ANIMAL HEALTH AND WELFARE

Detection, identification and treatment of Infectious Ovine Keratoconjunctivitis (Pink Eye) in sheep from a Western Australian pre-export feedlot

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Abstract

Infectious Ovine Keratoconjunctivitis (IOK), Pink Eye, has a significant impact on rejection rates of sheep for live export and thus the feedlot and live export industries in Western Australia. The study aimed to determine the flora and sensitivity to antibiotics of eyes from sheep showing clinical signs of IOK.

A diverse range of flora was isolated from eyes of sheep. Of the 352 eyes sampled, up to 37 bacterial species were detected, with the highest number of 28 found in affected eyes (those showing clinical signs of IOK), compared to 23 in unaffected eyes (those showing no clinical signs when the contra-lateral eye was affected), 11 in apparently healthy eyes (rejected sheep showing no signs of IOK in either eye but having mixed with IOK sheep) and 19 species in control sheep eyes (non reject healthy sheep showing no clinical signs of IOK in either eye).

In sheep in a pre-export feedlot, the most commonly isolated organism in affected, unaffected and apparently healthy eyes was *Moraxella ovis*.

Mycoplasma species was the second most commonly isolated organism from affected eyes and apparently healthy eyes, and this species was isolated less frequently in unaffected eyes. *Mycoplasma spp* were isolated at rates of less than 5% in control eyes.

Seven treatment regimes were tested. The greatest clinical improvement was obtained when sheep with clinical signs of IOK were treated with long-acting oxytetracycline at a dose of 20mg/kg bodyweight injected into the neck muscle and if clinical signs were still present after 4 days, a second injection at the same dose rate was administered.

Executive summary

Infectious Ovine Keratoconjunctivitis (IOK), Pink Eye, has an important impact on rejection rates of sheep for live export. The disease is estimated to be the cause of 0.5% of rejections at a sheep pre-export feedlot in Western Australia and is a serious economic and welfare concern.
Infectious Ovine Keratoconjunctivitis is an infectious disease that has been reported worldwide. Outbreaks commonly occur when sheep are in close contact with each other, for example during transportation or in a feedlot. Many risk factors have been suggested including the effects of a hot, dry and dusty environment and ultra-violet light has been suggested as a possible pre-disposing factor also. Reports from Europe state that sheep which show no clinical signs can still be carrying organisms implicated in IOK and are therefore able to transmit the infection to other sheep when in contact in the right environment for transmission. Eliminating the possible risk factors mentioned above from a pre-export feedlot in Western Australia is unlikely to be possible.

The study determined the flora of normal and IOK – affected sheep's eyes and the sensitivity to antibiotics of bacteria from eyes of sheep showing clinical signs of IOK. Using these results, a treatment trial was conducted comparing seven treatments for IOK which are used commonly in Western Australia.

Of the 352 eyes sampled, up to 37 bacterial species were detected, with the highest number of 28 found in affected eyes (those showing clinical signs of IOK), compared to 23 in unaffected eyes. (those showing no clinical signs when the contra-lateral eye was affected), 11 in apparently healthy eyes (rejected sheep showing no signs of IOK in either eye but having mixed with IOK sheep and been in the feedlot for up to 3 weeks) and 19 species in control sheep eyes (non reject healthy sheep showing no clinical signs of IOK in either eye).

In sheep in a pre-export feedlot, the most commonly isolated organism in affected, unaffected and apparently healthy eyes was *Moraxella ovis*. *Mycoplasma* species was the second most commonly isolated organism from affected eyes and apparently healthy eyes, and was isolated less frequently in unaffected eyes. *Mycoplasma spp* was isolated at rates of less than 5% in control eyes.

Seven treatment regimes were tested. The greatest clinical improvement was obtained when sheep with clinical signs of IOK were treated with long-acting oxytetracycline (Alamycin LA 300, Norbrook Lab. Aust. Pty Limited) at a dose of 20mg/kg bodyweight injected into the neck muscle followed by a second injection 4 days later at the same dose rate (Group 8). This treatment was found to be significantly better than treating with Cloxacillin 500mg/3g Ointment (Orbenin Eye Ointment, Pfizer Animal Health) (Group 2), giving no treatment (Group 3), oxytetracycline powder 200mg/g in the water (Group 4) or topical oxytetracycline in the form of an aerosol (Terramycin Pink Eye Aerosol, Pfizer Animal Health) (Group 7) or in the form of a powder (Terramycin Pink Eye Powder, Pfizer Animal Health) (Group 5). Treating with a combination of Oxytetracycline Powder (Terramycin Pink Eye Powder, Pfizer Animal Health) and injectable Oxytetracycline (Alamycin LA 300, Norbrook Lab. Aust. Pty Limited) (Group 6) was found to be significantly more effective than using topical oxytetracycline alone, either in aerosol (Terramycin Pink Eye Aerosol, Pfizer Animal Health) (Group 7) or powdered forms (Terramycin Pink Eye Powder, Pfizer Animal Health) (Group 5) or giving no treatment (Group 3). Giving no treatment (control sheep, Group 3) was found to be more effective than treating with topical oxytetracycline alone, either in aerosol (Terramycin Pink Eye Aerosol, Pfizer Animal Health) (Group 7) or powdered forms (Terramycin Pink Eye Powder, Pfizer Animal Health) (Group 5).

Those treatments found to be the least effective were Oxytetracycline 2mg/g (Terramycin Pink Eye Aerosol, Pfizer Animal Health) applied topically to the eyes twice daily for 5 days and

Oxytetracycline 20mg/g (Terramycin Pink Eye Powder, Pfizer Animal Health) applied topically twice daily to eyes for 5 days. These treatments were also amongst the most expensive of the treatments tested. Topical oxytetracycline powder plus injectable oxytetracyline was ranked second most effective treatment, however, it was also the most expensive; and so could not be recommended over the Oxytetracycline injectable (Alamycin LA 300, Norbrook Lab. Aust. Pty Limited) 20mg/kg given as a single intramuscular injection (Group 1).

The results of this study should benefit sheep (and goat) producers and the export feedlot industry.
Further research needs to be conducted to determine if virulent and non-virulent types of *Moraxella ovis* exist and whether the organism has a role in predisposing the eye to infection with *Mycoplasma conjunctivae*. Future work could also investigate virulence factors of *M. conjunctivae* and its role in disease progression and transmission. The possibility of a vaccine against one or both of *Moraxella ovis* or *Mycoplasma conjunctivae* could be considered.

The use of in-water oxytetracycline shows promise based on the small sample size. It is the researchers' recommendation that further experimental work be carried out on the oral administration of drug to assess its efficacy. It is recommended that an experiment using larger numbers of affected sheep and a higher dose rate of oxytetracycline (22mg/kg bodyweight of active ingredient) given daily in water for 5 days be carried out. The effect on rumen function and feed intake should be closely monitored.

Based on the findings of the bacteriological studies and seven treatment trials it is the researchers' recommendation that:
Sheep showing clinical signs of IOK (Pink Eye) should be treated with long-acting oxytetracycline at a dose of 20mg/kg bodyweight injected into the neck muscle and if clinical signs are still present after 4 days, a second injection at the same dose rate should be administered.

The time interval of the second injection should be related to the recommendation of the manufacturer of the particular proprietary preparation used. This might be shorter than the 4 days in the reported trial. The meat withholding period should be a factor considered when choosing a proprietary product.

Ideally, treatment should be initiated at the first signs of the disease; the early signs being epiphora and tear-staining below the eye.

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1 Background

Infectious Ovine Keratoconjunctivitis (IOK) is a contagious ocular disease of sheep seen throughout the world. It is most commonly called Pink Eye in Australia but has other regional colloquial names including contagious ophthalmia and “Snow blindness”. Although research to determine the causative agents of IOK and treatment options for the disease has been carried out throughout the world, little work has been done in the unique environment of a sheep feedlot and specifically a Western Australian sheep pre-export feedlot. IOK raises welfare concerns and is reported to have a significant impact on the sheep feedlot and export industries in Western Australia.

It is estimated that the export of live sheep from Australia generates $1.8 billion per year in gross domestic product and provides employment for 13,000 Australians. At present it is thought that approximately 0.5% of sheep are rejected for export due to IOK (Garry Robinson, Wellard Rural Exports Pty Ltd, pers. comm.). Those sheep that are rejected are treated and held in the feedlot until the next shipment, or if treatment is unsuccessful or not possible, euthanased and sold for pet meat. In both cases money is lost as a result of the disease being present.

Approximately 3.5 million live sheep are exported from Western Australia each year. These sheep are required to be held in a quarantine feedlot for an average of 5-7 days, depending on destination, prior to shipping. It is during this quarantine time that rejections can occur due to the spread of an infectious disease such as IOK. Animals are transported to the feedlot by road, in the case of the particular feedlot in the study this journey is a maximum of 8 hours. On arrival at the feedlot, sheep are drafted into groups depending on type of sheep, weight and breed. Animals are inspected on entry to the feedlot for signs of ocular disease. Sheep identified with signs of IOK are separated and treatment is given. If large numbers of sheep with ocular disease are found in a line, the animals are often returned to the farm of origin. Individual animals are treated with topical Cloxacillin ointment (Orbenin Eye Ointment, Pfizer Animal Health). Large numbers affected are treated with Oxytetracycline powder in the water at a dose of 2g powder per head per day based on the animal drinking 4 L of water a day. Any animals with chronic lesions on the eyes are deemed to have no commercial value and are sold for pet meat.

Research has been done worldwide looking at the risk factors involved for IOK. It is difficult to draw conclusions from the research because of the wide range of climatic conditions, geographical regions, sheep breeds and housing arrangements studied. A study published in 2007 (Lysnyasky *et al*) concluded that environmental and climatic factors worked synergistically with the infective agents resulting in conjunctivitis which then progressed onto keratoconjunctivitis. Outbreaks of IOK are commonly seen in the winter months throughout the northern hemisphere where it is postulated that housing animals over these months increases animal to animal contact thus allowing spread of infection (Akerstedt and Hofshagen 2004).

Studies in Croatia which has a Mediterranean climate, similar to SW Western Australia, found no seasonal pattern (Naglic *et al* 2000). Cooper (1967) found in New Zealand that outbreaks occurred throughout the year with an increase in autumn; Belloy *et al* (2003) found that outbreaks in alpine regions peaked during summer and autumn. These peaks of outbreaks were linked to a number of environmental factors including hot, dry, dusty and windy climatic conditions which are thought to lower the defences of the superficial eye.

It is known that close contact is required for the infection to spread (Hosie 1988). Suggestion has been made in the literature as to a possible stimulant effect of ultra-violet light (Hosie 1988). Anecdotal reports suggest that outbreaks of IOK are more common during the summer months at the feedlot in SW Western Australia. As this time of year is hot, dry and dusty, outbreaks would be expected based on the findings of previous studies throughout the world.
The project reported here was designed to identify agents responsible for IOK, potential risk factors and possible treatments suitable for use in a Western Australian sheep pre-export feedlot.

2 Project objectives

The project objectives were fourfold. These were to:

1. Establish the prevalence of Infectious Ovine Keratoconjunctivitis (IOK) and risk factors for sheep contracting IOK in a sheep pre- export feedlot.
2. Establish a practical grading system to reliably grade the severity of IOK (Appendix 1).
3. Collect samples from affected animals in order to isolate pathogens which might be responsible for causing the infection
4. Based on results of bacterial culture and sensitivity, establish a best practice treatment protocol for IOK.

