Final report

Risk factors, treatment and prevention options for pinkeye disease in cattle

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Abstract

Pinkeye (infectious bovine keratoconjunctivitis, IBK) is an economically important eye disease occurring in all cattle producing areas of Australia. This project aimed to update the status of frequency of occurrence of this disease, its economic significance, to identify risk factors for the disease and assess the efficacy of different treatments and vaccination with the goal to inform policy development for better prevention, control and management the disease at the producer level in the Australian beef industry.

A study was conducted to estimate the frequency of occurrence of the disease in Australia using the sales of prescription medications as a surrogate indicator. A survey of beef producers was conducted to identify the risk factors for the occurrence of the disease and to understand producers' perceptions about pinkeye treatments and the impact of the disease on farm productivity. Two randomised control trials were conducted: the first to evaluate the effectiveness of Piliguard® vaccine for naturally occurring pinkeye and the second to compare the effectiveness of currently available treatments. A pilot pharmacokinetic study was conducted to quantify antibiotic concentrations in bovine tears.

The annual incidence of pinkeye in Australia was estimated to be 10.25% (95% PI: 6.43, 16.97) and the Australian cattle industry is expected to lose A$ 9.67 million (95% PI: 8.56, 13.11) per annum just considering the cost of the three medications analysed. This indicates pinkeye has a higher economic impact in Australia than previously estimated. There was an increased risk of pinkeye on farms located in southern Australia, of smaller grazing area with cattle ≤ 2 years of age, and if respondents ranked their farms as having high fly levels, low rainfall, and high dust levels. The most used treatments for pinkeye were cloxacillin pinkeye ointments, followed by eye patches. The most common reason for not treating was that it was too difficult to treat individual animals. Producers ranked the pinkeye impact on farm productivity as high if they had cattle ≤ 2 years of age, treated for pinkeye more frequently, and as their herd size increased. The pinkeye vaccine was not found to be effective against naturally occurring pinkeye. After treating a calf with pinkeye ointment, cloxacillin was detected in tear samples but for a short period indicating increased dosing frequency is required.

This project generated evidence about disease risk factors, treatment and prevention options and highlighted ways to improve on-farm outcomes from pinkeye. Our studies stimulated renewed interest from the international research community in pinkeye disease. Findings and recommendations of this project would be valuable for the cattle industry, researchers, veterinarians and farmers, and will improve outcomes for cattle.

Executive summary

Background

Pinkeye is an economically important eye disease occurring in all cattle producing areas of Australia. Identified in MLA's priority list of endemic diseases for red meat industries, pinkeye can cause permanent scar and blindness, impact reproduction rates (bulls use sight to locate cows in oestrous), impact workplace health and safety (blind cattle can be dangerous), and be detrimental to animal welfare. It is debilitating and painful causing corneal ulceration, eyeball rupture, blindness, weight loss, injury and death by thirst, starvation or misadventure. Treatment and vaccination options for the disease are available but there is very limited information about their efficacy. Despite a large research effort into the disease for over 130 years (1), there has been no obvious improvement in on-farm outcomes.

This project was conducted to estimate the frequency of occurrence of pinkeye disease, better understand the impact of the disease, identify risk factors for the disease and evaluate the effectiveness of pinkeye vaccine and different treatments in managing the disease. The overall goal of the project was to generate evidence for the development of better prevention, control and management strategies for the disease in the Australian beef industry. We believe that the findings of the project would be valuable for Australian cattle producers, the cattle industry, researchers, and cattle veterinarians.

Objectives

The aim of this project was to update the status of frequency of occurrence of this disease, its economic significance, to identify risk factors for the disease and assess efficacy of different treatments and vaccination. Specifically, the objectives of this project were to:

1. Estimate the frequency of occurrence of the disease and impact on beef production in Australia
2. Identify animal and herd level risk factors for pinkeye occurrence
3. Evaluate the efficacy of different treatments on naturally occurring pinkeye
4. Evaluate the efficacy of the Piliguard® vaccine to prevent the disease

Methodology

The project employed a novel approach to estimate the frequency of occurrence and the impact of pinkeye based on Australian pinkeye drug sales data. An online survey was conducted to understand the risk factors of the disease and to better understand producers' perceptions about treating pinkeye and the impact of the disease on farm productivity. Two randomised control trials were conducted to evaluate the effectiveness of Piliguard® vaccine for pinkeye and to compare the effectiveness of currently available treatments to enable development of improved treatment guidelines for the disease. A pilot pharmacokinetic study was conducted to quantify antibiotic concentrations in bovine tears.

Results/key findings

The number of cattle affected by pinkeye each year in Australia was estimated to be 2.80 million (95% PI: 1.76, 4.65) or 10.25% (95% PI: 6.43, 16.97) of the entire Australian cattle herd each year.

The cattle industry losses A$ 9.67 million (95% PI: 8.56, 13.11) each year on the cost of three popular pinkeye medications alone. There was an increased risk of pinkeye on farms located in southern Australia, of smaller grazing area with cattle ≤ 2 years of age, and if respondents ranked their farms as having high fly levels, low rainfall, and high dust levels. Australian producers with smaller farm sizes, who yard their cattle more, and rate pinkeye as highly painful were more likely to treat for pinkeye more frequently. Pinkeye ointments containing cloxacillin were the most used treatment for pinkeye in Australia and the most common reason for not treating pinkeye was that it was too difficult to treat. Producers ranked pinkeye impact on farm productivity as high if they had cattle ≤ 2 years of age, if producers treated more frequently, and as their herd size increased. Pinkeye as an animal welfare issue was rated by the greatest number as high severity. The median amount reportedly spent on pinkeye in 2018 was $250 per farm. Reported spending increased as herd size and number of cattle affected increased, perception of the impact on farm productivity and animal welfare increased, if they treated more frequently, reported higher fly worry, if their herds contained Angus cattle, if they bred on farm, and if their farms were in southern Australia. Producers' attitudes to treatment and impact of pinkeye vary, but they are increasingly aware of the animal welfare implications. The first field trial of the only commercial pinkeye vaccine in Australia in seven herds in southwestern Queensland during two pinkeye seasons found the vaccine was not protective against naturally occurring pinkeye. After topical administration of cloxacillin into the lower conjunctival sac, cloxacillin reached peak concentration (834.24 μg/mL) at 1.42 hours, maintained a concentration > 1 μg/mL up to 17.33 hours but penicillin could not be detected in any tear sample.

Benefits to industry

The project updated information about the occurrence and economic significance of pinkeye and engendered renewed interest from the international research community in the disease. The project generated evidence about disease risk factors, treatment and prevention options and highlighted ways to improve on-farm outcomes of pinkeye.

Future research and recommendations

We recommend that the cattle industry to update economic models of disease impact based on project findings, increase funding for pinkeye control in southern Australia, develop guidelines for pinkeye treatment based on the project findings and educate producers about the study findings. To better manage pinkeye at the farm level, we encourage producers to control fly levels, monitor younger cattle more often and increase zebu content if it aligns with their breeding plan. We also recommend discontinuing outdated treatments and avoid spending on pinkeye vaccine.

Further work should be done to understand the influence of dust levels on pinkeye, finalise eye scores developed in the project and develop of an app for diagnosing pinkeye. Research should be done to develop a sensitive and specific diagnostic test for pinkeye and extending the pilot study done in this project to investigate pharmacokinetics of treatments.

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1. Background

Pinkeye, also known as infectious bovine keratoconjunctivitis (IBK), is the most significant ocular disease of cattle worldwide (2). It is considered to be caused by the bacterium *Moraxella bovis* as it remains the only organism that can reliably produce typical IBK in undamaged eyes in challenge studies (3), although the role of *Moraxella spp* in IBK is far from settled. Clinical signs of pinkeye are initially profuse lachrymation, photophobia and blepharospasm, followed by conjunctivitis and keratitis, with corneal ulceration. Pinkeye can lead to iridocyclitis, hypopyon and perforated cornea resulting in eyeball rupture; some will progress to buphthalmia or phthisis bulbi (2). Pinkeye is debilitating and painful (4) and can result in weight loss, injury and death by thirst, starvation or misadventure. Pinkeye can cause permanent scar and blindness, impact reproduction rates, workplace health and safety and animal welfare.

Pinkeye is a disease of major importance in all states of Australia and was listed 14th of 17 diseases of cattle in the 2015 Meat & Livestock Australia (MLA) report, *Priority list of endemic diseases for the red meat industries* (5). However, the incidence of pinkeye in Australia is not well known. Estimates of incidence range from 0.6% (5) to 90% (6) and vary with age and breed of cattle, between regions and over time. Spradbrow (6) reported the incidence of pinkeye in Queensland was extremely high, particularly in young stock during summer, with often more than 90% of calves and weaners affected. In another study, the incidence of pinkeye in weaner heifers in a Queensland herd from 1984 to 1986 was reported to be 43.1% in Hereford cross, 21.4% in Simmental cross, and 7.2% in Afrikander-Hereford cross cattle (7). In a nationwide postal questionnaire of beef and dairy producers of Australia, 81% of respondents reported occurrence of pinkeye between 1975 and 1979. The national pinkeye presence was estimated to be 4.5% in all cows and 10% in calves, with breed and climate significantly affecting prevalence (8, 9).

Despite numerous studies on pinkeye since first being described in 1889 (1), there are significant knowledge gaps on its impact on farm productivity, the on-farm costs, and disease consequences (10). The scant data available on herd-level impacts of pinkeye refer mostly to lower weaning weights in affected calves (11) and are mostly from the USA. Compared to healthy calves, pinkeye affected calves in the USA are reported to have shortfalls in weaning weights ranging from 7 (12) to 18 kg (13), with a study of 45,497 calf records reporting an average weaning weight deficit of 8.9 kg (14). Pinkeye majorly impacts cattle production and incurs significant costs in all states of Australia (15), but losses may vary substantially between regions and farms (16, 17) and are seldom explicitly quantified. The few Australian studies available that specify losses due to pinkeye are outdated. For example, a study found young *Bos taurus* (Hereford X Shorthorn) calves with pinkeye were on average 22.8 kg lighter at 15 months of age compared to those unaffected in a herd (18). Another study reported average daily gain (ADG) was decreased by up to 10 % in pinkeye affected cattle (7). Because pinkeye losses typically refer only to lower weaning weights, the full disease impact may be underestimated (11), thus there is a pressing need for better knowledge about all of the farm-level effects of pinkeye.

Information about risk factors for a disease is crucial for controlling a disease but there are remarkably few studies on pinkeye risk factors available in Australia or worldwide. Only one previous Australian study deals specifically with the risk factors of pinkeye. This was a targeted national survey of beef and dairy farmers conducted in 1979 by mailed questionnaire of 32 questions in four sections with 1458 respondents (8, 9). Two subsequent studies to assess the economic impact of common livestock diseases have included pinkeye (17, 19) and both rely heavily on findings of the former survey. Pinkeye is clearly multifactorial (20, 21), as animal susceptibility varies (11), and pathogenesis of field disease remains obscure (22, 23). Pinkeye risk factors can be categorized into the epidemiology triad of agents, host, and environment (11). Whilst *M. bovis* is considered the main cause, pinkeye may be a multi-agent disease (20, 21). Major host risk factors for pinkeye are cattle sub-species or breed, as well as age (11, 24) . Suspected environmental risk factors include geographical climate (11), season (25), ocular trauma (26), UV radiation (27), wind and ambient temperatures (25), rainfall (8, 28), dust (8), plant debris and pollens (29), congregation and crowding (17, 30), and flies (31). However, few of these risk factors have been properly assessed. Better knowledge of risk factors may improve outcomes for pinkeye on farm (32).

Like risk factors, there are significant gaps in our understanding of the treatment options for the disease. Pinkeye treatments reported to be effective include cloxacillin eye ointment, (33), subconjunctival injections of penicillin (34), and intramuscular injections of oxytetracycline (35). However, there is little information available to compare their efficacy. Two systematic reviews of antibiotic treatments of pinkeye have been published (36, 37). The 2006 review found a lack of randomisation and adequate controls in many of the published studies, with only nine randomised controlled trials available (37). In 2016, Cullen, Yuan (36) stated there were not enough suitable trials to allow meta-analysis of various antibiotic treatments of IBK. The authors found it was not possible to rank treatments by effectiveness due to lack of suitable data. The most common treatment for pinkeye is cloxacillin eye ointments- Orbenin® and Opticlox®, yet in vitro cloxacillin-sensitivity of *M bovis* is variable (McConnel, et al., 2007, Aust Vet J, 85: 70). Many cattle with pinkeye recover without treatment (Lane et al., 2015, Meat & Livestock Australia Report), complicating claims of efficacy of treatments ranging from turpentine flushing to saline washes. Sub-conjunctival antibiotic injection is recommended, yet such treatment was not better than 'no treatment' in one study (Allen, et al., 1995, J Am Vet Med Assoc. 206: 1200). Some beef producers claim eye patching alone is efficacious. Thus, further information is required about the effectiveness of various treatment options for naturally occurring field pinkeye.

Historically, attitudes of Australian farmers to treating pinkeye have differed which could, in part, be due to the variable nature of field disease (9). Australia is one of few countries with national data on pinkeye, and this is almost exclusively from an Australia-wide targeted survey of beef and dairy farmers conducted by mailed questionnaire in 1979 (8, 9). Despite being over forty years old, this seminal survey is also one of the few sources of information about farm-level approach to the treatment of pinkeye in Australia or worldwide. For example, it was reported that many beef farmers did not treat pinkeye because they accepted it as an inevitable part of cattle production. Of those farmers who did not treat, 60% said pinkeye does not do enough damage to bother treating, 43% said cost (or time) of handling cattle was too great, 11% said handling animals further spreads disease, 10% said animals recover without drugs, 9% said drugs are ineffective, 6% said handling diseased animals further weakens them, and 5% said drugs for treatment are too expensive (9). Little is known about current attitudes of farmers to treating pinkeye in their herds, even basic information like why some farmers treat more than others and what treatments are used. Whilst antibiotics are assumed to be the preferred treatment for pinkeye, with unprecedented access to online anecdotes and opinions, untested alternatives may also be used (38).

Coopers Bovilis Piliguard Pinkeye® vaccine (Intervet Australia Pty Ltd) is the only commercial pinkeye vaccine available in Australia. A serological survey of isolates of *M bovis* from naturally occurring pinkeye in Australia concluded that Piliguard may be a useful pinkeye management tool (39) and an MLA report suggested that an effective pinkeye preventative Piliguard® was available and assumed that it was used in 50%, 25% and 10% of highly, moderately and lowly affected southern herds, respectively (5). However, some other evidence suggests that pinkeye vaccines are ineffective (22, 40, 41), yet as at 2007 over 25 million doses of Coopers Piliguard Pinkeye Vaccine were sold in the USA in the preceding 8 years (42). Piliguard was first registered in USA in 1990 and in New Zealand and South Africa in 2000. According to the promotional brochure "*efficacy of the vaccine is accepted worldwide*", and "*efficacy has been proven internationally by controlled challenge studies, field trials and serology testing of vaccinated calves*", citing a 1990 challenge study; however, this study is 'commercial in confidence' and not available and there have been no trials on Piliguard conducted in Australia '*on ethical grounds*' (42).

This project was funded to update the status of frequency of occurrence of this disease, its economic significance, identify risk factors for the disease and assess the effectiveness of vaccination and different treatments. We conducted several studies to achieve these aims. Given the lack of recent data about the impact of this disease to the cattle industry, a study was undertaken to estimate the incidence and the treatment costs of the disease modelled on the sales of pinkeye medications in Australia. A comprehensive Australia-wide survey of cattle farmers was conducted to estimate risk factors associated with pinkeye (43), attitudes of Australian farmers to treating pinkeye and to estimate the impact of pinkeye on Australian farmers. In addition, two clinical trials were conducted: one to evaluate the effectiveness of Piliguard vaccine for pinkeye and the second to investigate the effectiveness of various treatments used for pinkeye. All this information is required to efficiently allocate resources, reduce unnecessary or improper use of antibiotics, better direct the research community, and ultimately improve pinkeye treatment outcomes for farmers and cattle.

2. Objectives

The overall aim of this project was to update the status of frequency of occurrence of this disease and evaluate treatment and prevention options to inform policy development for better prevention, control, and management the disease.

The objectives of this project were to:

1. Estimate the frequency of occurrence of the disease and compare the frequency between farms and feedlots.
2. Identify animal and herd level risk factors for pinkeye occurrence
3. Evaluate the efficacy of different treatments on naturally occurring pinkeye.
4. Evaluate the efficacy of the Piliguard® vaccine to prevent the disease.

In this report, we describe the studies conducted to achieve these objectives, summarise the results obtained and provide recommendations for making policy and practice decisions based on the findings. We expect that the findings from this study would be useful in making policy decisions regarding management and control of the disease in Australia.

3. Frequency of occurrence of pinkeye

3.1 Methodology

The purpose of this study was to use sales data of three popular products marketed for treatment of pinkeye to estimate the disease frequency and the treatment costs of the disease on the cattle industry in Australia.

3.1.1 Data collection and management

Detailed methods are presented in (16). Briefly, we first estimated the number of cattle that can be treated with a syringe or a can and then using the data of sales of pinkeye treatments and the total cattle population of Australia, estimated the incidence of pinkeye. Cost of the disease to the industry was estimated based on the sale price of pinkeye treatments and the total sale volume of these drugs.

Treatments listed under 'Pink eye' in the general index of the 2018 IVS, the Australian guide to veterinary medicines and products (44), are all antibacterial preparations: systemics Norfenicol® (Norbrook Laboratories Australia PL) and Nuflor LA injectable solution® (MSD Animal Health) both containing 300mg/ml florfenicol; and topicals Opticlox® Eye Ointment (Norbrook Laboratories Australia PL) and Terramycin® Pinkeye Aerosol and Powder (Zoetis Australia Pty Ltd). Other products registered for use in pinkeye are Orbenin® Eye Ointment (Zoetis Australia Pty Ltd) and injectable and oral Oxytetracylcine (OTC) (44). Please see Table 1 for further details.

Total monthly Australian sales data of Orbenin® Eye Ointment and Terramycin® Pinkeye Aerosol from March 2016 to April 2018, and of Opticlox® Eye Ointment from January 2015 to December 2017 were obtained from the manufacturers Zoetis Australia Pty Ltd and Norbrook Laboratories Australia P/L, respectively. Zoetis product data from March and April 2018 were excluded for consistency so that the dataset contained data for two years from March 2016 to February 2018. Australian total cattle population estimate was obtained from the Australian Bureau of Statistics: Agricultural Commodities (ABS, 2015). Average retail prices of cloxacillin eye ointments were estimated based on information supplied by practicing veterinarians from various states/territories of Australia in February and March 2019. Some veterinarians only sold Orbenin® Eye ointment or only Opticlox® Eye ointment, and some both. We obtained retail prices of Terramycin® Pinkeye Aerosol from rural produce retailers from six of the eight states and territories of Australia (excluding ACT and Tasmania) in February and March 2019. Sampling with replacement was conducted to generate a distribution of values of retail prices because there were only limited number of observations available.

Table 1. Information about the pinkeye products analysed in the study to estimate the cumulative incidence and cost of pinkeye in Australian cattle.

Product	Form	Active ingredient	Total active	Dose	Repeat	Other instructions	Estimated number of eyes that can be treated
Orbenin	3g syringe	166.67 mg/ml Benzathine cloxacillin	500mg	¼ to ½ contents of one syringe per eye	48-hour intervals if necessary	Advise to treat both eyes to prevent cross infection even if one eye is affected	2- 4 eyes per syringe
Opticlox	5g syringe	166.67 mg/ml Benzathine cloxacillin	833mg	15 to 30% contents of one syringe per eye	48-hour intervals if required	Advise to treat both eyes to prevent cross infection even if one eye is affected	3.3- 6.7 eyes per syringe
Terramycin pinkeye aerosol	125g aerosol can	2 mg/g Oxytetracycline	250mg	Spray for 2 seconds	3 times daily until complete resolution	Pack supplies approx. 40 applications of 2 sec duration	5- 40 eyes per can

3.1.2 Data analysis

Australian sales data were analysed to estimate the frequency of occurrence of pinkeye in Australia. The annual volume sold of each product was multiplied by the number of cattle that could be treated per unit to estimate the number of pinkeye affected cattle in Australia in a calendar year. Cumulative incidence was calculated by dividing the estimate of the number of pinkeye affected cattle per year with the total national cattle herd in Australia. To estimate the total annual cost to producers of three medications, the distributions of the retail price of Orbenin® and Opticlox® Eye ointments and Terramycin® aerosol were multiplied by their respective annual sales figures. Treatment costs for each product were then summed to estimate the overall annual treatment cost of pinkeye. Four sensitivity analyses were conducted to evaluate the impact of some of our assumptions on the number of cases of pinkeye and the cumulative incidence of the disease in Australia.

The estimates of the number of eyes treated per syringe/can were based on the amount of active ingredient and the usage recommendations for each product. It is recommended to treat both eyes of cattle with pinkeye, whether affected unilaterally or bilaterally, therefore we assumed number of cattle was half (i.e., multiplied by 0.5) of the number of eyes that could be treated per syringe/can. This estimate was multiplied by 0.80 to account for retreatment of a small proportion of cattle and for wastage (i.e., 20% used in treating a second time or wasted). We also assumed that only half of the cattle affected by pinkeye in Australia are treated but fitted a pert distribution by keeping mode at 0.5, min at 0.25 and max at 0.75 to take the variation into account. Months were classified into seasons as follows: Summer (December to February), Autumn (March to May), Winter (June to August) and Spring (September to November).

