposed work, it was found that many previous conclusions had no quantitative support or were limited in their ecological interpretation. Most importantly, they failed to provide a clear understanding of the constraints for the survival, development and spread of the species in regions where it may frequently fail to survive through winter and are therefore important to the definition of vector free areas. Sampling methodology was generally well developed although there was the potential for introducing new techniques and for refining those already in use. In each case, the sampling method adopted was the most efficient for obtaining the information required. Light traps were used to record activity at night and for the study of the species distribution. Truck traps were used to record flight activity during the day and sweep nets were used to record flight activity in small, specific locations. Emergence chambers were used to study the immature stages in the breeding habitat (dung). Adults resting in vegetation and ovipositing on dung were extracted for counting with a vacuum sampler. Further details on the specific use of these methods will be provided with each of the studies described below.

Several studies were carried out to provide a detailed understanding of the behaviour and ecology of *C. brevitarsis*. Emphasis was placed on determining where and when to sample, daily activity, larval distribution in dung, resting habitats and development in response to constant temperature. Special attention was given to the ability of *C. brevitarsis* to overwinter at the southern limits to its distribution.

Study 1.1 - **C. brevitarsis** ***in different farm habitats.***

Comparisons of *C. brevitarsis* numbers were made during the resting, flight and reproductive phases of activity in four basic on-farm habitats frequented by livestock. Ten basic habitats were vacuum sampled each fortnight for 12 months to establish the preferred resting habitats of *C. brevitarsis*. Three sampling methods were then used to quantitatively assess and compare numbers in the four most important habitats given in Table 1. Five replicates were taken in each habitat by light traps (individuals flying and feeding at night), a vacuum sampler (resting individuals) and using emergence chambers to rear larvae from dung. Selected results are shown in Table 1. Full details are given in publication 1.

Table 1. Total numbers of *C. brevitarsis* from three sampling methods (behavioural activities) in four on-farm habitats. Counts in columns separated by different letters are significantly different ($P < 0.05$).

	Sampling method (Behaviour)		
Habitat Type	Vacuum sampling (resting)	Light trapping (flight)	Dung sampling (reproduction)
Open pasture	3.6 b	67.3 a	49.8 a
Wooded pasture	1.6 c	46.4 ab	11.9 ab
Dam margins	14.4 a	26.7 b	62.6 a
Creek margins	0.8 c	4.8 c	4.4 b

Dam margins and open pasture were the preferred resting habitats of *C. brevitarsis*. The data also indicated that humidity was important during the resting phase and that the aggregation of midges around the margins of dams helped them to survive during dry periods. Numbers in vegetation near dams decreased as water levels rose and pasture conditions improved after rainfall.

Light trapping was most effective in open and wooded (campsite) pasture areas (Table 1) and reflected the distribution of cattle at night. Few adults were caught in light traps along the margins of creeks.

Most adults were reared from dung collected in open pasture especially in dung deposited near dams. There was a strong association between numbers in the resting and breeding habitats. However, contrary to previous beliefs neither resting or egg laying activity required the host cattle to be constantly close-by.

Study 1.2 - Effects of habitat during the resting phase.

In this experiment, numbers were compared after the broadly defined habitat areas of dam margins and open pasture (see Study 1.1) were divided into four discernible habitat (plant) types. Samples were collected with a vacuum sampler. The habitats are given in Table 2 together with a summary of *C. brevitarsis* counts and the population structure of the resting individuals. Full details are given in publication 2.

Table 2. Mean number, sex and parous ratios of *Culicoides brevitarsis* caught by vacuum sampling for 60 seconds in specific habitats associated with open pasture and dam margins. Means in the first column with different letters are significantly different ($P < 0.05$).

Habitat	*C. brevitarsis*		
	Total	**Sex ratio (♂:♀)**	**Parous ratio**
Dams Margins			
Tussocks (*Juncus*	44.3 ab	0.65	0.45
sp.)	28.7 bc	0.66	0.51
Grass			
Pasture	82.9 a	0.51	0.53
Tussocks (*Poa* sp)	3.9 c	-	0.55
Grass		χ^2 = NS	χ^2 = NS

C. brevitarsis was strongly influenced by the physical composition of the habitat, especially the presence of tussocks. The species was 21 times more abundant in grass tussocks than in equivalent areas of pasture grass; the larger the tussocks the more *C. brevitarsis* present. High numbers were also found in reed tussock and other grasses along the margins of dams. Reed tussocks were not occupied when dams were full and the base of the tussocks were submerged. Movements from the tussocks to the air above the tussocks were recorded around sunset when mating commonly occurs. Visual orientation was important for the relocation of tussocks as a resting habitat after these flights. The species apparently could not find the tussocks after sunset and was therefore spread more evenly over the pasture at night. A preference for tussocks as a resting site by *C. brevitarsis* would enable its presence to be detected quickly in the event of a disease outbreak. Control in outbreak areas could perhaps be enhanced by targeting tussocks with insecticides during the day. Tussock reduction by slashing

may be useful although this may simply cause a redistribution of midges in the remaining pasture.

Study 1.3 - Daily activity of C. brevitarsis.

This study was undertaken to describe and compare the different phases of activity by *C. brevitarsis* during the day at the southern limit of its distribution. Samples were taken at hourly intervals for most of the day with light traps (night only), a truck trap (5km transects) and a vacuum sampler (individuals on dung and in pasture vegetation). Numbers were analysed with respect to corresponding changes in temperature, humidity and light intensity. Numbers were analysed with respect to corresponding changes in temperature, humidity and light intensity. The experiment was intended to provide a basis for future investigations of specific activities, to establish the best times to sample and to explain behavioural interactions. A brief summary of the results here is given below. The results are given fully in publication 3.

Light and truck trap data supported work by other scientists and also indicated that behaviour involving flight was limited by temperature. Activity in the morning was documented for the first time and indicated that behaviour at this time was opposite to that recorded as the light changed around sunset. Light trap catches mainly recorded feeding activity and were significantly affected by the presence/absence of cattle. Numbers were highest one hour after sunset in April and May and then declined with temperature until activity ceased late in the night (Figure 1). This differed from previous descriptions of feeding activity being continuous and demonstrated the impact that temperature has on behaviour.

Truck trap catches appeared largely associated with mating activity. Numbers in truck traps were highest one hour before sunset and declined soon after dark as males ceased to be active. Catches early in the day included males again (mating) and decreased as the temperature increased until all flight activity had ceased by mid-morning.

Oviposition and resting activity was described for the first time. Females visited dung to lay eggs in morning and afternoon peaks. Their presence on dung was correlated with mating swarms (recorded by the truck trap), temperature and humidity. No females came to dung after sunset, again showing the species need for visual orientation. Resting on the ground occurred day and night over a wide range of temperatures and humidities.

This study provided valuable information on the species behaviour. It also enabled sampling to be timed and carried out to maximum effect. Further work will enable the

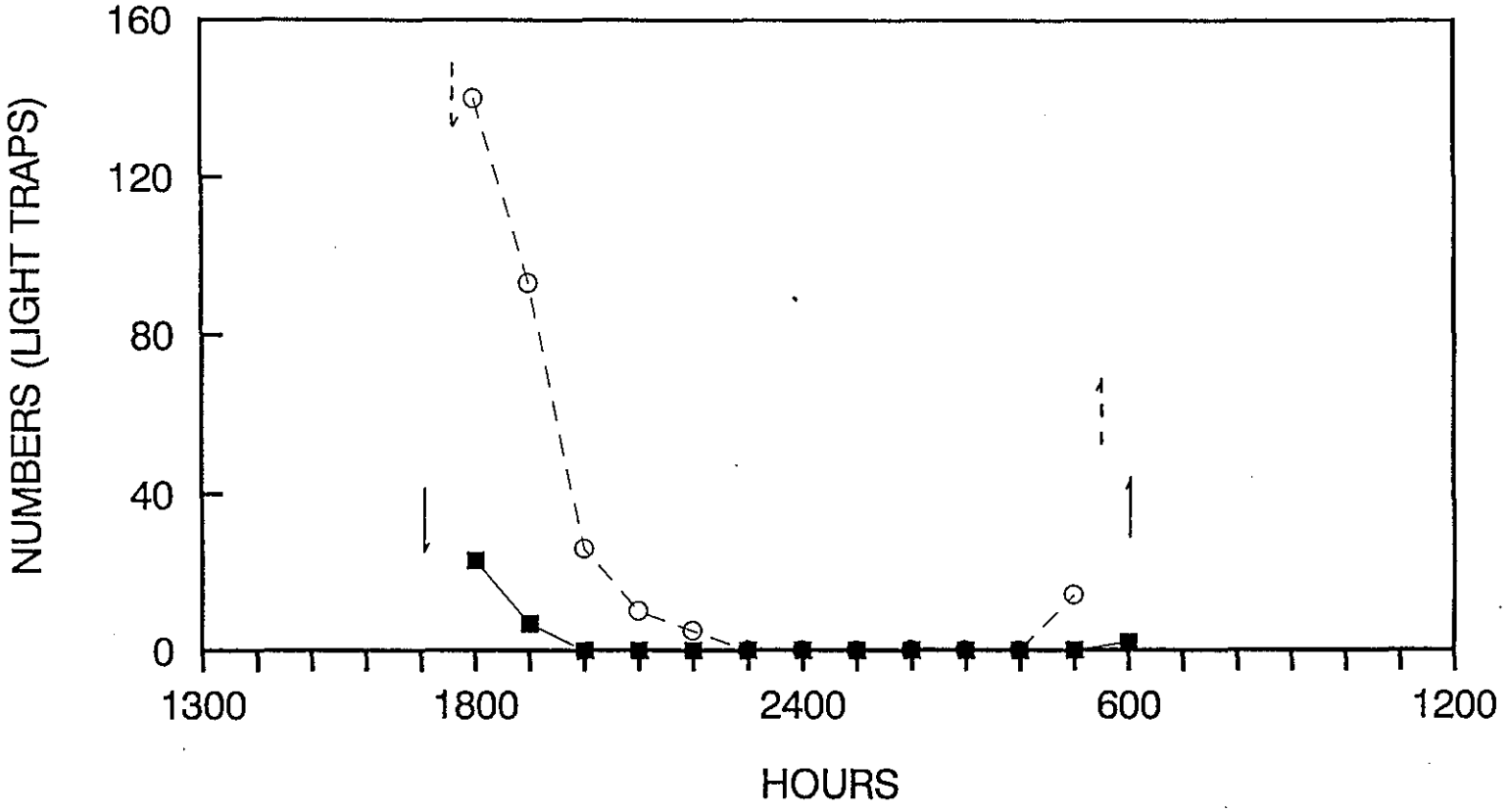


Figure 1. Daily pattern of total numbers of *Culicoides brevitarsis* caught each hour in light traps used once in April ○ and May ● 1993. Sunset and sunrise are indicated by arrows.

periods during the day when stock are at greatest risk to be defined more clearly.

Study 1.4 - Distribution of* C. brevitarsis *in bovine dung.

This study was designed to consider how to best quantify the development and survival of *C. brevitarsis* in the laboratory by sampling cores of dung in the field. It also enabled behavioural and spatial interactions between different species of fly to be considered by determining the positions of the immature stages at different times before adults started to emerge. Contrasts were made by dividing the dung into vertical and horizontal sections. The results of the study on *C. brevitarsis* are shown in Figure 2. Full details are given in publication 4.

C. brevitarsis laid most of its eggs on the top of the dung pat. After eggs hatched, immature stages were evenly distributed in a horizontal plane. However, vertical positions clearly changed as larvae spread progressively downwards until they were evenly distributed through the dung. These vertical movements were associated with the changes in moisture content as the dung dried and crusted on the upper surface. The study also provided information on four other species of fly breeding in dung. Patterns of their distribution were recorded for the first time. The information that this study provided has enabled the positioning and timing of core sampling to be established effectively. Information derived from this study was then used in Study 1.5.

Study 1.5 - Effects of constant temperature.

This study was undertaken to examine the effects of constant temperature on the emergence, development and survival of *C. brevitarsis* in bovine dung. It was necessary to enable the conclusions on *C. brevitarsis* survival derived in the field to be correctly interpreted and the results confirmed. This was vital to the understanding of events at its southern limits where temperatures may stop activity and prevent further spread of both vector and virus (See Study 1.6). The study was made in two parts summarised below and the full details are given in publication 5.

To conduct this research, the suitability of emergence chambers (designed to record numbers of adults emerging from dung) for use in the laboratory had to be determined. It was thought that this technique would avoid the problems of having to deal with a limited number of instars and of using artificial media encountered by other workers. In summary, data loggers were used to establish that the emergence chambers enabled: the natural breeding medium to be kept; relative humidity to be maintained at high levels; and temperature through the dung to be stabilised at the treatment temperature relatively quickly. This made them suitable for the experiments planned for the second part of the study.

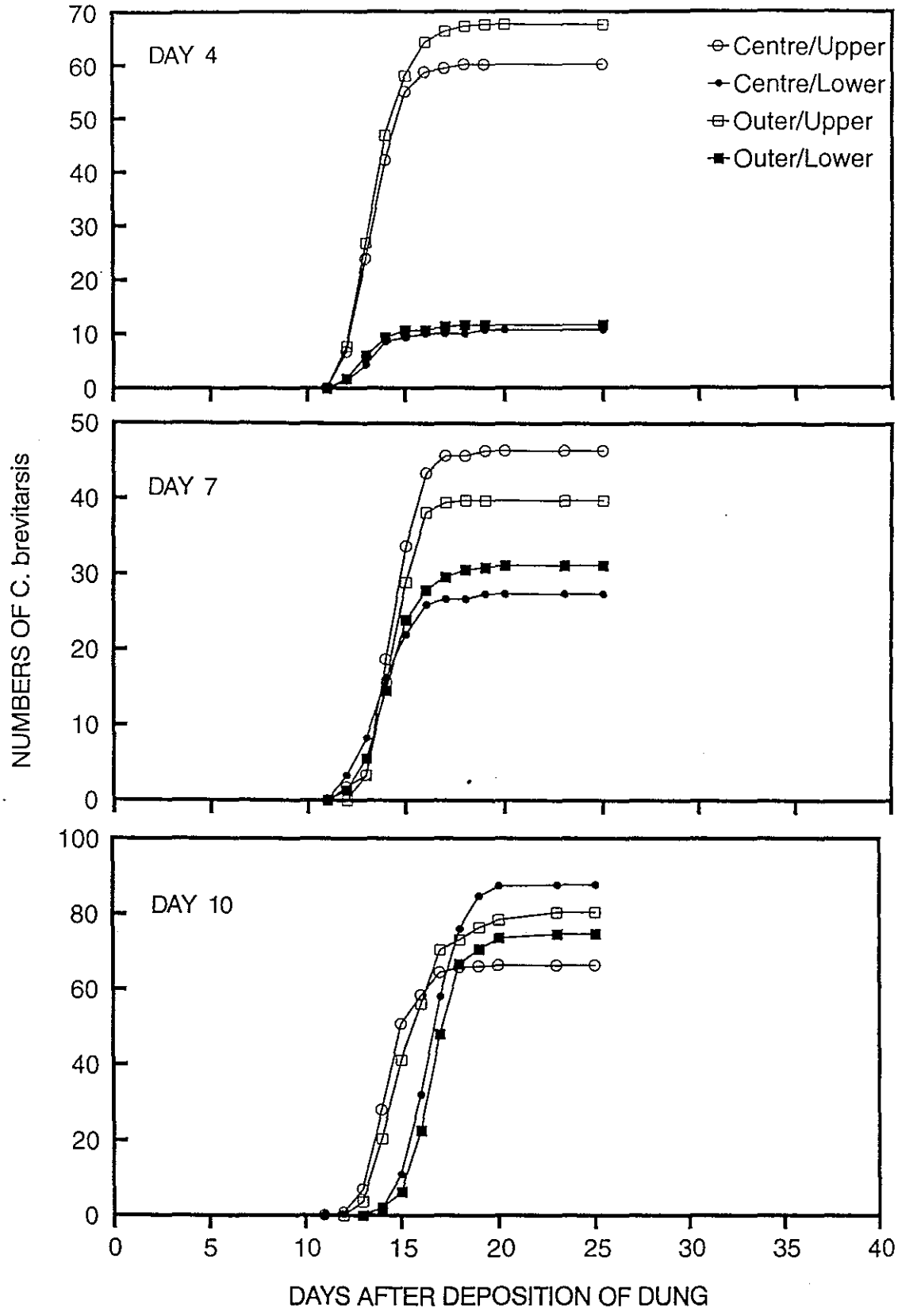


Figure 2. Distribution of *Culicoides brevitarsis* at three times after the deposition of bovine dung and after limiting field exposure to 2 days.

In the second part, a series of experiments were undertaken to assess the effects of different temperature regimes on *C. brevitarsis*. Samples were cores of dung derived as determined in Study 1.4 and kept in emergence chambers. A summary of the response by *C. brevitarsis* to constant and changed temperatures between the range of 5°C and 40°C is given in Table 3. Other details of these experiments, including the sequence of emergence with other dung breeding flies, can be found in publication 5.