2.1 Milestones

1. Receipt of progress report of sampling and determining the causative agents of IOK in a feedlot in SW Western Australia
2. Receipt of progress report detailing a practical grading system for IOK eye lesions
3. Receipt of progress report of determination of flora of a larger sample of normal eyes.
4. Receipt of progress report of determination of flora and sensitivity to antibiotics of eyes showing clinical signs of Infectious Ovine Keratoconjunctivitis (IOK)
5. Receipt of progress report describing risk factors involved in outbreaks of IOK and how they can be applied to a feedlot in SW Western Australia
6. Results of treatment trial using a selection of antibiotic treatments commonly used for treatment of IOK in Western Australia

3 Materials and methods

3.1 Animal ethics approval

All experimentation involving animals was carried out with approval from Murdoch University's Animal Ethics Committee (AEC R22159/08). The experiments detailed in this report were carried out during 2008 and 2009.

3.2 Determining Ocular Flora

This study aimed to show both the ocular flora found in a clinically normal eye and that found in an eye showing signs of IOK. This was done in 2 parts over a 2 year period.

In all cases, photographs of the eyes were taken prior to swabbing and the ear tag of the sheep was recorded. Photographs were used to develop a severity grading or scoring system (Appendix 1 – Humphries (2008)).

Eyes were sampled using a cotton tipped wire or plastic shafted swab which was inserted between the eye and lower lid, the lower lid held closed and the swab rotated several times between the two layers of conjunctiva.
The swab was plated onto horse blood Agar Plates in the manner following the instructions for plating to obtain isolated colonies and then the shaft cut and the swab placed into Mycoplasma Broth. Samples taken during the first part of the experiment were also rolled onto a glass slide for Chlamydia identification. Samples were kept cool and delivered to the Animal Health Laboratories, Department of Agriculture and Food Western Australia (DAFWA), South Perth, Western Australia for bacterial culture and identification. PCR was performed on Mycoplasma isolates to determine species.

Part one
On four separate occasions, samples were collected from sheep housed in a pre export feedlot in the south west of Western Australia. Sheep were randomly selected from animals that were rejected for live export and had spent up to 3 weeks in the feedlot co-mingling (varied between sampling days). In total, 250 eyes were sampled from 199 different sheep. Samples were taken from three groups of sheep as follows:

1. Affected – eye clinically affected by IOK (n = 178)
2. Unaffected – one eye clinically affected and contra-lateral eye unaffected (n = 52)
3. Apparently healthy – neither eye affected (n = 20)

The severity of infection varied, as did the length of time that each sheep had spent in the feedlot before sampling.

Results were analysed with SPSS version 15.0 with a Chi square test for independence being used to calculate significant difference. Odds ratios were calculated for individual organisms and were compared with the association with OKC and a logistic regression model was used to identify multiple strains of bacteria linked with lesions.

Part two
In order to further define the ocular flora associated with IOK it was deemed necessary to sample normal eyes from healthy sheep that had not been identified as reject animals and had not been co-mingling with affected animals. Sampling of these clinically normal eyes (control eyes) was undertaken at the same sheep pre-export feedlot in the southwest of Western Australia. Samples were collected from both eyes of 51 sheep (102 samples) that had no visible eye lesions or evidence of IOK. These sheep had been in the feedlot for approximately 5 days prior to sampling. The 51 healthy sheep were selected at random from a larger group of healthy animals.

3.3 Sensitivities

In vitro antimicrobial susceptibility testing of samples from IOK affected sheep was conducted at the DAFWA Animal Health Laboratories according to the Performance Standards for Antimicrobial Disk and Dilution Susceptibility for Bacteria Isolated from animals; approved standard third edition, volume 28, no. 8, Clinical and Laboratory Standards Institute. (http://www.clsi.org/source/orders/free/m31-a3.pdf accessed 19 10 2010). The standard agar disk method was used. Sensitivities of *Moraxella ovis* isolates to Neomycin, Penicillin,

Tetracycline, Cotrimoxazole/Trimethoprim (Cotrimoxazole) were recorded. *In vitro* antimicrobial sensitivity testing of Mycoplasma isolates was not available at the laboratory.

3.4 Treatment Trial

The aim of the study was to extend the work done previously on determining the ocular flora in the eyes of IOK affected sheep. Animals were recruited from those sheep rejected from shipments due to the presence of clinical signs of IOK at the same sheep pre-export feedlot from which the previous work had been done. Swabs were taken from both eyes of these sheep and submitted for both culture and sensitivity. Based on these results, the efficacy of various treatments for IOK was evaluated. The treatments chosen were those currently used at the feedlot and those licensed for use in Western Australia.

Eyes were examined prior to selection of the sheep and graded according to a scale created by previous work (Appendix 1). The scale was adapted to make the grades numerical, as was done by Konig (1983) to allow analysis of the results to be carried out. Sheep with a grade 2 (conjunctivitis) or 3 (clouding of the cornea) in both eyes were selected for the trial. All sheep were otherwise clinically healthy. Selected sheep were transported to the Murdoch University Veterinary School on-campus Farm where they were housed in pens in an open-sided shearing shed. This was done to enable regular treatments to be given and for ease of caring for and daily monitoring of the animals.

Both eyes were photographed on day one and swabbed as for the clinically normal eyes (see description in 3.2). Individual identification of each animal was also recorded. Sheep were then randomly allocated to a treatment or control group. A total of 80 Merino-cross sheep of mixed age were included in the trial group; one control group (n=10) and 7 treatment groups (n=10 for each).

Sheep in the control group were not treated and were photographed and graded on day 0, 4, 6, 8, 12, 18 and 20. Eyes were re-swabbed on day 10 and again at day 20 by the method described previously. Treatment groups were given treatments as detailed in Table 1. These sheep were photographed and graded on day 0, 4, 6, 8 and 10. Eyes were re-swabbed on day 10 by the method described previously. The treatment groups were not monitored to day 20 because it was not a practical option to continue treatment for this length of time.

Statistical analyses were performed using statistical package SPSS version 17. ANOVA with bonferroni corrections was used for normally distributed data. The Kruskal-Wallis ANOVA was used for continuous data that was not normally distributed. Paired t tests were used to measure changes in individual sheep between the start and the end of the trial.

Group	Given
1	Oxytetracycline injectable (Alamycin LA 300, Norbrook Lab. Aust. Pty Limited) 20mg/kg given as a single intramuscular injection into neck muscle. Dose calculated based on accurate sheep weights.
2	Cloxacillin 500mg/3g (Orbenin Eye Ointment, Pfizer Animal Health) administered at dose of 125mg per eye (one quarter of a tube applied as a streak to each eye).
3	No treatment
4	Oxytetracycline powder 200mg/g administered orally in the drinking water. 2g of powder(400mg drug)/head/day based on each sheep drinking 4L water/day.
5	Oxytetracycline 20mg/g, (Terramycin Pink Eye Powder, Pfizer Animal Health), applied topically twice daily to eyes for 5 days.
6	Oxytetracycline 20mg/g, (Terramycin Pink Eye Powder, Pfizer Animal Health). Applied topically to eyes twice daily for 5 days PLUS Oxytetracycline injectable 20mg/kg (Alamycin LA 300, Norbrook Lab. Aust. Pty Limited) given as single intramuscular injection into the neck muscle. Dose calculated based on accurate sheep weights.
7	Oxytetracycline 2.0mg/g, (Terramycin Pink Eye Aerosol, Pfizer Animal Health). applied topically to the eyes twice daily for 5 days.
8	Oxytetracycline (Alamycin 300 LA Norbrook Lab. Aust. Pty) at a dose of 20mg/kg on day 0 and day 4 by intramuscular injection into the neck muscle.* Dose calculated based on accurate sheep weights.
Notes	** Day of repeat dose would depend on the long-acting preparation used – refer recommendations for particular preparation*

Table 1 Treatments tested for control of Infectious Ovine Keratoconjunctivitis

4 Results and discussion

4.1 Results

4.1.1 Determining Ocular Flora

Part one

Many different organisms were cultured over the four separate collection days and these are presented in Table 2. In order to determine the ocular flora associated with clinical signs of IOK the eyes that were sampled were grouped as either affected eyes or normal eyes. Normal eyes were considered as those eyes not showing clinical signs of IOK (unaffected plus apparently healthy). The most common isolates grown from affected eyes were *Moraxella sp.* (n=150), *Bacillus sp.* (n= 107), *Mycoplasma* (n=84) and *Staphylococcus epidermidis* (n=69). The same four bacterial species were also the most commonly grown from normal eyes.

Table 2: The number of eyes which were either positive or negative for all bacteria isolated for both affected (n = 178) and normal (n = 72) eyes.

	IOK affected eyes		Normal eyes	
Isolated Bacteria	**Positive**	**Negative**	**Positive**	**Negative**
Moraxella sp.	150	28	64	8
Bacillus sp.	107	71	21	51
Mycoplasma	84	94	14	58
Staphylococcus epidermidis	69	109	19	53
Staphylococcus hyicus chromogens	34	144	13	59
Mannheimia haemolytica	27	151	13	59
Streptococcus pyogenes	27	151	12	60
Actinomyces pyogenes	19	159	0	72
Acinetobacter sp.	12	166	5	67
Moraxella/Neisseria sp.	12	166	2	70
Pseudomonas Shewanella	11	167	0	72
Arcanobacterium pyogenes	10	168	3	69
Staphylococcus haemolyticus	7	171	4	68
Pasteurella multocida	7	171	4	68
Staphylococcus aureus	5	173	2	70
Corynebacterim sp.	2	176	0	72
Neisseria sp.	4	174	0	4
Proteus	4	174	0	72
Staphylococcus chromogens	4	174	3	69
Staphylococcus intermedius	3	175	0	72
Pseudomonas aeruginosa	1	177	0	72

Table 3 shows the results of the Chi-squared analysis; that is, the number (and percentage) of eyes that grew a particular bacterial isolate compared to the total number of either affected (n=178) or normal (n=72) eyes sampled. Sheep infected with *Mycoplasma* species were significantly ($P < 0.001$) more likely to have IOK, with 47.2% of affected eyes having *Mycoplasma* species isolated from them. Eyes with *Mycoplasma* species were 3.63 times more likely to have IOK compared to eyes that did not have *Mycoplasma* species.

Bacillus species were isolated from 107 of the 178 affected eyes sampled (60.1%). Eyes with *Bacillus* species were found to be significantly more likely to have IOK compared to eyes that were negative for *Bacillus* species ($P < 0.001$). Eyes positive for *Bacillus* species were 3.6 times more likely to have IOK than uninfected eyes.

Moraxella ovis was the most common bacteria isolated from all of the samples collected. It was cultured in 150 of 250 eyes; there was no difference in prevalence between affected and normal eyes ($P = 0.35$). *Moraxella ovis* was grown from 84.3% of affected eyes versus 88.9% of normal eyes.

In contrast, for *Mycoplasma* and *Bacillus* species, there were significant differences between affected and normal eyes. *Bacillus* species were significantly more likely to be isolated from affected eyes compared with normal eyes ($P < 0.001$; 68% and 29% respectively). Similarly *Mycoplasma* species were isolated from 47% of affected eyes compared with 19% of normal eyes and this was significantly different ($P < 0.001$). The risk estimate for both *Bacillus* and *Mycoplasma* species shows that sheep eyes that had either of these two micro-organisms were 3.6 times more likely to have IOK.

Eyes that were infected with *Mycoplasma* and/or *Bacillus species* were also significantly more likely to have IOK ($P < 0.001$), with a risk estimate of 4.0. Seventy seven percent of all affected eyes had *Mycoplasma* or *Bacillus species* isolated from them, versus 45.1% of normal eyes.

Table 3: Results of chi-squared analysis for organisms isolated from both normal and affected eyes.

The number in brackets is the percent of total number of affected or normal eyes.