Input values used in the analyses are presented in Table 2. All analyses were conducted using R version 3.5.3 (45) employing R Base and mc2d packages.

Table 2. Input values used in the model to estimate the cumulative incidence and cost of pinkeye in Australian cattle. The distributions were included for some input values to account for variability and uncertainty using R statistical program (R core Team 2003).

Variable	**Input parameter**	**Distribution**	**Parameters**			**Justification**
Number of eyes treated per syringe/can			Min	Max		
	Orbenin	Uniform	2	4		Min and max values were estimated based on prescribing instructions (Table 1). Uniform distributions between these values were used as we had no information about the most likely value.
	Opticlox	Uniform	3.3	6.7		
	Terramycin	Uniform	5	40		
Proportion of cows treated in Australia			Min	Max	Mode	
	-	Pert	0.25	0.75	0.5	Pert distribution was used because information about min, mode and max values was available.
Number of units sold per year			Total			
	Orbenin	Fixed	732 864			Based on actual data
	Opticlox	Fixed	134 800			
	Terramycin	Fixed	27 755			
Total cattle herd	-	Fixed	27 400 000			Obtained from the Australian Bureau of Statistics: Agricultural Commodities (ABS, 2015).
Iterations			10000			

3.2 Results

Sales data indicate that 732,864 syringes of Orbenin® Eye Ointment, 27,755 cans of Terramycin® aerosol and 134,800 syringes of Opticlox® were sold each year in Australia (Table 3). Based on the input values presented in Table 2 and Figure 1, the annual quantity of these three products sold in Australia is sufficient to treat 1.39 million (95% PI: 1.00, 1.79) cattle with pinkeye. Adjusting the number for the proportion of cattle that are likely to be treated increases the number of pinkeye cases to 2.80 million cases (95% PI: 1.76, 4.65) (Figure 2).

Table 3. Sales of pinkeye medications in Australia based on two-year sales data for Orbenin eye ointment from March 2016 to February 2018, three-year sales data for Opticlox eye ointment from January 2015 to December 2017, and two-year sales data for Terramycin pinkeye aerosol from March 2016 to February 2018 obtained from drug manufacturers.

Month	Orbenin (Number of syringes)	Opticlox (Number of syringes)	Terramycin (Number of aerosol cans)
January	173472	22687	2841
February	92160	22907	3029
March	10368	13867	2100
April	576	11907	2106
May	4608	4553	646
June	0	3400	2300
July	79872	8140	3266
August	162048	5900	1176
September	3168	1720	839
October	13824	4300	2923
November	62784	13613	3293
December	129984	21807	3239
Total per annum	**732,864**	**134,800**	**27,755**

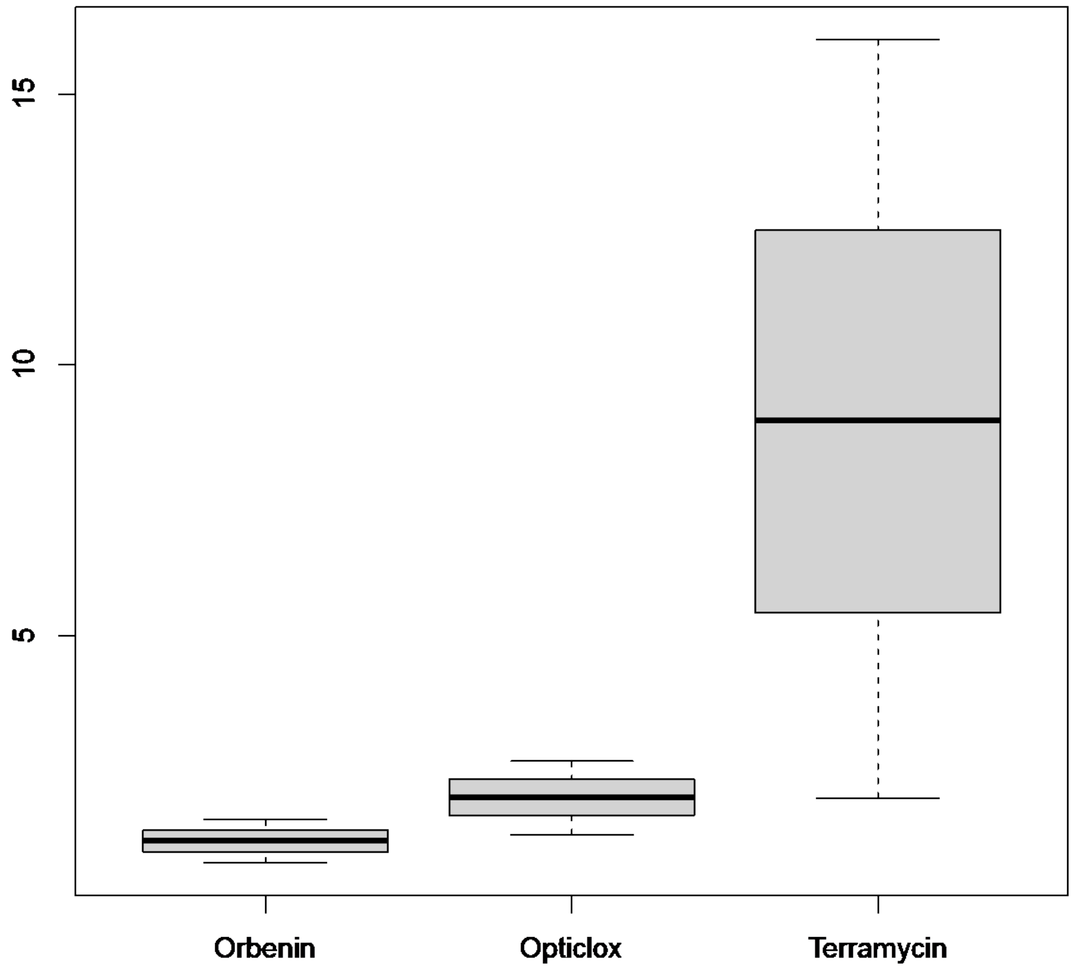


Figure 1. Number of cows treated per unit product based on recommended dose rates by drug manufacturers.

As of June 2015, the Australian cattle herd consisted of 27,400,000 cattle including 2,800,000 dairy cattle. Our modelling indicates that 10.25% (95% PI: 6.43, 16.97) of the entire Australian cattle herd is affected by pinkeye per year (Figure 2).

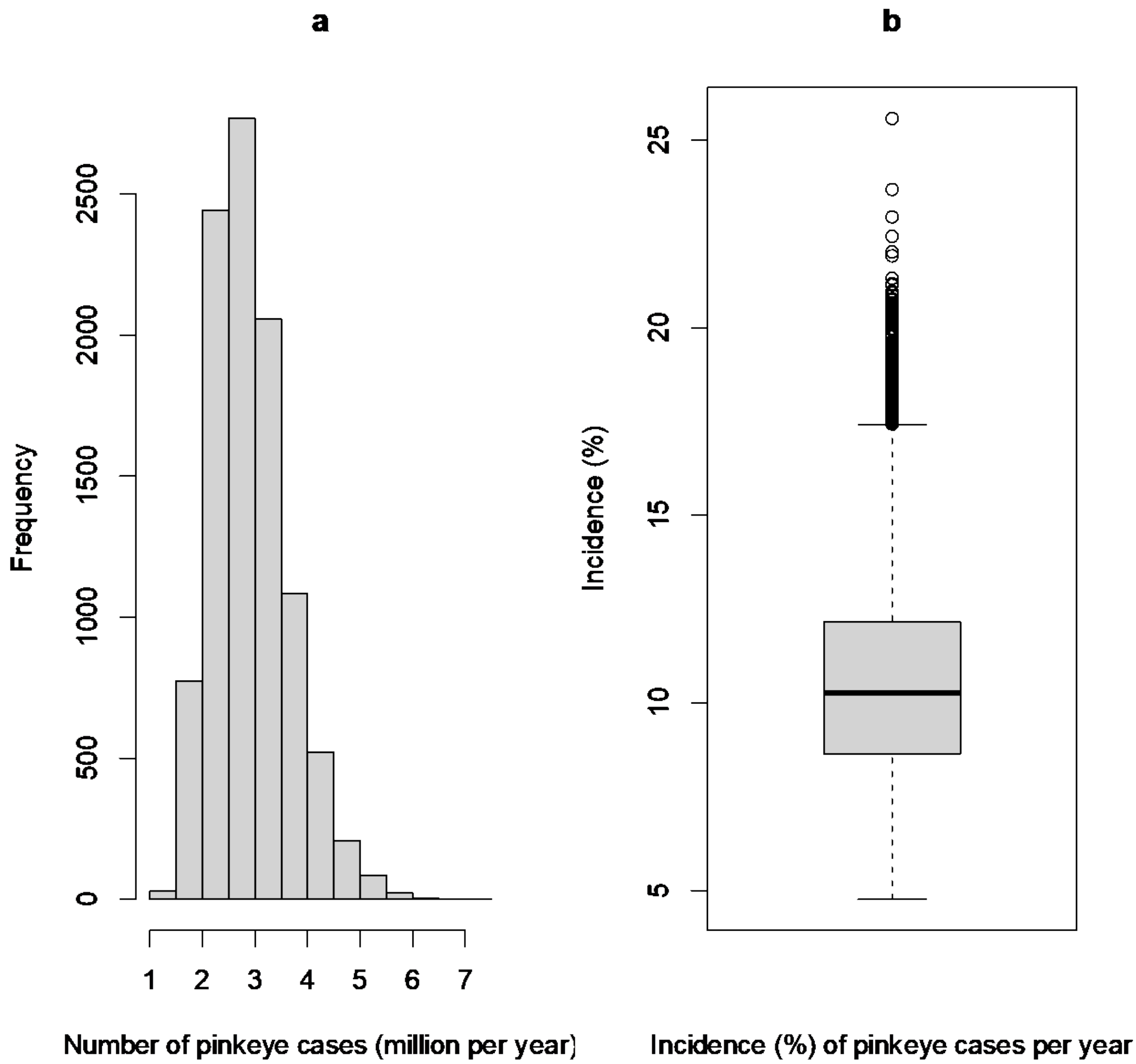


Figure 2. The estimated (a) number and (b) incidence of pinkeye cases in Australia based on the sales data of three pinkeye medications.

Sales data show a distinct seasonality, with sales of Orbenin®, Opticlox® and Terramycin® aerosol all being more common in summer (Figure 3). Sales of Opticlox® Eye Ointment were more common in New South Wales and Victoria (Figure 3).

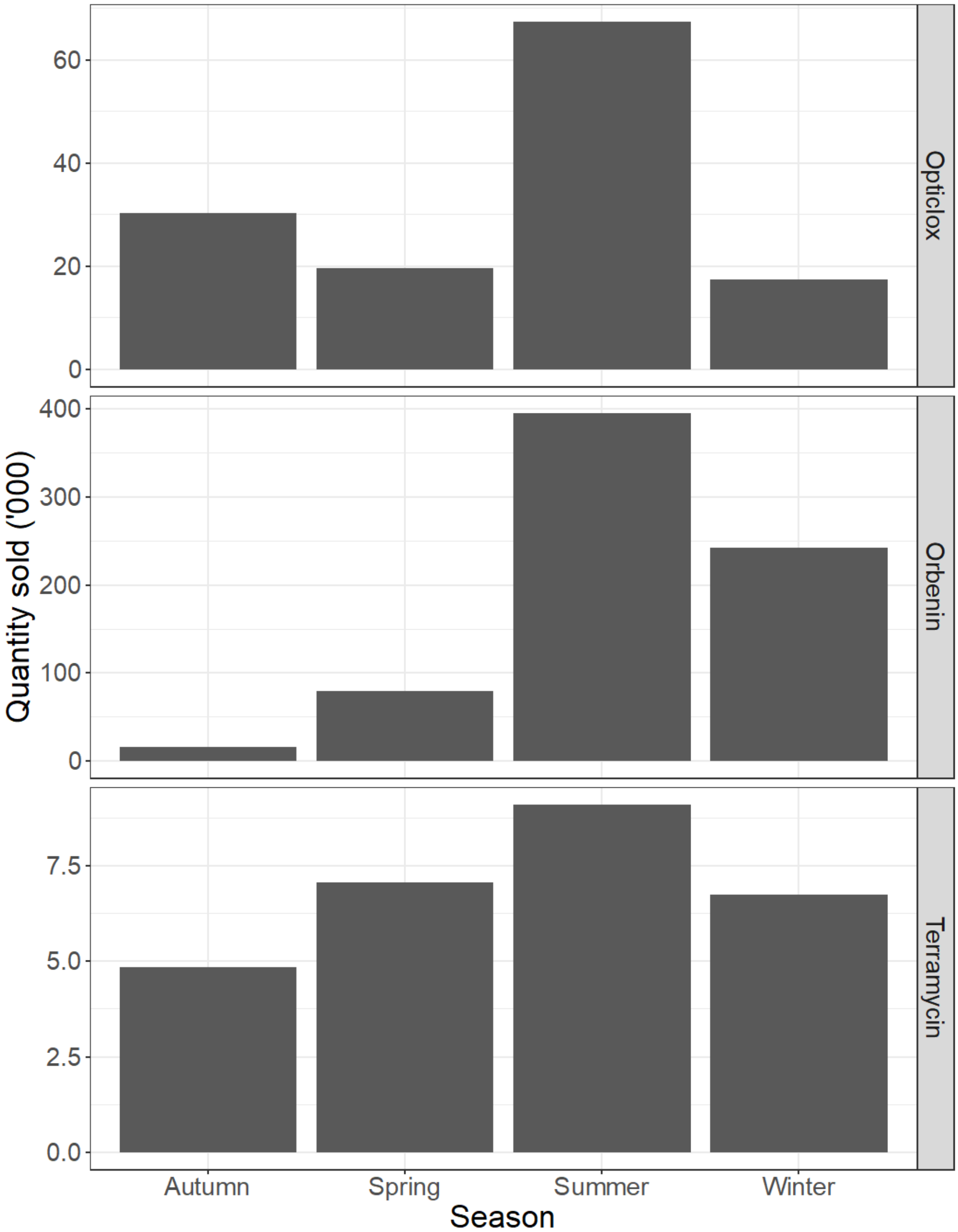


Figure 3. Sales of Orbenin, Terramycin and Opticlox *per annum* in Australia classified by season based on two-year sales data for Orbenin and Terramycin pinkeye spray from March 2016 to February 2018 and three-year sales data for Opticlox from January 2015 to December 2017 obtained from drug manufacturers.

The price per unit medication is shown in Figure 4. The total annual cost of the disease to the cattle industry was estimated to be A$ 9.67 million (95% PI: 8.56, 13.11). (Figure 5).

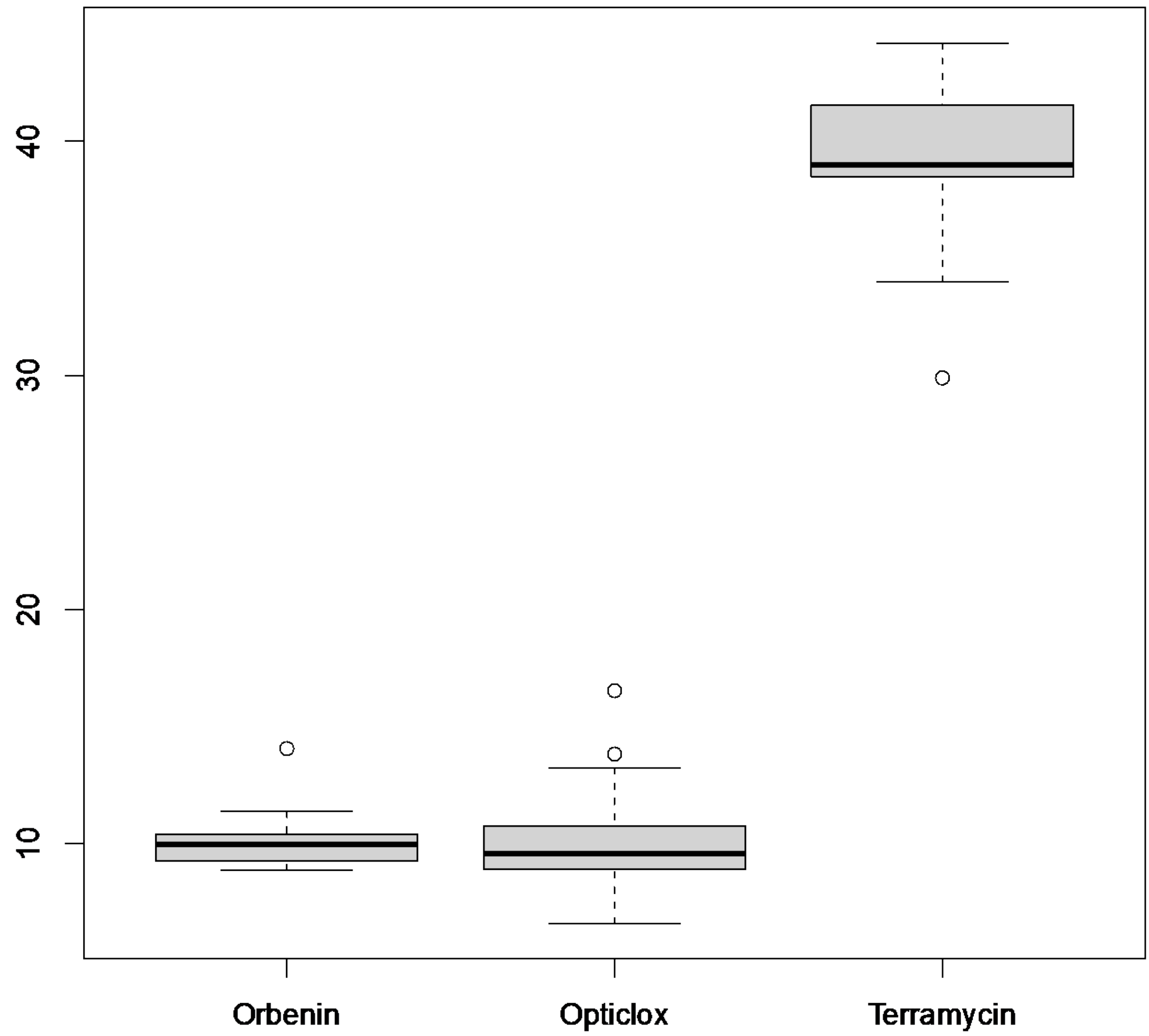


Figure 4. Price per unit medication obtained by survey of veterinary practices and retailers from different states of Australia.

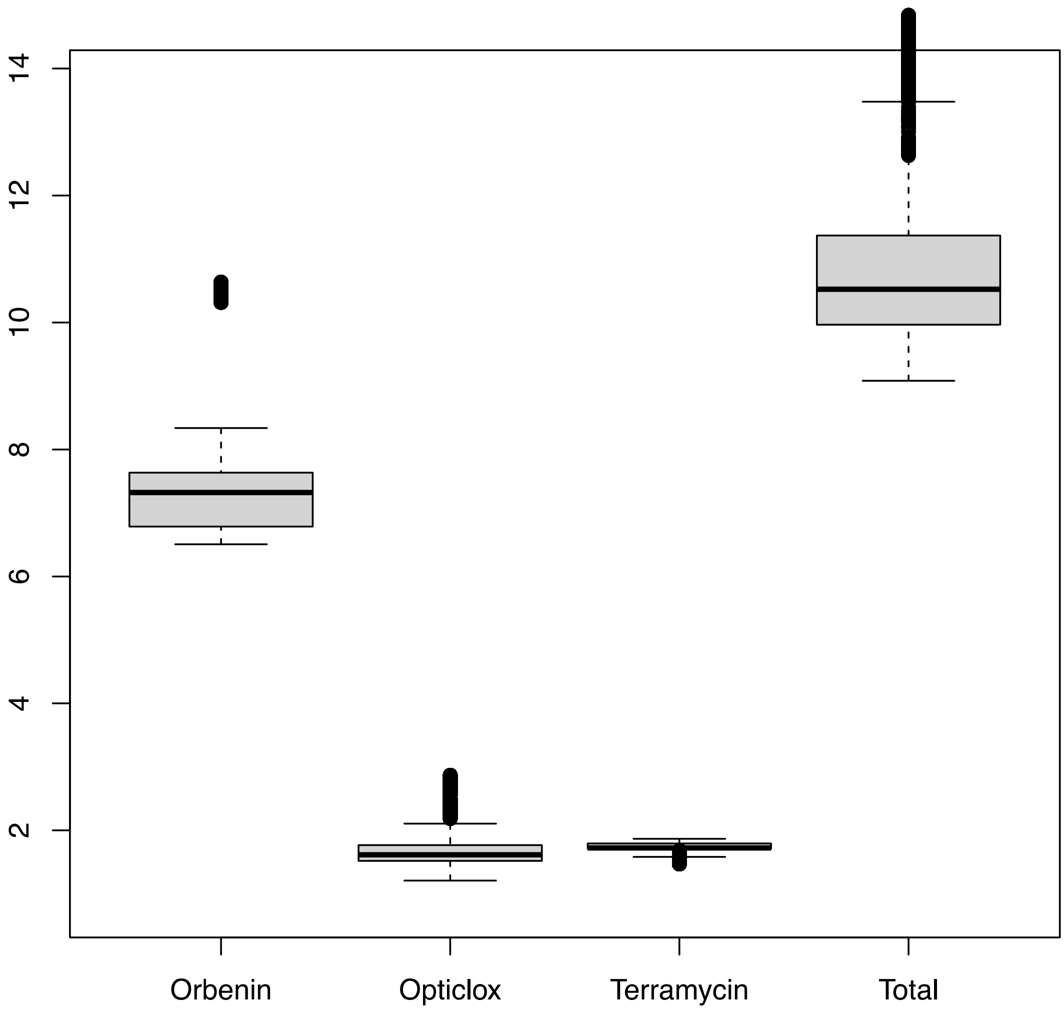


Figure 5. Estimated cost of pinkeye to the Australian cattle industry per annum.