The development and survival of *C. brevitarsis* was strongly influenced by both the pre-treatment temperature (i.e. the temperature in the field before the dung was placed at constant temperature) and the treatment temperatures. *C. brevitarsis* emerged within a temperature range of 17°C to 36°C with the greatest proportion of individuals at 25°C to 28°C. It developed and emerged faster as the temperature was increased until an upper limit was reached at 36°C. A few individuals also survived for 28 days at 40°C but did not emerge until the temperature was lowered. This would be important to the species survival in the northern half of Australia.

The critical lower temperature affecting survival was about 17°C and only small numbers of adults emerged slowly at this temperature. This led to two further experiments that were designed to consider the effects of:

1) Raising the critical temperature to a range of higher temperatures that would simulate different conditions that may be experienced in the field
2) Time on the emergence and survival of *C. brevitarsis* at the critical temperature.

In the first of these experiments, numbers increased immediately the temperature was raised above 17°C after 28 days at this temperature. More adults emerged the higher temperature was increased.

In the second of these experiments, the species survived for up to 50 days at 17°C. Adults emerged immediately and in significantly higher numbers when the temperature was raised to 28°C after 28 and 42 days at 17°C (Figure 3).

In addition to defining activity in response to temperature, three key factors were evident from this study:

- *C. brevitarsis* survived for considerable periods below temperatures that inhibited its development and emergence. Emergence was dependent on the temperature eventually being raised to a more favourable level.
- The species could not survive in dung for more than 50 days at a critical temperature of 17°C. At other temperatures, emergence was either complete before 50 days of did not occur at all.

Table 3 **Predicted total numbers of *C. brevitarsis* at constant temperatures. Means in columns separated by different letters are significantly different ($P < 0.05$).**

	Experiment					
	1		2		3	
Temp	28d	56d	28d	56d	28d	56d
5°C#	0	0				
12°C#	0	1.8				
15°C#			0	3.0a		
17°C#	6.5a	72.1a	0	15.0b		0
20°C#	67.0b	79.0a	4.2a	4.9a		
25°C	184.2c	191.0b	14.8b	14.8b	270.2a	270.2a
28°C			50.5c	50.5c	164.4b	164.4b
32°C					187.6b	187.6b
36°C					166.7b	166.7b
40°C#					0	0.4*

Treatments moved to 25°C after 28 days.
* Insufficient data for analysis.

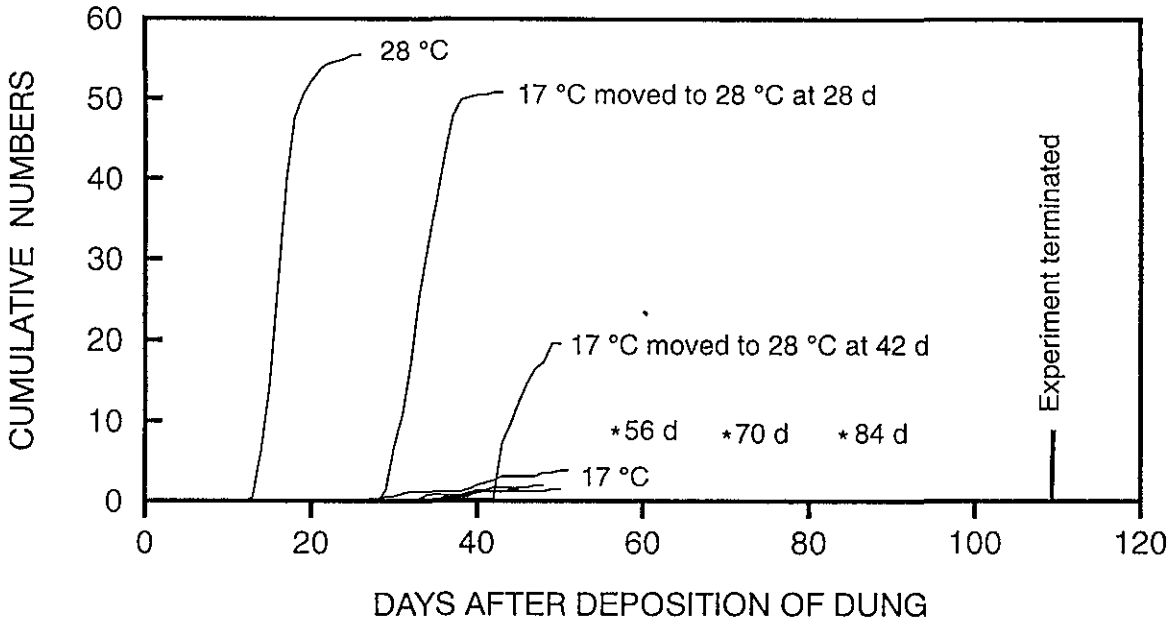


Figure 3. Cumulative emergence of *Culicoides brevitarsis* adults from bovine dung at 17°C, 28°C and after being held at 17°C for different periods (*) before being moved to 28°C.

- After 28 days and up to 42 days at the critical temperature, the surviving individuals responded immediately to a rise in temperature although their numbers progressively decreased over time until the 50 day limit was reached.

***Study 1.6 - Overwintering of* C. brevitarsis.**

This study was undertaken to investigate the capacity of *C. brevitarsis* to survive in the field through winter at the southern limits to its distribution in NSW. The study was carried out in two parts, the full details of which are given in publication 6.

The first part used data from a continuous on-farm assessment of the ecology of *C. brevitarsis* (see Study 1.7). Light trapping, vacuum sampling and dung sampling (emergence chambers) were used to determine numbers of the different stages in the life cycle throughout the year. Statistical relationships were derived between the numbers from mid-autumn to mid-spring in 1991 and 1992 and weather variables (temperature and rainfall) (Table 4).

C. brevitarsis numbers declined linearly towards winter in both years. The decline was associated with temperature but was independent of rainfall. Low activity thresholds were calculated from the regression equations. These thresholds were similar for the three sampling methods. Some activity carried over into winter. The species continued to survive in dung for a month after all adult activity had ceased. Similar relationships with temperature were obtained for four other *Culicoides* species. However, these species were capable of maintaining viable populations in an area where it was obviously difficult for *C. brevitarsis* to survive.

The second part of the study considered reproduction by *C. brevitarsis* and the development of immature stages in bovine dung as the dung aged in the field during autumn and winter. Dung was left in the field for varying (monthly) periods up to 6 months. The monthly samples of dung of different ages were returned to the laboratory where surviving individuals were allowed to emerge in emergence chambers held at 25^0C. No other *Culicoides* species were found in the dung. The results are given in Table 5 (September data are not given as no emergence occurred).

Numbers in dung decreased as the season progressed and as the dung aged. No individuals survived in dung that was more than two months old (one month when temperatures were below the thresholds after May). Emergence from aged dung occurred almost immediately after the dung was taken from the field and placed at a higher temperature. This was the first evidence that *C. brevitarsis* could overwinter

Table 4. Numbers of *C. brevitarsis* (mean ± SD) recorded from three sampling methods at Paterson in the 1991 and 1992 winter periods.

Species	April	May	June	July	August	September
Light trapping						
1991	13.3 (10.9)	17.9 (10.3)	5.0 (2.9)	0.2 (0.4)	0	0
1992	55.3 (94.0)	11.3 (19.9)	0.3 (0.5)	0	0	0
Vacuum sampling	3.6 (6.6)	3.0 (4.3)	0.9 (1.6)	0.1 (0.3)	0	0
1991	-	-	-	-	-	-
1992						
Dung sampling						
1991	24.5 (12.3)	12.5 (15.1)	4.3 (5.4)	0 .4 (0.3)	0.1 (0.2)	0
1992	28.3 (26.3)	3.3 (1.8)	0.04 (0.08)	0	0	0

Table 5. Total numbers of *C. brevitarsis* (mean ± SD), emerging from dung of different ages at monthly intervals at Tocal in the 1991 and 1992 winter periods (- = no sample).

Time of deposition	Time of sampling				
	April	May	June	July	August
April					
1991	21.5 (21.8)	-	1.4 (1.9)	0	0
1992	-	-	-	-	-
May					
1991		33.0 (36.9)	10.0 (17.9)	0	0
1992		19.6 (34.9)	1.4 (0.9)	0	0
June					
1991			10.4 (13.3)	0.2 (0.4)	0
1992			0	0	0
July					
1991				0.2 (0.4)	0
1992				0	0
August					
1991					0
1992					0

in the pupal/pre-pupal stage. Both the length of time that *C. brevitarsis* survived in dung and the immediate emergence of adults after temperatures were raised were consistent with the laboratory controlled temperature data in Study 1.5.

Study 1.7 - On-farm ecology.

In this study, data were taken for almost three years on the beef and dairy farms at the C.B. Alexander Agricultural College at Tocal. Different stages of the life cycle were monitored by two sampling methods at fortnightly intervals. The aim was to compare the effects of different pasture types, livestock, farm management practices and weather variables on the numbers and ecology of *C. brevitarsis*.

The main differences in the two farms concerned the dryland and irrigated production of pasture (especially during drought), stock type and differences in consistency and shape of the dung as a potential larval breeding medium. However, there were no major differences in the numbers of adults caught in light traps or reared from the dung on the two farms. No variations in *C. brevitarsis* numbers could be attributed to farm practices involving stock movements and pasture management. Some data were used selectively in Study 1.6 and many of the ecological principles tested in the other studies resulted from observations made here. It was concluded that beef or dairy farms could be used equally for monitoring *C. brevitarsis*.

Objective 2: Virus Monitoring Systems.

2.1. Development of rapid tests for virus antibodies.

Previous research conducted at EMAI has shown that the viruses belonging to the Simbu serogroup (Akabane, Aino, Peaton, Tinaroo and Douglas viruses) are transmitted to ruminants by *Culicoides brevitarsis* with a relatively high degree of efficiency, compared to other *Culicoides* borne arboviruses. Monitoring the distribution of Akabane virus is important because it is a virus that has an economic impact on the livestock industries both directly as a cause of disease (mainly in cattle) and indirectly through loss of trade either from areas where the virus is present or stock that have been infected. It has also been shown that construction of the composite pattern of the distribution of the Simbu viruses in any year provides a sensitive indicator of effective populations of *C. brevitarsis* (see Final Report, MRC Project DAN46). Normally individual virus neutralisation tests are used for detecting antibodies to each of the Simbu viruses. Neutralisation tests are conducted in cell cultures and hence are relatively expensive and take approximately one week to complete. The objectives of this section of the project are:

- to develop a rapid, inexpensive test which will singly detect antibodies to any of the Simbu viruses;
- to develop a similar test which will detect antibodies specifically to Akabane virus.

The technology most likely to offer promise is encompassed in the ELISA test, which can be conducted in a single day and is relatively inexpensive. Conventional ELISAs are usually specific to an animal species, so separate tests are required for cattle and sheep. However, the competition ELISA (cELISA) has the advantage that a single test can be used to test serum samples from any animal species, and samples from several species can be tested at the same time. The most critical component of the cELISA is probably the 'control' antibody which is used as the test 'indicator'. This antibody is preferably a monoclonal antibody, the binding characteristics of which do not vary over time, unlike polyclonal antibodies which are produced *in vivo*. Monoclonal antibodies can also be produced in vitro and their characteristics more readily permits test standardisation.

In addition to production and selection of suitable monoclonal antibodies, the other key factor in the development of the cELISA is the test antigen. However, the antigen specificity of the monoclonal antibody is the most critical component. There are antigens of the nucleoprotein ('N' protein) of the Simbu viruses which are shared and permit the grouping of these viruses. On the other hand, there are antigens of the 2

surface glycoproteins G1 and G2 which are involved in virus neutralisation and allow the individual Simbu viruses to be distinguished. In developing a Simbu group reactive test, the essential requirement is one or more monoclonal antibodies which bind to the shared antigens of the N protein. Conversely, for an Akabane-specific test, the preference is a monoclonal antibody which binds to the antigen(s) of the major glycoprotein G1. Further, this monoclonal should bind to a G1 antigen which is common to all of the strains of Akabane virus.

Progress towards the selection of suitable monoclonal antibodies was enhanced through collaboration with Dr H. Akashi, National Institute of Animal Health, Ibaraki, Japan. Dr Akashi kindly provided a panel of monoclonal antibodies, some with differing reactivity to the N protein of several Simbu viruses, others with reactivity to the Akabane G1.

Methodology.

Broadly, the approach adopted for both the Simbu group test and the Akabane-specific test was to develop a range of cELISAs using different monoclonal antibodies (with specificity for either the N or G1 proteins) and then to compare them with conventional tests. In the case of cELISAs with Simbu group reactivity, serum samples from animals known to have either been infected with different members of the Simbu group, or to have been free of infection with all of the viruses, were tested in the prospective Simbu group test. For the Akabane-specific cELISA, serum samples were tested from animals known to have either been infected with Akabane virus, or to be free of antibodies to Akabane viruses. Test formulations were chosen which had optimal sensitivity and specificity, that is, detected the maximal number of animals infected with the target viruses and did not produce a positive result for animals which had not been infected. After developing suitable tests they were then evaluated by testing samples from a serum bank from previous arbovirus monitoring, sera from 499 cattle in Tasmania (animals free of infection as a measure of specificity) and also prospectively by use in sentinel herd monitoring. In the later stages of the evaluation, samples were also tested from Western Australia, the Northern Territory and Queensland.

Results.

In the case of the Simbu group test, the preferred test incorporated a combination of 3 monoclonal antibodies. The combination of Mabs was necessary to provide sufficiently broad reactivity to correctly classify as positive, all sera from the serum

bank from animals known to have been infected with one or more of the Simbu viruses. Having established a prototype test format, the sera from animals in Tasmania were tested. A single sample gave a weak positive result. As these samples had originally been collected randomly for a bluetongue survey, it is possible that the positive serum had come from an animal imported from one of the northern mainland states. Alternatively, it is a false positive, which would result in a test specificity of 99.8%. The results of the sentinel herd monitoring for 1993/94 and 1994/95 also showed that the test had a high level of sensitivity and specificity. By comparison with the VNT results, it was shown that the Simbu group ELISA usually gave a positive result as soon as an animal seroconverted (ie an animal which had previously tested negative now gave a positive result, after infection with one or more of the Simbu viruses). Occasionally, individual animals failed to seroconvert in the Simbu cELISA as quickly as the VNT or, when infected with Akabane virus, the Akabane cELISA (see below). When resampled a month later, these animals have a positive Simbu ELISA result. An example of the pattern of seroconversion in individual animals is shown in Table 6. The test was used routinely in both the 1993/94 and 1994/95 arbovirus seasons. Seroconversion patterns on a herd basis throughout the state are shown in the next section.

The Akabane cELISA utilised a single monoclonal antibody. Initial testing showed a high level of agreement with the VNT, except on occasional samples with low VN titres (titres usually of 4 or 8). It has been recognised for many years that the VN test can give low titre false positive results so this should be considered when the ELISA test is evaluated. Whether such results were due to a lack of specificity of the VNT or a lack of sensitivity of the Akabane cELISA was best be resolved by testing samples from Akabane free areas. When this was done, the cELISA was proven to be reliable, that is, all of the sera with suspect low VN titres gave negative ELISA results. In this context, the Akabane ELISA had a specificity of 100%.

By definition of the Simbu group test, all sera which have antibody to Akabane virus should give a positive result in the Simbu cELISA. However, the converse need not be true, that is, not all sera testing positive in the Simbu test will necessarily be positive in the Akabane test. Therefore, in comparing and evaluating the Simbu and Akabane cELISAs, 2 groups of sera were chosen, those which had antibody to Akabane (as measured in the VN test) and those which had did not have Akabane antibody but did have antibody to one or more of the other Simbu viruses. All sera, from both groups, were found to be positive in the Simbu cELISA. However, only those sera which had Akabane antibody were positive in the Akabane cELISA.

TABLE 6:INDIVIDUAL ANIMAL RESULTS -
SIMBU & AKABANE cELISA and AKABANE NEUTRALISATION TEST.

	SIMBU cELISA					AKABANE cELISA				AKABANE VNT			
ID	**JAN**	**MAR**	**APR**	**MAY**	**JUN**	**MAR**	**APR**	**MAY**	**JUN**	**MAR**	**APR**	**MAY**	**JUN**
412	-	-	95	87	95	-	90	80	92	-	≥128	≥128	≥128
413	-	-	94	80	95	-	93	90	93	-	≥128	≥128	≥128
414	-	-	94	89	90	-	88	90	93	-	≥128	≥128	≥128
415	-	-	95	91	84	-	83	92	92	-	≥128	≥128	≥128
416	-	-	94	94	94	-	95	87	93	-	≥128	≥128	≥128
417	-	-	95	91	95	-	96	94	87	-	≥128	≥128	≥128
418	-	-	95	91	95	-	91	93	88	-	≥128	≥128	≥128
419	-	-	93	90	95	-	92	93	90	-	≥128	≥128	≥128
420	-	-	95	90	93	-	94	85	90	-	≥128	≥128	≥128
421	-	42	95	87	94	-	93	88	91	-	≥128	≥128	≥128

NOTES: **cELISA:** NEG (-) = <40% INHIBITION; 40-50%=INCONCLUSIVE; >50%=POSITIVE.
VNT: Titres >8 significant.