Organisms Isolated	Affected (%) n=178	Normal (%) n = 72	P Value	Risk Estimate
Moraxella sp.	150 (84.3)	64 (88.9)	0.346	0.670
Bacillus sp.	107 (60.1)	21 (29.2)	< 0.001	3.660
Mycoplasma	84 (47.2)	14 (19.7)	< 0.001	3.638
Staphylococcus epidermidis	69 (38.8)	19 (26.4)	0.064	1.766
Staphylococcus hyicus chromogens	34 (19.1)	13 (18.1)	0.848	1.072
Mannheimia haemolytica	27 (15.1)	13 (18.1)	0.573	0.812
Streptococcus pyogenes	27(15.2)	12 (16.7)	0.768	0.894
Actinomyces pyogenes	19 (10.7)	0 (0)	<0.005	NA
Acinetobactor sp.	12 (6.7)	5 (6.9)	0.954	0.969
Moraxella/Neisseria sp.	12 (6.7)	2 (2.8)	0.217	2.530
Pseudomonas shewanella	11 (6.2)	0 (0)	<0.05	NA
Arcanobacterium pyogenes	10 (5.6)	3 (4.2)	0.640	1.369
Pasteurella multocida	7 (3.9)	4 (5.6)	0.571	0.696
Staphylococcus haemolyticus	7 (3.9)	4 (5.6)	0.571	0.696

Different *Bacillus* strains were isolated from each eye sample. The results obtained on sampling days 2 and 4 displayed in Table 4. Although each *Bacillus species* isolate was not identified to species level, they are described according to the visual morphology of the colonies. A flat, dry, yellow strain was significant in causing IOK infection (P < 0.001) with 72.7% of affected eyes having this *Bacillus* strain isolated from them. Sheep with the flat, dry, yellow strain of *Bacillus sp.* were 5.2 more likely to have an IOK infection.

Table 4: *Bacillus* species showing affected eyes versus normal eyes, isolated from sample days two and four.

Isolated Bacteria	A (%)	N (%)	P value	Risk Estimate
Flat, dry, yellow	**57 (43.8)**	**4 (12.9)**	**<0.005**	**5.271**
Small dry yellow crenated	14 (10.8)	1 (3.2)	0.306	3.621
Grey/green colony	19 (14.6)	5 (16.1)	0.832	0.890

Moraxella species had the highest prevalence of all eyes sampled. Of all affected eyes, 150 were positive for some type of *Moraxella species*. Although *Moraxella ovis* was cultured from a high percentage of affected eyes, Table 5 shows that of the 82 samples collected on sample day 4, only one specific strain of *Moraxella ovis* was significantly associated with IOK. Sheep eyes infected with *Moraxella ovis* with smooth colonies that were non-auto agglutinating, were significantly more likely to have IOK than eyes that did not have this isolate ($P < 0.05$). Furthermore 53.1% of all affected samples were infected by this isolate and were 3.9 times more likely to be infected with IOK.

Another *Moraxella ovis* strain, which was crenated and had auto-agglutinating properties, was significantly more likely to be isolated from normal eyes ($P < 0.05$). However it was only isolated in 2 normal eyes.

Table 5: Affected and normal eyes displaying different strains of *Moraxella* spp collected on sample day four only

Isolated *Moraxella* strains	A (%) n= 64	N (%) n=18	P value
Rough colony, auto agglutinating	4(6.3)	4 (22.2)	0.066
Smooth colony, auto agglutinating	9 (14.1)	5 (27.8)	0.172
Smooth colony, non-auto agglutinating	**34 (53.1)**	**4 (22.2)**	**<0.05**
Umbonate colony, auto agglutinating	24 (37.5)	6 (33.3)	0.746
Umbonate, non-auto agglutinating	3 (4.7)	0 (0)	1.000
Crenated auto-agglutinating	**0 (0)**	**2 (11.1)**	**<0.05**
Non-Haemolytic	0 (0)	1 (5.6)	0.220

Part two

A total of 20 organisms were cultured from the 102 control eyes. Of these organisms, *Bacillus* species were the most commonly isolated with 87.3 % positive. *Moraxella ovis* was the second most commonly isolated organism with 38.2% followed by *Staphylococcus chromogens* with

33.3%. Only 4.9% of control animals were positive for *Mycoplasma* species and of these, only one was positively identified as *Mycoplasma conjunctivae* (Table 6).

Table 6: Microbial flora isolated from Control Eyes (n=102)

Organism	Positive N (%)	Negative N (%)
Moraxella ovis	39 (38.2)	63 (61.8)
Mycoplasma species	5 (4.9)	97 (95.1)
M. conjunctiva positive	4 (80)	
M.conjunctivae positive	1 (25.0)	
M.ovis + Mycoplasma sp.	3 (15)	
M.ovis + M.conjunctivae	1 (0.9)	
Staphylococcus epidermis	20 (19.6)	82 (80.4)
Bacillus species	89 (87.3)	13 (12.7)
Bacillus sp. Dry yellow	12 (11.8)	90 (88.2)
Staphylococcus chromogens	34 (33.3)	68 (66.7)
Streptococcus pyogenes	0 (0)	102 (100)
Arcanobacterium pyogenes	0 (0)	102 (100)
Mannheima haemolytica	2 (2)	100 (98)
Non-haemolytic *Moraxella* sp.	2 (2)	100 (98)
Gram-negative *cocco-bacilli*	0 (0)	102 (100)
Acinetobacter sp.	8 (7.8)	94 (92.2)
Unidentified GPR	0 (0)	102 (100)
Shewanella sp.	0 (0)	102 (100)
Pasteurella multocida	0 (0)	102 (100)
Unidentified	0 (0)	102 (100)
Corynebacterium sp.	0 (0)	102 (100)
Staphylococcus haemolyticus	38 (37.3)	64 (62.3)
Gram-negative diplococcic	0 (0)	102 (100)
Unidentified GNR	0 (0)	102 (100)
Staphylococcus aureus	3 (2.9)	99 (97.1)
Neisseria sp.	2 (2.0)	100 (98)
Proteus sp.	0 (0)	102 (100)
Unidentified GPC	0 (0)	102 (100)
Staphylococcus intermedius	0 (0)	102 (100)
Coliform	2 (2)	100 (98)
Pseudomonas aeruginosa	0 (0)	102 (100)
E.coli	0 (0)	102 (100)
Staphylococcus hyicus	0 (0)	102 (100)
Streptococcus Group G	1 (1)	101 (99)
Streptococcus species	4 (3.9)	98 (96.1)
Pseudomonas sp.	0 (0)	102 (100)
Aeorcoccus viridians	5 (4.9)	97 (95.1)
Micrococcus sp.	2 (2)	100 (98)
Kocuria varians	1 (1)	101 (99)
Enterococcus faecalis	0 (0)	102 (100)
Brevundimonas dimuta	13 (12.7)	89 (87.3)

GNR = Gram- negative rod; GPC= gram-positive cocci; GPR=gram-positive rod

Determining ocular flora

Over an 18 month time period, a total of 352 eyes were sampled. The sheep sampled varied in age, time in feedlot, time of year and disease status. Of the 352 eyes that were sampled, up to 37 bacterial species were found in eyes. Twenty eight species were found in affected eyes, compared to 23 species found in unaffected eyes, 11 species found in apparently healthy eyes and 19 species in control eyes.

Moraxella ovis was detected at the highest rate of all organisms for all eyes except control eyes, at a rate of 87.2% (n=156) of affected eyes, 88.2% (n=45) of unaffected eyes, 95.0% (n=19) of apparently healthy eyes and 38.2% (n=39) of control eyes.

Bacillus species, comprised of up to four colony morphology types, were isolated at the highest rate (87.3%) in control eyes.

Mycoplasma species was the second most commonly isolated organism in affected eyes at rates of up to 46.9%, compared to control eyes with an isolation rate of 4.9% (n=5) and was isolated in significant rates from unaffected and apparently healthy eyes. Of these, 69 (82.1%) *Mycoplasma* species cultured from affected eyes, and four species (80.0%) cultured from control eyes were tested for *M*. *conjunctivae* by PCR and identified 31 (44.9%) from affected eyes and one (25.0%) from control eyes (Refer Table 7). The non-*M*. *conjunctivae* Mycoplasma were not speciated.

The combined isolation of *Moraxella ovis* and *Mycoplasma* species in an eye occurred at the highest rate in affected eyes; 43.0% compared to <15.0% for both unaffected and control eyes, and 1.0% for apparently healthy eyes (Table 7)

Table 7. Microbial flora isolated from eyes affected (A) by IOK (n=179), unaffected (U) eyes (n=51), apparently healthy (AH) eyes (n=20) and control (C) eyes (n=102) showing the number and percentage of eyes that were positive or negative for each micro-organism

Organism	A eyes		U eyes		AH eyes		Control eyes	
	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)
Moraxella ovis	156 (87.2)	23 (12.8)	45 (88.2)	6 (11.8)	19 (95.0)	1 (0.05)	39 (38.2)	63 (61.8)
Mycoplasma species cultured	84 (46.9)	95 (53.1)	14 (19.7)	57 (80.3)	4 (20.0)	16 (80.0)	5 (4.9)	97 (95.1)
No. *Mycoplasma* tested by PCR	69 (82.1)		6 (60.0)		1 (25.0)		4 (80.0)	
M. conjunctivae positive	31 (44.9)		3 (50.0)		0 (0.0)		1 (25.0)	
Moraxella ovis + *Mycoplasma* sp.	77 (43.0)		3 (15.0)		1 (0.9)		3 (15.0)	
Moraxella ovis + *M. conjunctivae*	27 (39.1)		9 (17.6)		0 (0.0)[1]		1 (0.9)	
Eyes with *Moraxella ovis* only	81 (45.3)		26 (51.0)		15 (75.0)		37 (36.3)	
Eyes with *Mycoplasma* sp. only	8 (4.5)		2 (3.9)		1 (5.0)		3 (2.9)	
Staphylococcus epidermidis	72 (40.2)	107 (59.8)	19 (37.3)	32 (62.3)	0 (0.0)	20 (100)	20 (19.6)	82 (80.4)
Bacillus species	67 (37.4)	112 (62.6)	17 (33.3)	34 (66.7)	0 (0.0)	20 (100)	89 (87.3)	13 (12.7)
Bacillus sp. dry yellow	57 (31.8)	122 (68.2)	4 (7.8)	47 (92.2)	0 (0.0)	20 (100)	12 (11.8)	90 (88.2)
Staphylococcus chromogens	42 (23.5)	137 (76.5)	16 (31.4)	35 (68.6)	1 (5.0)	19 (95.0)	34 (33.3)	68 (66.7)
Streptococcus pyogenes	30 (16.8)	149 (83.2)	9 (17.6)	42 (82.4)	3 (15.0)	17 (85.0)	0 (0.0)	102 (100)
Arcanobacterium pyogenes	30 (16.8)	149 (83.2)	2 (3.9)	49 (96.1)	0 (0.0)	20 (100)	0 (0.0)	102 (100)
Mannheima haemolytica	28 (15.6)	151 (84.4)	8 (15.7)	43 (84.3)	4 (20.0)	16 (80.0)	2 (2.0)	100 (98.0)
Non-haemolytic *Moraxella* sp.	24 (13.4)	155 (86.6)	9 (17.6)	42 (82.4)	0 (0.0)	20 (100)	2 (2.0)	100 (98.0)
Gram-negative cocco-bacilli	17 (9.5)	162 (09.5)	6 (11.8)	45 (88.2)	0 (0.0)	20 (100)	0 (0.0)	102 (100)
Acinetobacter sp.	13 (7.3)	166 (92.7)	5 (9.8)	46 (90.2)	1 (5.0)	19 (95.0)	8 (7.8)	94 (92.2)
Unidentified GPR	12 (6.7)	167 (93.3)	3 (5.9)	48 (94.1)	0 (0.0)	20 (100)	0 (0.0)	102 (100)
Shewanella sp.	11 (6.1)	168 (93.9)	0 (0)	51 (100)	0 (0.0)	20 (100)	0 (0.0)	102 (100)
Pasteurella multocida	10 (5.6)	169 (94.4)	4 (5.5)	68 (94.5)	0 (0.0)	20 (100)	0 (0.0)	102 (100)
Unidentified	10 (5.6)	169 (94.4)	1 (1.9)	50 (98.1)	2 (10.0)	18 (90.0)	0 (0.0)	102 (100)
Corynebacterium sp.	8 (4.5)	171 (95.5)	0 (0)	51 (100)	0 (0.0)	20 (100)	0 (0.0)	102 (100)
Staphylococcus haemolyticus	7 (3.9)	172 (96.1)	3 (5.9)	48 (94.1)	1 (5.0)	19 (95.0)	38 (37.3)	64 (62.3)
Gram-negative diplococci	7 (3.9)	172 (96.1)	2 (3.9)	49 (96.1)	0 (0.0)	20 (100)	0 (0.0)	102 (100)