Sensitivity analyses did not detect a major impact of the input values except the input that allowed us to take into consideration that both eyes may not be treated in some proportion of cattle. An increase in this multiplier increased the number of cases and cumulative incidence as expected (Table 4).

Table 4. Sensitivity analysis for proportion of cattle with both eyes treated, varying wastage and herd size.

Sensitivity analysis	Number of cases (million)			Incidence		
	Median	2.5th percentile	97.5th percentile	Median	2.5th percentile	97.5th percentile
Varying both eyes treated multiplier[1]						
0.60	3.32	2.10	5.54	12.12	7.67	20.20
0.70	3.90	2.47	6.48	14.25	9.02	23.67
0.80	4.46	2.81	7.42	16.27	10.25	27.10
Varying wastage multiplier[2]						
0.70	2.44	1.53	4.09	8.91	5.58	14.93
0.80	2.81	1.75	4.64	10.25	6.43	16.97
0.90	3.14	1.98	5.23	11.45	7.22	19.08
Varying herd size						
-10%				11.37	7.13	18.84
-5%				10.77	6.76	17.85
5%				9.75	6.11	16.15
10%				9.31	5.84	15.41

[1] This multiplier was used to estimate the number of cattle from the number of eyes treated. A multiplier of 0.5 was used in the main analysis. It is recommended to treat both eyes of cattle with pinkeye, whether affected unilaterally or bilaterally, therefore we assumed number of cattle was half (0.5) of the number of eyes treated. Varying this multiplier from 0.6 to 0.8 takes into consideration that less cattle may have both eyes treated in these proportions.

[2] This multiplier was used to allow for some wastage of medication. A multiplier of 0.8 was used in the main analysis assuming a wastage of 20%.

4. Risk factors for pinkeye occurrence

4.1 Methodology

A comprehensive Australia-wide survey of cattle farmers was conducted to evaluate the risk factors for the occurrence of pinkeye. Responses were statistically analysed to provide insights into the mechanisms that influence the occurrence of pinkeye.

4.1.1 Questionnaire design and implementation

Detailed methods are presented in a paper published in Preventive Veterinary Medicine (43). Briefly, a minimum sample size of 385 was required to estimate the prevalence of pinkeye with 95% confidence and 5% precision. A questionnaire was designed with three sections: 1. Enterprise and farm management, 2. Breeding enterprise information, and 3. Pinkeye disease. The questionnaire was piloted with ten farmers and changes were made to the questionnaire following their feedback. Farmer bodies were asked to promote the survey and all Australian beef farmers were invited to participate by following a link at http://bit.ly/pinkeye-survey. The survey was open from January 22 until September 15, 2019. Ethics approval for the study was obtained from Human Research Ethics Committee at The University of Sydney (number 2018/914).

4.1.2 Data management and analysis

Data from Survey Monkey were downloaded in Microsoft Excel and subsequently analysed using R Studio (46). Data were cleaned and re-categorised using the dplyr package (47) with graphs developed using ggplot2 (48). A total of 1675 survey responses were collected, a subset of which was used for the inferential analyses (n = 999). Within the subset of data, the outcome chosen was the within-herd (animal-level) prevalence of pinkeye in 2018 at the respondent's herd. This outcome was derived from two questions *(a) What was the total number of cattle in your herd with pinkeye in 2018?* and *(b) What was the total number of cattle on your farm in 2018?* A total of 999 observations were available for this outcome variable. Twenty-six categorical risk factors were selected as explanatory variables Table 5.

To evaluate the association of pinkeye risk factors (explanatory variables) with the outcome, a series of univariable logistic regression models were firstly fitted. Explanatory variables with univariable P-values < 0.20 were then retained for multivariable analyses. Multicollinearity was assessed between these explanatory variables through correlation plots using the Spearman's Rank method. In the case of highly correlated variables ($r^2 \geq |0.7|$), the explanatory variable with the greater association with the response variable in univariable models was retained for multivariable models. All 26 variables had < 10% missing values thus were included in the analyses.

For multivariable analyses a manual forward stepwise selection was performed, with explanatory variables that returned a P-value < 0.05 retained for the final model. Variables eligible for multivariable analyses were then re-added individually to the final model to retest for non-significance. Any biologically relevant two-way interactions were tested in the final model. Potential confounders including *enterprise type* and *zebu content* in the herd were then added to the final model, as they resulted in parameter estimates differing by > 20% compared to the full model without confounders. Finally, goodness-of-fit of the final logistic regression model was assessed using the Hosmer-Lemeshow technique.

Table 5. Definitions and categories of the 26 pinkeye risk factor variables in a study conducted to investigate risk factors for pinkeye in Australian cattle.

Variable	Definition	Categories	Frequency	Percent
Farm characteristics				
Number of locations	Number of postcodes where cattle are kept	1[1]	884	89%
		2	92	9%
		3	22	2%
Farm location	Location of farm, either Northern (QLD, NT) or Southern (NSW, VIC, TAS, ACT, SA, WA) Australia	Northern[1]	173	17%
		Southern	824	83%
Farm enterprise	Type of farm enterprise	Breeding[1]	584	59%
		Mixed farm	232	23%
		Non-breeding	180	18%
Farm size	Total farm size in hectares	< 100 ha[1]	241	24%
		100 – 1000 ha	449	45%
		> 1000 ha	303	31%
Farm grazing area	Total area available for cattle grazing in hectares	< 100 ha[1]	278	28%
		100 – 1000 ha	448	45%
		> 1000 ha	264	27%
Farm uncleared area	Total area of uncleared land on the property in hectares	< 100 ha[1]	642	66%
		100 – 1000 ha	242	25%
		> 1000 ha	85	9%
Dust levels	Farmer-reported rating of dust on farm in 2018	None/Low[1]	217	22%
		Moderate	266	27%
		High	502	51%

Variable	Definition	Categories	Frequency	Percent
Fly levels	Farmer-reported rating of fly worry on farm in 2018	None/Low[1]	221	22%
		Moderate	383	39%
		High	387	39%
Long grass levels	Farmer-reported rating of long grass, thistles, or stubble in 2018	None/Low[1]	531	54%
		Moderate	333	34%
		High	121	12%
Rain levels	Farmer-reported rating of rainfall on farm in 2018	None/Low[1]	678	69%
		Moderate	222	23%
		High	82	8%
Temperature levels	Farmer-reported rating of average daily temperature on farm in 2018	None/Low[1]	30	3%
		Moderate	405	41%
		High	555	56%
UV light levels	Farmer-reported rating of UV light (solar radiation) on farm in 2018	None/Low[1]	48	5%
		Moderate	351	36%
		High	578	59%
Wind levels	Farmer-reported rating of wind on farm in 2018	None/Low[1]	77	8%
		Moderate	419	43%
		High	478	49%
Animal characteristics				
Breed of cattle	Main breeds in the cattle herd	British[1]	556	56%
		European	19	2%
		Zebu (*Bos indicus*)	42	4%
		Mixed breeds	382	38%

Variable	Definition	Categories	Frequency	Percent
Zebu content	Does your herd contain any *Bos indicus* cattle?	No[1]	755	77%
		Yes	231	23%
Cattle density	Population density defined as the total number of cattle on farm in 2018 over the total area available for grazing	High (≥ 1)[1]	358	36%
		Medium (0.5 – 0.99)	269	27%
		Low (0 – 0.49)	363	37%
Origin of cattle	Region from which cattle were sourced, either Northern (QLD, NT) or Southern (NSW, VIC, TAS, ACT, SA, WA) Australia	Northern[1]	155	16%
		Southern	802	84%
Number of sources	Number of locations from which cattle were sourced in 2018	Zero sources[1]	379	41%
		1 - 3 sources	459	49%
		4 + sources	93	10%
Times yarded	Number of times cattle were yarded in 2018	Zero to 5 times[1]	379	38%
		6 to 10 times	365	37%
		11 + times	253	25%
Grazing on native pasture or stubble	Do cattle graze on native pastures or stubble?	No[1]	361	36%
		Yes	637	64%
Breed on farm		No[1]	111	11%

Variable	Definition	Categories	Frequency	Percent
	Do you breed cattle on farm?	Yes	887	89%
Weaning season	Season that calves are weaned (derived from respondents answer to when their animals calved, weaned, and mated)	Both[1]	353	40%
		Cool weaning (Autumn and Winter)	138	16%
		Warm weaning (Spring and Summer)	389	44%
Weaning method	Method of weaning	Mix[1]	166	18%
		Other	109	12%
		Paddock	155	17%
		Yard	476	53%
Pinkeye characteristics				
Pinkeye season	Season in which pinkeye is most common in the herd	Cooler (Autumn & Winter)[1]	60	6%
		Multiple (Non-seasonal or mixed)	154	16%
		Spring-Summer	130	14%
		Summer	611	64%
Age affected	Most common age group affected by pinkeye in the herd	≤ 2 years old[1]	661	67%
		> 2 years old	108	11%
		Mixed ages	214	22%
When treated	Treatment of pinkeye in the herd	Never/sometimes[1]	199	20%
		Usually	199	20%

Variable	Definition	Categories	Frequency	Percent
		Always	586	60%

[1]Reference category for univariable and multivariable analyses

4.2 Results

We obtained a total of 1675 survey responses of which 31 were excluded: 29 for insufficient data and two from New Zealand. Of the remaining 1644 responses, 53 that nominated dairy farming as their main enterprise (*Q.2*) were included as many indicated that they farmed beef cattle as well. After cleaning and filtering, complete inferential data for the outcome, '*prevalence of pinkeye in the herd in 2018*' were available for 999 responses. Of the 999 farmer responses, most had one location where they kept their cattle, the majority had farms located in the southern parts of Australia: 51.7% were from New South Wales, 22.1% were from Victoria, 16.5% were from Queensland, 3.7% were from Western Australia, 2.2% were from South Australia, 1.9 % were from Tasmania, 0.07 % were from Northern Territory, 0.01% was from Australian Capital Territory and a further 1.0% owned farms in multiple states.

4.2.1 Pinkeye occurrence

This survey confirmed the widespread occurrence of pinkeye in Australia. Of those who answered section 3 only 5.9% had not seen pinkeye in their herd in the last five years (2014-2018). When asked '*How many years did you have pinkeye cases in your herd in the last 5 years?*' over a third (35.5%) reported having pinkeye every year during the last five years, 7.7% had pinkeye in four of the last five years, 13.8% in three of the last five years, 17.0% in two of the last five years, and 20.1% in one of the last five years. Frequency tables of explanatory variables are presented in Table 5 and descriptive numerical summaries for the proportion of the herd with pinkeye according to the 26 risk factors are presented in Table 6.

Table 6. Descriptive numerical summaries for the proportion of the herd with pinkeye in 2018 according to the 26 categorical risk factors in a study conducted to investigate risk factors for pinkeye in Australian cattle.

Explanatory variable	Categories	Min	First quartile	Median	Third Quartile	Max	Mean	SD
Number of locations	1	0	0.02	0.06	0.13	1	0.10	0.13
	2	0	0.02	0.05	0.09	0.83	0.10	0.14
	3	0.004	0.02	0.06	0.10	0.40	0.09	0.11
Farm location	Northern	0	0.01	0.04	0.09	0.72	0.07	0.09
	Southern	0	0.03	0.06	0.13	1	0.11	0.14
Farm enterprise	Breeding	0	0.02	0.05	0.13	1	0.09	0.13
	Mixed farm	0	0.03	0.07	0.14	0.80	0.11	0.13
	Non-breeding	0	0.03	0.06	0.13	0.88	0.11	0.13
Farm size	< 100 ha	0	0.02	0.08	0.16	0.88	0.13	0.15
	100 – 1000 ha	0	0.03	0.06	0.13	1	0.10	0.13
	> 1000 ha	0	0.02	0.04	0.10	0.80	0.08	0.11
Farm grazing area	< 100 ha	0	0.03	0.08	0.16	0.88	0.13	0.15
	100 – 1000 ha	0	0.03	0.06	0.13	1	0.10	0.13
	> 1000 ha	0	0.01	0.04	0.10	0.72	0.07	0.10
Farm uncleared area	< 100 ha	0	0.02	0.07	0.14	1	0.11	0.13
	100 – 1000 ha	0	0.02	0.05	0.10	0.80	0.08	0.11
	> 1000 ha	0	0.01	0.04	0.10	0.72	0.09	0.14
Dust levels	None/Low	0	0.01	0.04	0.10	0.88	0.08	0.12
	Moderate	0	0.02	0.05	0.11	1	0.09	0.12
	High	0	0.03	0.07	0.15	0.90	0.11	0.14
Fly levels	None/Low	0	0.01	0.04	0.10	0.56	0.09	0.12
	Moderate	0	0.02	0.05	0.12	1	0.09	0.13
	High	0	0.03	0.07	0.15	0.83	0.12	0.14
Long grass levels	None/Low	0	0.02	0.06	0.14	0.90	0.10	0.12
	Moderate	0	0.02	0.05	0.11	1.00	0.09	0.13
	High	0	0.03	0.07	0.15	0.83	0.12	0.15

Explanatory variable	**Categories**	**Min**	**First quartile**	**Median**	**Third Quartile**	**Max**	**Mean**	**SD**
Rain levels	None/Low	0	0.03	0.06	0.14	1.00	0.11	0.14
	Moderate	0	0.01	0.05	0.10	0.88	0.09	0.12
	High	0	0.01	0.03	0.08	0.56	0.07	0.10
Temperature levels	None/Low	0	0.01	0.03	0.07	0.16	0.05	0.05
	Moderate	0	0.02	0.05	0.11	0.88	0.09	0.11
	High	0	0.03	0.07	0.15	1	0.11	0.14
UV light levels	None/Low	0	0.01	0.05	0.11	0.77	0.09	0.13
	Moderate	0	0.02	0.05	0.11	0.83	0.08	0.10
	High	0	0.02	0.06	0.14	1	0.11	0.14
Wind levels	None/Low	0	0.02	0.07	0.13	0.53	0.10	0.10
	Moderate	0	0.02	0.05	0.13	1	0.10	0.13
	High	0	0.02	0.06	0.13	0.90	0.10	0.13
Breed of cattle	British	0	0.03	0.06	0.14	1	0.11	0.13
	European	0	0.02	0.06	0.11	0.18	0.07	0.06
	Zebu (*Bos indicus*)	0	0.00	0.01	0.03	0.72	0.04	0.12
	Mixed breeds	0	0.02	0.06	0.13	0.83	0.10	0.13
Zebu content	No	0	0.03	0.07	0.14	1	0.11	0.14
	Yes	0	0.01	0.04	0.10	0.72	0.07	0.10
Cattle density	High density	0	0.02	0.06	0.13	0.83	0.10	0.12
	Medium density	0	0.02	0.05	0.13	0.90	0.10	0.14
	Low density	0	0.02	0.05	0.14	1	0.10	0.14
Origin of cattle	Northern	0	0.01	0.03	0.10	0.72	0.07	0.10
	Southern	0	0.03	0.06	0.13	1	0.11	0.14
Number of sources	Zero sources	0	0.02	0.05	0.13	1	0.10	0.13
	1 - 3 sources	0	0.02	0.06	0.13	0.83	0.11	0.13
	4 + sources	0	0.02	0.06	0.11	0.50	0.09	0.10
Times yarded	Zero to 5 times	0	0.02	0.05	0.11	1	0.10	0.14
	6 to 10 times	0	0.02	0.06	0.13	0.83	0.10	0.13
	11 + times	0	0.03	0.06	0.15	0.77	0.10	0.11

Explanatory variable	Categories	Min	First quartile	Median	Third Quartile	Max	Mean	SD
Grazing on native pasture or stubble	No	0	0.02	0.05	0.12	1	0.10	0.14
	Yes	0	0.02	0.06	0.13	0.83	0.10	0.13
Breed on farm	No	0	0.03	0.07	0.13	0.80	0.12	0.14
	Yes	0	0.02	0.05	0.13	1	0.10	0.13
Weaning season	Both	0	0.02	0.05	0.13	0.90	0.10	0.13
	Cool weaning	0	0.02	0.05	0.12	0.80	0.10	0.13
	Warm weaning	0	0.02	0.05	0.13	1	0.10	0.13
Weaning method	Mix	0	0.02	0.06	0.13	0.60	0.09	0.11
	Other	0	0.02	0.06	0.13	0.50	0.09	0.11
	Paddock	0	0.02	0.06	0.17	0.88	0.12	0.17
	Yard	0	0.02	0.05	0.13	1	0.10	0.13
Pinkeye season	Cooler	0	0.02	0.06	0.13	0.53	0.09	0.10
	Multiple	0	0.03	0.06	0.11	0.9	0.10	0.15
	Spring-summer	0	0.03	0.06	0.13	1	0.12	0.15
	Summer	0	0.03	0.06	0.13	0.80	0.10	0.03
Age affected	≤ 2 years old	0	0.03	0.06	0.13	1	0.11	0.13
	> 2 years old	0	0.01	0.03	0.07	0.88	0.06	0.12
	Mixed ages	0	0.02	0.06	0.15	0.80	0.11	0.14
When treated	Never/sometimes	0	0.01	0.04	0.10	1	0.08	0.13
	Usually	0	0.03	0.06	0.13	0.88	0.10	0.12
	Always	0	0.02	0.07	0.13	0.90	0.11	0.13

4.2.2 Factors influencing pinkeye occurrence

Results of the univariable analyses which showed the association of the 26 risk factors with pinkeye prevalence in the herd in 2018 are presented in Table 7. Of the 26 variables, 20 were associated with the proportion of pinkeye in the herd. Of these, 18 variables were considered in the multivariable analyses after excluding two variables due to high collinearity.

Table 7. Univariable logistic regression models for the 26 pinkeye risk factors, with the proportion of pinkeye in the herd in 2018 as the response variable. Only variables that resulted in a P-value < 0.20 are reported.

Variable	Categories	Estimate	SE	Odds ratio	95% CI[a]		P-value
Number of locations	1	0.00		1.00			< 0.01
	2	-0.40	0.13	0.67	0.51	0.87	
	3	0.16	0.15	1.17	0.86	1.55	
Farm location	Northern	0.00		1.00			< 0.001
	Southern	0.50	0.10	1.65	1.36	2.01	
Farm enterprise	Breeding	0.00		1.00			0.02
	Mixed farming	0.23	0.10	1.26	1.03	1.55	
	Non-breeding	-0.18	0.14	0.83	0.63	1.09	
Farm size	< 100 Ha	0.00		1.00			< 0.01
	100 – 1000 Ha	-0.39	0.23	0.68	0.44	1.09	
	> 1000 Ha	-0.64	0.22	0.53	0.35	0.84	
Farm grazing area	< 100 Ha	0.00		1.00			< 0.001
	100 – 1000 Ha	-0.42	0.21	0.66	0.44	1.01	
	> 1000 Ha	-0.68	0.20	0.51	0.35	0.76	
Farm uncleared area	< 100 Ha	0.00		1.00			0.01
	100 – 1000 Ha	-0.17	0.11	0.84	0.68	1.04	
	> 1000 Ha	-0.36	0.11	0.70	0.56	0.87	
Dust levels	None/Low	0.00		1.00			< 0.001
	Moderate	-0.35	0.16	0.71	0.52	0.96	
	High	0.38	0.13	1.47	1.15	1.90	
Fly levels	None/Low	0.00		1.00			< 0.001
	Moderate	0.14	0.15	1.15	0.87	1.55	

Variable	Categories	Estimate	SE	Odds ratio	95% CI[a]		P-value
	High	0.52	0.14	1.68	1.28	2.23	
Long grass levels	None/Low	0.00		1.00			< 0.01
	Moderate	-0.30	0.10	0.74	0.60	0.90	
	High	-0.30	0.14	0.74	0.55	0.97	
Rain levels	None/Low	0.00		1.00			< 0.001
	Moderate	-0.46	0.12	0.63	0.50	0.79	
	High	-0.84	0.19	0.43	0.29	0.62	
Temperature levels	None/Low	0.00		1.00			< 0.01
	Moderate	0.33	0.28	1.39	0.83	2.51	
	High	0.57	0.27	1.78	1.08	3.18	
UV light levels	None/Low	0.00		1.00			< 0.01
	Moderate	-0.02	0.28	0.98	0.59	1.75	
	High	0.31	0.27	1.37	0.84	2.42	
Breed of cattle	British	0.00		1.00			< 0.001
	European	-0.26	0.75	0.77	0.12	2.62	
	Zebu	-1.09	0.30	0.34	0.18	0.58	
	Mixed breeds	-0.18	0.09	0.84	0.70	1.00	
Zebu content	No	0.00		1.00			< 0.001
	Yes	-0.51	0.10	0.60	0.49	0.73	
Origin of cattle	Northern	0.00		1.00			< 0.001
	Southern	0.46	0.10	1.59	1.30	1.95	
Number of sources	Zero sources	0.00		1.00			0.06
	1 - 3 sources	0.23	0.10	1.26	1.04	1.55	
	4 + sources	0.07	0.13	1.08	0.83	1.39	
Times yarded	Zero to 5 times	0.00		1.00			< 0.001
	6 to 10 times	0.18	0.10	1.19	0.97	1.46	
	11 + times	0.48	0.11	1.61	1.29	2.01	
Pinkeye season	Cooler (Autumn and winter)	0.00		1.00			0.02

Variable	Categories	Estimate	SE	Odds ratio	95% CI[a]		P-value
	Multiple (Non-seasonal or mixed seasons)	0.08	0.22	1.09	0.72	1.70	
	Spring-summer (Spring and Spring-Summer)	0.48	0.23	1.61	1.04	2.58	
	Summer	0.30	0.21	1.35	0.91	2.08	
	≤ 2 years old	0.00		1.00			
Age affected	> 2 years old	-1.14	0.35	0.32	0.15	0.59	< 0.001
	Mixed ages	0.08	0.10	1.08	0.88	1.33	
	Never/sometimes	0.00		1.00			
When treated1	Usually	0.24	0.13	1.28	0.99	1.64	< 0.001
	Always	0.39	0.11	1.48	1.2	1.83	

[a]Confidence intervals are provided on the odds-ratio scale.