The above results for the Simbu and Akabane ELISAs then suggest an economical strategy for monitoring for the Simbu viruses. As these viruses are efficiently transmitted by *C. brevitarsis*, the results of Simbu ELISA monitoring can in turn be used to define effective vector populations.

After defining suitable parameters for the 2 ELISA tests, they were applied on a broader front, by testing sera collected in Western Australia, the Northern Territory and Queensland during the National Arbovirus Monitoring Program (NAMP). A total of 34 sera were tested from Western Australia, 175 from the Northern Territory and 72 from Queensland. Although these samples were not subjected to VN tests for all of the Simbu viruses, they were usually tested for neutralising antibodies to Akabane and Douglas viruses. Usually, when samples gave a positive VNT result, they also were positive in the Simbu cELISA. On 3 occasions samples were classified as negative, giving a result on the upper limit of the negative range. Conversely, all of the other samples that gave negative results were from localities or at times, especially in WA, where Simbu activity was not expected.

There was a high level of agreement between the VN test and the Akabane cELISA. A single sample with a low VN antibody titre gave a negative result in the ELISA. There were again a few samples with low VN antibody titres (usually 4, which is considered to be of doubtful significance) which were negative in the ELISA. The specificity of these Akabane ELISA results was often supported by a negative result in the Simbu ELISA. When the sensitivity and specificity of the Akabane cELISA were determined against the panel of interstate sera, using the VNT results as the benchmark for classification of samples, it was found that the test had a sensitivity of 98.2% and a specificity of 99.2%.

The apparent lack of sensitivity with a small number of sera is not considered to be a great problem. The Akabane ELISA has other advantages, especially high specificity, that greatly outweigh this shortcoming. As a monitoring tool, the test appears to be extremely useful. There is no readily apparent explanation for the failure to detect several positive sera. As these sera were single animal samplings and not from sequential bleeds, it is not known if they would have given a positive result at a later stage in infection. Although the onset of the immune response to individual proteins varies in individual animals, it is unlikely that this is an explanation in this situation, as the neutralising antibody response is broadly directed against the same glycoproteins. It is more likely that this phenomenon is due to the occurrence of antigenically variant strains. Although little is known about the extent of antigenic diversity between strains of Akabane viruses, it is likely that such variation exists.

Evidence in this direction is provided by a single sentinel animal in NSW that never became positive in the cELISA on sequential sera collected over a 4 month period and yet had high VN antibody titres.

In summary, there was generally a high level of agreement between the Simbu and Akabane ELISAs and also with the corresponding VN tests. It was found that in many situations the Simbu cELISA could be used as a broad screening test, as an indicator of infection with one of the Simbu viruses. This would readily allow mapping of effective populations of *C. brevitarsis*. Subsequent application of the Akabane cELISA to these positive sera would accurately determine the distribution of Akabane virus. The concurrent use of the tests in this way significantly reduces the total number of tests to be done, and hence lowers costs substantially, especially when up to 5 separate VN tests could be required otherwise. The sensitivity and specificity of the Akabane cELISA also make this test suitable for screening of animals (of any species) for export, again lowering costs to the livestock industries.

2.2. Monitoring of Virus Distribution.

An essential part of this project was also to continue monitoring sentinel cattle for infection with key arboviruses of economic significance. Akabane, Bluetongue and Bovine Ephemeral Fever viruses were included because of their role as either pathogens or direct causes of loss of trade. The non-Akabane Simbu viruses were included for epidemiological purposes, as sensitive indicators of effective vector populations. Monitoring for the other orbiviruses from the EHD and Palyam groups was continued because of their ability to induce antibody which is cross-reactive in the Bluetongue AGID test as to act as low grade pathogens. (Overseas, viruses from both groups can be significant pathogens.) A previous MRC project DAN 46 had shown that monitoring of sentinel cattle was an accurate and efficient method for defining the distribution of arboviruses on an annual basis. One of the recommendations from DAN 46 was that there was an immediate need for the establishment of an integrated national monitoring program which has a sure financial footing and is funded independently of research allocations. Following deliberations between the stakeholders, the States, Commonwealth and the sheep and cattle industries, it was agreed in 1993 to establish a co-ordinated National Arbovirus Monitoring Program (NAMP). This program is now funded through a cost sharing agreement between each of the stakeholders. A number of the sentinel herds engaged in this project were also utilised as monitoring sites for the NAMP. There were additional sites which were selected to complement the vector ecology studies and to assist the development and evaluation of models for predicting vector distribution.

Methodology.

The specifications and sampling techniques for sentinel herds and the range of tests conducted have been described in detail in Final Report for DAN 46. Briefly, groups of young animals, usually 6-9 months old in October/November were sampled at monthly intervals from October/November to June inclusive. Serum samples were tested for antibodies to Bluetongue and other orbiviruses (EHD and Palyam groups), Akabane and other Simbu viruses and to Bovine Ephemeral Fever Virus. Virus isolation, specifically directed towards Bluetongue, was carried out on blood samples from all cattle which were infected with Bluetongue.

In some instances, sheep were also sampled on or near sentinel cattle properties, at irregular intervals, depending on the availability of suitable properties and commitments of field staff. Sheep sera were tested for Bluetongue and Akabane.

The results of sentinel herd monitoring in NSW are often summarised in Tables and maps. When interpreting data from these tables, it should be remembered that animals have generally become infected at some stage during the month preceding the month in which seroconversions were recorded. Further, the maps could potentially depict a broader distribution of some viruses than actually occurred, as they are simply a graphical representation on a Rural Lands Protection Board basis, following sampling at a single point.

Results.

1992/93 Arbovirus Year.

During the 1992/93 arbovirus season, samples were collected from sentinel cattle at 26 sites in NSW. The locations were chosen to provide a complete coverage of the arbovirus endemic area of NSW and also to adequately cover the arbovirus free areas in Southern and Western NSW. The main features of this period was the return of a relatively normal pattern of Akabane transmission, at least as far as the Central Coast and Lower Hunter Valley. There was also a low to moderate level of Bluetongue virus infection on the Far North Coast and in the Manning River basin. The details are as follows:

Akabane Viruses.

During the 1992/93 arbovirus season, there was widespread transmission of Akabane virus. Infection was first detected on the North Coast during February and was found south to the Hunter Valley during March and April (See Table 7.) There was,

TABLE 7: SENTINEL HERD RESULTS 1992-93.

AKABANE VIRUS NEUTRALISATION TEST.

LOCATION	JAN	FEB	MAR	APR	MAY	JUN
LISMORE	0	0	10	10	10	10
CASINO	0	8	8	8	8	8
GRAFTON	0	NS	10	10	10	10
COFFS HARBOUR	0	10	10	10	10	10
DORRIGO	0	0	2	2	2	2
KEMPSEY	0	1	10	10	10	10
TAREE	0	0	4	10	10	10
GLOUCESTER	0	0	6	10	10	10
PATERSON	0	0	0	10	10	10
SINGLETON	0	0	0	0	0	0
SCONE	0	0	0	0	0	0
RICHMOND	0	0	0	0	0	0
CAMDEN	0	0	0	0	0	0
NOWRA	0	0	0	0	0	0
BODALLA	0	0	0	0	0	0
BEGA	0	0	0	0	0	0
MUDGEE	0	0	0	0	0	0
TAMWORTH	0	0	0	0	0	0

OTHER LOCATIONS: INVERELL, MOREE, BOURKE, COBAR, DUBBO, COWRA, WAGGA, YANCO, WENTWORTH.

(NS= Not Sampled)

however, limited spread of the virus westward along the Hunter Valley, with an absence of infection at Singleton (Figure 4.). This transmission pattern leaves a significant number of susceptible cattle along the margin of the endemic area. It is of some interest that, after an absence of infection the previous season, many susceptible cattle were infected on the North Coast and yet there were few deformed calves. One explanation for this observation could be that the virus strain was one of low pathogenicity.

Other Simbu Viruses

There was a high incidence of Douglas virus transmission throughout the regions in which Akabane transmission also occurred. Infections were occasionally found beyond the Akabane limits (eg in the Singleton district). There were also sporadic seroconversions to Aino virus in the Manning region and lower North Coast.

Bluetongue Virus.

During the latter half of the 92/93 season (March to June), between the North Coast and Hunter-Manning regions sentinel cattle were infected with several of the orbiviruses. There was a moderate incidence of Bluetongue serotype 1 infection on the far North Coast, with an independent focus of transmission in the Manning region (See Table 8 and Figure 5). Bluetongue viruses were isolated on two occasions. The virus, although identified as a Type 1 virus, appeared to be antigenically remote from the reference Type 1 virus.

EHD and Palyam Group viruses.

There was concurrent low-moderate level transmission of EHD virus on the North Coast and in the Manning region and Palyam virus infections from the North to Central Coast between March and June. The seroconversion rates with each of the orbiviruses, including Bluetongue, was much lower than with Akabane virus. Ten isolates of EHD virus serotype 5 were obtained, including one from a cow with an ephemeral fever-like illness. During the period of EHD and Palyam transmission, there were sporadic cases of this EF-like illness.

Bovine Ephemeral Fever Virus.

Although a mild ephemeral fever-like illness was reported in some northern and central coastal areas, there was no Ephemeral fever virus transmission detected in NSW this season, either in sick animals or sentinels.

Figure 4: Distribution of Akabane Virus: 1992-93.

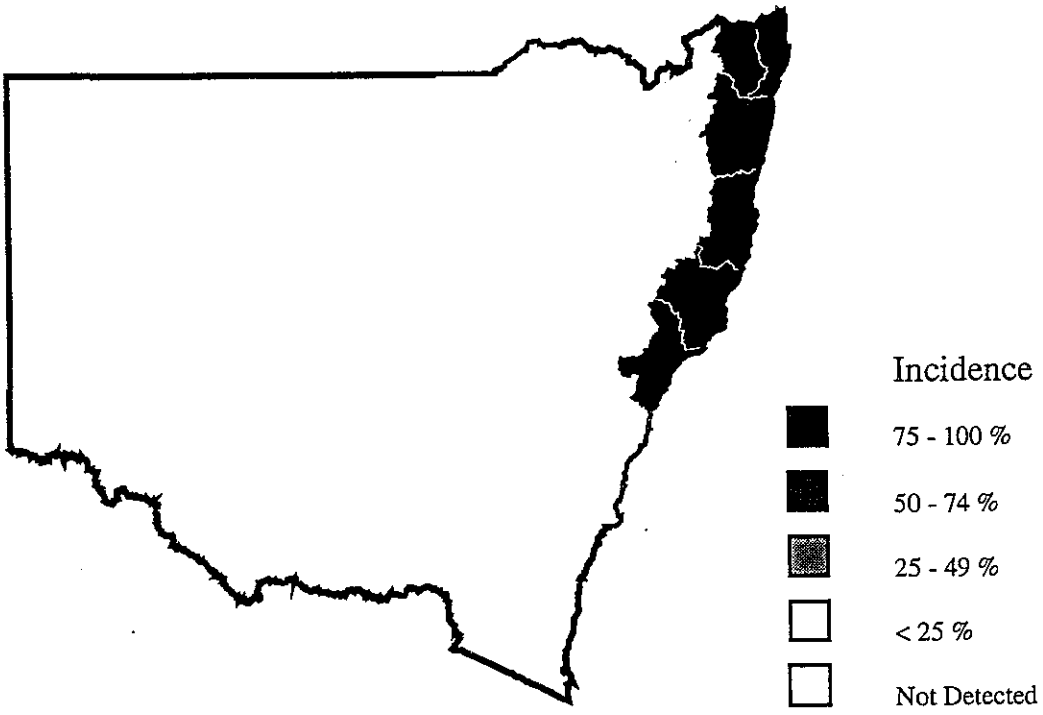


TABLE 8: SENTINEL HERD RESULTS 1992-93.

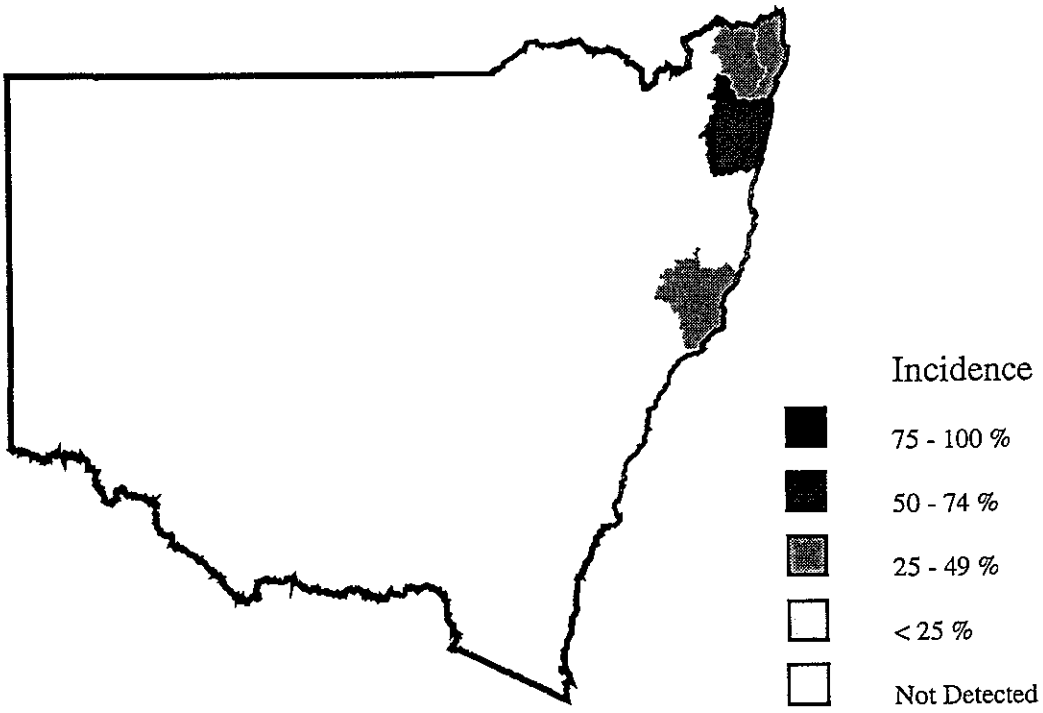
BLUETONGUE VIRUS cELISA.

LOCATION	JAN	FEB	MAR	APR	MAY	JUN
LISMORE	0	0	1	1	4	3
CASINO	0	0	0	3	3	3
GRAFTON	0	0	0	0	1	7
COFFS HARBOUR	0	0	0	0	0	0
DORRIGO	0	0	0	0	0	0
KEMPSEY	0	0	0	0	0	0
TAREE	0	0	0	0	0	4
GLOUCESTER	0	0	0	0	0	0
PATERSON	0	0	0	0	0	0
SINGLETON	0	0	0	0	0	0
SCONE	0	0	0	0	0	0
RICHMOND	0	0	0	0	0	0
CAMDEN	0	0	0	0	0	0
NOWRA	0	0	0	0	0	0
BODALLA	0	0	0	0	0	0
BEGA	0	0	0	0	0	0
MUDGEE	0	0	0	0	0	0
TAMWORTH	0	0	0	0	0	0

OTHER LOCATIONS: INVERELL, MOREE, BOURKE, COBAR, DUBBO, COWRA, WAGGA, YANCO, WENTWORTH.

(NS= Not Sampled)

Figure 5: Distribution of Bluetongue Virus: 1992-93.



1993/94 Arbovirus Year.

During the 1993/94 arbovirus season, sentinel cattle were sampled at 24 sites in NSW and sheep were sampled at 5 locations. These main features of this arbovirus season were the extensive transmission of both Bluetongue and Bovine Ephemeral Fever viruses.

Bluetongue.

Bluetongue transmission appeared to spread out from a focus on the Mid North Coast at Coffs Harbour very early in the season (December/January, Table 9) and spread mainly southwards, eventually reaching Camden where there were seroconversions in early June, consistent with infection during May (Figure 6). A prevalence of bluetongue antibody of 63% (14/22) was found in sentinel sheep at Gloucester at the end of the season. There were no reports of disease. The prevalence of Akabane antibody in these sheep was >95% (21/22), confirming a high attack rate by midges.

There were 9 isolations of bluetongue viruses from blood samples. The serotype of Bluetongue virus involved was again Type 1.

EHD and Palyam Group viruses.

There was a moderate to high incidence of EHD infections commencing in January (seroconversions in February) on the North Coast (Coffs Harbour to Lismore) and moving south to and through the Hunter Valley during March and April. Infection with the Palyam viruses followed a similar transmission pattern and incidence to Bluetongue but was more widespread, reaching Nowra in the south.

Ephemeral Fever.

There was extensive Ephemeral Fever virus transmission, with separate foci of infection commencing in the Hunter Manning and on the far North Coast. The disease was particularly severe at Paterson in the Hunter Valley with 9/10 sentinels very sick and all seroconverting within 1 month. The other notable feature was the spread up the Hunter Valley beyond Scone and to Dubbo. (See Figure 7)

Akabane virus.

There was a lower level of Akabane infection than usual and spread occurred later in the season. However, the virus did move further south than usual, resulting in the infection of animals in both the Camden and Illawarra districts (Figure 8). A limited number of deformed calves and sheep were born during Spring in these areas and the Upper Hunter Valley.

TABLE 9: SENTINEL HERD RESULTS 1993-94.