Table 7. continued

Organism	A eyes		U eyes		AH eyes		Control eyes	
	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)
Unidentified GNR	7 (3.9)	172 (96.1)	3 (5.9)	48 (94.1)	0 (0.0)	20 (100)	0 (0.0)	102 (100)
Staphylococcus aureus	6 (3.3)	173 (96.7)	2 (3.9)	49 (96.1)	0 (0.0)	20 (100)	3 (2.9)	99 (97.1)
Neisseria sp.	4 (2.2)	175 (97.8)	0 (0)	51 (100)	0 (0.0)	20 (100)	2 (2.0)	100 (98.0)
Proteus sp.	4 (2.2)	175 (87.7)	0 (0)	51 (100)	0 (0.0)	20 (100)	0 (0.0)	102 (100)
Unidentified GPC	4 (2.2)	175 (97.8)	2 (3.9)	49 (96.1)	0 (0.0)	20 (100)	0 (0.0)	102 (100)
Staphylococcus intermedius	3 (1.7)	176 (98.3)	0 (0)	51 (100)	0 (0.0)	20 (100)	0 (0.0)	102 (100)
Coliform	2 (1.1)	177 (98.9)	1 (1.9)	50 (98.1)	0 (0.0)	20 (100)	2 (2.0)	100 (98.0)
Pseudomonas aeruginosa	1 (0.6)	178 (99.4)	0 (0)	51 (100)	0 (0.0)	20 (100)	0 (0.0)	102 (100)
E. coli	1 (0.6)	178 (99.4)	2 (3.9)	49 (96.1)	0 (0.0)	20 (100)	0 (0.0)	102 (100)
Staphylococcus hyicus	0 (0)	179 (100.0)	0 (0)	51 (100)	2 (10.0)	18 (90.0)	0 (0.0)	102 (100)
Streptococcus Group G	0 (0)	179 (100.0)	0 (0)	51 (100)	0 (0.0)	20 (100)	1 (1.0)	101 (99.0)
Streptococcus species	0 (0)	179 (100.0)	3 (5.9)	48 (94.1)	1 (5.0)	19 (95.0)	4 (3.9)	98 (96.1)
Pseudomonas sp.	0 (0)	179 (100)	0 (0)	51 (100)	0 (0.0)	20 (100)	0 (0.0)	102 (100)
Aerococcus viridans	0 (0)	179 (100)	0 (0)	51 (100)	0 (0.0)	20 (100)	5 (4.9)	97 (95.1)
Micrococcus sp.	0 (0)	179 (100)	0 (0)	51 (100)	0 (0.0)	20 (100)	2 (2.0)	100 (98.0)
Kocuria varians	0 (0)	179 (100)	0 (0)	51 (100)	0 (0.0)	20 (100)	1 (1.0)	101 (99.0)
Enterococcus faecalis	0 (0)	179 (100)	0 (0)	51 (100)	1 (5.0)	19 (95.0)	0 (0.0)	102 (100)
Brevundimonas dimuta	0 (0)	51 (100)	0 (0)	51 (100)	0 (0.0)	20 (100)	13 (12.7)	89 (87.3)

*Note: The mycoplasma sample for one unaffected eye was contaminated therefore only 71 unaffected eyes were cultured for *Mycoplasma* species. GNR=Gram-negative rod; GPC=Gram-positive cocci; GPR=Gram-positive rod; Na = not applicable; nc = not calculated; No. = Number; [1]Only one of the four Mycoplasma isolated were able to be tested by PCR.

In the 131 affected eyes, where the morphology of *Moraxella ovis* isolates was examined, , *Moraxella ovis* was isolated from 85.5% of eyes and 42.7% contained *Moraxella ovis* only (no *Mycoplasma* species). *Mycoplasma* species were isolated from 48.9% of affected eyes and 6.1% of affected eyes had *Mycoplasma* only without *Moraxella ovis* isolated (Table 8). In control eyes, 38.2% of eyes had *Moraxella ovis* with 35.3% containing *Moraxella ovis* and no *Mycoplasma* species. *Mycoplasma* species were isolated from 4.9% of control eyes with 2.9% containing *Mycoplasma* species and no *Moraxella ovis*.

The severity of clinical signs was recorded for the same 131 affected eyes. Regardless of organisms harboured, a lesion rating of 3 was recorded for 71.0% (n=93) of eyes (Table 9). A similar result was obtained for eyes that had *Mycoplasma* species, *M*. *conjunctivae*, and *Moraxella ovis*, or a combination of these organisms. The lesions in eyes that had *Mycoplasma* species only (no *Moraxella ovis*) or *M*. *conjunctivae* only had a lesion rating of 3 for 75.0% of eyes compared to 66.1% of eyes with a lesion rating of 3 for eyes that had *Moraxella ovis* only. For eyes that had *Moraxella ovis* (with or without *Mycoplasma* species) 81.5% had a lesion rating of 3 for non-auto-agglutinating *Moraxella ovis*, compared to 72.2 % for smooth colony type, 60.0% for rough colony type and 61.2% for auto-agglutinating cells.

Table 8. *Mycoplasma* species and relationship with morphology type of *Moraxella ovis* for IOK affected eyes (n=131) and control eyes (n=102)

Organism	Affected eyes Positive N (%)	Control eyes Positive N (%)
Mycoplasma sp. cultured	64 (48.9)	5 (4.9)
Eyes with no *Mycoplasma* sp. cultured	67 (51.1)	97 (95.1)
Mycoplasma tested by PCR	53 (82.8)	4 (80.0)
Mycoplasma conjunctivae positive	24 (58.5)	1 (25.0)
M. conjunctivae negative	29 (22.1)	3 (75.0)
Moraxella ovis	112 (85.5)	39 (38.2)
Eyes with no *Moraxella ovis*	16 (12.2)	63 (61.8)
Moraxella ovis auto-agglutinating	49 (37.4)	26 (25.5)
Moraxella ovis non-auto-agglutinating	25 (19.1)	11 (10.8)
Eyes with both *Moraxella ovis* auto & non-auto-agglutinating	39 (29.7)	1 (1.0)
Moraxella ovis smooth surface	108 (82.4)	27 (69.2)
Moraxella ovis rough surface	30 (22.9)	13 (33.3)
Moraxella ovis smooth surface auto-agglutinating	75 (57.3)	15 (38.5)
Moraxella ovis smooth surface non-auto-agglutinating	65 (49.6)	12 (30.8)
Moraxella ovis rough surface auto-agglutinating	29 (22.1)	13 (33.3)
Moraxella ovis rough surface non-auto-agglutinating	1 (0.8)	0 (0.0)
Mycoplasma sp + *Moraxella ovis*	57 (43.5)	1 (1.0)
M conjunctivae + *Moraxella ovis*	20 (15.3)	1 (1.0)
Mycoplasma sp. + *Moraxella ovis* auto-agglutinating	21 (16.0)	1 (1.0)
M. conjunctivae + *Moraxella ovis* auto-agglutinating	14 (10.7)	0 (0.0)
Mycoplasma sp. + *Moraxella ovis* non-auto-agglutinating	12 (9.2)	0 (0.0)
M. conjunctivae + *Moraxella ovis* non-auto-agglutinating	14 (10.7)	1 (1.0)
Mycoplasma sp. + *Moraxella ovis* smooth surface	54 (41.2)	0 (0.0)
M. conjunctivae + *Moraxella ovis* smooth surface	19 (14.5)	1 (1.0)
Mycoplasma sp. + *Moraxella ovis* rough surface	14 (10.7)	1 (1.0)
M. conjunctivae + *Moraxella ovis* rough surface	5 (3.8)	0 (0.0)

Table 8 continued

Organism	Affected eyes Positive N (%)	Control eyes Positive N (%)
Mycoplasma + *Moraxella ovis* smooth auto-agglutinating	38 (29.0)	0 (0.0)
M. *conjunctivae* + *Moraxella ovis* smooth auto-agglutinating	12 (9.2)	0 (0.0)
Mycoplasma + *Moraxella ovis* smooth non-auto-agglutinating	34 (26.0)	1 (1.0)
M. *conjunctivae* + *Moraxella ovis* smooth non-auto-agglutinating	14 (10.7)	1 (0.0)
Mycoplasma + *Moraxella ovis* rough auto-agglutinating	14 (10.7)	1 (1.0)
M. *conjunctivae*+ *Moraxella ovis* rough auto-agglutinating	5 (3.8)	0 (0.0)
Mycoplasma + *Moraxella ovis* rough non-auto-agglutinating	0 (0.0)	0 (0.0)
M. *conjunctivae*+ *Moraxella ovis* rough non-auto-agglutinating	0 (0.0)	0 (0.0)
No. of eyes with *Mycoplasma* sp. only	8 (6.1)	3 (2.9)
No. of eyes with *M*. *conjunctivae* only	4 (3.1)	0 (0.0)
No. of eyes with *Moraxella ovis* only	56 (42.7)	36 (35.3)

Note: In this table *Mycoplasma* species includes those identified as *M*. *conjunctivae*

Table 9: Severity of IOK in affected eyes (n=131)