In total, six variables were significant in the final model (Table 8). A further two variables, *Zebu content* and *Farm enterprise* were forced into the model as they were found to be confounders. Pinkeye prevalence was significantly greater on southern-located farms with smaller grazing areas and in cattle two years of age or less with no zebu content. The prevalence was also significantly greater on farms where farmers reported high fly levels compared to those reporting moderate levels, and those reporting moderate fly levels had more than those reporting none/low fly levels. Similarly, those reporting low levels of rain had significantly higher pinkeye prevalence than those reporting moderate and high levels. However, whilst pinkeye prevalence was also significantly greater on farms reporting high dust levels compared to moderate and none/low dust levels, moderate dust levels were protective compared to none/low dust levels. The Hosmer and Lemeshow goodness-of-fit test indicated that the fit of the binomial logistic regression model was good (P = 0.23).

Table 8. Final multivariable logistic regression model, excluding the two-way interactions, for the response variable 'proportion of the herd with pinkeye in 2018' in a study conducted to investigate risk factors for pinkeye in Australian cattle.

Variable	Categories	Estimate	SE	Odds ratio	95% CI[a]		P-value
Intercept		-2.36	0.26	0.10	0.06	0.16	< 0.001
Farm location	Northern	0.00		1.00			<0.001
	Southern	0.39	0.12	1.48	1.18	1.88	
Farm enterprise	Breeding	0.00		1.00			0.16
	Mixed farming	0.05	0.10	1.05	0.87	1.27	
	Non-breeding	-0.25	0.15	0.78	0.58	1.03	
Farm grazing area	< 100 Ha	0.00		1.00			<0.001
	100 – 1000 Ha	-0.41	0.20	0.66	0.45	0.99	
	> 1000 Ha	-0.69	0.19	0.50	0.35	0.75	
Dust levels	None/Low	0.00		1.00			<0.001
	Moderate	-0.37	0.15	0.69	0.52	0.93	
	High	0.09	0.14	1.09	0.84	1.43	
Fly levels	None/Low	0.00		1.00			<0.001
	Moderate	0.10	0.14	1.10	0.85	1.45	
	High	0.46	0.13	1.58	1.23	2.06	
Rain levels	None/Low	0.00		1.00			<0.001
	Moderate	-0.28	0.12	0.76	0.59	0.96	
	High	-0.72	0.19	0.49	0.33	0.70	
Zebu content	No	0.00		1.00			0.01
	Yes	-0.25	0.11	0.78	0.63	0.97	
Age affected	≤ 2 years old	0.00		1.00			<0.001
	> 2 years old	-1.05	0.31	0.35	0.18	0.61	
	Mixed ages	0.02	0.11	1.02	0.83	1.25	

[a]Confidence intervals for the odds-ratios.

5. Attitudes of cattle producers towards pinkeye treatment

5.1 Methodology

This study was conducted to understand the perceptions and practices of Australian farmers regarding the treatment of pinkeye: factors influencing when farmers treat pinkeye, treatments used and considered effective, and reasons for not treating. We used questionnaire responses from the survey reported in Section 4.1 above to investigate on-farm experiences with treating pinkeye: the factors influencing when Australian farmers treat, the treatments they use and consider effective, and reasons for not treating. This information is required to efficiently allocate resources, reduce unnecessary or improper use of antibiotics, better direct the research community, and ultimately improve pinkeye treatment outcomes for farmers and cattle.

5.1.1 Data management and analysis

Survey data were cleaned and re-categorised, when necessary, using the dplyr package with graphs developed using ggplot2. Responses to the survey question: “Rank any of the following treatments for pinkeye you have used” were graphically presented (number of responses varied according to treatment), with treatments ranked as ‘Not effective’ or ‘Low effectiveness’ merged to ‘None/Low’, treatments ranked as ‘Highly effective’ or ‘Extremely effective’ merged to ‘High’ and treatments ranked as ‘Moderate effectiveness’ remaining the same. Responses to “If you do not treat pinkeye why not?” (n = 216, 21.9%) were also graphically presented.

5.1.2 Descriptive data analysis

A subset of data was selected for inferential analyses, which involved complete responses for the chosen outcome “when do you treat pinkeye?” (n = 985), where answers were categorised as ‘sometimes/never’, ‘usually’, or ‘always’. The category of ‘sometimes/never’ was created by combining the questionnaire responses of ‘sometimes’, ‘rarely’, ‘never’, ‘only if severe’, ‘only once a significant number of herds affected’ and ‘only if animals are handy, already yarded’, whereas the categories of ‘always’ and ‘usually’ already had sufficient numbers and were therefore not recategorized.

A total of 15 farm-level explanatory variables were considered for analyses, including 14 categorical variables (Table 9) and one continuous variable, namely the “Proportion of herd affected by pinkeye in 2018”. For the categorical variables, contingency tables were created using the ‘tabyl’ function in the janitor package (49) to determine the distribution of the data (proportions and counts) across each category. The distribution of explanatory variable categories across outcome categories (always, usually, sometimes/never) were also explored.

Table 9. Definitions, categories, counts, and proportions associated with the 14 categorical explanatory variables used in the univariable and multivariable models.

Variable	Definition	Categories	N (%)
Farm location	Location of farm, either Northern (QLD, NT) or Southern (NSW, VIC, TAS, ACT, SA, WA) Australia	Northern	172 (17.5)
		Southern	811 (82.5)
Farm enterprise	Type of farm enterprise	Breeding	571 (58.2)
		Mixed farm	237 (24.1)
		Non-breeding	174 (17.7)
Farm size	Total farm size in hectares	< 100 ha	218 (22.5)
		100 – 1000 ha	441 (45.5)
		> 1000 ha	311 (32.1)
Angus as a main breed	Does your herd have Angus cattle as a main breed?	No	316 (32.1)
		Yes	669 (67.9)
Zebu content	Does your herd contain *Bos indicus*?	No	749 (77.1)
		Yes	223 (22.9)
Cattle density	Population density defined as the total number of cattle on farm in 2018 over the total area available for grazing	High density (≥ 1)	344 (35.7)
		Medium density (0.5 – 0.99)	257 (26.7)
		Low density (0 – 0.49)	362 (37.6)
Times yarded	Number of times cattle were yarded in 2018	Zero to 5 times	372 (37.8)
		6 to 10 times	363 (36.9)
		11+ times	248 (25.2)
Breed on farm	Do you breed cattle on farm?	No	104 (10.6)
		Yes	880 (89.4)
Weaning method	Method of weaning	Mix	162 (18.1)
		Other	104 (11.6)
		Paddock	151 (16.8)
		Yard	480 (53.5)
Age affected		≤ 2 years old	671 (68.2)

Variable	Definition	Categories	N (%)
	Most common age group affected by pinkeye in the herd	> 2 years old	101 (10.3)
		Mixed ages	212 (21.5)
Ranking of pain induced by pinkeye	Perceived pain caused by pinkeye ranked according to quantiles	Q1 (0 – 5)	242 (25.0)
		Q2 (5 – 7)	241 (25.0)
		Q3 (7 – 8.5)	241 (25.0)
		Q4 (8.5 – 10)	241 (25.0)
Fly levels	Farmer-reported rating of fly worry on farm in 2018	None/Low	207 (21.2)
		Moderate	382 (39.1)
		High	387 (39.7)
Animal welfare effect	Perceived effect of pinkeye on animal welfare	None/Low	107 (11.4)
		Moderate	167 (17.9)
		High	661 (70.7)
Farm productivity effect	Perceived effect of pinkeye on farm productivity. Ranked according to quantiles.	Q1 (0 – 1)	244 (25.0)
		Q2 (1 – 2.3)	243 (25.0)
		Q3 (2.3 – 5)	243 (25.0
		Q4 (5 – 10)	243 (25.0)

5.1.3 Factors associated with pinkeye treatment

A series of univariable ordinal logistic regression models were constructed using the ‘polr’ function in the MASS package (50) to investigate the association of the 15 explanatory variables with the outcome variable, “when do you treat pinkeye?”. Likelihood-ratio χ^2 tests of significance were conducted, with variables that resulted in p-values < 0.20 retained for multivariable analyses.

Collinearity between pairs of explanatory variables was checked using Spearman Rank correlations, with none of the variables exceeding an r^2 of 0.7. All variables also had < 10% missing values thus were retained for further analyses. Variables with p values < 0.20 were considered for multivariable analyses, with a manual forward stepwise selection procedure performed with variables added sequentially based on their p-values (lowest p-value first). Variables achieving p < 0.05 were retained for the final model. Non-significant variables from the stepwise procedure were retested in the final model to confirm their non-significance, with no variables re-added to the final model. Potential confounders farm enterprise, Angus as a main breed and zebu content were added to the final model, if the parameter estimates of the other variables in the model differed by > 20%. Biologically relevant two-way interactions were tested in the final model. The proportional odds assumption was also checked for the final model using the ‘brant’ test function of the brant package (51).

5.2 Results

Of the full dataset of 1675 survey responses, 31 were excluded, including two from New Zealand and 29 with insufficient data, leaving 1644 responses. Of the 1644 survey responses, 985 had completed the question “when do you treat pinkeye?” and were therefore used in the inferential analyses. The majority of these respondents were from farmers with smaller sized farms (≤ 1000 hectares in size) located in southern Australia. Two thirds listed Angus as a main breed while just under a quarter had *Bos indicus* (zebu) content in their herds. The majority were from cattle breeding enterprises and almost 90% bred cattle on farm (Table 9).

5.2.1 Pinkeye treatments

Treatments that farmers used for pinkeye and a ranking of their perceived effectiveness are displayed in Figure 6. The highest number of respondents (n = 861) had used pinkeye ointment that contains cloxacillin (Orbenin® Eye Ointment - Zoetis Australia Pty Ltd; or Opticlox® Eye Ointment - Norbrook Laboratories Australia P/L), followed by eye patches (n = 637), pinkeye spray (n = 623), fly control (n = 507), pinkeye powder (n = 408), Piliguard® pinkeye vaccine (Coopers’ Bovilis Piliguard® pinkeye vaccine - Intervet Australia Pty Ltd known as MSD Animal Health Australia) (n = 341), injectable antibiotics (n = 303), other eye ointments (n = 246), called the vet (n = 243), subconjunctival injection (n = 224), Vitamin ADE (n = 219), kerosene or turpentine (n = 106), and oral antibiotics (n = 80). The treatment ranked as highly effective by the highest percentage of those who had used it was subconjunctival injection (n = 152, 68%), followed by pinkeye ointment (n = 565, 66%), eye patches (n = 406, 64%), injectable antibiotics (n = 175, 58%) and called the vet (n = 123, 51%). All other treatments were ranked highly effective by less than half of those who had used them. Kerosene or turpentine was ranked highly effective by only 14% (n = 15) of respondents that had used it, but 68% (n = 72) of respondents found it to have none/low effectiveness for the treatment of pinkeye. Other treatments used that were ranked as none/low effectiveness included oral antibiotics (n= 46, 58%) and pinkeye powder (n = 185, 45%). Farmers were also invited to list, but not rank the effectiveness of, other treatments they had used for pinkeye. These included ensuring good animal nutrition, removing them from dusty paddocks, putting sulphate in water, and treating eyes with dry cow therapy ointments, sandalwood ointment, sugar, clean water, betadine, zinc sulphate and baking powder.

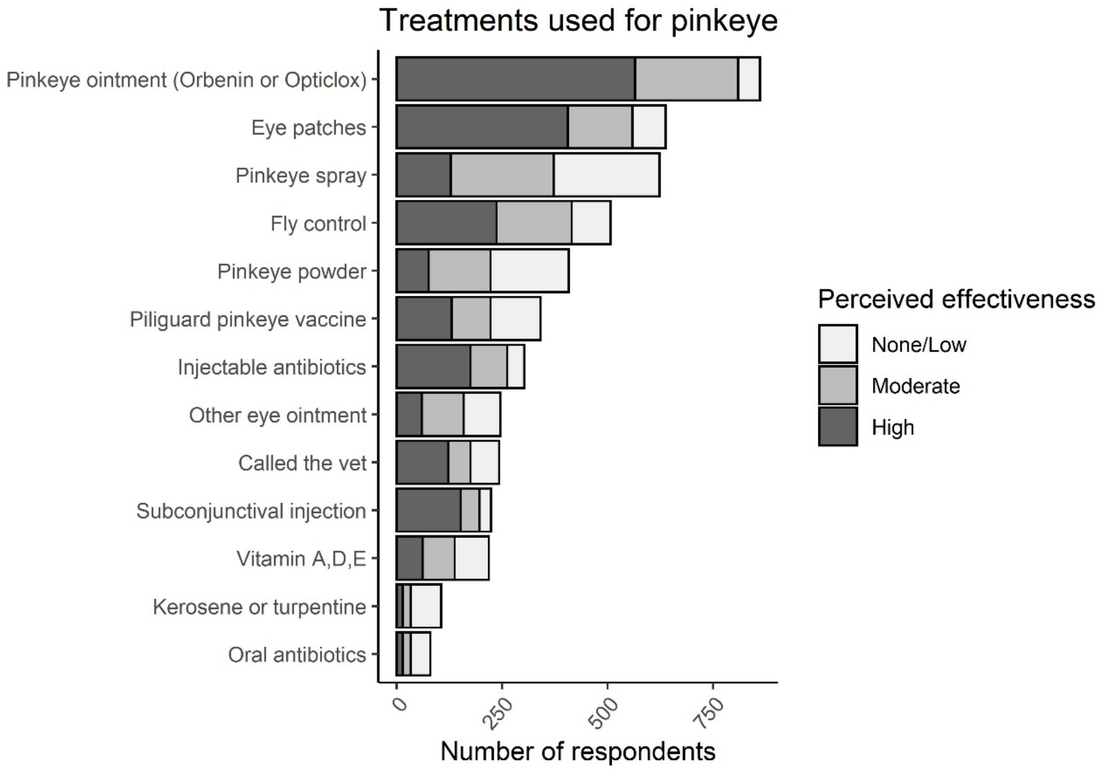


Figure 6. Stacked bar chart for the treatments that farmers used for pinkeye including a ranking of their perceived effectiveness. Number of responses varied according to each treatment and are indicated by the length of bars. Responses were received within the survey "Risk factors, treatment and prevention of pinkeye disease" in Australia, in 2019.

5.2.2 Reasons for not treating pinkeye

Of the 216 respondents who answered, "If you do not treat pinkeye why not?", 28% said that it is too difficult to treat individuals. One participant from the Northern Territory further explained that they did not treat pinkeye because it takes two to three months to muster their herd. A further 26% of respondents said cattle recover without treatment, 15% reported pinkeye does not cause enough problem to warrant treatment, 11% said treatments do not change the outcome, 7% believed treatments do not work and a further 4% said that treatment is too costly (Figure 2). Other reasons for not treating pinkeye included yarding animals for treatment would spread the problem to other healthy animals, their farms had an antibiotic-free status, there was a focus on prevention of pinkeye rather than treatment, and there were time and labour constraints associated with mustering, yarding, and treating the cattle.

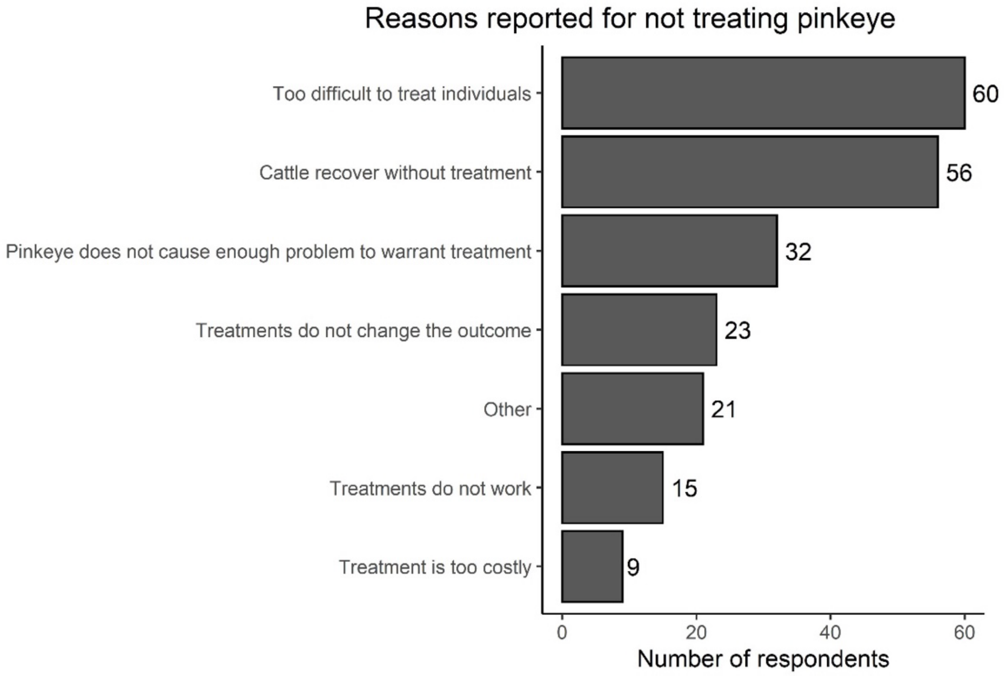


Figure 7. Bar chart documenting the reasons that farmers do not treat pinkeye in their cattle. Based on the responses of 216 cattle owners, who reported not treating for pinkeye in the 2019 survey for "Risk factors, treatment and prevention of pinkeye disease" in Australia.

5.2.3 Factors influencing pinkeye treatment

After cleaning and filtering, complete data for the outcome "when do you treat pinkeye?" were available for 985 responses. Crude associations between the outcome "when do you treat pinkeye?" and the 14 categorical explanatory variables are presented in contingency tables (See **Table 10** and **Table 11**).

Table 10. Contingency table for outcome (when do you treat pinkeye) and the six farm demographic-related categorical explanatory variables.

		When do you treat pinkeye?		
Variable	Category	Never/sometimes (Row %)	Usually (Row %)	Always (Row %)
Farm location	Northern	52 (30.2)	29 (16.9)	91 (52.9)
	Southern	139 (17.1)	177 (21.8)	495 (61.0)
Farm enterprise	Breeding	126 (22.1)	108 (18.9)	337 (59.0)
	Mixed farm	42 (17.7)	61 (25.7)	134 (56.5)
	Non-breeding	23 (13.2)	36 (20.7)	115 (66.1)
Farm size	< 100 ha	20 (9.2)	36 (16.5)	162 (74.3)
	100 – 1000 ha	59 (13.4)	98 (22.2)	284 (64.4)
	> 1000 ha	111 (35.7)	69 (22.2)	131 (42.1)
Angus as a main breed	No	59 (18.7)	60 (19.0)	197 (62.3)
	Yes	133 (19.9)	146 (21.8)	390 (58.3)
Zebu content	No	128 (17.1)	167 (22.3)	454 (60.6)
	Yes	62 (27.8)	37 (16.6)	124 (55.6)
Cattle density	High	45 (13.1)	68 (19.8)	231 (67.2)
	Medium	41 (16.0)	53 (20.6)	163 (63.4)
	Low	103 (28.5)	81 (22.4)	178 (49.2)

Table 11. Contingency table for outcome (when do you treat pinkeye) and the eight farm management-related categorical explanatory variables.