BLUETONGUE VIRUS cELISA.

LOCATION	JAN	FEB	MAR	APR	MAY	JUN
LISMORE	0	0	0	0	0	1
CASINO	0	0	0	0	0	0
GRAFTON	0	0	0	NS	0	0
COFFS HARBOUR	10	10	10	10	10	10
DORRIGO	0	0	1	NS	2	3
KEMPSEY	0	1	5	10	10	10
TAREE	0	0	0	3	NS	5
GLOUCESTER	0	1	4	10	10	10
PATERSON	0	0	0	6	10	10
SINGLETON	0	0	0	1	7	7
SCONE	0	0	0	0	7	7
RICHMOND	0	0	0	0	0	2
CAMDEN	0	0	0	0	0	0
NOWRA	0	0	0	0	0	0
BODALLA	0	0	0	0	0	0
BEGA	0	0	0	0	0	0
MUDGEE	0	0	0	0	0	0
TAMWORTH	0	0	0	0	0	0

OTHER LOCATIONS: INVERELL, MOREE, BOURKE, COBAR, DUBBO, COWRA, WAGGA, YANCO, WENTWORTH.

(NS= Not Sampled)

Figure 6: Distribution of Bluetongue Virus: 1993-94.

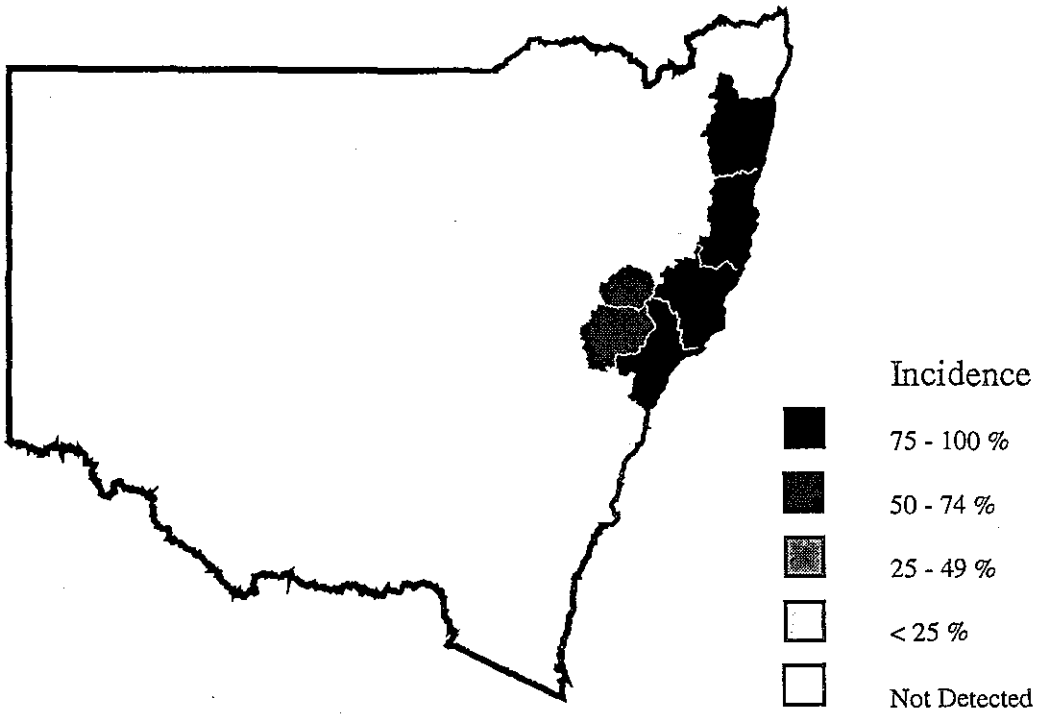


Figure 7: Distribution of Bovine Ephemeral Fever Virus:1993-94.

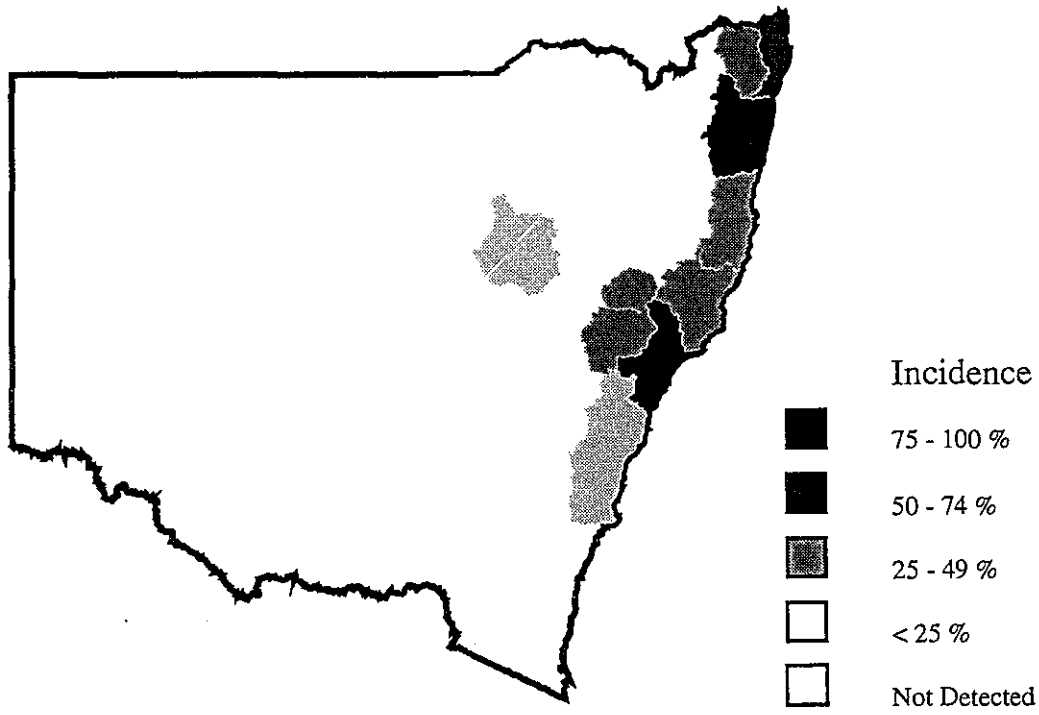
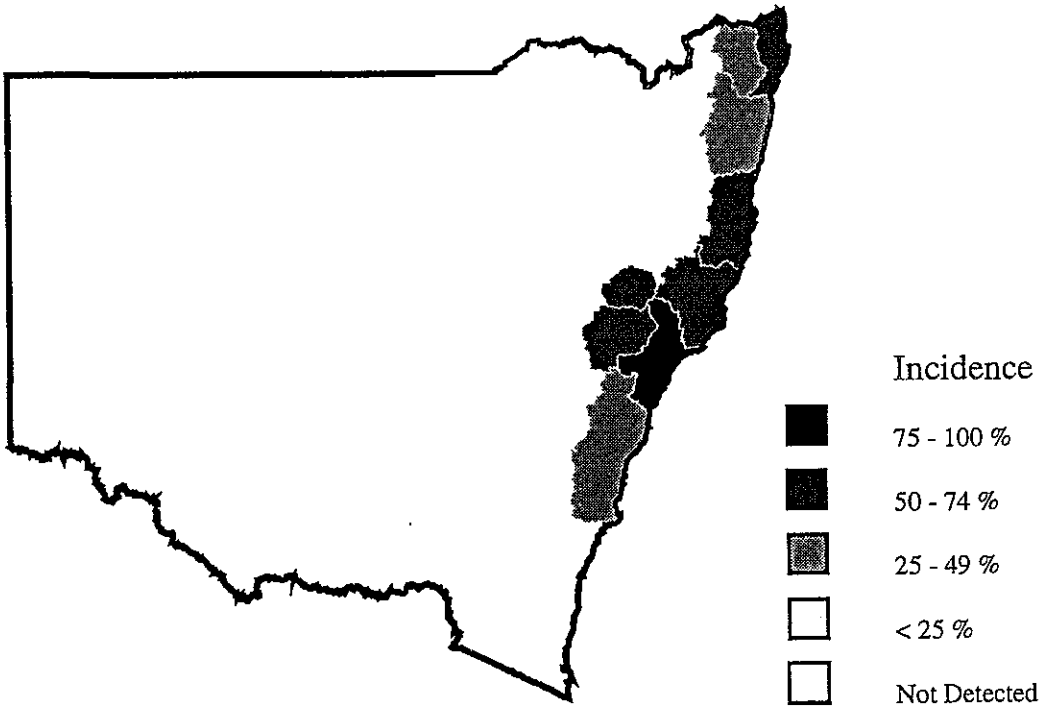


Figure 8: Distribution of Akabane Virus: 1993-94.



Simbu viruses.

The Simbu group was introduced for evaluation as a monitoring tool. Seroconversions were detected throughout the coastal endemic area, commencing in December at Casino on the Far North Coast, with a progressive southward pattern with the southern limit being Nowra, with a single animal seroconverting in June. Most seroconversions occurred between January and April. Almost all animals seroconverted in coastal herds south as far as Camden. A relatively unusual feature was that there was less dominance of 1 or 2 Simbu viruses, with moderate incidences of seroconversions to each of the viruses, Aino, Douglas, Peaton and Tinaroo.

1994/95 Arbovirus Year.

During the 1994/95 arbovirus season, blood samples were collected from sentinel cattle at 22 sites in NSW, the locations chosen to provide a complete coverage of the arbovirus endemic area of NSW and also to adequately cover the arbovirus free areas in Southern and Western NSW. The main features were the limited transmission of Akabane virus and the lack of infection with both Bluetongue and Bovine Ephemeral Fever viruses. These results are not unexpected with the widespread and severe drought conditions. Unfortunately, the drought prevented continuous sampling of animals in a number of locations, as they were sold or agisted, in some cases due to lack of water as well as feed.

Akabane virus.

In herds where transmission occurred, the incidence of Akabane infection was relatively normal (>90%) but the geographical distribution was restricted and spread occurred later in the season (Compare Table 10 with Table 7). The virus did not spread south of the Hunter Valley and only moved inland through the valley as far as Maitland (Figure 9). No seroconversions were recorded at Singleton or further inland.

Other Simbu Viruses.

The Simbu Group ELISA was used routinely for monitoring throughout the year. Seroconversions co-incided with the first occurrence and incidence detected in the Akabane ELISA (See Tables 11 & 12). Consequently, later infections with other Simbu viruses were masked, except in locations where there was no Akabane transmission. Neutralisation tests also indicated infections with Peaton and Douglas viruses. Results in herds in the endemic area confirmed that both Peaton and Douglas virus transmissions were widespread.

TABLE 10: SENTINEL HERD RESULTS 1994-95.

AKABANE VIRUS NEUTRALISATION TEST.

LOCATION	JAN	FEB	MAR	APR	MAY	JUN
LISMORE	NS	2	10	10	10	10
CASINO	0	NS	0	10	10	10
GRAFTON	NS	0	0	3	7	8
COFFS HARBOUR	0	NS	0	0	8	8
DORRIGO	0	NS	0	0	0	0
KEMPSEY	0	NS	0	8	10	10
TAREE	0	0	0	NS	NS	NS
GLOUCESTER	0	0	0	0	7	10
PATERSON	0	0	0	10	10	10
SINGLETON	0	NS	0	0	0	0
SCONE	0	0	0	0	NS	0
RICHMOND	0	0	0	0	0	0
CAMDEN	0	0	0	0	0	0
NOWRA	0	0	0	0	0	0
BODALLA	0	0	0	0	0	0
BEGA	0	0	0	0	0	0
MUDGEE	0	0	0	0	0	0
TAMWORTH	0	NS	0	0	NS	NS

OTHER LOCATIONS: INVERELL, MOREE, BOURKE, COBAR, DUBBO, COWRA, WAGGA, YANCO, WENTWORTH.

(NS= Not Sampled)

Figure 9: Distribution of Akabane Virus: 1994-95.

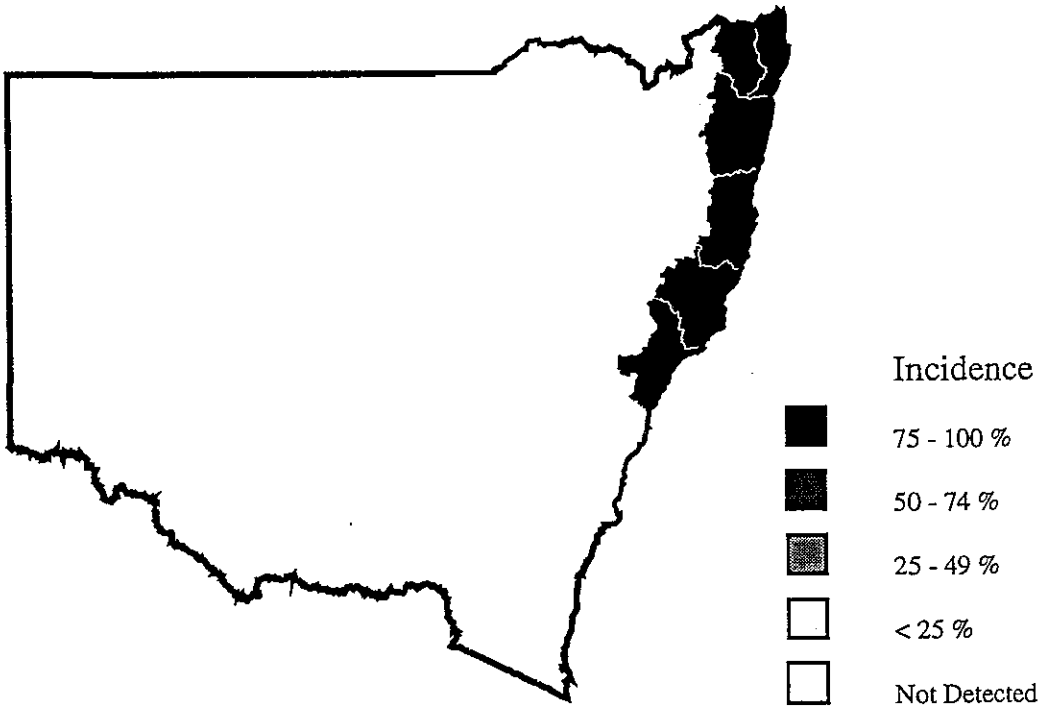


TABLE 11: SENTINEL HERD RESULTS 1994-95.

SIMBU SEROGROUP cELISA.

LOCATION	JAN	FEB	MAR	APR	MAY	JUN
LISMORE	NS	0	10	10	10	10
CASINO	0	NS	0	9	10	10
GRAFTON	NS	0	0	7	7	8
COFFS HARBOUR	0	NS	0	0	8	8
DORRIGO	0	NS	0	0	0	0
KEMPSEY	0	NS	0	9	10	10
TAREE	0	0	2	NS	NS	NS
GLOUCESTER	0	0	0	0	8	10
PATERSON	0	0	0	10	10	10
SINGLETON	0	NS	0	0	0	0
SCONE	0	0	0	0	NS	0
RICHMOND	0	0	0	0	0	0
CAMDEN	0	0	0	0	0	0
NOWRA	0	0	0	0	0	0
BODALLA	0	0	0	0	0	1
BEGA	0	0	0	0	0	0
MUDGEE	0	0	0	0	0	0
TAMWORTH	0	NS	0	0	NS	NS

OTHER LOCATIONS: INVERELL, MOREE, BOURKE, COBAR, DUBBO, COWRA, WAGGA, YANCO, WENTWORTH.

(NS= Not Sampled)

TABLE 12: SENTINEL HERD RESULTS 1994-95.

AKABANE cELISA.

LOCATION	JAN	FEB	MAR	APR	MAY	JUN
LISMORE	NS	0	10	10	10	10
CASINO	0	NS	0	9	10	10
GRAFTON	NS	0	0	7	7	8
COFFS HARBOUR	0	NS	0	0	8	8
DORRIGO	0	NS	0	0	0	0
KEMPSEY	0	NS	0	9	10	10
TAREE	0	0	0	NS	NS	NS
GLOUCESTER	0	0	0	0	8	10
PATERSON	0	0	0	10	10	10
SINGLETON	0	NS	0	0	0	0
SCONE	0	0	0	0	NS	0
RICHMOND	0	0	0	0	0	0
CAMDEN	0	0	0	0	0	0
NOWRA	0	0	0	0	0	0
BODALLA	0	0	0	0	0	0
BEGA	0	0	0	0	0	0
MUDGEE	0	0	0	0	0	0
TAMWORTH	0	NS	0	0	NS	NS

OTHER LOCATIONS: INVERELL, MOREE, BOURKE, COBAR, DUBBO, COWRA, WAGGA, YANCO, WENTWORTH.
(NS= Not Sampled)

Ephemeral Fever.

There was no Ephemeral Fever virus transmission detected by sentinel cattle but there was a separate small focus of infection in the Narrabri area. There were no sentinel cattle in this area in 1994/5.

Bluetongue.

There was no Bluetongue transmission in NSW. The nearest infections detected by NAMP were in central coastal Queensland.

EHD and Palyam Group viruses.

There was a high incidence of Palyam infections confined to the Far North Coast, commencing in April (seroconversions in May and June). There was no evidence of infection with viruses belonging to the EHD serogroup.