Organism	Rating 0 No. (%)	Rating 1 No. (%)	Rating 2 No. (%)	Rating 3 No. (%)	Rating 4 No. (%)
No of eyes with lesions (n=131)	1 (0.8)	15 (11.5)	18 (13.7)	93 (71.0)	4 (3.1)
All *Mycoplasma* sp. (n=64)	0 (0.0)	4 (6.3)	7 (10.9)	49 (76.6)	4 (6.3)
M. conjunctivae (n=24)	0 (0.0)	2 (8.3)	1 (4.2)	18 (75.0)	3 (12.5)
Eyes with *Mycoplasma* only (n=8)	0 (0.0)	0 (0.0)	1 (12.5)	6 (75.0)	1 (12.5)
Eyes with *M. conjunctivae* only (n=4)	0 (0.0)	0 (0.0)	0 (0.0)	3 (75.0)	1 (25.0)
Moraxella ovis (n=112)	1 (0.9)	12 (10.7)	15 (13.4)	81 (72.3)	3 (2.7)
Eyes with *Moraxella ovis* smooth (n=108)	0 (0.0)	12 (11.1)	15 (13.9)	78 (72.2)	3 (2.8)
Eyes with *Moraxella ovis* rough (n=30)	1 (3.3)	5 (16.7)	6 (20.0)	18 (60.0)	0 (0.0)
Eyes with *Moraxella ovis* auto-agglutinating (n=49)	1 (2.0)	7 (14.3)	9 (18.4)	30 (61.2)	0 (0.0)
Eyes with *Moraxella ovis* non-auto-agglutinating (n=27)	0 (0.0)	1 (3.7)	2 (7.4)	22 (81.5)	2 (7.4)
Eyes with *Moraxella ovis* only (n=56)	1 (1.8)	8 (14.0)	10 (17.5)	37 (66.1)	0 (0.0)
Moraxella ovis + all *Mycoplasma* sp. (n=57)	0 (0.0)	4 (7.0)	5 (8.8)	44 (77.2)	3 (5.3)
Moraxella ovis + *M. conjunctivae* (n=20)	0 (0.0)	2 (10.0)	0 (0.0)	15 (75.0)	3 (15.0)
Moraxella ovis smooth + *M. conjunctivae* (n=19)	0 (0.0)	2 (10.5)	0 (0.0)	14 (73.7)	3 (15.8)
Eyes with no *Mycoplasma* sp. (n=67)	1 (1.5)	11 (16.4)	11 (16.4)	44 (65.7)	0 (0.0)
Eyes with non-*M. conjunctivae Mycoplasma* (n=27)	0 (0.0)	2 (7.4)	3 (11.1)	21 (77.8)	1 (3.7)
Eyes with no *Moraxella ovis* (n=16)	0 (0.0)	3 (18.8)	2 (12.5)	10 (62.5)	1 (0.1)
Eyes with no *Moraxella ovis* & no *Mycoplasma* sp. (n=11)	0 (0.0)	3 (27.3)	1 (9.1)	7 (63.6)	0 (0.0)

Note: In this table *Mycoplasma* species includes those identified as *M. conjunctivae* unless stated otherwise

4.1.2 Sensitivities

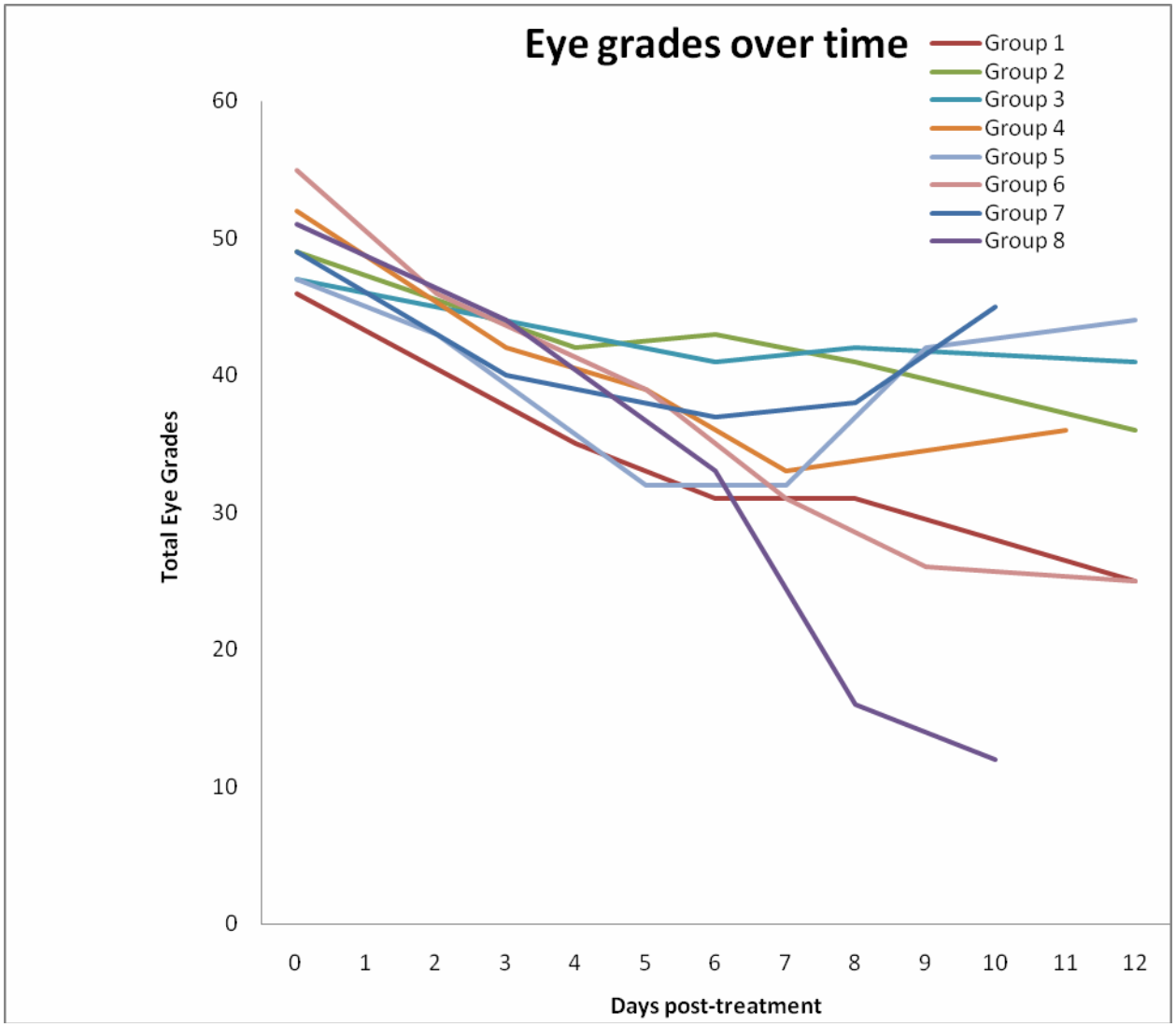
Bacterial cultures performed pre and post-treatment isolated a wide range of bacteria as listed in Table 7. Studies throughout the world have identified *Mycoplasma conjunctivae* and *Moraxella ovis* as the main organisms involved in IOK (Hosie 1988 and Dagnall 1994), therefore this study focused on the sensitivity of these organisms to treatments. *Mycoplasma* sensitivity testing was not available at the laboratory to which samples were submitted, so only *Moraxella* isolates were tested.

Resistance to Neomycin was seen with a small number of isolates; however this was described as borderline resistance. All isolates were sensitive to Penicillin, Tetracycline and Cotrimoxazole/Trimethoprim (Cotrimoxazole).

4.1.3 Treatment Trial

All sheep continued to eat and behave normally throughout the duration of the experiment. Each eye, left and right, was graded according to the grading scale developed by Humphry (2008) which was modified to make grades numerical as was done by Konig (1983). Treatments were administered as described in Table 1 (Refer section 3.4 above). The left and right eye scores were added to give group scores for both eyes and the change from day 0 to the end of the trial is shown in Graph 1. A one-way ANOVA applied to the difference between the total scores at day 0 and the total scores at the end of the trial for the different groups showed a significant difference between the groups ($p<0.001$). It was found that the order of improvements with the greatest reduction first and the least improvement last were Group 8, 6, 1, 4, 2, 3, 7 and 5. Group 8 was found to be significantly better than Groups 2, 3, 4, 5 and 7, and Group 6 was significantly better than Groups 3, 5, and 7. Therefore the study showed that Group 3, the control group, was significantly worse than Groups 6 and 8. No significant difference was noted between Groups 8 and 6. Costs of treatments were calculated and are included in Table 10 below. The most expensive treatment was the combination of oxytetracycline given by intramuscular injection and topical powder. The least expensive treatment was oral medication (oxytetracycline powder in drinking water).
Table 10 gives the treatments ranked in order of efficacy and includes the cost per head and the significances (P values).

Graph 1: Effect of treatments - eye grades over time



Group 1: Single Alamycin LA 300™ injection 20mg/kg. **Group 2**: Orbenin Eye Ointment™. **Group 3**: No treatment. **Group 4**: Oxytetracycline powder in the water. **Group 5**: Terramycin Pink Eye Powder™. **Group 6**: Terramycin Pink Eye Powder™ plus Alamycin LA 300™ 20mg/kg single injection. **Group 7**: Terramycin Pink Eye Spray™. **Group 8**: Two injections Alamycin LA 300™ 20mg/kg 4 days apart.

Rank	Treatment	Treatment cost (per head)	P value
1	**Group 8** Oxytetracycline (Alamycin 300 LA Norbrook Lab. Aust. Pty) at a dose of 20mg/kg on day 0 and day 4 by intramuscular injection into the neck muscle.	$3.64	<0.001
2	**Group 6** Oxytetracycline 20mg/g, (Terramycin Pink Eye Powder, Pfizer Animal Health). Applied topically to eyes twice daily for 5 days plus Oxytetracycline injectable 20mg/kg given as single intramuscular injection. (Alamycin LA 300, Norbrook Lab. Aust. Pty Limited).	$14.12	<0.001
3	**Group 1** Oxytetracycline injectable (Alamycin LA 300, Norbrook Lab. Aust. Pty Limited) 20mg/kg given as a single intramuscular injection into the neck muscle.	$1.82	<0.005
4	**Group 4** Oxytetracycline powder 200mg/g administered orally in the drinking water, 2g/head/day based on each sheep drinking 4L water/day.	$0.15	<0.05
5	**Group 2** Cloxacillin 500mg/3g (Orbenin Eye Ointment, Pfizer Animal Health) administered at dose of 125mg per eye (one quarter of a tube applied as a streak to each eye) .	$3.58	<0.05
6	**Group 3** No treatment.	$0.00	<0.01
7	**Group 7** Oxytetracycline 2.0mg/g, (Terramycin Pink Eye Aerosol, Pfizer Animal Health) applied topically to the eyes twice daily for 5 days.	$11.50	Not significant
8	**Group 5** Oxytetracycline 20mg/g, (Terramycin Pink Eye Powder, Pfizer Animal Health) applied topically twice daily to eyes for 5 days.	$12.30	Not significant

Table 10: Rank, cost and P values of treatment groups

All groups showed a significant reduction in *Moraxella ovis* autoagglutinating (P<0.05) and *Mycoplasma spp* (P<0.05), indicating that regardless of treatment chosen there was a reduction in the growth of *M. ovis* and *Mycoplasma spp* over the time of the trial.
No significant difference was seen in *Moraxella ovis* non-agglutinating between the groups.
For *Moraxella ovis* autoagglutinating, the change is less in Group 3 than Group 8 (P<0.041) and for *Mycoplasma spp* the change in Group 3 is more than Group 8 (P<0.033), indicating that giving no treatment will be more effective at reducing *Mycoplasma spp* growth than treating with topical oxytetracycline aerosol however the opposite is true for *Moraxella ovis* auto-agglutinating growth.

4.2 Discussion

4.2.1 Determining normal ocular flora in sheep eyes
This part of the study investigated the bacterial flora isolated from sheep eyes affected by IOK, unaffected by IOK, apparently healthy eyes, and eyes deemed control eyes due to no clinical signs of IOK and minimal time in the pre-export feedlot. Despite sampling occurring at different times of year and on different days the results highlight the broad range of organisms present in the eyes of sheep. The most commonly isolated organism in affected, unaffected and apparently healthy eyes was *Moraxella ovis*. *Mycoplasma* species was the second most commonly isolated organism from affected eyes and apparently healthy eyes and was isolated less frequently in unaffected eyes. Mycoplasma was isolated at rates of less than 5% in control eyes. Sampling at different times of year repeatedly showed that *Moraxella ovis* and *Mycoplasma spp* were the most commonly isolated organism in affected animals. These results agree with other research where these two organisms predominated in IOK (Åkerstedt and Hofshagen, 2004). *Moraxella ovis* could be associated with severe clinical signs of IOK and again confirmed research by Dagnall (1994) that found that the smooth colony type of *Moraxella* ovis produced more severe lesions.