Variable	Category	When do you treat pinkeye? Never/sometimes (Row %)	Usually (Row %)	Always (Row %)
Times yarded	Zero to 5 times	104 (28.0)	81 (21.8)	187 (50.3)
	6 to 10 times	60 (16.5)	81 (22.3)	222 (61.2)
	11 + times	28 (11.3)	44 (17.7)	176 (71.0)
Breed on farm	No	13 (12.5)	18 (17.3)	73 (70.2)
	Yes	179 (20.3)	188 (21.4)	513 (58.3)
Weaning method	Mix	37 (22.8)	36 (22.2)	89 (54.9)
	Other	18 (17.3)	18 (17.3)	68 (65.4)
	Paddock	16 (10.6)	36 (23.8)	99 (65.6)
	Yard	110 (22.9)	99 (20.6)	271 (56.5)
Age affected	≤ 2 years old	130 (19.4)	149 (22.2)	392 (58.4)
	> 2 years old	25 (24.8)	20 (19.8)	56 (55.4)
	Mixed ages	36 (17.0)	37 (17.5)	139 (65.6)
Ranking of pain induced by pinkeye	Q1 (0 – 5)	59 (24.4)	60 (24.8)	123 (50.8)
	Q2 (5 – 7)	49 (20.3)	49 (20.3)	143 (59.3)
	Q3 (7 – 8.5)	39 (16.2)	49 (20.3)	153 (63.5)
	Q4 (8.5 – 10)	43 (17.8)	46 (19.1)	152 (63.1)
Fly levels	None/Low	41 (19.8)	46 (22.2)	120 (58.0)
	Moderate	86 (22.5)	79 (20.7)	217 (56.8)
	High	63 (16.3)	80 (20.7)	244 (63.0)
Animal welfare effect	None/Low	21 (21.5)	21 (19.6)	63 (58.9)
	Moderate	38 (22.8)	33 (19.8)	96 (57.5)
	High	125 (18.9)	147 (22.2)	389 (58.9)
Farm productivity effect	Q1 (0 – 1)	58 (23.8)	41 (16.8)	145 (59.4)
	Q2 (1 – 2.3)	50 (20.6)	50 (20.6)	143 (58.8)
	Q3 (2.3 – 5)	44 (18.1)	60 (24.7)	139 (57.2)
	Q4 (5 – 10)	39 (16.0)	55 (22.6)	149 (61.3)

Results of the univariable ordinal logistic regression models to evaluate the association of each explanatory variable with the outcome “when do you treat pinkeye?” are presented in Table 12. Twelve of the 15 variables were significant at the liberal cut-off value of 0.20 and eligible for multivariable analyses. These variables were “farm location” (p = 0.005), “farm enterprise” (p = 0.09), “farm size” (p < 0.001), “zebu content” (p = 0.03), “cattle density” (p < 0.001), “times yarded” (p < 0.001), “breed on farm” (p = 0.01), “weaning method” (p = 0.02), “proportion of herd affected by pinkeye in 2018” (p = 0.13), “age affected” (p = 0.12), “ranking of pain induced by pinkeye” (p = 0.02), and “fly levels” (p = 0.11). “Angus as a main breed”, “animal welfare” and “farm productivity effect” were not significantly associated with the outcome.

Table 12. Univariable ordinal logistic regression models for the 15 explanatory variables, with the response variable derived from the question “when do you treat pinkeye?” where responses were either ‘sometimes/never’, ‘usually’ or ‘always’. SE = Standard error; CI = Confidence intervals.

Variable	Categories	Estimate	SE	Odds ratio	95 % CI of odds ratio		p-value
Farm location	Northern[1]	Ref					0.005
	Southern	0.46	0.16	1.59	1.15	2.19	
Farm enterprise	Breeding[1]	Ref					0.09
	Mixed farming	-0.01	0.15	0.99	0.74	1.34	
	Non-breeding	0.37	0.18	1.45	1.03	2.06	
Farm size	< 100 ha[1]	Ref					< 0.001
	100 – 1000 ha	-0.45	0.18	0.64	0.44	0.90	
	> 1000 ha	-1.48	0.19	0.23	0.16	0.33	
Angus as a main breed	No[1]	Ref					0.28
	Yes	-0.15	0.14	0.86	0.66	1.13	
Zebu content	No[1]	Ref					0.03
	Yes	-0.33	0.15	0.72	0.54	0.97	
Cattle density	High[1] (≥ 1)	Ref					< 0.001
	Medium (0.5 – 0.99)	-0.17	0.17	0.84	0.60	1.17	
	Low (0 – 0.49)	-0.81	0.15	0.45	0.33	0.60	
Times yarded	Zero to 5 times[1]	Ref					< 0.001
	6 to 10 times	0.51	0.14	1.67	1.26	2.21	
	11 + times	0.94	0.17	2.57	1.85	3.59	
Breed on farm	No[1]	Ref					0.01
	Yes	-0.53	0.22	0.59	0.38	0.90	

Variable	Categories	Estimate	SE	Odds ratio	95 % CI of odds ratio		p-value
Weaning method	Mix[1]	Ref					0.02
	Other	0.42	0.25	1.52	0.93	2.51	
	Paddock	0.52	0.22	1.69	1.09	2.63	
	Yard	0.04	0.18	1.04	0.74	1.47	
Proportion of herd affected by pinkeye in 2018		0.79	0.54	2.21	0.79	6.55	0.13
Age affected	≤ 2 years old[1]	Ref					0.12
	> 2 years old	-0.18	0.21	0.84	0.56	1.26	
	Mixed ages	0.27	0.16	1.31	0.96	1.81	
Ranking of pain induced by pinkeye	Q1 (0 – 5)[1]	Ref					0.02
	Q2 (5 – 7)	0.31	0.18	1.37	0.97	1.93	
	Q3 (7 – 8.5)	0.51	0.18	1.67	1.18	2.37	
	Q4 (8.5 – 10)	0.47	0.18	1.60	1.13	2.28	
Fly levels	None/Low[1]	Ref					0.11
	Moderate	-0.08	0.17	0.92	0.66	1.28	
	High	0.22	0.17	1.24	0.89	1.73	
Animal welfare effect	None/Low[1]	Ref					0.83
	Moderate	-0.06	0.24	0.94	0.58	1.51	
	High	0.04	0.21	1.04	0.69	1.55	
Farm productivity effect	Q1 (0 – 1)[1]	Ref					0.73
	Q2 (1 – 2.3)	0.03	0.18	1.03	0.73	1.47	
	Q3 (2.3 – 5)	0.02	0.18	1.02	0.72	1.45	
	Q4 (5 – 10)	0.18	0.18	1.20	0.84	1.71	

[1]Reference category for univariable analyses

Results from the final multivariable model for when farmers treat pinkeye are presented in Table 13. The model satisfied the proportional odds assumptions, with all probabilities > 0.05. In total, three variables were significant in the final model; namely “farm size” (p < 0.001), “times yarded” (p < 0.001) and “ranking of pain induced by pinkeye” (p < 0.01). Specifically, farmers with smaller farm sizes were more likely to “always/usually” treat their cattle for pinkeye, as were those who yarded their cattle more often, and those that rated pinkeye as highly painful. There were no confounding variables identified in the final model and the interaction between farm size and times yarded was not significant (P = 0.23), therefore excluded from the final model

Table 13. Final multivariable ordinal logistic regression model based on 952 observations for the response variable derived from the question “when do you treat pinkeye?” where responses were either ‘sometimes/never’, ‘usually’ or ‘always’. SE = Standard error; CI = Confidence intervals.

Variable	Categories	Estimate	SE	Odds ratio	95 % CI of odds ratio		p-value
Intercept	1|2	-1.53					
Intercept	2|3	-0.39					
Farm size	< 100 ha[1]	Ref					< 0.001
	100 – 1000 ha	-0.55	0.19	0.58	0.40	0.83	
	> 1000 ha	-1.46	0.19	0.23	0.16	0.34	
Times yarded	Zero to 5 times[1]	Ref					< 0.001
	6 to 10 times	0.37	0.15	1.44	1.07	1.94	
	11 + times	0.79	0.18	2.20	1.55	3.14	
Ranking of pain induced by pinkeye	Q1 (0 – 5)[1]	Ref					< 0.01
	Q2 (5 – 7)	0.39	0.18	1.47	1.03	2.11	
	Q3 (7 – 8.5)	0.60	0.19	1.83	1.27	2.64	
	Q4 (8.5 – 10)	0.57	0.19	1.77	1.23	2.55	

[1]Reference category for multivariable analyses

6. The impact of pinkeye on Australian cattle farms

Pinkeye has been described in the scientific literature for over 130 years, yet there is little information available on its consequences at the farm level. This investigation was conducted based on the survey reported in Section 4.1 above to update knowledge on the impact of pinkeye on Australian cattle farms by exploring how farmers rate the impact of pinkeye on farm productivity, how much they report spending on pinkeye, and their observations about other economic, management and welfare consequences of pinkeye on-farm. Factors influencing the impact and money spent were also investigated.

6.1 Methodology

6.1.1 Data management and analyses

From a list of possible consequences of pinkeye, respondents were asked to select those that were relevant to their enterprise and to also rank their severity. To ensure adequate counts for each category, the categories of 'None' and 'Low' were merged to "None/Low', 'High' and 'Extremely high' to 'High', whilst 'Moderate' remained unchanged, and 'N/A' was excluded. These results were then graphed using ggplot2 (48).

Respondents were also asked to rank the impact of pinkeye on their farm productivity on a sliding scale from '0: No significant impact' to '10: Severe production limiting'. Responses to this continuous outcome were categorized into quartiles, where Q1 = 0 to 0.8; Q2 = 0.9 to 2.0; Q3 = 2.1 to 4.8 and Q4 = 4.9 to 10. Eleven explanatory variables were analyzed for this outcome, including one continuous and 10 categorical variables (Table 14). A series of univariable ordinal logistic regression models were constructed using 'polr' function in 'MASS package' (Venables and Ripley, 2002) to determine the association between the 11 explanatory variables and the outcome "Impact on farm productivity". For the multivariable analyses, a manual forward stepwise selection procedure was applied, with variables added sequentially based on their p-values (lowest first). Variables with p-value < 0.05 were retained for the final multivariable model. Potential confounders, 'farm enterprise', 'Angus as a main breed' and 'zebu content' were tested in the final model and re-added if they resulted in parameter estimates differing by > 20 % to the model without confounders.

Another outcome 'money spent' was created based on this question: "How much money did you spend on pinkeye in 2018?". Responses for this continuous variable were filtered to only include observations where pinkeye was present in 2018 and where a value ≥ $0 was supplied or could be inferred from comments provided (n = 779). Examples of responses excluded were 'not sure', 'don't know', 'not much', 'minimal', 'a lot' and 'thousands of dollars'. Further, many respondents mentioned additional costs like culling of affected cattle, labour and loss of body weight but did not specify an amount, so we were unable to estimate money spent. Fifteen explanatory variables were modelled against this outcome, including two continuous variables that were log transformed and 13 categorical variables (Table 14). Categorical variables were categorized based on quartiles. A series of univariable linear regression models were constructed using 'lm' function to determine the association between the 15 explanatory variables and the outcome "Money spent". The multivariable model building technique was similar to that described above, with variables added sequentially based on the lowest p-values.

Table 14. Definitions and categories of explanatory variables used in univariable and multivariable models for the outcomes (1) "Rank the impact of pinkeye on your farm productivity" and (2) "How much money did you spend on pinkeye in 2018?"

Explanatory variable	Definition	Categories	Outcome variable
Farm location	Location of farm, either Northern (QLD, NT) or Southern (NSW, VIC, TAS, ACT, SA, WA) Australia	Northern Southern	1; 2
Farm enterprise	Type of farm enterprise	Breeding Mixed farming Non-breeding	1; 2
Grazing on native pasture or stubble	Do cattle graze on native pastures or stubble?	No Yes	1
Farm size	Total farm size in hectares	< 100 ha 100 – 1000 ha > 1000 ha	1; 2
Angus as a main breed	Does your herd have Angus as a main breed?	No Yes	1; 2
Zebu content	Does your herd contain *Bos indicus*?	No Yes	1; 2
Herd size	Total number of cattle on farm in 2018, log transformed	*Continuous*	1; 2
Breed on farm	Do you breed cattle on farm?	No Yes	1; 2
Number of cattle affected by pinkeye in 2018	Number of cattle affected by pinkeye in 2018, derived from number of cattle affected by pinkeye in 2018, divided by total herd size in 2018. Variable log transformed.	*Continuous*	2
Pinkeye season	Season when pinkeye is most common in the herd	Cooler (winter and autumn) Multiple (Non-seasonal and mixed) Spring-summer	1; 2

Explanatory variable	Definition	Categories	Outcome variable
		Summer	
Age affected	Most common age group affected by pinkeye in the herd	≤ 2 years old > 2 years old Mixed ages	1; 2
Ranking of pain induced by pinkeye	Perceived pain caused by pinkeye ranked according to quartiles	Q1: 0 to 5 Q2: >5 to 7 Q3: >7 to 8.5 Q4: >8.5 to 10	2
Fly levels	Farmer-reported rating of fly worry on farm in 2018	None/Low Moderate High	2
Animal welfare effect	Perceived effect of pinkeye on animal welfare	None/Low Moderate High	2
Impact on farm productivity	Impact of pinkeye on farm productivity (quartiles based on ranking from 0 – 10, where 0 is no significant impact and 10 is severe production limiting).	Q1: 0 to 1 Q2: >1 to 2.1 Q3: >2.1 to 5.0 Q4: >5.0 to 10	2
When treated	Treatment of pinkeye in the herd	Never/sometimes Usually Always	1; 2

6.2 Results

Of 1675 survey responses, 31 were excluded: two from New Zealand and 29 with insufficient data, leaving 1644 responses, but the number of suitable responses varied between survey questions. Of those that answered (1640), 1346 (82.1 %) were southern and 294 (17.9 %) were northern cattle farmers. None of the variables had > 10 % of their data missing.

6.2.1 Consequences of pinkeye on farm enterprises

The consequences of pinkeye on farm enterprises with rankings of their perceived severity are displayed in Figure 1. The greatest number of respondents nominated that pinkeye resulted in significant treatment expense (n = 1028), followed by decreased sale value of cattle and farm profits (n = 1015), decreased weight gain of affected cattle (n = 1015), restricted movement, disruption to farm routine (n = 1012), workplace health and safety issue (n = 1012), animal welfare issue (n = 993), mismothering of calves (n = 912), and affects bull fertility (n = 862).

Animal welfare issue was rated by the greatest number of farmers overall as being high severity (n = 691) a pinkeye consequence with high severity (n = 691), followed by decreased sale value of cattle and farm profits (n = 561). Similarly, as a proportion of those who chose them, animal welfare issue was rated high severity by the most (70 %), followed by decreased sale value of cattle and farm profits (55 %) and decreased weight gain of affected cattle (n = 525, 52 %). All other consequences of pinkeye were rated as high severity by less than half of those who nominated them. Most respondents perceived pinkeye to have none to low consequence on mismothering of calves (n = 705, 77 %) and on bull fertility (n = 637, 74 %).

Farmers had the opportunity to specify other consequences of pinkeye on their enterprises, but not rank them. Of the 53 participants who provided a free-response, 21 reported that there were significant time and labor costs associated with pinkeye treatment. Others noted impact on salability of pinkeye-affected animals, that may be withheld from sale or culled, that could be costly particularly for stud or show animals. One respondent stated they lost $90,000 in bull sales in a previous year due to pinkeye. Three respondents noted death as a possible consequence and four stated pinkeye was not only a welfare issue for cattle but stressful for farmers too.

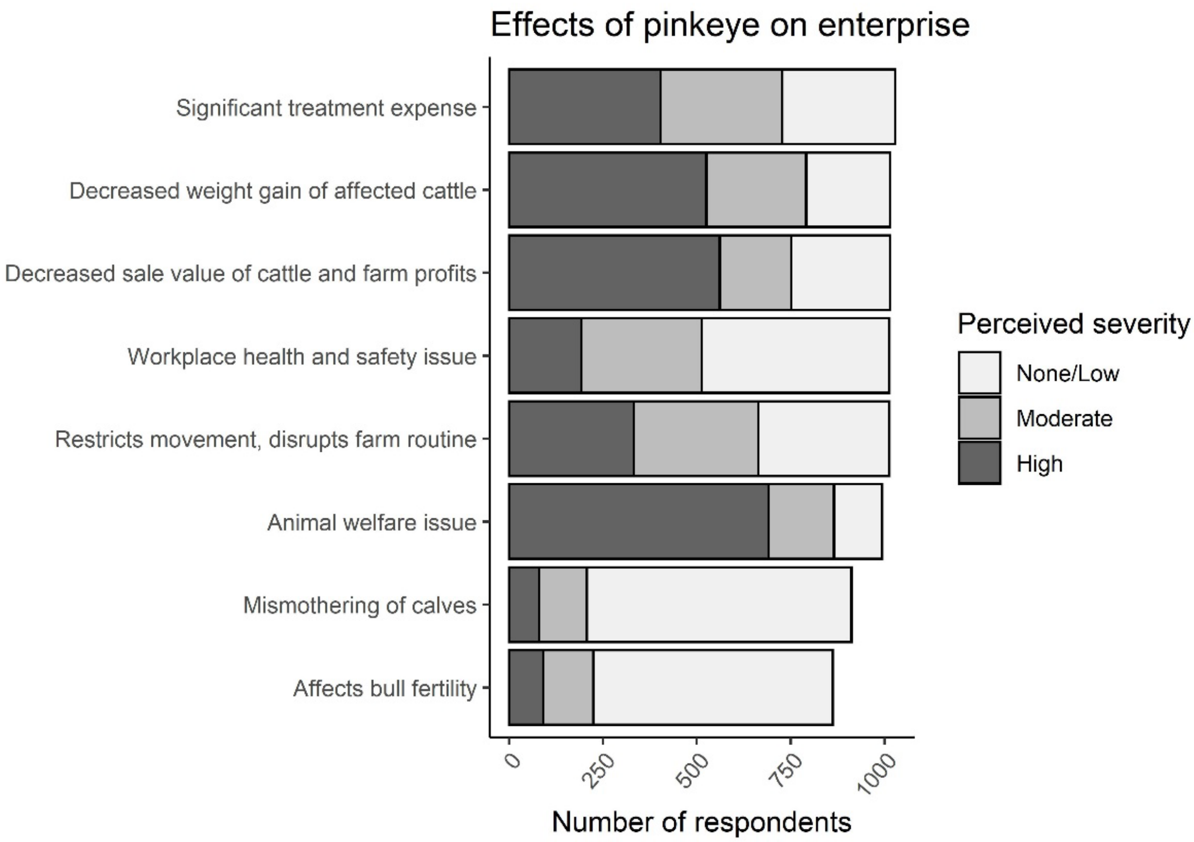


Figure 8. Stacked bar chart for farmer-reported consequences of pinkeye with ranking of perceived severity. Number of responses for each on-farm consequence vary.

6.2.2 Impact on farm productivity

After cleaning and filtering, complete data for the outcome "Rank the impact of pinkeye on farm productivity" were available for 1035 responses. Counts and proportions of the ten categorical explanatory variables tested for this outcome are presented in Table 15. Of these, 82 % (n = 852) of respondents farmed in southern regions of Australia, 45 % (n = 456) had farms 100 to 1000 ha in size, 58 % (n = 595) reported breeding as their main farm enterprise, and 89 % (n = 918) bred animals on their farm.

Results of univariable ordinal logistic regression models to evaluate the association of each explanatory variable with the outcome 'Impact on farm productivity' are presented in Table 16. Eight variables were significant at the liberal p-value of 0.20 and were therefore eligible for multivariable analyses.

Table 15. Frequencies and proportions for the 10 categorical explanatory variables tested for the outcome 'Impact on farm productivity' based on the question, "Rank the impact of pinkeye on your farm productivity".

Explanatory variable	Categories	Q1 (Row %)	Q2 (Row %)	Q3 (Row %)	Q4 (Row %)
Farm location	Northern	56 (30.9)	46 (25.4)	42 (23.2)	37 (20.4)
	Southern	198 (23.2)	207 (24.3)	221 (25.9)	226 (26.5)
Farm enterprise	Breeding	153 (25.7)	139 (23.4)	151 (25.4)	152 (25.5)
	Mixed farm	51 (20.3)	63 (25.1)	64 (25.5)	73 (29.1)
	Non-breeding	48 (25.8)	50 (26.9)	50 (26.9)	38 (20.4)
Grazing on native pasture or stubble	No	88 (23.7)	97 (26.1)	95 (25.6)	91 (24.5)
	Yes	166 (25.0)	156 (23.5)	169 (25.5)	172 (25.9)
Farm size	< 100 ha	73 (29.9)	63 (25.8)	55 (22.5)	53 (21.7)
	100 – 1000 ha	111 (24.3)	106 (23.2)	125 (27.4)	114 (25.0)
	> 1000 ha	67 (20.8)	82 (25.5)	81 (25.2)	92 (28.6)
Angus as a main breed	No	99 (28.9)	80 (23.3)	89 (25.9)	75 (21.9)
	Yes	155 (22.4)	173 (25.0)	176 (25.4)	188 (27.2)
Zebu content	No	187 (24.0)	191 (24.5)	193 (24.8)	208 (26.7)
	Yes	64 (26.3)	60 (24.7)	68 (28.0)	51 (21.0)
Breed on farm	No	35 (30.2)	26 (22.4)	29 (25.0)	26 (22.4)
	Yes	219 (23.9)	226 (24.6)	236 (25.7)	237 (25.8)
Pinkeye season	Cooler	16 (27.1)	20 (33.9)	12 (20.3)	11 (18.6)
	Multiple	29 (17.6)	46 (27.9)	40 (24.2)	50 (30.3)
	Spring-summer	24 (17.5)	32 (23.4)	38 (27.7)	43 (31.4)
	Summer	155 (24.6)	149 (23.7)	170 (27.0)	155 (24.6)
Age affected	≤ 2 years old	147 (21.6)	159 (23.3)	196 (28.8)	179 (26.3)
	> 2 years old	39 (36.8)	30 (28.3)	27 (25.5)	10 (9.4)
	Mixed ages	56 (24.1)	64 (27.6)	40 (17.2)	72 (31.0)
When treated	Never/sometimes	66 (31.1)	58 (27.4)	46 (21.7)	42 (19.8)
	Usually	35 (16.9)	52 (25.1)	64 (30.9)	56 (27.1)
	Always	145 (24.2)	141 (23.5)	151 (25.2)	163 (27.2)

Table 16. Univariable ordinal logistic regression models for 11 explanatory variables for the outcome 'Impact on farm productivity' based on the question "How much money did you spend on pinkeye in 2018?". SE = standard error; CI = Confidence intervals. P-values of significant variables are bolded.