Composite Patterns of Arbovirus Transmission.

A technique has been developed whereby "effective" vector populations can be defined. At any sentinel location, each animal is scored as either positive or negative, depending on whether it had been infected with any one (or more) of the *Culicoides*-borne viruses (ie any of the Simbu or orbiviruses). Ephemeral Fever seroconversions were not included in this classification of animals because of the likelihood that the principal vector of BEFV is a mosquito. Maps of *Culicoides*-borne arbovirus distribution and incidence were then prepared (Figures 10, 11 & 12). These arbovirus distribution patterns were found to be entirely consistent with known "normal" limits of Culicoides populations. The data generated for each of the *Culicoides* -borne viruses was used each year to produce composite patterns of virus transmission and hence, the likely distribution of effective vector populations. These maps will be presented in the next section for comparison with the vector modelling projections.

Summary.

Each year, even under severe drought conditions, there was one or more of the Simbu viruses transmitted, usually at a high level. The drought conditions did however limit the geographical distribution of the Simbu viruses to an area significantly within the bounds of the endemic area, especially at the southern and western limits. These viruses appear to be transmitted more efficiently and consistently than any of the other viruses. In most years there were usually 2 Simbu viruses spread

Figure 10: Distribution of Arboviruses: 1992-93.

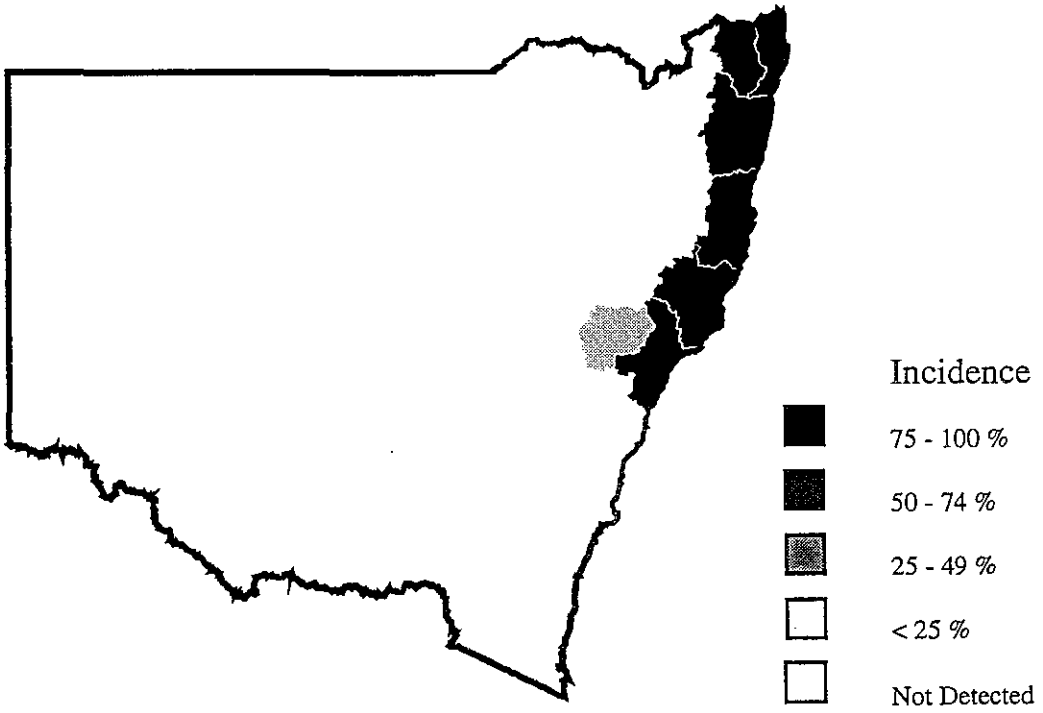


Figure 11: Distribution of Arboviruses: 1993-94.

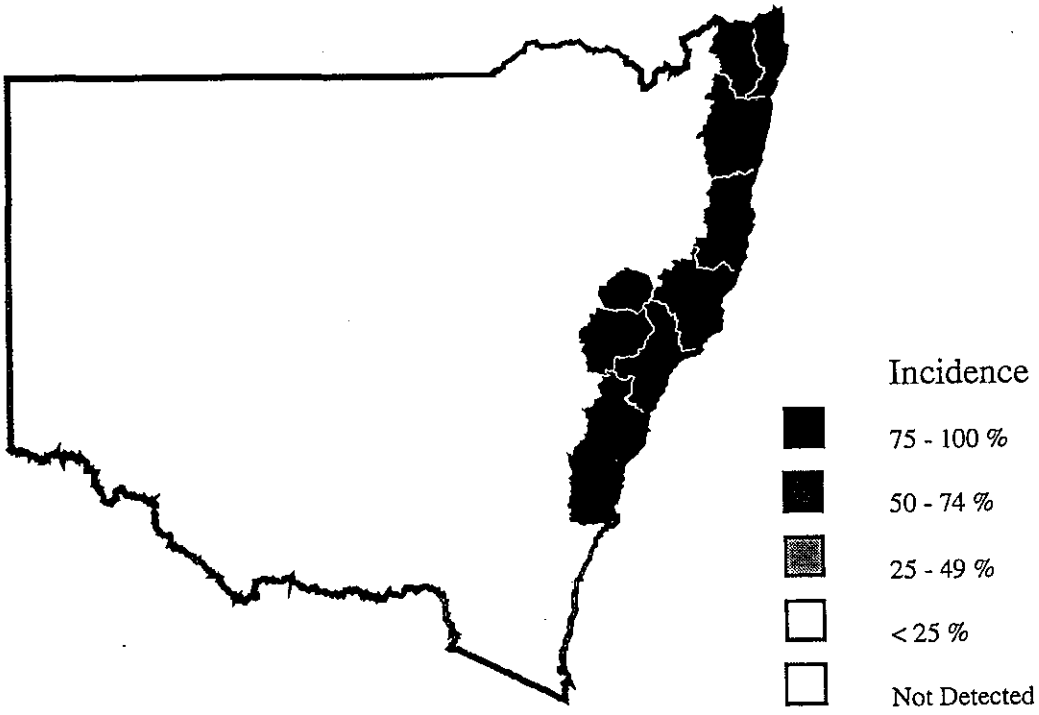
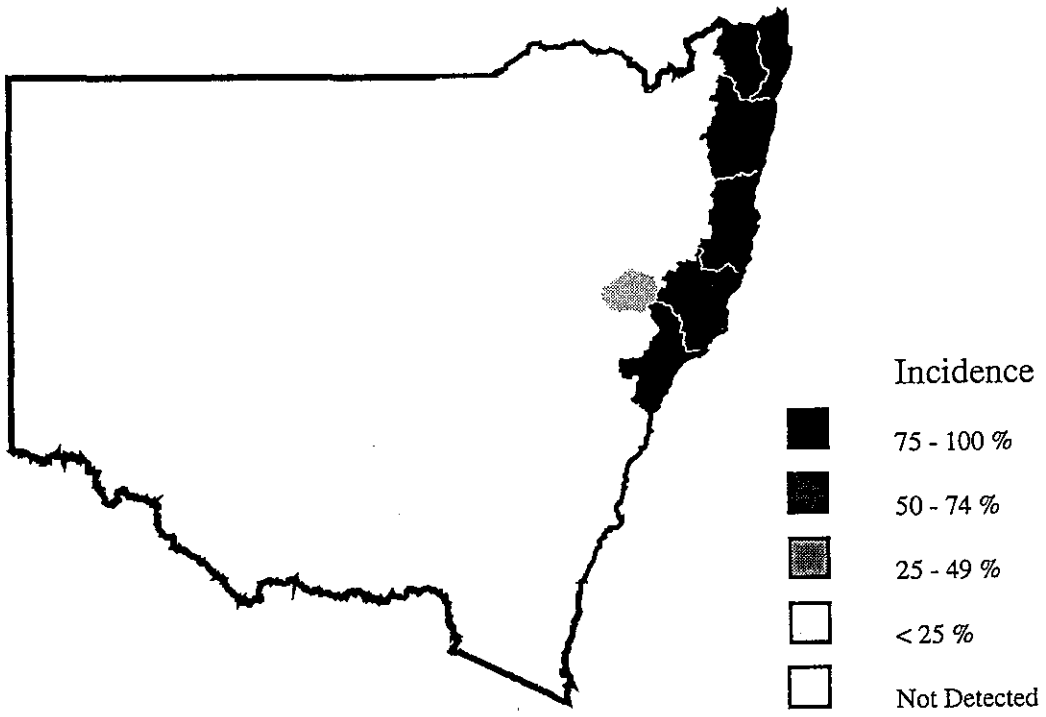


Figure 12: Distribution of Arboviruses: 1994-95.



at a high incidence, however, there was usually a lag of a month or two before the transmission of the second virus. The pattern in 1993/94 was relatively unusual in that several of the Simbu viruses were spread concurrently at a moderate incidence.

After the low, (sometimes undetected) levels of transmission of viruses from the Bluetongue and EHD groups in the 3 years preceding this study, there was relatively frequent infection with viruses from these groups. The pattern of transmission of Bluetongue viruses (Serotype 1) in 2 successive years (1992/93 and 1993/94) was very similar to the pattern of 1988/89 and 1989/90, where in the first year infection was limited to the north coast and was followed by widespread infection in the second year. There was also a high incidence of EHD infection (serotype 5) in 1993/94 concurrently with the high incidence of Bluetongue viruses. In the final year, the only orbiviruses to be transmitted were viruses of the Palyam group which were spread with relatively high frequency after a low to moderate incidence in the preceding two years.

A widespread epidemic of Ephemeral Fever was also observed in the 1993/94 year, emanating from foci in the Hunter and Manning River Valleys. Disease in the Lower Hunter Valley appeared to be particularly severe. There appeared to be a clear pattern of movement from the Hunter Valley inland to Dubbo. The pattern of the occurrence of disease and seroconversion rates are quite different to that of the *Culicoides*-borne viruses and consistent with a probable mosquito vector.

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Objective 3: Forecasting Vector/Virus Distribution.

The aim of this phase of the project was to be able to understand and predict the seasonal presence of *C. brevitarsis* throughout NSW. Currently this is done by considering "brevitarsis lines" that enclose areas in which there is a possibility that *C. brevitarsis* may develop populations capable of transmitting viruses. These lines do not necessarily indicate that the species will actually be present.

The study area was from Taree to Moruya on the central and southern coastal plains and extended up the Hunter Valley to the western slopes (Mudgee) and northern tablelands (Tamworth) in seasons 1990-93. The study was further extended to the north coast in seasons 1993- 95. Associations between vector and viruses were considered from concurrent records taken over these years.

Study 3.1 - Last occurrence and survival during winter.

This study was aimed at adding more data to the linear relationships implied in Study 1.6 and expressing them as statistical models. Activity thresholds (temperature °C) derived from these models could then be used with average (historical) temperature data to predict the month when activity should cease in a particular area. The thresholds, applied in combination with a two consecutive month maximum survival period (see Study 1.6) could also be used to estimate the probability that the species could survive winter. The full results of this study are given in publication 7.

The temperatures at which *C. brevitarsis* was no longer present (activity thresholds) were found to be 17.5°C, 13.2°C and 8.1°C for maximum, average and minimum temperatures respectively. Greatest statistical accuracy was obtained from the minimum temperature threshold and this was used in subsequent calculations. The results are summarised and compiled in maps of NSW (Figure 13). Estimates were made at 60 main centres (i.e. locations of District Agronomist offices) and are treated as representative of the location (although some variations could be expected within a district. Estimates were also made at 10 key centres along the coast where weather records are kept. The maps show:

1) The month that *C. brevitarsis* should last occur. These data can be used to define the time at which livestock should no longer be at risk of infection at the end of the season
2) The number of months that temperatures are below the minimum threshold. These data can be calculated to give the probability of survival throughout the state. (i.e. there is zero probability of survival when the temperature is below the thresholds for more than three months). Localities where temperatures are highest and where

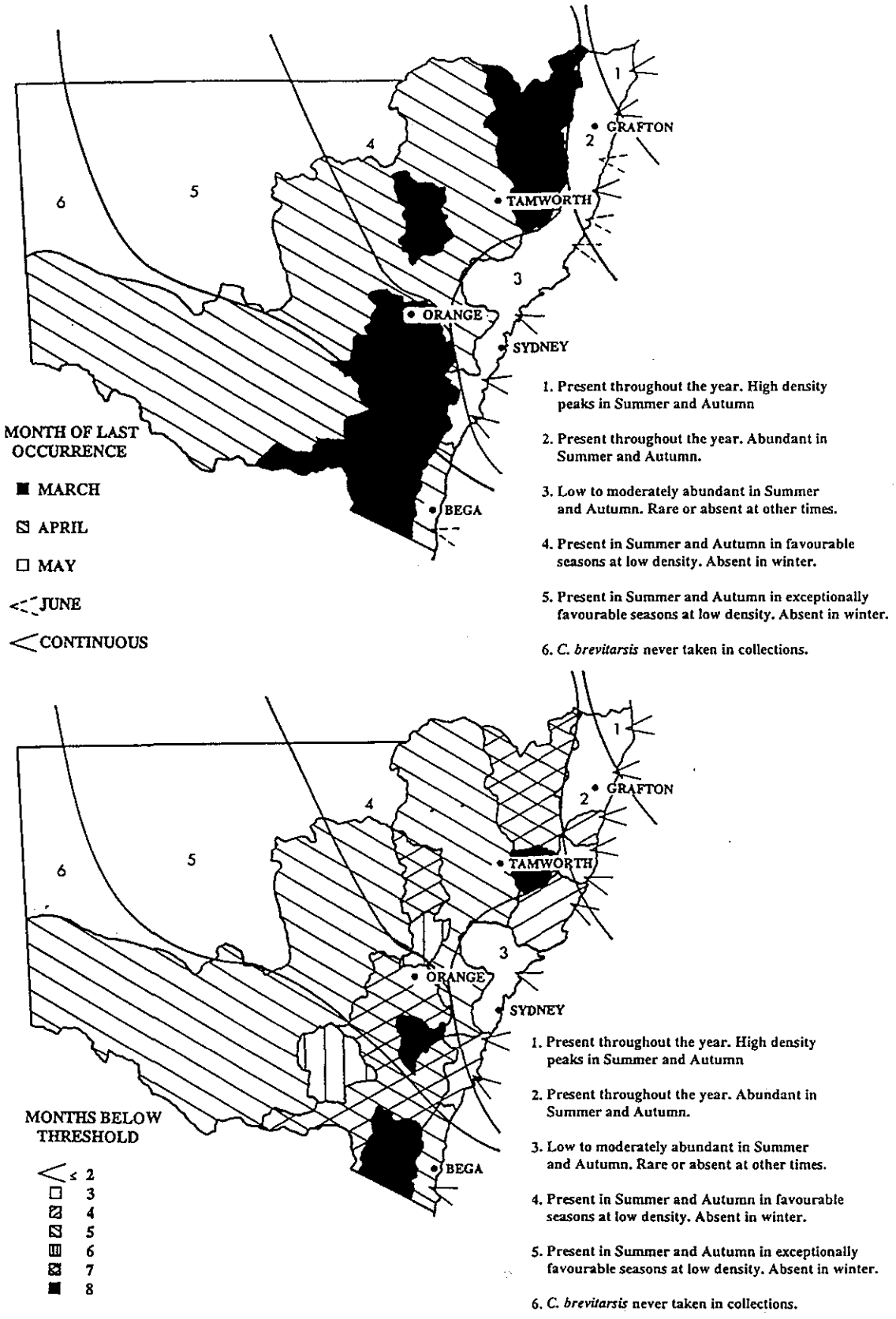

Figure 13. Areas of NSW showing the month when *Culicoides brevitarsis* should last occur and the number of months when temperatures are below activity thresholds. Maps are overlaid with modified 'brevitarsis lines'.

the chance of survival is greatest were evident along the coast and in the north west.

It is essential to recognise that the predictions for western NSW should be treated with caution. This region was not studied as part of this research program. It is also well documented that *C. brevitarsis* has great difficulty in reaching these areas because of distance and the fact that low rainfall adds to the inhibition caused by temperature. The maps have therefore been overlaid with "brevitarsis lines" which now designate six (6) regions. Modifications have been made to the original lines in the following cases:

1) The western line designating region 2 has been moved eastwards to coincide with the north-eastern escarpment. The chance of presence and survival west of the escarpment on the northern tablelands is extremely low.
2) A new region (3) is proposed. This is based on information derived from this study and can be termed the marginal area.

It is proposed that the currently accepted "brevitarsis lines" should still apply to western NSW. It is only in extremely favourable years that *C. brevitarsis* has reached region 5 while the species has never been recorded in region 6. In addition two other potentially important conclusions were made from this study.

1) Sites very close to the coast (as far south as the Victorian border and beyond) were potential areas for foci for *C. brevitarsis*. These areas extended less than 10km inland. The significance of these areas urgently requires further consideration as they could alter our concepts of the vector's distribution and therefore the epidemiology of the viruses it transmits.
2) Probability data were also estimated to simulate an arbitrary rise in temperature of 2°C (e.g. the proposed effect of global warming resulting from the greenhouse effect). Even such a small rise could significantly alter the potential distribution of *C. brevitarsis,* change strategies for disease control and affect the designation of vector free areas. For example, a 2°C rise in temperature at Nowra could change the probability of survival from 0.8% to 90.0%.