It is estimated that during the wetter and cooler months approximately 0.03% of sheep arriving at the feedlot will have signs of IOK. This increases to 0.4% during the hot and dry months (Tim Counsel Wellard Rural Exports Pty Ltd pers. comm.)

This study showed that eyes showing no clinical signs could still harbour significant organisms for IOK and thus be a risk to other sheep. Naglic *et al* (2000) reported severe ocular disease in native sheep in Croatia following the importation of 6,000 sheep from Australia and New Zealand – these native sheep had no history of ocular disease and the new sheep had been quarantined for 30 days before being distributed all over Croatia. During the quarantine period no signs of ocular disease were observed. The report of Naglic *et al* (2000) is interesting because no disease was reported in the case of native sheep and imported sheep housed in adjacent pens – disease spread appeared to be when imported sheep were exposed to native sheep during the time of grazing common pasture. Naglic *et al* (2000) reported *Mycoplasma conjunctivae* could be found in animals showing signs of IOK and those that appeared to have no clinical signs, but it was always found in sheep in flocks where IOK was present.

Further research needs to be conducted to determine if virulent and non-virulent types of *Moraxella ovis* exist and whether the organism has a role in predisposing the eye to infection with *M. conjunctivae*.

Future work should also investigate virulence factors of *M*. *conjunctivae* and their role in disease progression and transmission (Buller *et al* 2010 submitted for publication).

A total of 37 bacterial species were isolated in the study. The significance of a number of these species is not fully known. Spradbrow (1968) found that *Micrococcus spp*., *Streptococcus spp*., *Achromobacter spp*., *Corynebacterium spp*., *Bacillus spp*., and *Moraxella spp*. were found in the ovine conjunctival sac of a healthy sheep showing no clinical signs of ocular disease. *Bacillus spp*. are commonly found in the environment, in particular dust (N. Buller pers. comm.). The initial work reported in this study found high rates of isolation of a particular *Bacillus spp*. in infected eyes. The researchers believe that this is likely to be a secondary agent; whether this entered the eye via dust contamination or was already in the eye, is unknown.

4.2.2 Sensitivities

The antibiotic sensitivity study focused on testing the sensitivities of *Moraxella* isolates only. It was found that, of the drugs tested, resistance was observed only to Neomycin and this was borderline resistance. Neomycin is not a drug that is commonly used in sheep medicine in Western Australia. Licensed injectable forms are available (Neomycin Sulfate Injection Jurox™, Neomycin-Penicillin 100/200 Intervet/Schering-Plough Animal Health™) however none have licensing for use as a treatment for IOK in sheep. The efficacy of Neomycin at achieving adequate levels in the lacrimal fluid following systemic administration is unknown, however

Dihydrostreptomycin (another aminoglycoside) has been found to have poor penetration into the lacrimal fluid following intra-muscular injection (Nouws and Kong 1983). All isolates were sensitive to tetracyclines which supports their use in the treatment of IOK.

4.2.3 Treatment Trial

Numerous treatments have been recommended for Infectious Ovine Keratoconjunctivitis. Many of these are no longer used, for example treatments suggested by stockmen to the researchers as being effective for IOK included kerosene and cod liver oil. Kerosene was not tested because the Material Safety Data sheets for kerosene listed eye irritation as a potential adverse health effect (MSDS Science Stuff, Inc). Cod liver oil used in the 1930's as an effective treatment for external infections of the eyes in humans was not tested in this study.

Moraxella ovis and *Mycoplasma spp* were apparently eliminated in all treatment groups and the control group. The use of the topical powder treatment gave a better result than no treatment for *Moraxella ovis.* The use of the topical powder treatment gave a poorer result than no treatment for *Mycoplasma spp.*

Studies conducted in the United Kingdom by Hosie (1988) indicated that a combination of injectable and topical chlortetracycline powder therapy resolved clinical signs. Animals in Group 6 received this treatment during the trial. Analysis of the results found that these animals showed the second greatest clinical improvement following treatment (of the treatments studied). It was found that giving the topical treatment of oxytetracycline powder in addition to the systemic oxytetracycline dose was better than a single systemic injection at giving clinical improvement in the eye. This is an interesting finding as any aqueous topical treatment has been found to have a half-life of a few minutes in the lacrimal fluid (Ward and Clark 1991) therefore it would be expected that its effect would be minimal. When the oxytetracycline powder was used on its own in Group 5 animals, these animals

showed the least improvement clinically and were found to be clinically worse following the trial than those in Group 3 which had received no treatment.

The topical oxytetracycline treatments (both powder and aerosol) were found to resolve clinical signs during application of the treatment, however, as the graph shows (Graph 1, section 4.1.3), clinical signs re-appeared following cessation of treatment and in many individuals, the clinical signs were worse than on initial presentation. Animals in Group 7 and Group 5 received topical aerosol spray and powder respectively and neither group demonstrated a significant clinical improvement following treatment. These groups had the smallest clinical improvement of all groups, including Group 3 which received no treatment.

Normal corneal anatomy plus the constant washing from the tear film prevent high levels of drug penetration of the cornea (Davidson 2009). McConnel *et al* (2007) working on the treatment of Infectious Keratoconjunctivitis in cattle stated that the tear half-life of aqueous topical preparations was short and any antimicrobials sprayed into the eye were likely to be washed away by the tear film within a few minutes. Davidson (2009) also stated that the contact time for topical ophthalmic solutions and suspensions is short and only about 1 to 10% of the drug is absorbed by the corneal stroma. As a result of these pharmacokinetic principles, frequent applications are required to maintain minimum inhibitory concentrations in the ocular tissue. It was also found that sheep showed aversion to topical treatments, in particular to the Terramycin Pink Eye Aerosol™. Animals were reluctant to move into the race for treatments or examination following commencement of treatment with the aerosol. Based on the results of the trial it was found that those animals that received no treatment showed a significantly greater clinical improvement than those treated with topical oxytetracyclines. As a treatment protocol, both powder and aerosol topical treatments are labour intensive and therefore impracticable for use on a large number of animals, aside from the issues of efficacy and aversion to application seen in this trial. This confirmed Davidson's (2009) findings that drugs manufactured in powder form are irritating and have low drug bioavailability and are not recommended for application to eyes.

Orbenin™, cloxacillin, has been advocated as a treatment for infectious keratoconjunctivitis in both sheep and cattle. In cattle eyes, the effects of cloxacillin have been well documented (Buswell, 1983; Daigneault, 1990) and the drug has been shown to be effective. Unlike the aqueous medications discussed previously, cloxacillin is in an ointment base and this formulation provides an increased contact time with the ocular tissue due to the increased viscosity of the preparation and the slow release of the drug from droplets which have been found to settle in the inferior cul-de-sac (McConnel *et al*, 2007). However, Infectious Bovine Keratoconjunctivitis is known to be caused by *Moraxella bovis* only, an organism which has good sensitivity to cloxacillin. In IOK however, the involvement of *Mycoplasma* will limit the efficacy of cloxacillin because this drug has limited to no effect due to the absence of a cell wall in these organisms; penicillins act on receptors in the cell wall of bacteria to cause bacterial death (Rang *et al*, 1999). Hosie (1988) found that a single dose of Aureomycin Powder ™ (Chlortetracycline) was more effective than cloxacillin ointment at treating clinical IOK in hill sheep in the United Kingdom. Sheep in Group 2 received treatment with cloxacillin ointment. Of those animals treated with topical

preparations, these sheep showed the greatest clinical improvement. However overall, animals in Group 2 showed the fifth greatest clinical improvement. Given the increased labour effort required in applying the treatment and lower levels of drug bioavailability weighed against that of more effective treatments, cloxacillin ointment could not be recommended as a treatment of choice.

In-water medications would appear to be the least labour intensive and therefore the most suitable treatment protocol for use on large numbers of animals as occurs in a sheep pre-export feedlot. However the use of in-water or in-feed medications makes control of dosing difficult; some animals are likely to be under-dosed and potentially some over-dosed. It was found during the treatment trial that, on average, the sheep were drinking 2 litres of medicated water per head per day which would deliver a sub-therapeutic dose according to the manufacturer's guidelines. Davidson (2009) recommended a dose of 22mg/kg once daily for 5 days for oral administration of tetracycline – this is double the dose rate used in our study (880mg for a 40kg sheep versus the 400mg active ingredient we used). Normal levels of water intake for sheep are known to be around 4 litres per day; however this can vary widely as a result of a number of factors including weather, diet, water quality and access to water. Although a clinical improvement was seen in the sheep in Group 4 (oxytetracycline in the water) (Graph 1, Section 4.1.3) shows that this improvement was less than seen with other treatment protocols. Animals in Group 4, oxytetracycline in the water, showed the 4th greatest clinical improvement. These animals improved more so than those in the control group and those receiving topical medications. At present there are no in-water or in-feed oxytetracycline preparations licensed for use in sheep in Australia. Use of such medications on board a livestock carrying vessel is not controlled by on-shore regulations; however use in animals in a pre-export feedlot would be subject to standard AVPMA regulations. Although the use of in-water antibiotic medication shows promise, the effect on rumen microflora is unknown; it could potentially cause diarrhoea, have an impact on the animal's appetite and could lead to other diseases, e.g. Salmonellosis.

The use of systemic antibiotic therapy for IOK has been studied throughout the world. Systemic antibiotic therapy is usually given by way of an intramuscular injection of a long acting antibiotic preparation. This limits the labour requirements for treatment and has been found to be effective (Hosie *et al*, 1995). Hosie *et al* (1995) found that a single injection of long-acting oxytetracycline at a dose of 20mg/kg body weight halted signs and produced a rapid clinical cure of severe conjunctivitis. Konig (1983) also found a single injection of long-acting oxytetracycline to be effective in treating clinical cases.

The current study compared the efficacy of both a single injection and that of two injections 4 days apart. Animals in Group 8 were found to show the greatest clinical improvement of all animals in the treatment trial. Animals in Group 1 were found to show the third greatest clinical improvement. There was no statistical difference between the improvement seen in sheep treated with one, or two, oxytetracycline injections. Both groups showed a highly significant clinical improvement following treatment ($p<0.005$ Group 1 and $p<0.001$ Group 8).

Cost must be a consideration when choosing a treatment for use in sheep in a feedlot. The least expensive treatment was oral medication (powder in drinking water), see Table 10 Section 4.1.3, however this was not the most effective of the treatments tested.

It was noted that all animals in the treatment trial continued to eat and drink as normal. Axelson (1961) reported that feed intakes can be reduced and animals will lose weight as a result of infection with IOK. The sheep in the trial showed only moderate signs of IOK. It would be expected that sheep with severe clinical signs would have limited visibility and would be likely to be disorientated and may struggle to locate feed and water troughs.

4.2.4 Risk Factors

Although the researchers were unable to fully ascertain the risk factors leading to IOK in a pre-export feedlot in SW Western Australia, it is possible to comment on the published literature and observations made during the study. As discussed in section 1, possible risk factors involved in IOK have been commented on by researchers in many parts of the world.

IOK is an infectious disease that can be spread amongst sheep by a number of routes: physical eye-to-eye contact (Hosie, 1988, Egwu *et al* 1989); nasal discharge (Beveridge 1942) or vectors (Jones 1991). A number of vectors may be responsible for transmitting a variety of diseases. In the case of IOK insects, and in particular flies, are the most commonly associated vector. Jones (1991) found that flies were responsible for spreading *M conjunctivae* between domestic and wild animals in Switzerland. Beveridge (1942) also postulated that outbreaks were more commonly seen in Australia during the summer months as a result of the high populations of flies at this time of year.