Variable	Categories	Estimate	SE	Odds ratio	95 % CI		P-value
Farm location	Northern[1]	0.00		1.00			**0.01**
	Southern	0.36	0.15	1.44	1.08	1.92	
Farm enterprise	Breeding[1]	0.00		1.00			**0.13**
	Mixed farm	0.20	0.13	1.22	0.94	1.59	
	Non-breeding	-0.14	0.15	0.87	0.65	1.17	
Grazing on native pasture or stubble	No[1]	0.00		1.00			0.84
	Yes	0.02	0.12	1.02	0.82	1.28	
Farm size	< 100 ha[1]	0.00		1.00			**0.03**
	100 – 1000 ha	0.28	0.14	1.32	1.00	1.74	
	> 1000 ha	0.41	0.15	1.51	1.12	2.03	
Angus content	No[1]	0.00		1.00			**0.02**
	Yes	0.27	0.12	1.30	1.03	1.65	
Zebu content	No[1]	0.00		1.00			0.20
	Yes	-0.17	0.13	0.85	0.65	1.09	
Herd size in 2018	-	0.31	0.09	1.37	1.15	1.63	**< 0.001**
Breed on farm	No[1]	0.00		1.00			0.21
	Yes	0.22	0.18	1.25	0.88	1.78	
Pinkeye season	Cooler[1]	0.00		1.00			**0.03**
	Multiple	0.59	0.27	1.80	1.06	3.05	
	Spring-summer	0.68	0.28	1.98	1.15	3.41	
	Summer	0.34	0.24	1.40	0.87	2.25	
Age affected	≤ 2 years old[1]	0.00		1.00			**< 0.001**
	> 2 years old	-0.82	0.19	0.44	0.30	0.63	
	Mixed ages	-0.07	0.14	0.93	0.71	1.22	
When treated	Never/sometimes[1]	0.00		1.00			**0.002**
	Usually	0.60	0.17	1.82	1.30	2.57	
	Always	0.41	0.14	1.51	1.14	2.00	

[1]Reference category for univariable analyses

The final ordinal multivariable logistic regression model for the farmer-reported impact of pinkeye on farm productivity is presented in Table 17. The model satisfied the proportional odds assumptions, with all probabilities > 0.05. 'Zebu content', 'Angus content' and 'Farm enterprise' were added to the final model, as they were confounders.

Farmers with older cattle (> 2 years old) were less likely to rank the impact of pinkeye on farm productivity as high compared to those with younger cattle (≤ 2 years old). Farmers that treated their cattle usually or always were more likely to rank impact on farm productivity high compared to those that never or only sometimes treated. Finally, as the herd size in 2018 increased, so too did ranking the pinkeye impact on farm productivity as high.

Table 17. Final multivariable ordinal logistic regression model for the outcome 'Impact on farm productivity'. Confidence intervals are provided on the odds-ratio scale. SE = standard error; CI = Confidence intervals.

Variable	Categories	Estimate	SE	Odds ratio	95 % CI		P-value
	Q1|Q2	-0.27					
	Q2|Q3	0.89					
	Q3|Q4	2.0					
	≤ 2 years old[1]	0		1			
Age affected	> 2 years old	-0.74	0.20	0.48	0.33	0.70	< 0.001
	Mixed ages	-0.02	0.14	0.98	0.74	1.30	
	Never/sometimes[1]	0.00		1.00			
When treated	Usually	0.61	0.18	1.84	1.29	2.63	0.002
	Always	0.46	0.15	1.58	1.17	2.14	
Herd size in 2018	-	0.23	0.10	1.25	1.03	1.52	0.02
	Breeding[1]	0.00		1.00			
Farm enterprise	Mixed farm	0.24	0.14	1.28	0.97	1.68	0.08
	Non-breeding	-0.15	0.16	0.86	0.63	1.17	
Angus content	No[1]	0.00		1.00			0.19
	Yes	0.17	0.13	1.18	0.92	1.51	
Zebu content	No[1]	0.00		1.00			0.64
	Yes	-0.07	0.14	0.94	0.71	1.23	

[1]Reference category for multivariable analyses

6.2.3 Money spent

After cleaning and filtering, complete inferential data for the outcome “Money spent” were available for 779 responses. Of the 779 responses suitable for analyses, the median amount spent per farm was $250.00 (min: $0.00, Q1: $67.50, Q3: $600, max: $25,000). For the inferential analyses, the outcome “Money spent” was log-transformed because it was right skewed.

Summary statistics of the outcome by the 13 categorical explanatory variables tested for this outcome are presented in Table 18. Of these, 83 % (n = 644) of respondents farmed in southern regions of Australia, 46 % (n = 357) had farms 100 to 1000 ha in size, 58 % (n = 449) reported breeding as their main farming enterprise, and 89 % (n= 694) bred animals on their farm.

Table 18. Descriptive summary statistics for the untransformed outcome “Money spent”, based on the question “How much money did you spend on pinkeye in 2018?” against the 13 categorical explanatory variables. SD = standard deviation.

Explanatory variable	Categories	n	Percent (%)	Min	First quartile	Median	Third quartile	Max
Farm location	Northern	134	17.2	0.0	50.0	200.0	496.0	10000.0
	Southern	644	82.8	0.0	100.0	250.0	600.0	25000.0
Farm enterprise	Breeding	449	57.8	0.0	60.0	250.0	600.0	25000.0
	Mixed farming	180	23.2	0.0	100.0	300.0	712.0	8000.0
	Non-breeding	148	19.1	0.0	50.0	150.0	500.0	8000.0
Farm size	< 100 ha	177	22.9	0.0	50.0	100.0	300.0	1200.0
	100 – 1000 ha	357	46.1	0.0	70.0	200.0	500.0	8000.0
	> 1000 ha	240	31	0.0	100.0	500.0	1425.0	25000.0
Angus as a main breed	No	246	31.6	0.0	50.0	200.0	500.0	10000.0
	Yes	533	68.4	0.0	100.0	250.0	700.0	25000.0
Zebu content	No	586	76.1	0.0	80.0	224.0	600.0	25000.0
	Yes	184	23.9	0.0	50.0	250.0	532.0	10000.0
Breed on farm	No	84	10.8	0.0	47.5	100.0	425.0	5000.0
	Yes	694	89.2	0.0	86.0	250.0	600.0	25000.0
	Cooler	49	6.4	0.0	80.0	200.0	300.0	10000.0

Explanatory variable	Categories	n	Percent (%)	Min	First quartile	Median	Third quartile	Max
Pinkeye season	Multiple	127	16.5	0.0	100.0	250.0	600.0	25000.0
	Spring-summer	100	13	0.0	100.0	400.0	800.0	8000.0
	Summer	495	64.2	0.0	60.0	200.0	600.0	7000.0
Age affected	≤ 2 years old	544	69.8	0.0	100.0	250.0	700.0	25000.0
	> 2 years old	75	9.6	0.0	12.5	100.0	250.0	2200.0
	Mixed ages	160	20.5	0.0	71.2	250.0	500.0	6000.0
Ranking of pain induced by pinkeye	Q1: 0 to 5	253	32.9	0.0	50.0	200.0	500.0	5000.0
	Q2: >5 to 7	156	20.3	0.0	88.0	200.0	562.0	8000.0
	Q3: >7 to 8.5	180	23.4	0.0	100.0	300.0	800.0	8000.0
	Q4: >8.5 to 10	180	23.4	0.0	100.0	300.0	1000.0	25000.0
Fly levels	None/Low	174	22.5	0.0	36.2	100.0	250.0	6500.0
	Moderate	286	37	0.0	60.0	250.0	600.0	25000.0
	High	313	40.5	0.0	100.0	300.0	959.0	8000.0
Animal welfare effect	None/Low	85	11.5	0.0	25.0	100.0	300.0	7000.0
	Moderate	136	18.4	0.0	50.0	200.0	562.0	6500.0
	High	519	70.1	0.0	100.0	300.0	750.0	25000.0
Impact on farm productivity	Q1: 0 to 1	228	29.5	0.0	30.0	100.0	312.0	5400.0
	Q2: >1 to 2.1	161	20.9	0.0	50.0	200.0	500.0	10000.0
	Q3: >2.1 to 5.0	230	29.8	0.0	100.0	300.0	738.0	10000.0
	Q4: >5.0 to 10	153	19.8	0.0	200.0	500.0	1500.0	25000.0
When treated	Never/sometimes[1]	136	17.6	0.0	27.5	128.0	500.0	8000.0
	Usually	162	20.9	0.0	76.2	250.0	700.0	5000.0
	Always	476	61.5	0.0	100.0	250.0	600.0	25000.0

Results of the univariable linear regression models to evaluate the association of each explanatory variable with the outcome 'Money spent' are presented in Table 19. Fourteen of the 15 variables were significant at p-value of 0.20 and eligible for multivariable analyses, with "Zebu content" the only non-significant variable at this liberal cut-off value.

Table 19. Univariable linear regression models for the 15 explanatory variables for the outcome 'Money spent', where responses were log transformed. SE = Standard error; CI = Confidence intervals.

Explanatory variable	Categories	Estimate	SE	Lower CI	Upper CI	P value
Farm location	Northern	4.80	0.16	4.49	5.11	**0.003**
	Southern	0.53	0.18	0.18	0.87	
Farm enterprise	Breeding	5.25	0.09	5.08	5.42	**0.004**
	Mixed farm	0.30	0.16	-0.02	0.62	
	Non-breeding	-0.38	0.17	-0.72	-0.03	
Farm size	< 100 ha	4.56	0.14	4.30	4.83	**< 0.001**
	100 – 1000 ha	0.68	0.17	0.35	1.00	
	> 1000 ha	1.19	0.18	0.84	1.54	
Angus as a main breed	No	4.92	0.12	4.68	5.15	**0.001**
	Yes	0.47	0.14	0.20	0.75	
Zebu content	No	5.27	0.08	5.12	5.42	0.48
	Yes	-0.11	0.16	-0.42	0.20	
Herd size in 2018		0.46	0.04	0.37	0.55	**< 0.001**
Breed on farm	No	4.69	0.20	4.29	5.08	**0.004**
	Yes	0.62	0.21	0.21	1.04	
Number of cattle affected by pinkeye in 2018		0.70	0.04	0.62	0.77	**< 0.001**
Pinkeye season	Cooler	4.90	0.26	4.38	5.41	**0.18**
	Multiple	0.51	0.31	-0.10	1.12	

	Spring-Summer	0.62	0.32	-0.01	1.25	
	Summer	0.32	0.28	-0.22	0.86	
Age affected	≤ 2 years old	5.43	0.08	5.28	5.59	**< 0.001**
	> 2 years old	-1.43	0.22	-1.86	-0.99	
	Mixed ages	-0.27	0.16	-0.59	0.05	
Ranking of pain induced by pinkeye	Quartile 1	4.82	0.13	4.57	5.08	**< 0.001**
	Quartile 2	0.23	0.19	-0.13	0.60	
	Quartile 3	0.65	0.19	0.29	1.02	
	Quartile 4	0.87	0.19	0.50	1.24	
Fly levels	None/low	4.44	0.14	4.17	4.71	**< 0.001**
	Moderate	0.83	0.17	0.49	1.17	
	High	1.23	0.17	0.89	1.56	
Animal welfare effect	None/low	4.41	0.20	4.02	4.81	**< 0.001**
	Moderate	0.58	0.26	0.07	1.08	
	High	1.01	0.22	0.59	1.44	
Farm productivity effect	Quartile 1	4.45	0.13	4.20	4.70	**< 0.001**
	Quartile 2	0.50	0.18	0.15	0.85	
	Quartile 3	1.12	0.18	0.76	1.47	
	Quartile 4	1.59	0.18	1.24	1.94	
When treated	Never/sometimes	4.63	0.16	4.32	4.94	**< 0.001**
	Usually	0.71	0.21	0.29	1.13	
	Always	0.75	0.18	0.40	1.11	

[1]Reference category for univariable analyses

The final multivariable ordinal logistic regression model for money farmers reported spending on pinkeye in 2018 is presented in Table 20. There were nine explanatory variables in this final model that explained 38.21 % of the variation in the money spent (based on the adjusted R2). Farmers reported spending more money on pinkeye in 2018 as their herd size and number of cattle affected by pinkeye in 2018 increased. Also, as the reported level of fly worry increased from low to moderate and high, so did the money spent on pinkeye. Similarly, money spent on pinkeye increased as the perceived impact of pinkeye on farm productivity increased across the quartiles. If farmers ranked the animal welfare issue of pinkeye as higher, their reported expenditure on pinkeye increased. Additionally, if their herds contained Angus cattle as a main breed, if they treated pinkeye more frequently, if their farms were in southern regions of Australia, and if they bred cattle on farm, their reported expenditure on pinkeye increased. The model assumptions of normality and homoscedasticity of the residuals were approximated after log transformation.

Table 20. Final multivariable ordinal logistic regression model for the outcome "Money spent", which was log transformed. Estimates are provided as estimated marginal means on the back-transformed scale. SE = standard error; CI = Confidence intervals.

Explanatory variable	Categories	Estimate	SE	Lower CI	Upper CI	P value
Intercept		0.32	0.40	-0.46	1.09	
Number of cattle affected by pinkeye in 2018		0.54	0.06	0.43	0.65	< 0.001
When treated	Usually	0.76	0.18	0.41	1.12	< 0.001
	Always	1.06	0.16	0.76	1.37	
Fly levels	Moderate	0.50	0.15	0.21	0.79	< 0.001
	High	0.68	0.15	0.38	0.98	
Farm productivity effect	Quartile 2	0.30	0.16	-0.01	0.61	0.01
	Quartile 3	0.39	0.17	0.07	0.72	
	Quartile 4	0.61	0.17	0.27	0.95	
Farm location	Southern	0.35	0.16	0.05	0.66	0.02
Breed on farm	Yes	0.47	0.19	0.10	0.83	0.01
Angus as a main breed	Yes	0.28	0.12	0.04	0.53	0.02
Animal welfare effect	Moderate	0.21	0.21	-0.21	0.63	0.03
	High	0.44	0.18	0.08	0.80	
Herd size in 2018		0.13	0.06	0.02	0.24	0.02

[1]Reference category for multivariable analyses

7. Effectiveness of pinkeye treatments

This study was done to compare the effectiveness of five pinkeye treatments. Animal ethics approval for this work was obtained and the study commenced on 16 October 2019. A payment of AUD $500 was offered for their voluntary and confidential involvement to partially compensate producers for their time in herding animals.

7.1 Methods

7.1.1 Study population, unit of interest and eligibility criteria

The trial relied on natural occurring pinkeye disease in beef calves less than one year old. Cattle from farms that have previously experienced naturally occurring pinkeye were invited to be part of the treatment trial during the “pinkeye season” of 2019-2020. The study population were beef farms in the Goondiwindi region of northern NSW and southern Queensland. Farms meeting the following study inclusion criteria were enrolled in the study: individually identified calves, at least 3 cases of acute pinkeye in the herd, affected animals yarded, and suitable farm facilities to secure animals to allow close individual examination and treatment. An individual animal eye was the unit of concern.

7.1.2 Sample size

Assuming that 95% of the treated and 80% of the untreated cattle suffering from pinkeye would get well and a design effect of 1.5, a sample size of 122 per group (i.e., a total sample size of 610) was required to estimate the difference of 15% with 80% power and 5% level of significance. The sample size would increase to 158 (i.e., a total sample size of 790) assuming a design effect of 2.

7.1.3 Treatment allocation

In this trial we compared the effectiveness of five different treatments. Healthy eyes with no ocular lesions were not treated (0: Healthy eye, not treated). Animals with active pinkeye were allocated to one of the five treatments described in Table 21.

A single investigator (MK) assessed all the cattle. The diagnosis of pinkeye was made on clinical signs. Treatments were allocated in order from 1 to 5 as the animals came into the cattle crush (chute), except for those excluded from the comparison treatment trial. As the trial progressed less animals were assigned treatment 5 (no treatment) on ethical grounds. Blinding of outcome at assessment was achieved because the farmers, investigator, and assistant were all unaware of the treatment cattle had received at enrolment, except for patching that could be seen. Animal ID tags did not indicate the treatment.

Animals that were at risk of losing an eye or being permanently blinded due to pinkeye were withdrawn from the trial and given a ‘rescue’ treatment, which consisted of a combination of the trial treatments, on welfare grounds.

Table 21. Treatments administered to the calves enrolled in the treatment study.

Treatment	Definition and Treatment administration
0: Negative control	*No lesion apparent in the eye.* The eye was not treated unless the animal received oxytetracycline or a combination of treatments involving oxytetracycline due to the other eye being affected.
1: Cloxacillin ointment	*Cloxacillin ointment Orbenin® or Opticlox® eye ointments repeated once at 48 to 72 hours.* Topical benzathine cloxacillin eye ointment repeated as recommended at 48 to 72 hours. Cloxacillin eye ointment used was either Opticlox Eye Ointment®, 833 mg cloxacillin as benzathine salt per 5 g syringe (Norbrook BN 9063-20B Exp Feb-2021); or Orbenin Eye Ointment® 500 mg cloxacillin as benzathine salt per 3g syringe (Zoetis BN 71909700 Exp 12/2020).
2: Subconjunctival injection	*Subconjunctival (SJ) injection of 1 ml penicillin/dexamethasone* SJ injection of 1 ml 50:50 procaine penicillin 30mg/ml: dexamethasone 5mg/ml. SJ injection was a 1ml subconjunctival pinkeye injection of a pinkeye mix into the upper eyelid space, either through the eyelid skin (trans-palpebral) or through the upper conjunctiva with 22 g ¾ inch needle and 2.5 ml syringe. Pinkeye mix was a combination of 50 ml propercillin and 50 ml dexapent in a 100 ml mix. Administration of 1 ml equates to 300 mg of procaine penicillin and 5 mg dexamethasose. We combined a 1:1 mix of Ilium Propercillin injection containing procaine penicillin 300 mg/ml (BN 2000869A Exp Feb 22) and Ilium Dexapent injection containing 5 mg/ml dexamethasone sodium phosphate (BN 200714 Exp Jul 22 5mg/ml).
3: Oxytetracycline	*Oxytetracycline by intramuscular (IM) injection (systemic: it treats both eyes).* IM OTC injection at 10 mg/kg. IM OTC injection was 10 mg/kg intramuscular injection of Coopers Engemycin 100® Oxytetracycline hydrochloride 100mg/ml (BN A816A01 Exp 02-2021).
4: Eye patch	*Patch concealing the eye.* Eye patching only. Eye patches used were Leader Products Pty Ltd Pinkeye Patches® consisting of a box of 6 patches and 125 ml Pinkeye Patch Adhesive distributed by Leader Products Pty Ltd Head Office Victoria, Hume Highway, Craigieburn Victoria 3064
5: Positive control	*Active pinkeye in the eye. The animal was not treated.*
6: Combination of treatments	*Combination of treatments varied with clinical presentation.* Combination treatment was given if an eye was deemed too severe or excluded from the trial for other reasons, for example some other eye issue deemed not to be pinkeye. These animals were treated as the chief investigator thought clinically appropriate. The cut-off deemed to be too severe to be enrolled was an eye with a fluorescein positive corneal ulcer ≤ 5 mm in diameter.

7.1.4 Follow up

The study commenced when cattle were first diagnosed and treated for pinkeye. They were revisited and assessed weekly for a minimum of 2 weeks following treatment with most herds being assessed weekly for 3 weeks after treatment. We aimed to visit farms on day 0, day 2, day 7, day 14 and finally on day 21 but the original follow up plans were modified due to the impact of drought. Some cattle had to be sold due to drought and studies have been completed on day 14.

7.1.5 Clinical assessments

At enrolment (day 0), and on subsequent farm visits, data were collected about the herds e.g., herd environment, herd management; about the cattle e.g., breed, coat colour, sex, age, weight; and about their eyes. To collect animal-level data, animals were inspected, and their heads baled if necessary. At enrolment before treatment was administered, and at revisits, cattle were assessed in a crush (shute) with their heads caught in a head bale. Each eye was examined by close inspection employing clinical ophthalmology techniques. All assessments and ocular examinations were made by the same experienced registered cattle veterinarian (MK).

Pinkeye scores

To facilitate data collection and recording, scores were devised from clinical experience (MK) and a review of available literature. The amount of periocular pigmentation was measured as a p-score from 0 (no dark periocular pigmentation) to 4 (complete black periocular pigmentation) (Table 1). The extent of tear overflow (epiphora) was recorded using a t-score from 0 (no tear overflow) to 3 (wet tear tracks extending down the full length of the face). The stage and severity of eye lesions were recorded using an eye-score (i-Score) where lesions were categorised as acute (A), chronic (C), or recovered (R), with a severity score of 0 (no lesion) to 5 (catastrophic lesion) in each category (Table 22).

Schirmer tear test

In other animal species, volume of tear produced is measured by a standardised Schirmer tear test (STT), but they are not commonly reported for cattle. We conducted a pilot study to see if a STT would be valuable as a clinical measure for eye disease of cattle.

A STT I was performed on a number of eyes in the method of Wieser, Tichy, and Nell (2013) by placing a standardised sterile litmus paper strip in central aspect of lower conjunctival sac for one minute. No chemical sedation, local nerve blocks, or topical anaesthetics were used for a STT1. The STT strips have a notch a few millimetres from end of the strip that goes in the eye. Strips are bent at this notch and placed carefully over the lower eyelid so they may hook and be held in position. The tears wick up the paper strip and tear production is recorded in mm/minute immediately upon removal as the length of wetted paper including rounded end beyond the notch.

Fluorescein staining

Technique for fluorescein staining of individual cattle corneas was to place the fluorescein-impregnated strip (JorVet I-Glo Fluorescein Sodium Ophthalmic Strips Code J-1191), 1mg Fluorescein Sodium per strip (Lot OUF171001 Made Oct-2017 Exp Sep-2022) wetted with Salinaax ®Eye Wash or saline onto the surface of the cornea and examine in shade or under a black-out sheet and illuminate with blue torch light torch and magnification as required.