Study 3.2 - Distribution and seasonal movements of C. brevitarsis *and its association with arboviruses.*

This study was conducted to examine the seasonal movements, establishment and increase in numbers of *C. brevitarsis* in the study area. Data collected prior to the commencement of this project have been included to allow a more substantial comparison of vector and virus data. Light trap samples were taken at selected sites

from October to May in 1990-91 to 1994-95.

1990-93: From 1990-1993, the most northerly trap was located at Taree. Data were analysed to consider:

1) The first occurrence of *C. brevitarsis* at sampling sites
2) The distribution of its total numbers, sexes and parous stages at the different sites
3) Associations between *C. brevitarsis* and virus transmissions.

The results from this period are rather complex and a full account is given in publication 8. A summary is given below.

There was a distinct pattern of progression from the Manning region to the Hunter Valley and down the southern coastal plain each season (Figure 14). Distributions were compared by averaging data from sites within six zones. These zones were separated by the month that *C. brevitarsis* was recorded at a site for the first time. Numbers of adults and generations were always similar within the zones and this allowed the data to be combined for analysis . The species was most abundant in autumn with abundance and the number of generations becoming progressively lower away from the Manning region. The number of generations ranged from seven in zone 1 to zero in zone 6. Average generation time was 4.1 weeks. Temperatures affected population growth and were suitable for varying periods within the study area. Wind patterns consistent with the direction of the species' progression occurred for most of the season. However, there were no clearly defined effects of rainfall.

The incidence of infection of cattle with Akabane and Palyam viruses was high in both 1990-91 and 1992-93. Peaton virus was the only virus detected at a high incidence in 1991-92. The first occurrences of the viruses occurred an average 3-4 months after the vector although they were positively associated with its first occurrence at sampling sites.

Many factors relating to the dispersal of *C. brevitarsis* and its association with the transmission of Akabane and Palyam group viruses were evident. The most important of these are further summarised below.

- Distinct patterns of progression were recorded each season. These patterns were consistent with gradual and relatively slow movements by adult midges.
- The midge was first recorded at Taree in the Manning region in November each year. It was considered that this was due to reinfestations from further north as all the evidence suggested that *C. brevitarsis* failed to survive in the study area.
- Movements occur when environmental conditions are favourable for sustained activity and an increase in population density. Movement was probably aided by

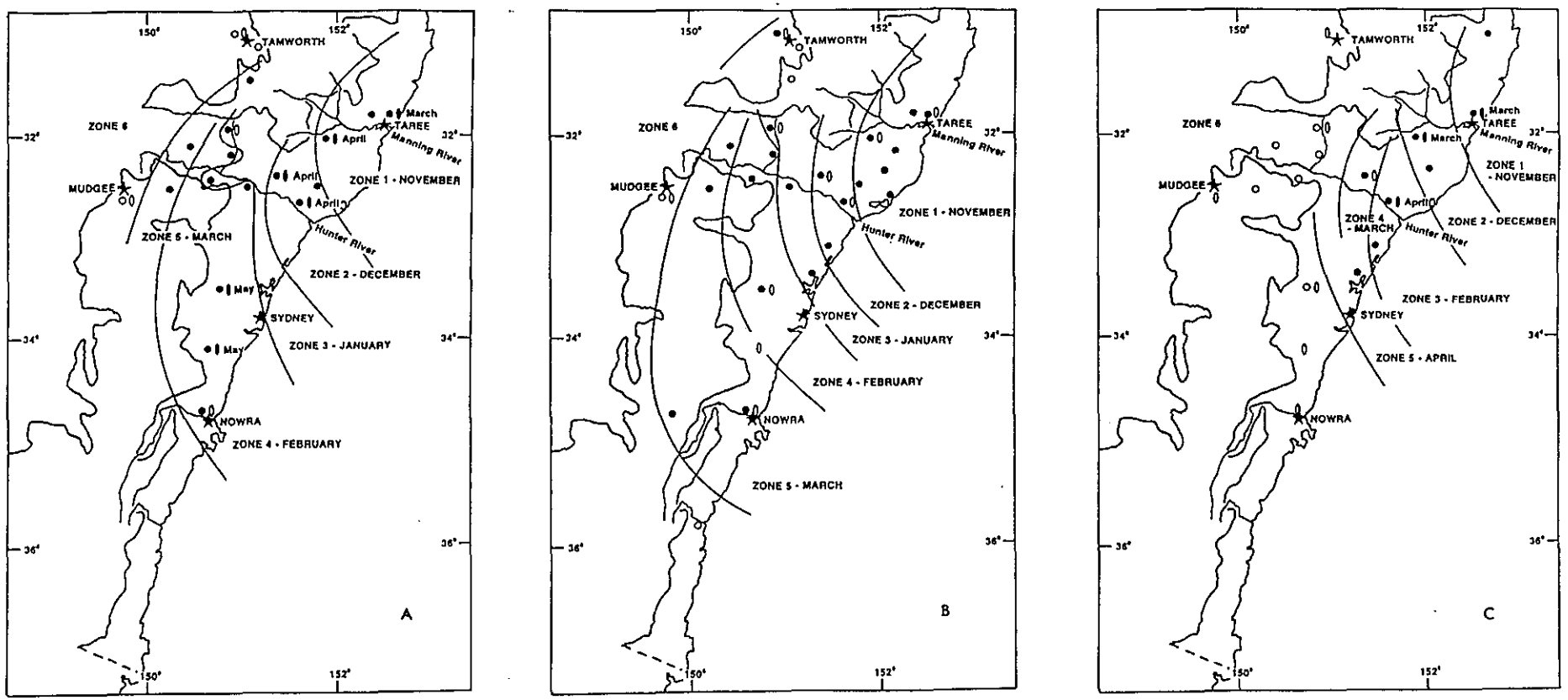

Figure 14. Monthly spread of *Culicoides brevitarsis* (● present, ○ absent) and Akabane virus (⬮ present, ⬯ absent) in A) 1990 - 91, B) 1991 - 92 and C) 1992 - 93. Zones are based on the first occurrence of *C. brevitarsis*. Month of first occurrence of Akabane is given at each site.

wind currents which were favourable for the type of dispersal that we recorded. This is different from the occasional rapid and relatively long distance movements described by others. Wind patterns were variable and would also have allowed northerly movements by the vector.

- There was a close relationship between movement of the vector and the spread of Akabane virus despite there being considerable delay between their first occurrences.
- Akabane virus was not present at high incidence each year. In 1991-92 there was a high incidence of Peaton virus . It is possible that the early occurrence of Peaton virus limited Akabane virus transmission due to the phenomenon of "interference" at the vector level. When interference occurs, the earlier infection of a vector with another virus, usually closely related, prevents the vector becoming infected with a second virus. This can have implications for the occurrence of disease as the proportion of Akabane-susceptible livestock would have increased.
- The time of occurrence of *C. brevitarsis* was relatively consistent each year. It therefore appeared possible that its dispersal could be modelled and the models used to predict future events.

1993-94:- This study was an extension of the work carried out from 1990-93. Areas on the north/mid-north coast were added and a slightly reduced number of sites south of Taree were retained. The most northerly light trap was located at Casino. Full details of the study and results are given in publication 9.

The occurrence of *C. brevitarsis* was recorded progressively from endemic areas on the north coast to Nowra in the south and in the Hunter Valley west to Scone (Figure 15). The species was again first present at Taree in November. Dispersal of *C. brevitarsis* south of Taree was then consistent with the three previous seasons except for its presence at Paterson in September. To be present at Paterson in September, the species must have survived through the preceding winter. This is the first documented evidence of the development of a focus of the vector in the study area although it had been suspected previously by others. Weather conditions (i.e. higher than average temperatures and rainfall during winter) favoured the species survival. However, its early presence at Paterson had no obvious effect on the broad pattern of its dispersal or on the occurrence of virus.

Akabane and bluetongue viruses were recorded and were found only within the recorded limits of the vector's distribution (Figure 16). These viruses started from foci near Coffs Harbour (See Table 8). Their first occurrences at light trap sites (determined from seroconversions in sentinel cattle at selected sites) were associated

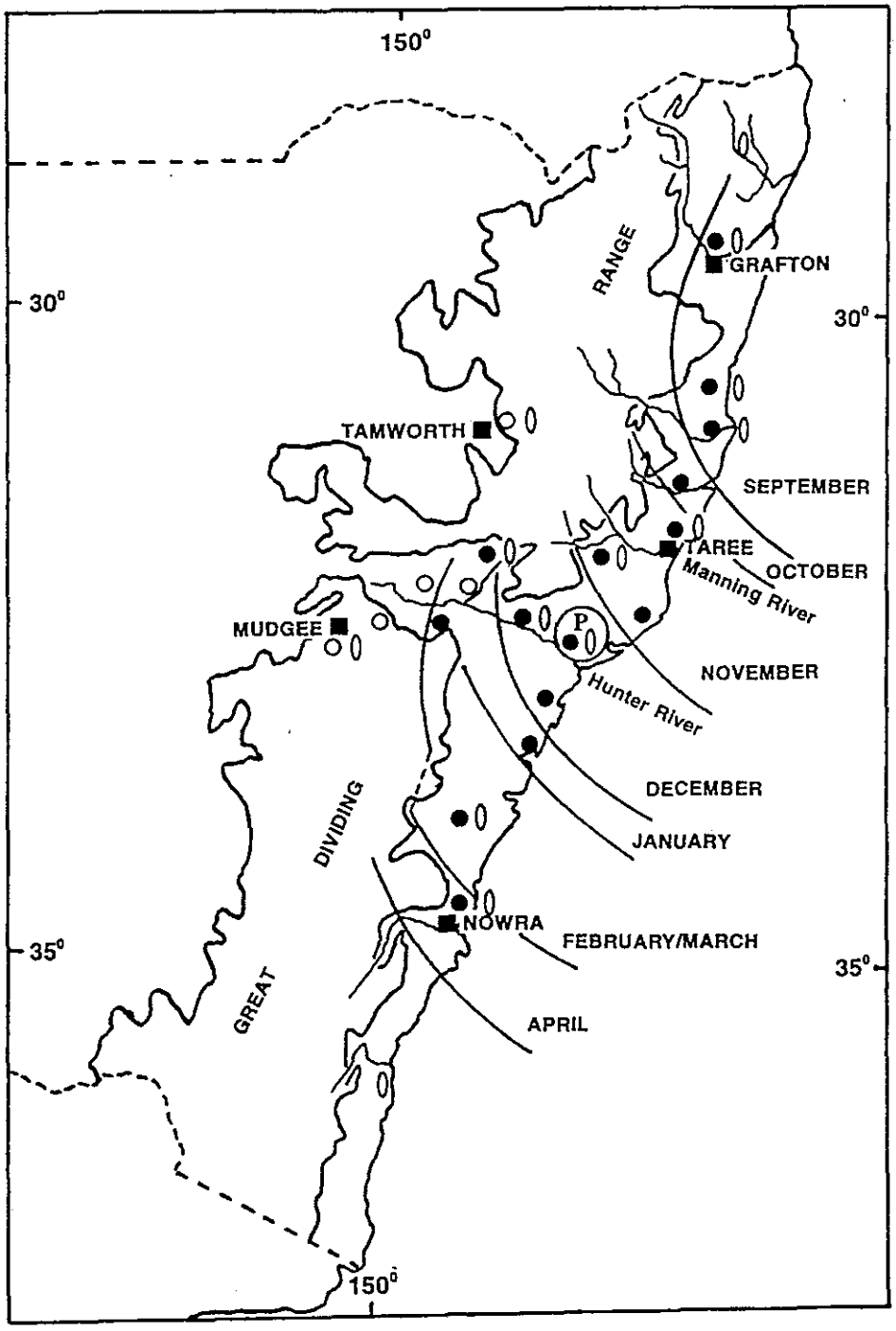

Figure 15. Light trap (circles) and sentinel herd locations (ellipses) with the first occurrence (month) of *Culicoides brevitarsis* (● present, ○ absent) in eastern NSW in 1993 - 94. Large circle around Paterson (P) indicates the first occurrence of *C. brevitarsis* in September. ■ = major centres for reference.

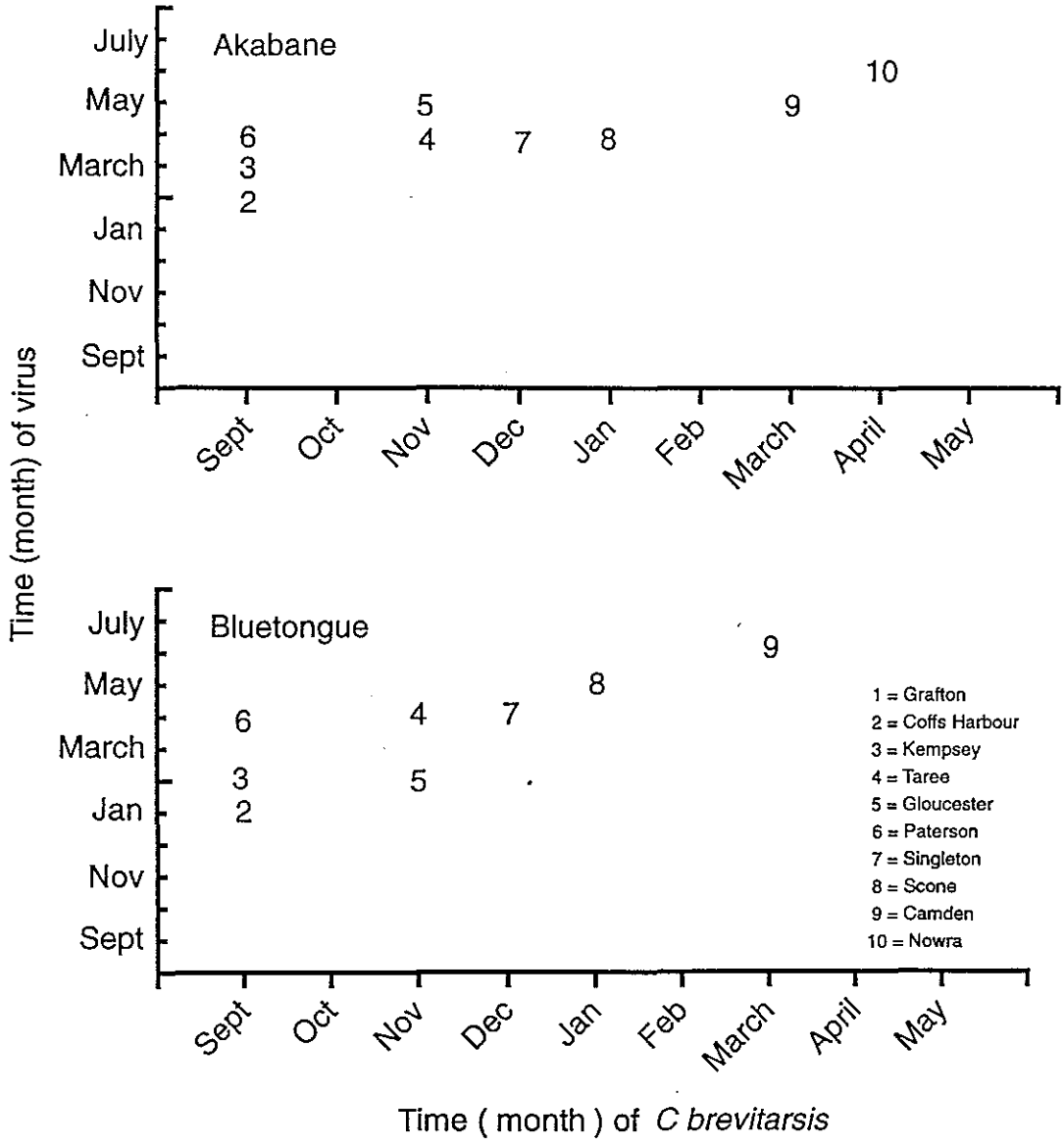

Figure 16. Months that *Culicoides brevitarsis* and two arboviruses first occurred at vector/ virus sampling locations in eastern NSW in 1993 - 94. Locations are arranged (1 to 10) in ascending order of distance from Grafton on the north coast.

with the dispersal of *C. brevitarsis* but were not associated with each other (i.e. they spread independently despite originating in approximately the same area). There were delays of 2 to 7 months between the times that the vector and the viruses were first recorded. The delays decreased away from the points of focus and were negatively associated with the initial dispersal of the vector.

Bovine ephemeral fever virus was also recorded in 1993-94. Infection started in the Hunter Manning region, initially near Taree. At Paterson infection was widespread and at high incidence and there was some evidence that the early transmission of the virus was associated with the presence of *C. brevitarsis*. However, bovine ephemeral fever was also found as far west as Dubbo, well beyond the observed distribution of *C. brevitarsis*. This data again confirms that ephemeral fever virus is spread by vectors other than *C. brevitarsis*. Mosquitoes are believed to be the principal vectors.

1994-95:- This study continued the emphasis on sampling from the north and mid-north coasts. The progression of *C. brevitarsis* in this season can be seen in Figure 17.