Prevalence of infections is higher in the summer at the pre-export feedlot than during the winter, 0.4% compared to 0.03%. Beveridge (1942) reported that in Australia, IOK was most prevalent during Spring, Summer and Autumn.

Cooper (1967) reported that droving and yarding could be factors which might pre-dispose to sheep developing IOK. Hosie (2000) described transportation as an additional factor. It is likely that the increase in animal to animal contact during yarding and droving would lead to spread of disease. Grieg (1989) described trough feeding and feeding hay out in hay racks as predisposing to the spread of IOK. There is contradicting information in the literature regarding the effect of stocking rates. Hosie (2000) stated that IOK is most easily spread when animals are housed or trough feed, whereas Cooper (1967) found that stocking rate made no difference. It is possible that this finding could be related more to sheep at pasture than those in housing.

All ages of animals are susceptible to the IOK. Egwu (1989) found that lambs suffered a milder and more transient infection. Hosie (2000) also states that disease in young lambs is generally very mild. Beveridge (1942) reported that outbreaks in Australia were more common, and signs more severe, in weaners.

Janovsky (2001) identified that clinically healthy sheep can be carriers of *Mycoplasma*

conjunctivae, and Hosie (2000) discussed the possibility of sheep remaining carriers for 3 months. It is likely that the introduction of new animals could lead to IOK as found by Naglic (2000) in Croatia.

When the information taken from the literature is applied to a pre-export feedlot situation it is not surprising that outbreaks of IOK occur. The feedlot will often receive up to 50000 sheep from a number of property sources over a few days. These sheep will be mustered and yarded at the property of origin prior to being transported by road for up to a maximum of 8 hours before arriving at the feedlot. On arrival sheep are unloaded and drafted into groups based on a number of traits including animal type, weight and absence or presence of horns. These groups of sheep are then housed in covered, raised pens and trough fed pellets until the quarantine period is over, at which stage the sheep are loaded and moved by road truck to the wharf for loading onto a vessel for export. It is likely that further mixing will occur at the wharf and on the vessel. Throughout the process the sheep are commonly in close contact with both sheep from the same property of origin and those from different properties. Added to this are the hot, dry and dusty conditions that are commonly associated with SW Western Australia and the high number of flies and almost all the described risk factors are present.

5 Success in achieving objectives

The project underwent a change in management with Associate Professor Helen Chapman taking over from Dr David Beatty as chief investigator and Dr Fraser Murdoch as investigator. Dr Nicky Buller (Department of Agriculture and Food WA) has been responsible for microbiology and Professor Ian Robertson for statistical analyses.

All the stated objectives have been achieved either fully (objectives 2, 3 and 4) or in part (objective 1).

Objective 1 has not been fully achieved. A questionnaire was produced to ascertain the risk factors for IOK. Staff at the sheep pre-export feedlot were asked to complete this questionnaire, however to date no completed forms have been returned to the researchers and advice has been received that the information requested would be difficult to obtain, thus was not pursued.

It has proved difficult to trace animals back through to farms of origin to ascertain risk factors throughout the process. Further work is required to complete this objective; however it is the researchers' opinion that, given the nature of the operation, it is unlikely that identifying risk factors will assist in reducing the prevalence of IOK in a pre-export feedlot. There is a large amount of published information, much of which has been referred to in this report, which highlights that housing animals closely in hot, dry and dusty conditions will significantly increase the prevalence of IOK – a situation which is unavoidable in a pre-export feedlot or on board a livestock carrier.

Objectives 2 and 3 have been successfully achieved. A paper will be submitted for publication detailing the results of the research looking at ocular flora in clinically normal sheep and those with IOK signs, in addition to a paper on the treatment trial, subject to appropriate permission from MLA.

The initial ocular flora work was detailed in *Humphry, P*: "Causative agents of ovine keratoconjunctivitis in a Western Australian feedlot". Honours. Murdoch University, Perth, 2008.

Objective 4 was also successfully completed. Analysis of the results identified which treatment protocols warrant further investigation. It is hoped that MLA will grant permission for the authors to publish the results of the treatment trials in a scientific journal.

6 Impact on meat and livestock industry – now and in five years time

6.1 Welfare

IOK has welfare implications especially if sheep become blind as a consequence of bilateral eye infections. The condition is likely to be painful in the stages of corneal oedema, and ulceration however throughout the study all animals continued to eat and drink as normal.

Severely affected sheep may stop eating and lose weight if untreated. Interest in livestock welfare is likely to increase and the expectation is that diseases are treated or prevented and that suffering is minimised.

6.2 Rejections for sale or export

It is estimated that the current loss to the industry through rejections at the feedlot due to IOK is 0.5%. Effective treatment of this disease could reduce this loss.

7 Conclusions and recommendations

7.1 Conclusions

Infectious Ovine Keratoconjunctivitis (IOK), Pink Eye, has a significant impact on rejection rates of sheep for export (rejection rate of 0.5% per year) and thus the feedlot and export industries in Western Australia. The study aimed to determine the flora and sensitivity to antibiotics of eyes showing clinical signs of IOK.

Of the 352 eyes that were sampled, up to 37 bacterial species were found in eyes, with the highest number of 28 found in affected eyes, compared to 23 species found in unaffected eyes, 11 species found in apparently healthy eyes (Part 1) and 19 species in control eyes (Part 2).

Results showed that in sheep in a pre-export feedlot, the most commonly isolated organism in affected, unaffected and apparently healthy eyes was *Moraxella ovis*.

Mycoplasma species was the second most commonly isolated organism from affected eyes and apparently healthy eyes and was isolated less frequently in unaffected eyes. *Mycoplasma spp* was isolated at rates of less than 5% in control eyes.

Clinical signs were also seen in eyes that did not harbour *M. conjunctivae* indicating that other organisms, including *Moraxella ovis*, may have a role in IOK.

Seven treatment regimes were tested. The order of clinical improvements was related to the different treatments.

Group 8 treatment (Oxytetracycline (Alamycin 300 LA Norbrook Lab. Aust. Pty at a dose of 20mg/kg on day 0 and day 4 by intramuscular injection) was found to be significantly better than Groups 2 (Cloxacillin ointment), 3 (No treatment), 4 (Oxytetracycline powder in the water), 5 (Oxytetracycline powder in the eye) and 7 (Oxytetracycline spray). Group 6 (Oxytetracycline powder in the eye and a single oxytetracycline injection) treatment was significantly better than Groups 3 (no treatment), 5 (Oxytetracycline powder in the eye) , and 7 (Oxytetracycline spray). Therefore the study shows that Group 3, the Control group, was significantly worse than Groups 6 and 8.

Table 10 (Section 4.1.3) also shows the difference in costs associated with a course of treatment for each group. It is interesting to note that those treatments found to be the least effective, Groups 5 and 7, were amongst the most expensive of those tested. Although Group 6 was ranked second most effective treatment, it was the most expensive treatment; and it would be difficult to advise its use over the treatment given to Groups 8 or 1 based on cost alone.

7.2 Recommendations

Further research needs to be conducted to determine if virulent and non-virulent types of *Moraxella ovis* exist and whether the organism has a role in predisposing the eye to infection with *Mycoplasma conjunctivae*. Future work could also investigate virulence factors of *M*. *conjunctivae* and their role in disease progression and transmission. The possibility of a vaccine against one or both of *Moraxella ovis* or *Mycoplasma conjunctivae* could be considered.

The use of in-water oxytetracycline shows promise based on the small sample size. It is the researchers' recommendation that further experimental work be carried out on the oral administration of oxytetracycline to assess its efficacy. It is recommended that experiments be carried out using a larger number of sheep and a higher dose rate of oxytetracycline (22mg/kg bodyweight of active ingredient) given daily in water for 5 days. As previously discussed, the use of this medication would be an off-label use of this product and consultation with the AVPMA would be required before a recommendation could be made to use this treatment protocol commercially. However because of the potential detrimental effect on normal gut microflora, in-water medication may be contraindicated and should only be considered for IOK affected sheep.

Based on the findings of the bacteriological studies and seven treatment trials it is the researchers' recommendation that:
Sheep showing clinical signs of IOK (Pink Eye) should be treated with long-acting oxytetracycline at a dose of 20mg/kg bodyweight injected into the neck muscle and if clinical signs are still present after 4 days, a second injection at the same dose rate should be administered. The time interval of the second injection should be related to the recommendation of the manufacturer of the particular proprietary preparation used. This might be less than the 4 days required for the product used in the reported trial. The meat withholding period should also be a factor considered when choosing a proprietary product.
Ideally, treatment should be initiated at the first signs of the disease; the early signs being epiphora and tear-staining below the eye.

8 Bibliography

Åkerstedt J. Hofshagen M: Bacteriological Investigation of Infectious Keratoconjunctivitis in Norwegian Sheep. *Acta Veterinaria Scandinavica* 2004, 45, 19-26

Axelsen A: Effect of Contagious Opthalmia on multiple lambing and sheep liveweight. *The Australian Veterinary Journal*. 1961, 37, 60-62

Humphry, P: Causative agents of ovine keratoconjunctivitis in a Western Australian feedlot. Honours. Murdoch University, Perth, 2008.

Belloy L, Janovsky M, Vilei E, Pilo P, Giacometti M and Frey J: Molecular Epidemiology of *Mycoplasma conjunctivae* in Caprinae: Transmission across the species in natural outbreaks. Applied and Environmental Microbiology 2003 69 (4) 1913-1919

Beveridge WIB: Investigations on contagious opthalmia of sheep with special attention to the epidemiology of infection by *Rickettsiae conjunctivae*. *The Australian Veterinary Journal*. 1942, 18, 155-164

Buller, NB et al.: An investigation of eye microflora found in sheep with infectious keratoconjunctivitis (Pink eye) in a West Australian feedlot. (Submitted for publication).

Buswel JFl, Hewett GR: Single topical treatment for bovine keratoconjunctivitis using benzathine cloxacillin. *Veterinary Record* 1983, 113:2626, 621-622.

Cooper BS: Contagious conjunctivo-keratitis (C.C.K.) of sheep in New Zealand. *New Zealand Veterinary Journal* 1967, 15, 79-84

Dagnall, GJR: The role of *Branhamella ovis*, *Mycoplasma conjunctivae* and *Chlamydia psittaci* in conjunctivitis of sheep. *British Veterinary Journal, 1994,* 150, 65-71.

Daigneault J, George LW: Topically applied benzathine cloxacillin for the treatment of experimentally induced infectious bovine keratoconjunctivitis. *American Journal of Veterinary Research*, 1990, 51, 376-80.

Davidson HJ: Opthalmic therapeutics. In *Current Veterinary Therapy, Food Animal Practice*, DE Anderson, DM Rings, Elsevier, USA, 2009, 445-448.

Egwu G, Faull W, Bradbury J, Clarkson M: Ovine Keratoconjunctivitis: a microbiological study of clinically unaffected and affected sheep eyes with special reference to *Mycoplasma conjunctivae*. *Veterinary Record*, 1989, 135, 253-256

Grieg A: Ovine Keratoconjunctivitis. *In Practice,* 1989, 11, 3, 110-113

Humphries P: Causative agents of ovine Keratoconjunctivitis in a Western Australian feedlot. Honours Thesis. Murdoch University, Perth, Western Australia.

Hosie BD: Keratoconjunctivitis in a hill sheep flock. *Veterinary Record*, 1988, 122, Issue 2, 40-43.

Hosie BD, *Greig A*: Role of oxytetracycline dehydrate in the treatment of mycoplasma associated ovine keratoconjunctivitis in lambs. *British Veterinary Journal*, 1995, 151, 83-88.