Table 22. Eye scores developed in the project to make objective measurements for the effectiveness of pinkeye treatments.

Score	Definition and categories
P score	*Periocular pigment score is a measure of the eyelid margin pigmentation of the eye* 0 = No dark periocular pigment. 1 = < 50 % (< 180 degrees) dark periocular pigment. 2 = ≥ 50 % (≥ 180 degrees) dark periocular pigment or complete lighter periocular pigment (for example tan or yellow not brown or black). 3 = 100 % pigment not black (i.e., brown). 4 = 100 % black pigment dark
T score	*Tear score is a measure of tear overflow (epiphora), after Shugart et al. (1979). Tears are wet to touch, not dried tracks.* 0 = Normal tearing confined to medial canthus, no epiphora (overflow of tears). 1 = Tear track from medial canthus to ≤ 2cm down face. 2 = Tear track to facial groove of maxilla (upper jawbone), roughly a line from upper lip to ear base. 3 = Tear track extending beyond maxillary groove or very broad large quantity of tear.
Acute eye score	*Pinkeye eye lesion score for acute cases of pinkeye (active inflammation < 2 weeks duration)* A0 = Normal eye, no clinical signs. A1 = Acute conjunctivitis without corneal diseases. A2 = Mild acute corneal disease without visible corneal ulceration or, if visible, as a well-demarcated (sharp-edged), either blue opacity (oedema) by naked eye or green fluorescein stain, < 5mm diameter. A3 = Moderate acute corneal disease, visible corneal ulceration, that may be less well-demarcated, of blue opacity or green fluorescein stain ≥ 5mm in diameter. A4 = Severe acute corneal disease, corneal distortion, keratoconus, descemetocele. A5 = Catastrophic acute corneal disease, corneal rupture, collapsed eye (phthisis bulbi, buphthalmia (pop-eye), permanent loss of functional organ.
Chronic eye score	*Pinkeye lesion score for chronic cases of pinkeye (active persistent or chronic non-resolving/indolent inflammation of ≥ 2 weeks duration that is neither acute (A) nor resolved (R)) is similar to acute stages but of ≥ 2 weeks duration as indicated by having any corneal opacity colour or colours other than solely blue, including yellow (pus), white (fibrous scar), black (melanin), and almost always red (blood), this may be visible as granulomatous inflammation and/or corneal vascularisation.* C1 = Chronic (of ≥ 2 weeks duration) conjunctivitis without corneal disease. C2 = Mild chronic corneal disease without visible corneal ulceration or, if visible, not solely blue opacity by naked eye including yellow (pus), white (fibrous scar), black (melanin), and red (blood) and/or green fluorescein stain uptake in an area < 5 mm diameter. C3 = Moderate chronic corneal disease similar to above affecting area ≥5 mm in diameter. C4 = Severe chronic corneal disease, corneal distortion, keratoconus, descemetocele. C5 = Catastrophic chronic corneal disease, corneal rupture, collapsed eye (phthisis bulbi, buphthalmia (pop-eye), permanent loss of functional organ.

Score	Definition and categories
Resolved eye score	*Inactive or resolved pinkeye scar with no evidence of active inflammation; no corneal vascularisation, no other red, blue, or yellow colour, rather white and sometimes black (melanin)* R1 = Minimal corneal scar (grey/white) as flat opacity ≤ 3 mm in diameter or slight haziness (nebula), animal sighted. R2 = Mild corneal scar (grey/white) as flat opacity >2mm ≤ 5mm in diameter (macular) or moderately haziness of cornea (nebula), animal sighted. R3 = Moderate corneal scar (grey/white) as flat opacity > 5 mm in diameter, (macular or leukoma) or heavy haziness of cornea (nebula), moderately affecting sight R4 = Severe corneal scar (grey/white) opacity and/or corneal distortion or derangement, keratoconus, severely affecting sight. R5 = Catastrophic corneal scar, completely opaque grey/white cornea (leukoma), phthisis bulbi (collapsed eye) or buphthalmia ('popeye'), severely distorted eye and/or total loss of sight.

7.1.6 Data management and analysis

Herd-level data were recorded manually on farm and subsequently entered onto Microsoft Excel®. All animal-level data were recorded crush-side on a Tru-Test XRS2® Stick reader (Datamars Australia Pty Ltd). The reader was used to scan and capture each animal's unique electronic identification (EID). For the few animals with no EID, a visual identification (VID) was manually entered. All farms had weigh scales and some could Bluetooth® weights directly to the stick reader. If not, weights were entered manually. All other data were entered manually onto the stick reader and subsequently entered onto Microsoft Excel®.

Data were analysed to compare the effectiveness of various treatments by estimating and comparing the proportions of animals in each of the five groups that recovered after the treatment within 3 weeks of being treated.

7.2 Results

Data analysis for these this component of the project is currently underway. Data cleaning and management has taken more time than we had anticipated due to changes in treatment classifications of some animals over time. We aim to provide the results of this trial by the end of February in a supplement to the report.

8. Quantification of antibiotic concentrations in bovine tears

The putative cause of pinkeye is the Gram-negative bacterium *Moraxella bovis* (Loy, Hille, Maier, & Clawson, 2021) and antibiotics are the preferred treatment (O'Connor & Kneipp, 2021; Sheedy et al., 2021). However, specific medications used, and their route of administration vary from country to country and farm to farm (Alexander, 2010; Kneipp, Green, Govendir, Laurence, & Dhand, 2021; O'Connor & Kneipp, 2021). In the United States of America, subconjunctival (SJ) injections containing penicillin are amongst the most popular treatments for pinkeye (Angelos, 2015). SJ penicillin is also popular in New Zealand (Preeni Abeynayake, 1984) and a study of the level of antibiotic detected in tears of cattle after SJ injection in that country indicated their use was valid (P Abeynayake & Cooper, 1989). SJ injections are not as popular in Australia as some other treatments for pinkeye however were consider an effective treatment by 68% of those that used them (Kneipp et al., 2021). The most popular pinkeye treatment nominated by respondents to an Australia-wide survey on pinkeye was cloxacillin eye ointments and the majority (67%) thought they were an effective treatment (Kneipp et al., 2021). They are available by prescription only (Kneipp, Govendir, Laurence, & Dhand, 2020). Australian cattle farmers spent $9.76M per annum on two cloxacillin ointments and a pinkeye spray (aerosol) containing oxytetracycline (available without prescription) (Kneipp et al., 2020).

Despite their widespread use for pinkeye treatment in Australia, there is little data available on levels of antibiotic achieved in the tears of cattle following either, topical application of cloxacillin eye ointments or SI injection of procaine penicillin. Therefore, we conducted a pilot pharmacokinetic (PK) study to determine the level of antibiotic in the tears of a calf after one eye was treated with topical cloxacillin eye ointment and the other with subconjunctival pinkeye mix containing procaine penicillin. High performance liquid chromatography (HPLC) was used to determine the level of antibiotic in the tears of each eye of the calf over a 72H period after receiving these two treatments.

8.1 Methods

8.1.1 Topical cloxacillin ointment

Tear sample information

Animals: Santa X red bull calf approximately 6 weeks old (mild mucopurulent discharge from right eye)

Treatment: The right eye was treated with topical with approximately 1g (one third of a 3g syringe) of Orbenin Eye Ointment® (Zoetis Australia Pty Ltd, Rhodes, NSW. Batch number A1945702, Exp 06/2023) containing 166.67mg/ml benzathine cloxacillin into the lower conjunctival sac. The dose of Orbenin Eye Ointment® applied topically was approximate. The recommended dosage instructions are to instil ¼ to ½ contents of one 3g syringe, that contains 500mg benzathine cloxacillin, per eye (Zoetis Australia Pty Ltd, Rhodes, NSW).

Collection of tear samples: Tear samples were collected at numerous time points after cloxacillin administration, from 16th April to 19th April 2021. Samples were transported over 48 hours on dry ice from Goondiwindi Qld to The University of Sydney, Camperdown NSW, and stored at – 80 ° Celsius until the samples were analysed.

Cloxacillin concentration in tears assay

A high-pressure liquid chromatography (HPLC) assay was developed to detect cloxacillin.

For quantification of cloxacillin concentrations, a Shimadzu Nexera XR LC system (*Rydalmere, NSW*) was used. The isocratic mobile phase involved a mixture of 50 mM potassium phosphate buffer (pH 2.15) and acetonitrile (MeCN) in a ratio of 55:45, *v/v*. The stationary phase was a ODS Hypersil, 150 x 4.6 mm, 5 µm (*Thermo Fisher Scientific, Macquarie Park, NSW*) column. The flow rate was kept at 1 mL/min and column oven (CTO-40 C) was maintained at 30 °C throughout the analysis. Cloxacillin was monitored at a UV-wavelength of 225 nm through a photo diode array detector (SPD-M40). Retention time of cloxacillin was 4.5 min, where total assay run time was 10 min.

Prior to analysis, tear sample and calibration samples were cleaned by a simple protein precipitation technique. Briefly, 50 µL (or 25 µL depending on its availability) of sample was mixed with the same volume of MeCN, vortexed, and centrifuged at the speed of 14,000 x g for 10 min. After centrifugation, 10 µL of supernatant was injected to the HPLC system. For the 'standard' curve, concentrations ranging from 0.4 to 4000 ug/mL of cloxacillin were added to plasma. Based on the back calculation of the standard curve, observed accuracies of the quality control (QC) samples were within 20 % (81 to 113 %).

8.1.2 Procaine penicillin injection

Tear sample information

Animals: Santa X red bull calf approximately 6 weeks old (mild mucopurulent discharge from right eye)

Treatment: The left eye was administered an injection using a 22g ¾ inch needle through the upper eyelid conjunctiva with 1mL of subconjunctival pinkeye mix (equating to 300 mg of procaine penicillin and 5mg dexamethasose and 0.65mg of atropine sulphate). Pinkeye mix was a combination of 20ml Ilium Propercillin® injection (Procaine Penicillin 300mg/ml) BN 2000869A Exp Feb 22, 20ml Ilium Dexapent® injection (5mg/ml Dexamethasone Sodium Phosphate) BN 200714 Exp Jul 22, and 10ml Ilium Atrosite® injection (0.65 mg/ml Atropine Sulphate) BN 200636 Exp Jun 22. Ilium products distributed by Troy Animal Healthcare: 37 Glendenning Road, Glendenning NSW 2761.

Collection of tear samples: Tear samples collected from 16th April to 19th April (R0 – R18). The samples were transported on dry ice from Goondiwindi, Queensland to The University of Sydney, New South Wales over 48 hours and stored at – 80 ° Celsius until the samples were analysed. The HPLC assay was developed to detect penicillin and procaine penicillin. A few of these samples were initially screened on arrival at The University of Sydney and penicillin could not be detected in the tear samples. Due to the greater Sydney Covid19 lockdown the samples were not all analysed until the last week of September 2021.

Penicillin assay

HPLC condition: For analysis of penicillin and procaine penicillin, Shimadzu Nexera XR LC system (*Rydalmere, NSW*) was used. The isocratic mobile phase involved a mixture of 80% ammonium acetate: 20% acetonitrile, pH 4.8. The stationary phase was a ODS Hypersil, 150 x 4.6 mm, 5 µm (*Thermo Fisher Scientific, Macquarie Park, NSW*) column. The flow rate was kept at 1 mL/min at ambient temperature. Penicillin was monitored at UV-wavelength of 220 nm through photo diode array detector (SPD-M40). Retention time of cloxacillin was 2.26 min, where total run time was 10 min.

Sample preparation: Prior to analysis, 50 uL of tear sample was mixed with 50 uL of acetonitrile. After vortexing, the samples were centrifuged at 14, 000 x g for 10 min, 50 uL of supernatant was further mixed with 50 uL of mobile phase. Finally, 20 uL was injected into the HPLC system.

8.2 Results

8.2.1 Topical cloxacillin ointment

This HPLC analysis required a minimum tear sample volume of 25 µL. Due to limited volume of some samples (< 25 µL), some samples (R11, R15, R16, and R17) were not analysed. There was inaccurate measurement of tear volume on R6. The retention time of cloxacillin was 4.5 min as illustrated in Figure 9, Figure 10 and Figure 11. Cloxacillin was not detected in the baseline (R0) sample. Based on the times of these samples collected, apparent maximal cloxacillin concentration (C_{max}) of 834.24 µg/mL was reached 1.42 hours after the topical administration and declined in bi-phasic manner. Cloxacillin was quantifiable (> 0.4 µg/mL) up to 17.33 hours, and detectable up to 25.25 hours as presented Table 23 and Figure 12. A previous study indicated that cloxacillin reached its peak lacrimal fluid concentration between 30 to 45 min (range 963 to 3,256 µg/mL) and cloxacillin activity was not detectable in the lacrimal fluid after 36 hours of topical administration (55). This study's earliest tear sample was collected at 1.42 hours. The cloxacillin *in-vitro* MIC_{50} and MIC_{90} of *Moraxella bovis* (*M. bovis*) isolates were 2 µg/mL and >32 µg/mL, respectively (56) as presented in Table 24. Another *in-vitro*-study reported 42.4% of hemolytic *M. bovis* isolates were inhibited with 2 µg/mL, whereas 87.9% inhibited with 8 µg/mL of cloxacillin (57).

Cloxacillin concentrations determined in this study exceeded an MIC_{50} & MBC_{50} > 2 ug/mL for *M. bovis* (when using Maboni et al, 2015 data) for approximately 5 hours. As cloxacillin is a time dependent antibiotic, the dosing frequency for a susceptible gram-negative isolate is estimated as Time > MIC > 50% (58). It is possible that one administration of cloxacillin may kill outright 50% of the *M. bovis* isolates or the dosing frequency should be less than 10 hrs.

However, limitations on any interpretation are that the pathogens on the cornea in Australian cattle are unknown, and consequently, the cloxacillin MIC to inhibit these pathogens are also unknown.

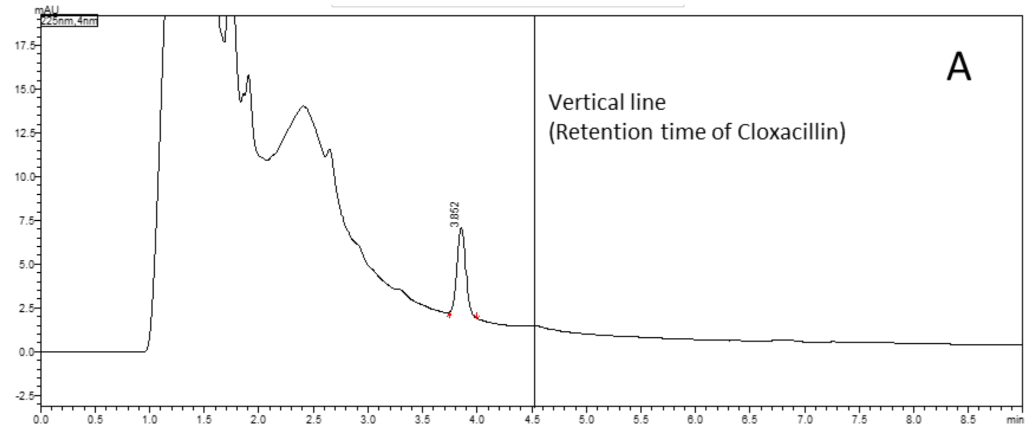


Figure 9. Chromatogram of R0 sample

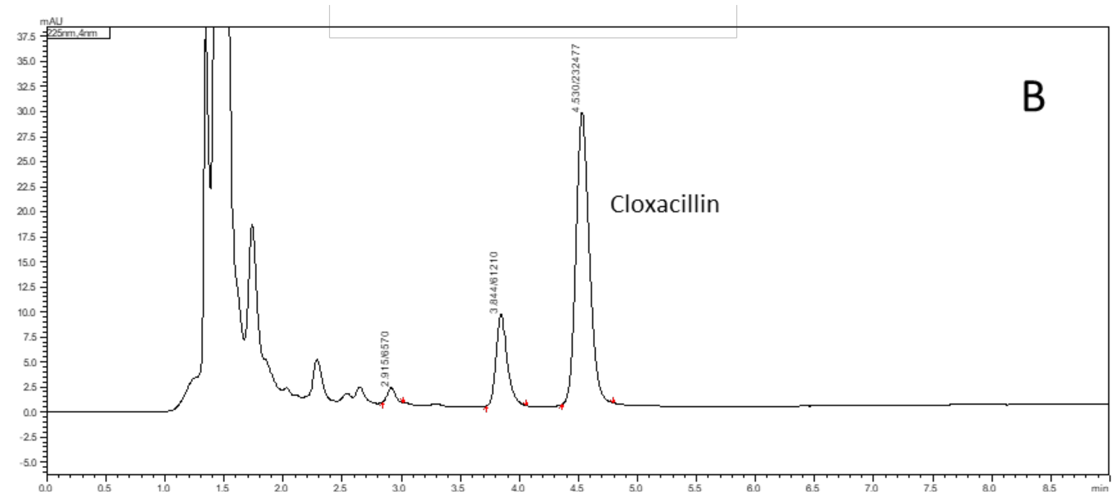


Figure 10. Chromatogram of R3 sample

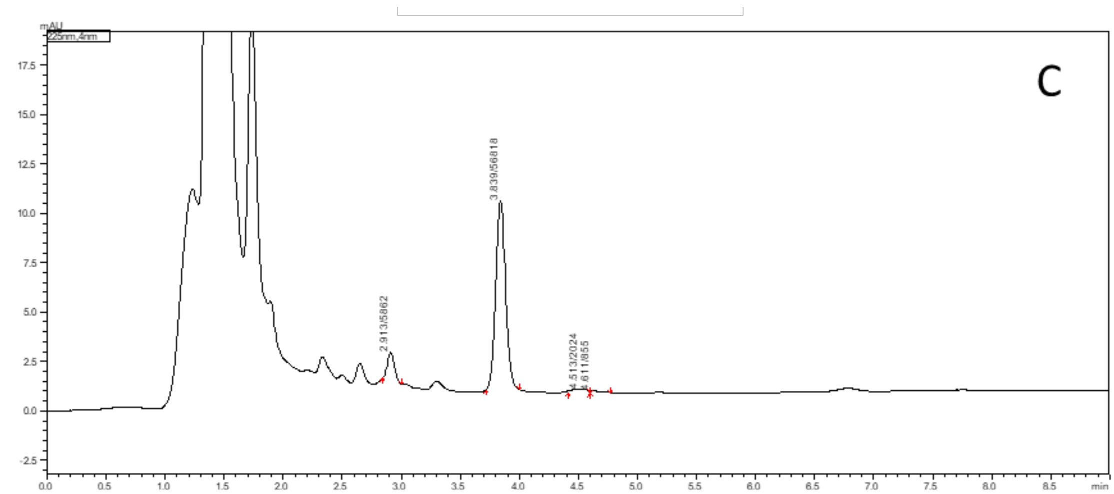


Figure 11. Chromatogram of R3 sample

Table 23. Concentrations of cloxacillin in bovine tear samples

	Sample	Date	Time	(tear) vol. analysed (µL)	hr	µg/mL
bag 1	R0	16/04/2021	14:10:00	50	**0**	**0**
bag 1	R1	16/04/2021	15:35:00	50	**1.42**	**834.24**
bag 1	R2	16/04/2021	16:35:00	50	**2.42**	**160.60**
bag 1	R3	16/04/2021	17:20:00	25	**3.17**	**35.34**
bag 1	R4	16/04/2021	18:35:00	25	**4.42**	**4.09**
bag 2	R5	16/04/2021	19:25:00	50	**5.25**	**1.76**
bag 2	R6	16/04/2021	20:25:00	> 50*	**6.25**	**2.82**
bag 2	R7	17/04/2021	07:30:00	50	**17.33**	**1.34**
bag 2	R8	17/04/2021	11:20:00	50	21.17	<LOQ (0.10)
bag 2	R9	17/04/2021	15:25:00	50	25.25	<LOQ (0.02)
bag 1	R10	17/04/2021	19:20:00	Not available	29.17	Not available
bag 2	R11	17/04/2021	23:20:00	25	33.17	0
bag 2	R12	18/04/2021	07:30:00	25		0
bag 2	R13	18/04/2021	11:20:00	25		0
bag 2	R14	18/04/2021	14:20:00	50		0
bag 2	R15	18/04/2021	18:20:00	Not available		Not available
bag 2	R16	19/04/2021	07:20:00	Not available		Not available
bag 2	R17	19/04/2021	11:20:00	Not available		Not available
bag 2	R18	19/04/2021	14:30:00	50		0

Note: *measurement was not accurate

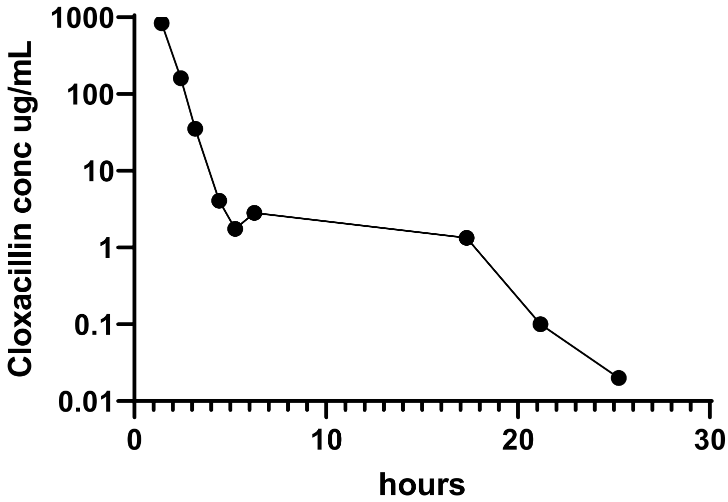


Figure 12. Semi log change in cloxacillin concentration over time

Table 24. Reported cloxacillin minimum inhibitory concentration (MIC ug/mL) and minimum bactericidal concentration (MBC) ug/ml to inhibit *Moraxella bovis* spp.