The coastal area was severely drought affected in the 1994 half of the season. As a consequence, distribution on the north coast, where the species is regarded as being endemic, was not as clear as in 1993-94. *C. brevitarsis* was more focal, with some locations not recording the species until later in the season (e.g. Grafton in December compared with September in 1993-94). The pattern south of Taree was similar to the previous four seasons of monitoring. Limits to its distribution were Nowra in the south and west at Scone in the Hunter Valley. Associations with viruses were unclear as transmissions and spread were limited to the lower Hunter region.

Several important conclusions were made from these years of study. They have particular relevance to our understanding of events in areas at the southern and western limits to *Culicoides* distribution in NSW.

- Data for the distribution of the vector south from Taree were relatively consistent over 5 years. This enhanced the chance that dispersion could be modelled and the models used to predict the time when *Culicoides brevitarsis* could be expected in an area.
- Normal movement is gradual and occurs relatively slowly.
- Preliminary data suggest that presence at Taree results from the dispersal of *C. brevitarsis* from endemic areas on the north coast. This would require confirmation from several more years of data.
- Under the relatively dry conditions we have experienced between 1990 and 1995, *C. brevitarsis* did not reach the inland slopes and tablelands via the Hunter Valley.

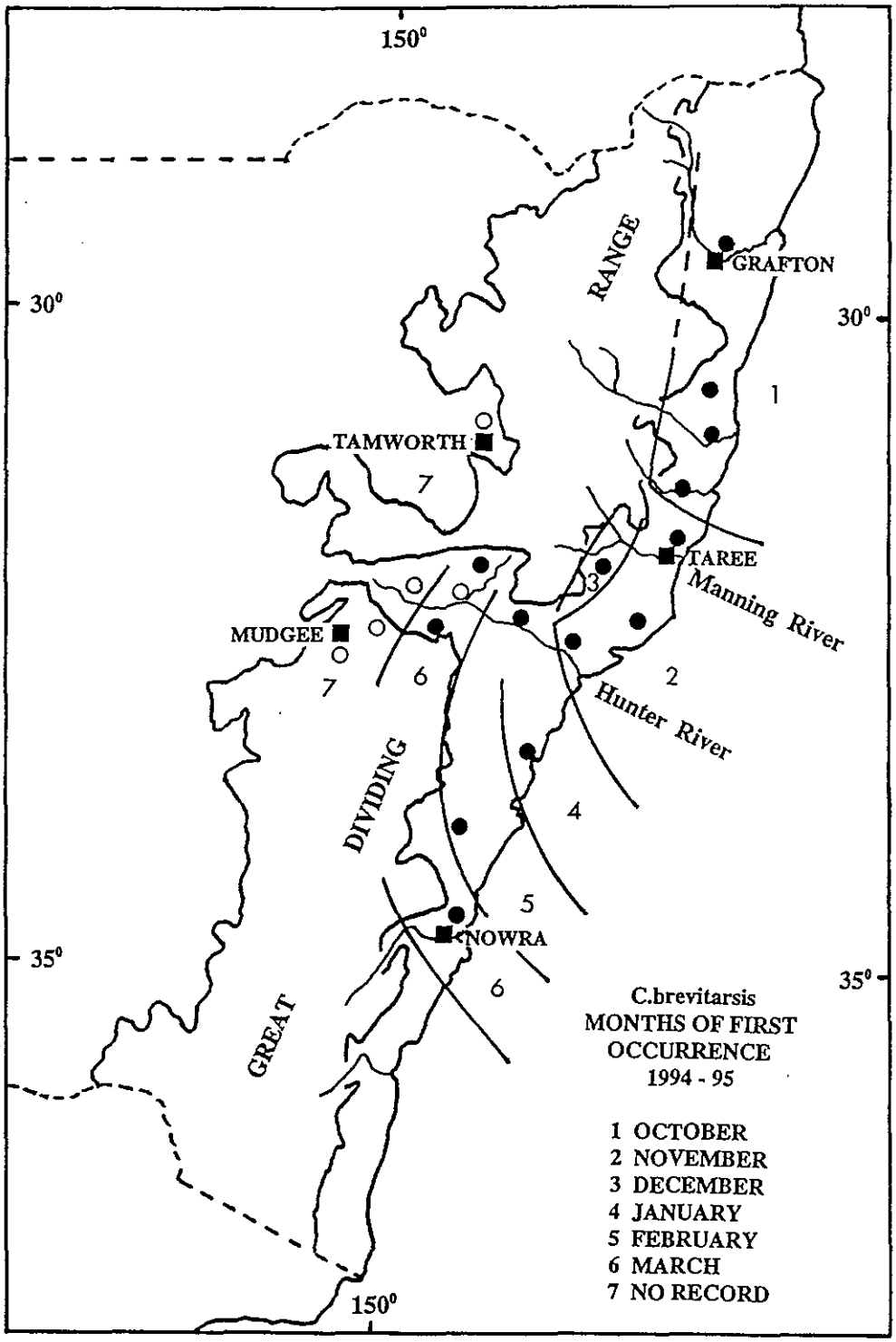

Figure 17. Light trap locations with the first occurrence of *Culicoides brevitarsis* (● present, ○ absent) in eastern NSW in 1994 - 95. ■ = major certres for reference.

- There is evidence to indicate that the dynamics of transmission of viruses differs and that viruses spread independently of each other. In fact, there is some evidence that there may be antagonism ("interference") when 2 viruses enter the vector population at about the same time. Further, with differences in factors such as vector competence, variations between the patterns of spread of 2 or more viruses are to be expected.
- The concurrent spread of *C. brevitarsis* and bluetongue virus is described for the first time in eastern Australia.
- There was conclusive evidence that *C. brevitarsis* can sometimes survive in foci close to the southern limit to its distribution. This has long been suspected and was predicted in study 3.1. However, survival in foci may not be as important in this area as expected. For example:
  - In 1993-94 the *C. brevitarsis* population developed slowly in the focal area at Paterson and only expanded significantly in numbers when immigrating adults were also expected at the site.
  - The apparent over-wintering of *C. brevitarsis* at Paterson (as evidenced by the isolated focal detection of midges at Paterson in September) did not change the spread of any of the viruses even though Akabane had been present at the end of the season in 1992-93 (see Figure 18).
  - There is no evidence to date suggest that viruses have been retained and transmitted by surviving (overwintering) populations of the vector.

  Perhaps only in very favourable seasons could these foci be important, when rapid amplifications in insect numbers result in significant populations.
- Associations between vector and virus have enabled a number of hypotheses explaining the epidemiology of the viruses to be made:
  - During winter viruses are distributed in foci in endemic areas even though the vector may be spread more uniformly.
  - Viruses may not be present when *C. brevitarsis* disperses initially. In some years they may not be present in the vector population at all, or transmission occurs at levels below the limits of the detection system.
  - Viruses appear to spread once a threshold population of the vector is capable of sustaining transmission.
  - There is a significant delay between the times that the vector is observed and virus infections are first recorded. This may have a major impact on the vectors ability to transmit viruses because there may be insufficient time for transmission at the end of the season when declining temperatures limit and stop vector activity.
  - Viruses appear to be spread more quickly or more efficiently later in the season. This could be aided by continuous migrations of *C. brevitarsis* with the number and proportion of infected individuals increasing as conditions

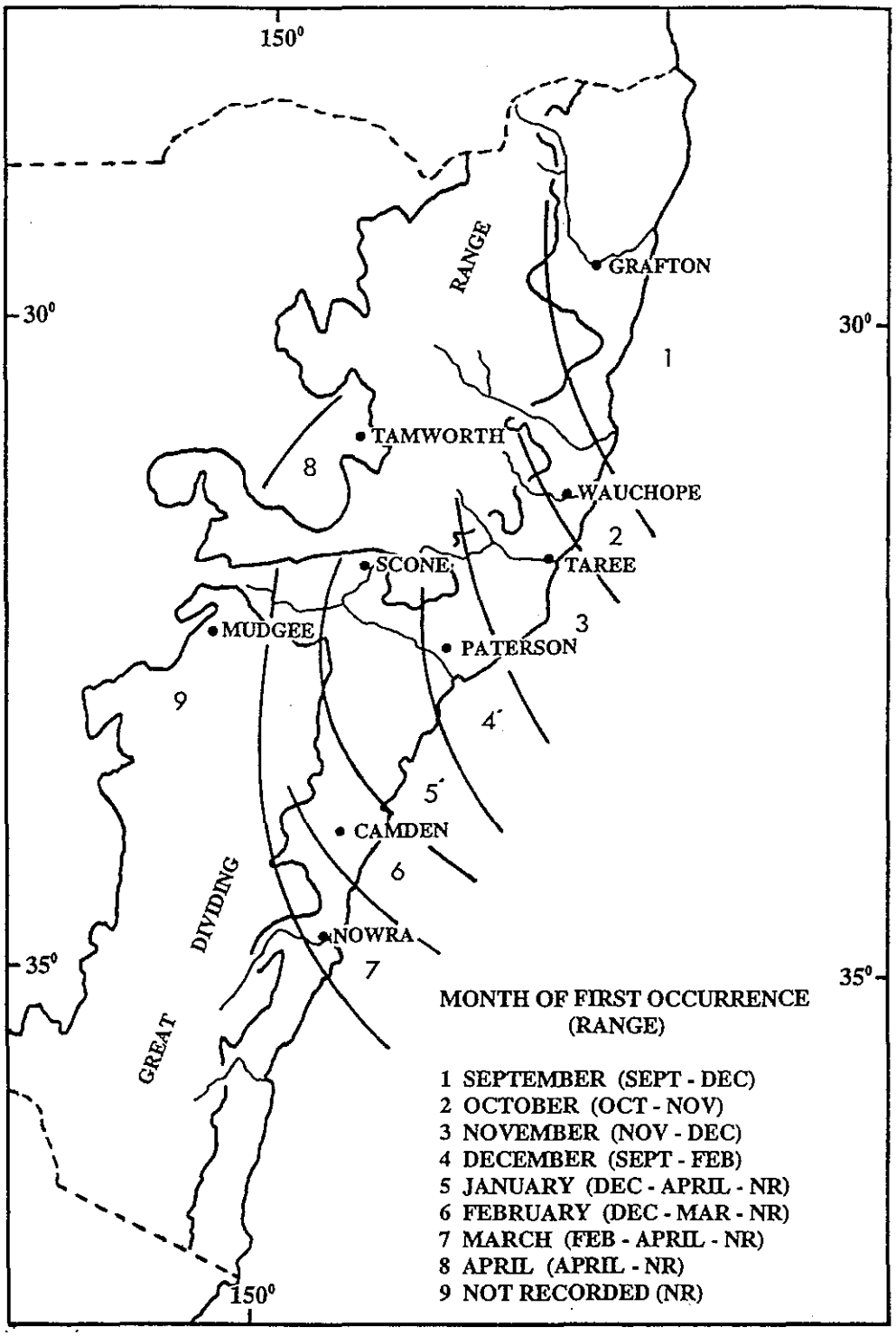

Figure 18. Expected first occurrence (month plus a range of possible variations given in brackets) of *Culicoides brevitarsis* in eastern NSW.

improve the vectors chances of surviving and establishing in new locations.

- Associations between vector and viruses would have to be attempted over several more years to allow predictions to be made confidently. Further, there are other factors that further confound virus/vector interactions. These include immunity in the host population and interactions (possibly interference) between 2 or more viruses that may enter the population concurrently.

Study 3.3 - Modelling distribution and population growth

Our aim was to provide:

1) A model describing the progression/dispersal of *C. brevitarsis* in eastern New South Wales. In this study, we concentrated our model on the area from Taree to the Hunter Valley and southern coastal plain.
2) A model to describe population growth at each sampling site in the study area. Site models were tested to see if they can be combined to represent population growth in the zones describing the dispersal of the species.
3) A comparison of our model of dispersal and the GROWEST model that has been used to predict the "brevitarsis lines" in the study area.

***Study 3.3.1.1 - Static model of the distribution and dispersal of* C. brevitarsis.**

The average month of first occurrence of *C. brevitarsis* over the five seasons 1990-1995 was calculated at all sampling sites. Sites where the species was present in the same month were allocated to zones (Figure 18) similar to those presented as the seasonal zone data in Figures 14, 15 and 17. The information on the mid-north and north coasts are based on a limited number of seasons and require several more seasons for validation.

Figure 18 is seen as a practical way of representing the time that *C. brevitarsis* would be expected to occur within the study area. Some variation may be expected from year to year. However, based on the differences we recorded, the variation would most likely result in the later arrival of *C. brevitarsis* or in the species not being present at all.

In a second analysis, distance from Taree was used to predict the month of first occurrence of *C. brevitarsis* in a simple linear regression in which 74.5% of the variance in the month of occurrence was accounted for by the following model:

Month = 0.754 + 0.0133 Distance

Predictions fell within the zones of the static model (Figure 18) when plotted on a distribution map.

***Study 3.3.1.2 - Dynamic model of the distribution and dispersal of* C. brevitarsis.**
The model was developed in three phases. Firstly, a preliminary model was to be developed using the data on the time of first occurrences of *C. brevitarsis* at each site for the three seasons from 1990/91 to 1992/93. This was then to be used to predict probable events in the study area in the subsequent two seasons 1993/94 and 1994/95 based on the first occurrence of *C. brevitarsis* at Taree. In the third phase, data from the five years were to be used to form a composite model.

Two models were considered at the preliminary phase. The aim was to first model the dispersal of *C. brevitarsis* based on environmental factors. This would represent an ecological interpretation of dispersal where dispersal was defined as the distance travelled by the midge in one day. The model describing this was then derived using the following information:

1. Month in which most of the dispersal between two locations would have taken place.
2. Wind direction from the original site to the destination site (e.g. from north, the north-east, east etc.)
3. Wind frequency = the number of occasions the wind blew from the recorder direction (as in 2 above) during the relevant month (from 1 above).
4. Wind "amount" = wind frequency x wind speed.
5. Monthly average temperature
6. Monthly total rainfall.

The preliminary model (model 1) has accounted for 85.7% of the variation of dispersal. Wind "amount' was excluded from the model as high wind speeds appeared to have a negative effect on dispersal. This is consistent with previous knowledge that flight is inhibited above a wind-speed threshold. This model confirmed that the observed seasonal movements by *C. brevitarsis* in the 1990 to 1993 seasons were dependent on suitable environmental conditions and that these conditions were relatively consistent in each of the years of study. However, it was considered that the efficiency of model 1 as a predictive tool would still be limited by the user not knowing future weather events. A second model (model 2) was therefore developed using distance, direction and year by dividing the sites into groups dependent on a specific wind direction to move the species to the sites. The fitted model was:

$$\log_e (\text{days}) = a + b \times \log_e (\text{distance})$$

The model accounted for 90.2% of the variation of the data.

First occurrences in the seasons 1993-95 were examined retrospectively and were found to be within the ranges predicted by the preliminary models. Data from these years were added to the data from model 1 which was also refined to include the following:

1. Movement in different directions being dependent on pivotal sites which account for geographical factors (e.g. mountain barriers and urban areas) which prevented direct dispersal through the region.
2. Month as a measure of time to better relate to the monthly weather data.
3. Estimations of the speed (= distance/time) of dispersal through the region.

Using Taree as the starting point, the natural logarithm of the speed of dispersal of *C. brevitarsis* was modelled using multiple regression of year, average temperature, total rainfall, wind information (direction and speed) at the pivotal points plus their interaction. The model accounted for 74.7% of the variation in log speed. The time in months that *C. brevitarsis* would be at different distances away from Taree was then calculated from the speed of dispersal estimated from the regression model. As an example, the speed that *C. brevitarsis* moved up the Hunter Valley towards Mudgee was estimated at 42km/month in 1990/91. The time taken to reach Mudgee would have been 7.3 months after being at Taree in November. This meant that the vector would possibly have arrived in Mudgee in May-June at which time low temperatures would have prevented any activity or establishment. *C. brevitarsis* adults were not recorded in light traps located at Mudgee in 1990/91 (or in any other season).

The environmental models make it possible for dispersal to be considered retrospectively by using historical weather data. This may help our understanding of past events but has limited use for predictive purposes as future weather events are unknown. However, each of the models we developed describe the first occurrence of *C. brevitarsis* occurring at sites within the range of months given in Figure 18. This figure would therefore be the most practical and effective way of predicting the dispersal of *C. brevitarsis* from Taree through the Hunter Valley and down the southern coastal plain. Data taken over several more seasons should be added to strengthen and validate the current models. Data from seasons with more favourable weather conditions must be included.

Study 3.3.2 - Modelling population growth.

The study was aimed at providing a model that could be used to estimate population density at different times after *C. brevitarsis* is first recorded in an area. Such a model would be important if densities at which vector populations are capable of moving

successfully and/or sustaining virus transmissions could be established. Data at sites were successfully modelled as population curves. Generations were included by using a sine-wave analysis. However, these data could not be applied for predictive purposes because:

- Models were site and season specific, i.e. a composite model could not be developed to predict population growth across the study area in each season. Predictions at individual sites were dependent on future weather conditions and unknown site factors.
- We were unable to establish consistent associations between vector numbers and virus transmissions from the field data (See publication 8). If these exist, they may have to be determined under controlled laboratory conditions using susceptible livestock.
- It was impossible to determine densities at which migrations of vectors may begin and be sustained from our study.