Hosie BD: Infectious Keratoconjunctivitis. In *Diseases of Sheep*. Martin W and Aitken I. Blackwell Science Ltd. 2000, 301-304

Janovsky M, Frey J, Nicolet J, Belloy L, Goldschmidt-Clermont E and Giacometti M: Mycoplasma conjunctivae is self-maintained in the Swiss domestic sheep population. *Veterinary Microbiology*. 2001, 83, 11-22.

Jones G : Infectious Keratoconjunctivitis. In *Diseases of Sheep*. Martin W and Aitken I. Oxford Blackwell.1991, 214-217

Konig CDW,: “Pink eye” or “zere oogjies” or keratoconjunctivitis infectiosa ovis (KIO). Clinical efficacy of a number of antimicrobial therapies. *Veterinary Quarterly*, 1983, 5, 127-130.

*Lysnyansky I, Levisohn S, Bernstein M, Kenigswald G, Blum S, Gerchman I, Elad D and Brenner J :*Diagnosis of *Mycoplasma conjunctivae* and *Chlamydophila* spp in an episode of conjunctivitis/keratoconjunctivitis in lambs. *Israel Journal of Veterinary Medicine ,*2007 62 (3-4)

McConnel CS, *Shum L*, *House JK:* Infectious bovine keratoconjunctivitis antimicrobial therapy, Australian Veterinary Journal, 2007, 85, 65-69.

MSDS Science Stuff, Inc http://www.sciencestuff.com/msds/C1955.html (accessed 19-10-10)

Naglic T, Hajsig D, Frey J, Seol, B, Busch K, Lojkic M : Epidemiological and microbiological study of an outbreak of infectious keratoconjunctivitis in sheep. *The Veterinary Record*, 2000, 147, 72-75.

Nouws JFM, Konig CDW: Penetration of some antibiotics into the lacrimal fluid of sheep, Veterinary Quarterly, Vol. 5, No. 3, 114-121, July 1983

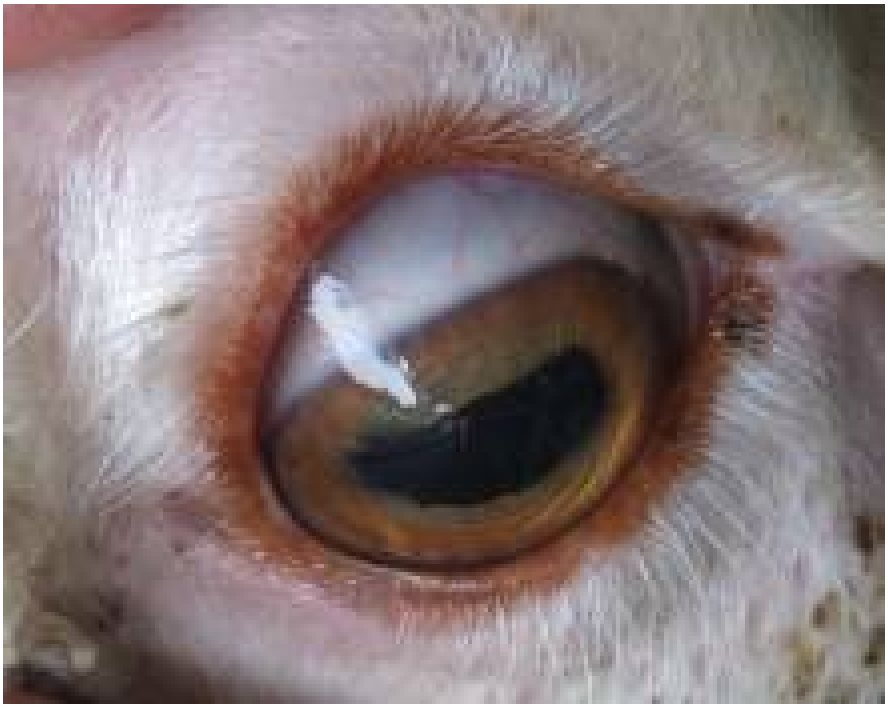
Rang HP, Dale, MM, Ritter JM: Pharmacology. Churchill Livingstone, Edinburgh, 1999, pp. 690-691.

Spradbrow P:Experimental infection of the ovine cornea with *Neisseria ovis*. *Veterinary Record*, 1971, 88, 615-616

Ward DA, *Clark ES*: Ocular Pharmacology. *Veterinary Clinics of North America: Food Animal Practice* – 1991, 7, 779-791.

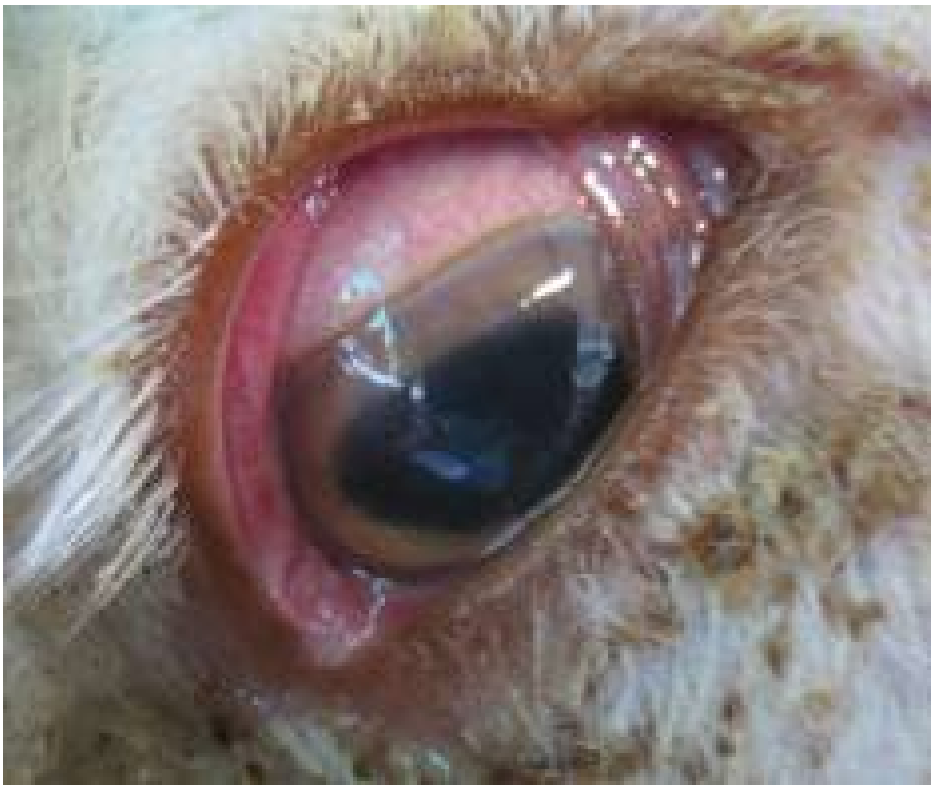
9 Appendices

9.1 Appendix 1 Clinical presentation of IOK and grading system

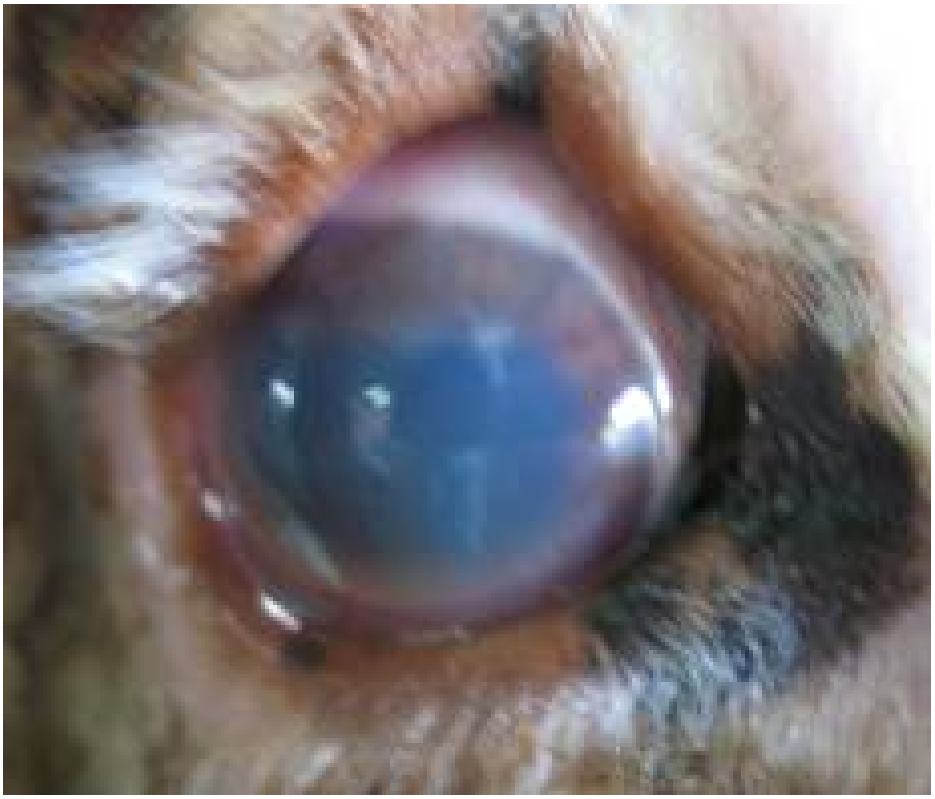


Grade 0 – normal eye

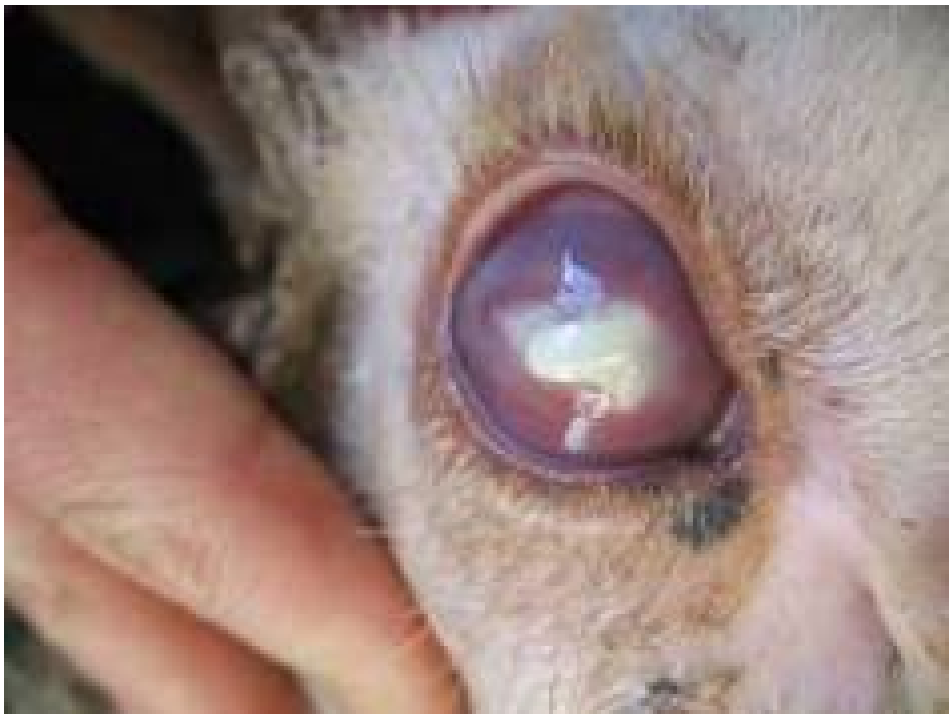
Grade 1 - Epiphora (weeping eye)



Grade 2 - Conjunctivitis and scleral injection



Grade 3 - Corneal oedema (clouding of the cornea)



Group 4 – Corneal ulceration

Group 5 – Corneal neovascularisation

Group 6 – Chronic eye damage