Study 3.3.3 - Comparison of distribution models

The GROWEST model has been used to define the southern limits of distribution and abundance of *C. brevitarsis* in south-eastern Australia. Essentially, the model is dependent on the survival of *C. brevitarsis* (at least in foci) throughout the area up to the limits to its distribution.

Our data indicate that survival is commonly restricted to endemic areas on the north and possibly the mid-north coast of NSW. Our models of distribution are therefore based on dispersal over areas that are essentially vector free until the time that movements by the vector are stopped by environmental conditions at the end of the season.

This research aims to test the two theories explaining the distribution of *C. brevitarsis* by comparing predictions made from both models with actual data. This study is being carried out in co-operation with Durno Murray who devised the current method of defining the distribution of *C. brevitarsis* within "brevitarsis lines". Unfortunately the computer programming used by Dr Murray is virtually obsolete and has had to be extensively modified so that this comparative study can be undertaken. This has taken substantially more time than originally expected and has delayed the actual scientific comparisons. Consequently, it has been decided to present these results as an appendix to this report when the work has been completed, rather than delay the presentation of this report.

GENERAL DISCUSSION.

A number of strategies may be implemented to minimise problems associated with arboviruses transmitted by the biting midge, *Culicoides brevitarsis*. These include using vaccines, treating stock with insecticides, housing stud animals and defining periods/areas that are vector free. To do this successfully, producers and exporters of livestock must be able to understand and predict the seasonal presence and behaviour of the midge. This may be achieved by their knowing the following:

- The probability that *C. brevitarsis* can survive at any particular location from year to year, i.e. where the species cannot survive, its presence is dependent on migrations from some other location
- The time when *C. brevitarsis* is likely to be first present at their location
- The time when the vector is most likely to be feeding during the day
- The time when the vector is no longer present or active at the end of the season
- The relationships between the vector and viruses.

In concert, this information would provide the basis for making decisions on disease prevention and define vector/virus free zones for exports. In particular, not only is it possible to define geographical areas which are annually free of vectors but also define areas which are temporally free of vector activity. This "window" of vector freedom can be a period as short as 2-3 months or can be as long as 8-10 months, depending on the season and location. However, in the promotion of virus and vector free areas, extreme care should be taken to distinguish between Bovine Ephemeral Fever virus and the *Culicoides*-borne viruses. With a probable mosquito vector, Ephemeral Fever can be found well beyond the limits of the *Culicoides* infested area, occasionally in South Australia or even Victoria.

The probability of *C. brevitarsis* surviving through winter in NSW can be determined from Figure 13 (months below threshold) (see also Table 1 in Publication 7). Areas where there are three months or less below temperature thresholds inhibiting activity have some probability that survival may occur.

- The probability of survival is highest on the far north coast and in pockets close to the coastline.
- The inference that survival can occur in western NSW may be ignored because factors other than temperature affect presence in this area.
- Survival beyond the normal endemic area may occur occasionally when,
  - Average monthly temperatures are not below the thresholds for more than two consecutive months (see study 1.6), and
  - There are short periods of warm weather (several days above the threshold

temperatures) during winter months (see study 3.2).

- Predictions made from these data (and the predictions on the month *C. brevitarsis* should last occur) are dependent on temperature and are not location dependent. It is therefore possible to apply them to locations in other Australian states and to countries like New Zealand where *C. brevitarsis* is a potential pest. As in western NSW, predictions elsewhere require clarification and must be considered in relation to the ability of the vector species to migrate and to the possible effects of environmental factors other than temperature (e.g. rainfall).

The month that *C. brevitarsis* can be expected to be found for the first time in eastern NSW is given in Figure 18. This is a static model of the vector's dispersal based on five years of data. It is the most practical way of representing the dispersal of *C. brevitarsis* and of demarcating the end of the vector free period and the start to the period when livestock would most likely be at risk. Information on first presence could possibly be augmented by predictions of population growth after establishment. This would aid the decision process and extend the virus free period if it can be shown when the vector populations are capable of sustaining virus transmissions and causing disease outbreaks (i.e. if virus activity is dependent on the density of the vector). Conversely, these data can be used to define times of vector freedom ("Temporal Freedom") within a normally endemic area. Definition of a free area in this way would expand the pool of livestock eligible for export when arbovirus infections are a limiting factor. Further, the lag phase between re-occurrence of the vector and first virus transmission adds a buffer and increased confidence in the safety of a zone defined by the concept of "Temporal Freedom".

There may be some variations in the time that *C. brevitarsis* may be first present at any location:

- At the limits of vector distribution, late presence (arrival due to migration) is the more likely to be encountered. Complete absence in a particular year is also possible. The chance of the vector not being present would increase away from endemic areas on the north coast.
- Even in "endemic" areas, late presence is possible especially under drought conditions, i.e. the number of foci in which the vector survives is reduced. The vector must therefore spread within the endemic area to locations where it may survive in normal seasons.
- Early presence is possible if the species can occasionally survive through winter. This early presence is most likely to occur at a focal location. It may not be as important to the spread of the vector or viruses as first thought (see Study 3.2 - 1993-94).

- Early presence and presence beyond predicted limits are occasionally possible when weather conditions are extremely favourable [e.g. Murray and Kirkland (1995). *Aust. Vet. J.* 72:56] and may be due to "abnormal" long distance dispersal by wind.

The time when *C. brevitarsis* should cease its activity throughout NSW can be ascertained from Figure 13 (month of last occurrence). This information can be used to demarcate the expected end to the period when livestock are at risk and the start of the vector free period.

- Almost all activity by *C. brevitarsis* in NSW could be expected to cease or be limited after May.
- Adult activity may occasionally extend into June at some coastal locations (see Study 1.6). The longer *C. brevitarsis* remains active at the end of the season, the greater is the chance that there would be less than two consecutive months below the temperature thresholds which inhibit survival. The potential for overwintering then increases.
- Activity in western NSW is subject to the same limitations of interpretation placed on the ability of the species to survive from year to year.
- Predictions can also be made for the possible cessation of activity in other states, as these data are also temperature and not site dependent. However, the potential for the vector to actually be present at a site and the effects of local environmental factors (e.g. temperature and rainfall) on establishment and development must be considered.

Conclusions

The data generated during this project provides an understanding of the factors affecting the dispersal, activity and survival of *C. brevitarsis*. Further, information is now available on the probability of the likelihood of survival of this midge in both coastal and inland areas. Concepts that will help us to understand the epidemiology of viruses through their relationship with the vector have been developed. Some concepts have an immediate application; others require additional research before conclusions can be made confidently. However:

- Viruses are likely to be maintained in foci (mechanism unknown) in the 'endemic' area even though the vector may be spread more widely ie all surviving vector populations may not retain the viruses. Vector distribution in the 'endemic' area may also be inconsistent especially when conditions are adverse (e.g. drought affected). It would therefore be difficult to establish

starting points for monitoring and predicting movements by both the vector and the virus from within the 'endemic' area.

- Virus presence is inconsistent and, in some years, particular viruses may be absent. Monitoring of vectors may therefore have little significance unless viruses are monitored concurrently.
- Akabane and bluetongue viruses spread in a pattern similar to that of *C. brevitarsis*. They also appear to spread independently of each other.
- Relationships between vector and virus could be further expanded if techniques could be developed to rapidly identify viruses in *C. brevitarsis* caught in light traps. This has been done with some viruses and other species of *Culicoides*. However, these techniques must be amenable to processing of large numbers of samples quickly and economically. If successful, the procedure could become part of a monitoring program (e.g. The NAMP). Vector and virus movements could be detected quickly and warnings given.
- Viruses were recorded in sentinel herds 2 to 7 months after the vectors were first present (referred to as "delay period"). It does not appear that the viruses are present when the vector disperses initially (or transmission is below the limits of detection). The relationships we have established to describe the dispersal of *C brevitarsis* are believed to reflect continuous and similar movements throughout the season.
- It was shown that the "delay period" to virus transmission was negatively related to the dispersal of *C. brevitarsis*. This was consistent with the expectation that establishment, population growth and higher proportions of infected individuals causing the viruses to be spread more quickly and effectively would increase as the season progressed and conditions became more favourable. Therefore, once virus presence is confirmed (and more data validate the associations between vector and virus), it may be possible to predict when virus would be expected in areas beyond a designated starting point.

In addition to the availability of predictive models which will optimise vector monitoring, low cost, sensitive rapid diagnostic tests to detect antibodies to key viruses have been developed. These can be used to confirm predictions of vector distribution and to delineate effective vector populations. These trends may given a valuable insight into the potential for an epidemic of Bluetongue if a pathogenic Bluetongue virus entered NSW.

In any season, the combined distribution of the Simbu viruses illustrates the range of the effective *C. brevitarsis* population in that year and also the extent to which Bluetongue virus could have been spread. In a similar manner, the transmission times

for these viruses may indicate the earliest time for the first occurrence of an epidemic of Bluetongue and the duration of a potential epidemic. It is considered that these would be probable limits for a potential epidemic, especially as Bluetongue virus is likely to be transmitted less efficiently. In reality, a Bluetongue epidemic would be likely to commence later and for there to be a lower incidence.

These studies have shown that there are distinct seasonal patterns of arbovirus transmission in Southern Australia and that it is feasible to define vector/virus free regions by the use of a model combined with strategic vector collections and by monitoring sentinel cattle for a range of viruses borne by *C. brevitarsis*. Maps of the limits of arbovirus and vector distribution can then be accurately defined from a composite of the distribution of the *Culicoides* borne viruses. Further, data can be provided on the probability of vector survival at a specified location and the likely length of vector-free periods within current vector areas. As a consequence, it is practical to develop and promote the concept of virus and vector free regions on both geographical and temporal bases to support trade from previously restricted areas. No longer do southern state boundaries need to be southern limits of *Culicoides* borne viruses.

However, in the promotion of virus and vector free areas, extreme care should be taken to distinguish between Bovine Ephemeral Fever virus and the *Culicoides*-borne viruses. With a probable mosquito vector, Ephemeral Fever can be found well beyond the limits of the *Culicoides* endemic area, occasionally in South Australia or even Victoria.

ACHIEVEMENT OF OBJECTIVES

All of the objectives for this project were clearly achieved. While a component of one objective was to compare a computer forecasting system for *C. brevitarsis* with an older model produced by CSIRO, it was not possible to carry out these comparisons in the manner originally planned. This was due to the need to re-write the original CSIRO computer programs in software compatible with modern computer systems. However, the breadth of the ecological studies was significantly greater than originally envisaged and several predictive models were developed and evaluated. In respect of the individual objectives, the following is a statement of achievements. Full details are documented in the preceding description of results.

1. **"Determine the ecological constraints for the survival, increase and spread of *Culicoides brevitarsis* in temperate Australia";**

A range of potential habitats for *Culicoides brevitarsis* were sampled using different collection methods throughout the day and night. Data were collected on the movements and abundance of midges at different times of the day and related to changes in temperature and humidity. Subsequently, extensive studies were conducted to determine the effects of temperature on the long-term survival and subsequent development into adults of midge larvae in cattle dung. Much of this data has contributed to the definition of the potential of the midge to survive from one year to the next (over-wintering). It is now possible to determine the number of months that the midge can survive in different regions throughout NSW and also the last month that is conducive for midge survival in that location. This information can be used to provide advice for disease control on the probability of a disease outbreak in a given area and, if disease occurs, the length of time that transmission could continue.

2. **"Develop low cost sensitive systems for the definition of virus distribution";**

In the virology components of the project, new diagnostic tests for the detection of antibodies to viruses of the Simbu serogroup were successfully developed. Two separate competitive ELISA tests were evaluated, one to detect antibodies to any of the five viruses in this group which infect farm livestock in Australia, the other to detect antibodies specifically to Akabane virus, the most significant virus in this group. These tests were shown to be sensitive and specific when compared with conventionally used virus neutralisation tests. However, the cELISA tests can be conducted in one day compared to 5 for the VN test and cost about one quarter of the cost of the VN test.

3. **"Refine a computer forecasting system for virus/vector distribution".**

From the studies conducted towards the achievement of Objective 1, the factors affecting the survival, increase and spread of *Culicoides brevitarsis* have been determined and supported the development of predictive models. From this data, models were successfully developed which can be used to:

- predict the month in which *C. brevitarsis* could first enter a district;
- predict the dispersal of the midge in eastern NSW;
- determine the probability of survival of *C. brevitarsis* through the winter.

Unlike the CSIRO 'GROWEST' model, there was no need for a continuous input of data to constantly predict development of the vector population using complex computer programming.

As a consequence of these forecasting systems, vector monitoring can be conducted in a more efficient manner and ultimately generate data which will allow vector free

zones to be defined more accurately. preliminary associations were also established between the dispersal of vectors and the spread of viruses in a region.

INTELLECTUAL PROPERTY

While this is a substantial body of information and "know how" which has been gained during the course of this project, there is none that could be considered commercially sensitive and needing protection. All data from this project should be considered to be in the "Public Domain".

IMPACT OF RESEARCH: 1996

Although the results of this project only recently been transferred beyond the research phase for implementation by end-users, there have already been modest benefits to the livestock industries of Australia. These early benefits have come through reduced costs to the National Arbovirus Monitoring Program (NAMP) as the Akabane and Simbu ELISA tests have been introduced for routine use in the NSW components of NAMP. It is expected that these tests will be implemented on a nationwide basis in the next 12 months.

The immediate use of these tests, combined with the vector monitoring and modelling, allows the distribution of effective vector populations to be defined. This information is regularly used to provide current data to support livestock exports. As the boundaries of the vector free areas are more accurately defined, and supported by a greater body of recent data, our trading partners will have greater confidence in the limits of these zones. As OIE moves towards to introduction of concepts of regional freedom from arboviruses, rather than more restrictive state and national freedom, more livestock producers will gain access to valuable export markets.

In the area of improved export opportunities, there have already been modest improvements by virtue of reduced testing of individual animals on the basis of originating from an area with a period of arbovirus freedom which has been demonstrated by sentinel animal and vector monitoring. Livestock agents and exporters, having previously purchased stock and then had them rejected on the basis of prior infection with an arbovirus, can now purchase stock for export with a higher degree of confidence. They can achieve this by selecting stock from populations of known arbovirus status, reducing rejection rates and ill-feeling between owners and agents alike. Being able to offer groups of livestock of known arbovirus status prior to testing also improves the reputation of Australia as a country which can reliably supply disease-free stock. Excessive culling of prospective export stock has in the past aroused the suspicion and concern of importers that there may be disease among the imported livestock.

An understanding of variations in the patterns of transmission of both Akabane and Ephemeral viruses, and hence variations in population immunity, has allowed advice to be given to producers on the strategic use of vaccines to minimise the impact of epidemics and make the most economical use of vaccines.

IMPACT OF RESEARCH: 2001

The cELISA tests developed for detecting antibodies to Akabane virus and the Simbu serogroup should be in routine use in diagnostic laboratories throughout Australia by the end of 1997. A recommendation has already been made that these tests should be used for NAMP testing in 1997. Within a few years, it is expected that these would be accepted as alternative tests for export certification. As a consequence, the costs

of certification testing will be greatly reduced and potentially could provide producers with access to additional markets, from which they are currently excluded because the costs of VN tests and the time delay to obtain results are prohibitive.

With the successful development of computer models of vector survival and distribution, it is expected that there will be a markedly reduced demand for routine vector surveillance. On the other hand there will be a capability to rapidly predict impending epidemics and define vector free areas more precisely. These capabilities will be of significant benefit to the Australian livestock industries in coming years when it is expected that there will be a need for current, annually generated data to maintain access to key export markets and to gain entry to new markets.

Within a five year period it is expected that Australian Animal Health authorities, particularly through AQIS and the Federal DPIE, will be able to gain greater acceptance of our newly defined virus/vector free areas. As a consequence, there should be a reduced need for the testing and certification of individual livestock and greater emphasis placed on regional certification.

To date, there have been only a few opportunities to promote the concept of virus and vector free areas in the international marketplace. On the limited number of occasions that these opportunities have arisen, our trading partners have been impressed with our ability to monitor livestock populations and define arbovirus distributions. There is already acceptance of our monitoring systems, to the extent that other countries are adopting similar programs. For example, the Virology laboratory at EMAI is participating in a collaborative project with the Chinese Ministry of Agriculture to assist with the development of a sentinel herd program in China to study the epidemiology of Bluetongue. As our trading partners appreciate the value and accuracy of these monitoring systems, Australia should see significant benefits through less restrictive access to valuable markets.

ACKNOWLEDGMENTS

We are indebted to the owners of the sentinel herds for their untiring assistance and willingness to have animals sampled with little direct benefit to their own enterprise. This co-operation was forthcoming despite significant disruption to their normal daily operations. We are similarly indebted to the farmers who operated light traps for vector collections. The co-operation of the various field veterinarians has also been invaluable. Finally, our thanks to the staff of the Virology laboratory at EMAI for their expert technical assistance during the preparation, testing and storage of samples and to the research staff at HRS, Gosford, who participated in the vector ecology studies.

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