	N	MIC	MIC_{50}	MIC_{90}	MBC_{50}	MBC_{90}
Haemolytic *Moraxella bovis* (Webber et al., 1982)	66	Mean 6.42; range 1.00 - > 128				
Non-haemolytic *M. bovis* (Webber et al., 1982)	18	Mean 2.94; range 1.00 – 8.00				
M. bovis (Maboni et al., 2015)	11		2	32	2	32
Moraxella bovoculi (Maboni et al., 2015)	12		0.06	>32	0.125	>32
Moraxella ovis (Maboni et al., 2015)	12		0.5	1	2	4

Weber et al., University of Missouri. USA

Maboni et al., Universidade Federal de Santa Maria, Santa Maria, Brazil

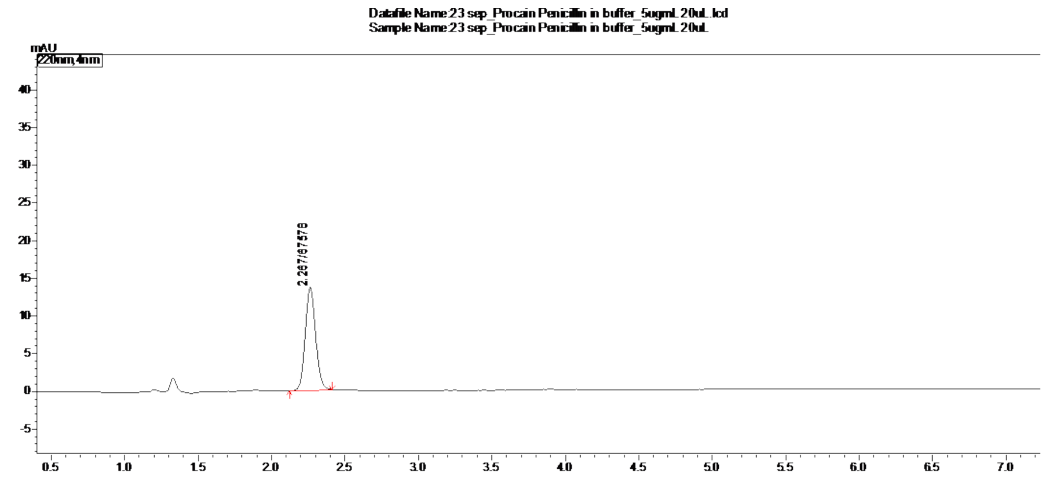
8.2.2 Procaine penicillin injection

The assay could detect low concentrations of the penicillin and procaine penicillin standards. The assay could not detect any penicillin in any of the samples (Figure 13).

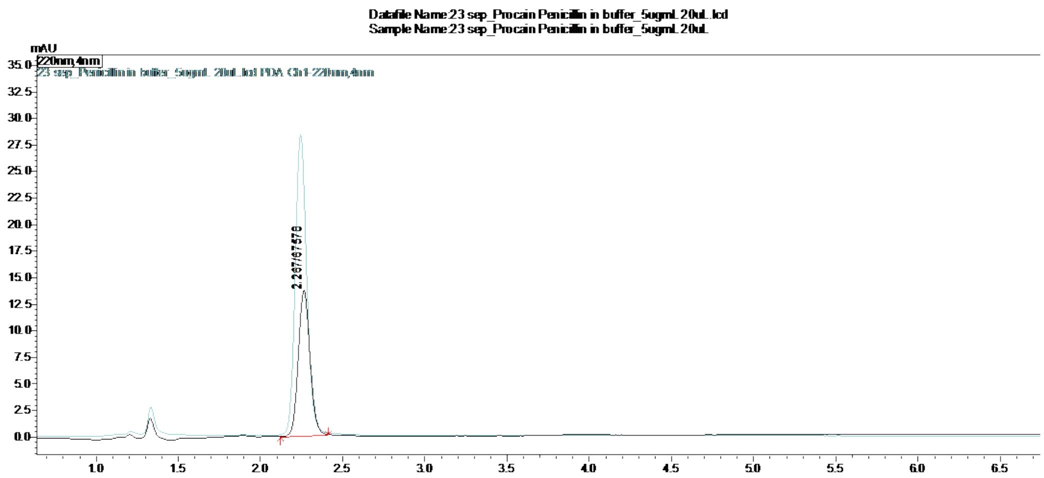
Previous researchers have described the pharmacokinetic profile of penicillin in calf tears when administered by subcutaneous injection using a less sensitive assay than the one used here (59, 60). A difference in the results of that of Abeynayake et Cooper 1989, and this study was that the Abeynayake injection had the antibiotics and other actives in an oily base. when this current study used a water base. If repeating this aspect of the study, describing the pharmacokinetic profile of penicillin in a formulation with an oily base, is warranted.

8.3 Conclusions

This study has developed sensitive assays to measure cloxacillin and penicillin concentrations in bovine tears. From this pilot study we suspect that some preparations such as cloxacillin ointment may achieve *M. bovis* MIC, but the concentration is not maintained above MIC for sufficient time between doses. For these drugs PK-PD studies are required to give evidence-based dose regimens. For other treatment such as a pinkeye SJ injectable mix in a water base are not likely to achieve sufficient concentration in tears to be efficacious. Further studies to investigate the level of pharmacological agents in the tears of cattle are required to improve outcomes from pinkeye.



5ug/mL procaine benzylpenicillin (MW 516), retention time: 2.26 min
HPLC condition: 80 % ammonium acetate: 20 % MeCN, pH 4.8
Column: Supelco Hypersil ODS 5um C18 150 x 4.6
Flow rate: 1mL/min, UV: 220nm



5ug/mL procaine benzylpenicillin (MW 516) (**Black**), 5 ug/mL penicillin (MW 334) (**Green**)
HPLC condition: 80 % ammonium acetate: 20 % MeCN, pH 4.8
Column: Supelco Hypersil ODS 5um C18 150 x 4.6
Flow rate: 1mL/min, UV: 220nm

Figure 13. A: analytical standard of procaine benzylpenicillin, B: analytical standard of penicillin and procaine benzyl penicillin

9. Effectiveness of the Piliguard® vaccine

This study was condcuted to evaluate the effectiveness of Piliguard® vaccine under real-life conditions in extensive beef herds in Australia. Animal ethics approval for this work was obtained and the study commenced November 1, 2019. The trial is an independent evaluation of the only commercial vaccine (Coopers Bovilis Piliguard Pinkeye vaccine®) available in Australia as a management tool to control naturally occurring pinkeye disease.

9.1 Methods

9.1.1 Study design

Assuming that 5% of the vaccinates and 15% of the non-vaccinates get the disease, a sample size of 295 per group (a total sample size of 590) was required to estimate the difference of 10% in proportions between the two groups with 80% power and 5% level of significance and for a design effect of 2.

The trial occurred over two spring-summer seasons, 2019-2020 and 2020-2021, in the Goondiwindi region of southwest Queensland. Farms with suitable facilities and meeting the following study inclusion criteria were invited to participate in the study: (a) the herd size should be at least 50 breeding cows; and (b) pinkeye should be endemic in the farm, i.e., the farm should routinely get cases of pinkeye almost every year. Free vaccinations and a payment of AUS$500 was offered for their involvement as a partial compensation for their time for herding animals.

We aimed to enrol young calves <1 year of age on the enrolled farms. Animals were excluded from the study; (a) if they were previously affected by pinkeye, (b) had any other ocular disease, (c) if they were unhealthy, or (d) if they were less than 1 week of age. At enrolment (day 0), and on subsequent farm visits, data were collected about the herds e.g., herd environment, herd management; about the cattle e.g., breed, coat colour, sex, age, weight; and about their eyes. Calves with evidence of eye lesions were excluded.

9.1.2 Treatment allocation

Calves enrolled were allocated to one of two treatments:

a) Single 2ml dose of Piliguard® given by subcutaneous or intramuscular injection into the side of the anterior third of the neck using 15mm x 16gauge needle and Philips vaccinator.

b) No treatment.

Treatments were allocated using a systematic random approach and administered blinded to all calves eligible. To allow for the herd immunity effect, different proportions of animals in the mobs were vaccinated, approximately 50% on farm 1 and farm 4, 90% on farm 2, and 75% on farm 3. For the mobs with approximately 50% vaccinated every second calf was vaccinated. In the other mobs, calves were vaccinated randomly to achieve the approximate 75% and 90% vaccinated proportions.

9.1.3 Follow up

The study commenced when calves were treated and was to be completed six months later, however some enrolled animals were lost to follow up due to forced sale and deaths and it is possible that no further data will be available. Following treatment enrolled animals were inspected weekly or intermittently depending on farm practicalities and the impact of drought. Data about pinkeye recorded, including occurrence of pinkeye, severity (i-Score) and duration of disease.

9.1.4 Sampling

Data recorded from individual animals at enrolment included breed, sex, weight, coat colour, and for each eye, p-Score (periocular pigmentation score), t-Score (tear score), whether fluorescein stain was taken-up by the cornea, and i-Score (a devised pinkeye score). Only animals with no fluorescein stain take-up and/or an i-Score of zero in both eyes were enrolled. At revisits calves were assessed through a race and crush and if any eye lesion was visible, were caught in a head bale and each eye examined by close inspection employing clinical ophthalmology technique including fluorescein stain as required. Data collected included weight, t-Score, whether fluorescein stain was taken-up, and i-Score. All animals with pinkeye lesions had digital photos recorded of, in order, their left eye, right eye, and head. Some were lost to follow up due to forced sale and deaths.

9.1.5 Data management and analysis

Data were collated in Microsoft Excel and then imported into RStudio version 1.3.1093, an integrated development environment for R (34). Data from the various herds were collated, cleaned and recategorised where necessary using the tidyverse package (35).

Effect of various demographic factors on vaccine effectiveness was evaluated using logistic regression. Eight categorical explanatory variables were explored besides the treatment group. These included six animal-level variables; angus breed (yes, no), sex (female, male), weight at enrolment categorised based on quartiles (Q1 ≤ 91kg, Q2 = 91.1 to ≤ 113kg, Q3 = 113.1 to ≤ 155kg, Q4 ≥ 155.1kg), p-Score (full pigmentation, not full pigmentation), and coat colour (black, other).

A series of univariable generalised linear mixed models (GLMMs) with binomial distribution were fitted using the lme4 package (61). In these models, the outcome was 'pinkeye' (yes, no), and the random effect was herd to control for repeated observations from the same herd. The eight explanatory variables were tested against this outcome individually, with variables achieving p-value < 0.20 considered for multivariable analyses. For the multivariable analyses, a manual forward stepwise selection procedure was applied, with variables added sequentially based on their p-values (lowest first). Variables with p-value < 0.05 were retained for the final multivariable model. The treatment variable was forced in the model regardless of its p-value.

Odds ratio of the treatment variable from the final logistic regression model was used to estimate vaccine effectiveness.

9.2 Results

Data were available from 775 animals from seven herds, but after excluding older animals >12 months old not meeting the inclusion criteria, observations from 717 animals from six herds were used for all analyses reported here. However, analyses were also conducted on the full dataset after including the older animals again to verify the results.

Herd-level demographics of the six farms remaining in this study are presented in Table 25, and animal-level characteristics per herd are presented in . One farmer reported always treating for pinkeye, but the others treated never or rarely. Five herds were on mixed farming enterprises, and the remaining two on a breeding farm. Complete data were available for 717 cattle enrolled from six herds over two pinkeye seasons, four herds of 456 calves (> 1 week and < 1 year old) in 2019-2020 and two herds of 261 calves (≥ 1 year old), in 2020-2021.

Table 25. Herd-level demographics of the seven herds enrolled in the pinkeye vaccination study.

	Herd					
	A	B	C	D	E	F
Trial year	2019	2019	2020	2020	2019	2019
Season enrolled	Spring	Spring	Spring	Summer	Spring	Spring
Main breed	Shorthorn	Angus	Angus	Angus	Angus cross	Angus cross & Hereford cross
Farm size	Medium	Large	Large	Small	Large	Large
Enterprise type	Mixed farming	Mixed farming	Mixed farming	Breeding	Mixed farming	Mixed farming
How often do you treat pinkeye?	Never /rarely	Never /rarely	Never /rarely	Never /rarely	Never /rarely	Always
Do cattle graze on native grass or stubble?	No	No	No	Yes	Yes	No

Table 26. Comparison of animal-level characteristics between control and vaccinated animals per herd.

Variable	Herd	Categories	Control	Vaccinated	p-value
Angus breed	A	No	36	61	N/A
	B	Yes	62	164	N/A
	C	Yes	112	111	N/A
	D	Yes	8	30	N/A
	E	No	2	23	0.78
		Yes	8	54	
	F[1]	No	14	25	0.11
		Yes	5	2	
Coat colour	A	Other	36	61	N/A
	B	Black	62	164	N/A
	C	Black	112	111	N/A
	D	Black	8	30	N/A
	E[1]	Black	9	77	0.12
		Other	1	0	
	F	Black	15	22	1
		Other	4	5	
Sex of cattle	A	Female	19	34	0.94
		Male	17	27	
	B	Female	33	87	1
		Male	29	77	
	C	Female	59	48	0.20
		Male	53	63	
	D	Female	1	21	0.01
		Male	7	9	
	E[1]	Female	3	36	0.50
		Male	7	41	
	F	Unknown	19	27	N/A
Weight at enrolment (average kg)[2]	A		80.19	85.09	0.20
	B		152.67	138.06	0.001
	C		89.41	88.47	0.69
	D		221.63	237.47	0.40
	E		144.25	142.22	0.84
	F		112.05	102.96	0.16
P-score	A	Not full	36	61	N/A
	B	Full	62	164	N/A
	C	Full	112	111	N/A
	D[1]	Not full	0	1	1
		Full	8	29	
	E[1]	Not full	1	4	0.47
		Full	9	73	
	F	Not full	5	5	0.79
		Full	14	22	

[1]Fisher's exact test performed due to low counts in some categories

Pinkeye was common in both years, with 26 % (183/717) of the cattle enrolled having the disease during the trial, 21% (94/456) in 2019-2020 and 32 % (89/261) in 2020-2021 (Table 27). No adverse reactions to vaccination were reported.

Table 27. Distribution (counts and proportions) of pinkeye across the control and vaccinated calves of the six enrolled farms.

		Herd details						
Herd ID		**A**	**B**	**C**	**D**	**E**	**F**	Total
Year		2019	2019	2020	2020	2019	2019	
Number of animals inspected		116	272	224	84	140	55	891
Number of animals used		97	226	223	38	87	46	717
Number of times herd assessed		2	5	5	2	2	4	20
Time between first and last visit		35	65	210	115	75	18	
		Treatment groups (vaccinated or controls)						
Number vaccinated		61	164	111	30	77	27	470
Number of controls		36	62	112	8	10	19	247
Herd proportion vaccinated		63%	73%	50%	79%	89%	59%	66%
		Pinkeye disease status						
Number of accumulative pinkeye		21	54	68	21	14	5	183
Herd proportion affected by pinkeye		0.22	0.24	0.30	0.55	0.16	0.11	0.26
Number free of pinkeye		76	172	155	17	73	41	534
		Treatment group and disease status of vaccinated animals						
Vaccinated	Pinkeye	14	38	37	19	13	4	125
	No pinkeye	47	126	74	11	64	23	345
Vaccinated with pinkeye /Total vaccinated		14/61	38/164	37/111	19/30	13/77	4/27	125/470
Proportion vaccinated with pinkeye		0.23	0.23	0.33	0.63	0.17	0.15	0.27
		Treatment group and disease status of unvaccinated animals						
Controls	Pinkeye	7	16	31	2	1	1	58
	No pinkeye	29	46	81	6	9	18	189
Controls with pinkeye /Total controls		7/36	16/62	31/112	2/8	1/10	1/19	58/247
Proportion controls with pinkeye		0.19	0.26	0.28	0.25	0.10	0.05	0.24

The univariable binary logistic mixed-effect model results are presented in Table 28. At the liberal cut-off value of p-value < 0.20, only two variables, namely treatment group and sex of cattle were associated with the outcome 'pinkeye' (yes/no).

Table 28. Results of the univariable binary logistic mixed-effects models where herd was fitted as a random effect.

Predictor	Term	Estimate	SE	Statistic	p-value
Coat colour	(Intercept)	-1.06	0.28	-3.72	0.57
	Other	-0.33	0.58	-0.57	
Sex of cattle	(Intercept)	-0.84	0.27	-3.15	0.11
	Male	-0.29	0.18	-1.61	
Angus breed	(Intercept)	-1.36	0.37	-3.64	0.41
	Yes	0.35	0.43	0.83	
Weight at enrolment	(Intercept)	-0.98	0.34	-2.87	0.41
	Quartile 2	0.09	0.23	0.38	
	Quartile 3	-0.42	0.33	-1.29	
	Quartile 4	-0.24	0.39	-0.61	
Treatment	(Intercept)	-1.04	0.28	-3.80	0.19
	Control	-0.25	0.19	-1.31	
P-score	(Intercept)	-0.99	0.29	-3.46	0.25
	Partial pigmentation	-0.65	0.56	-1.15	
Herd proportion vaccinated	(Intercept)	-1.39	0.38	-3.63	0.30
	Low	0.07	0.56	0.13	
	Medium	0.79	0.56	1.43	

In the final model where treatment was fitted as a fixed effect and herd as a random effect, the odds of having pinkeye was the same for control and vaccinated cattle (p-value 0.19). Similarly, after adjusting for the weight at enrolment and age of cattle in the model, there was no effect of treatment group (Table 29). The Hosmer-Lemeshow goodness of fit test indicated that the final model fit well (p-value 0.81). Results were quite similar after including older animals that did not meet the inclusion criteria (Table 30).

Table 29. Results of the final binary logistic mixed-effects models where herd was fitted as a random effect. The first model fitted contains treatment as a fixed effect to control for differences in vaccinated cattle and controls. The second model fitted additionally contains cattle weight at enrolment and sex as fixed effects to control for their confounding effects.

Model fitted	Explanatory variables	Category	Estimate	SE	Odds ratio	95 % confidence interval	p-value
After adjusting for the herd effect		Intercept	-1.30	0.30			
	Treatment	Control*	0.00		1.00		0.19
		Vaccinated	0.25	0.19	1.29	0.88, 1.87	
After adjusting for sex and weight besides the herd effect		Intercept	-0.88	0.39			
	Treatment	Control*	0.00		1.00		0.29
		Vaccinated	0.21	0.20	1.23	0.84, 1.81	
	Weight at enrolment	Q1*	0.00		1.00		0.69
		Q2	0.09	0.25	1.09	0.67, 1.77	
		Q3	-0.04	0.33	0.96	0.51, 1.81	
		Q4	-0.33	0.38	0.72	0.34, 1.52	
	Sex	Female*	0.00		1.00		
		Male	-0.27	0.18	0.76	0.53, 1.09	0.13

*Reference category

Table 30. Results of the final binary logistic mixed effects after reincluding excluded older animals. models. The first model fitted contains treatment as a fixed effect to control for differences in vaccinated cattle and controls. The second model fitted additionally contains cattle weight at enrolment and age as fixed effects to control for their confounding effects.

Model fitted	Explanatory variables	Category	Estimate	SE	Odds ratio	95 % confidence interval	p-value
After adjusting for the herd effect		Intercept	-1.53	0.32			
	Treatment	Control*	0.00		1.00		0.085
		Vaccinated	0.30	0.18	1.37	0.96, 1.97	
After adjusting for sex and weight besides the herd effect		Intercept	-1.13	0.40			
	Treatment	Control*	0.00		1.00		0.15
		Vaccinated	0.28	0.19	1.32	0.91, 1.93	
	Weight at enrolment	Q1*	0.00		1.00		0.45
		Q2	0.01	0.24	1.01	0.63, 1.62	
		Q3	-0.27	0.32	0.76	0.41, 1.41	
		Q4	-0.06	0.38	0.57	0.28, 1.20	
	Age	<1 year*	0.00		1.00		0.66
		≥1 year	-0.35	0.80	0.71	0.15, 3.35	

*Reference category

10. Conclusions

10.1 Key findings

Incidence and impact

- IBK was defined as a herd disease affecting only the eyes of cattle with an average herd morbidity of > 2% in calves and >0.6% in cows, spreading rapidly through a herd within 30 days, seen as acute keratoconjunctivitis including ≥10% with corneal ulcers (62)..
- The annual incidence of pinkeye in Australia was estimated to be 10.25% (95% PI: 6.43, 16.97).
- The Australian cattle industry is expected to lose A$ 9.67 million (95% PI: 8.56, 13.11) per annum just considering the cost of the three medications analysed.

Risk factors

- Farmer-reported fly levels were associated with pinkeye occurrence indicating that efforts to control flies is warranted. Insects, particularly flies, are long suspected of having a role or even directly causing pinkeye. Ocular damage by face fly may predispose to pathogen invasion.
- Farmer-reported higher rain levels were protective against pinkeye. Survey respondents that ranked their farm as having low rain levels had a higher proportion of pinkeye in their herds compared with those that ranked their farm as having moderate and high rain levels, respectively.
- The relationship between dust levels and pinkeye prevalence was ambiguous in our results.
- Our results confirmed the risk of pinkeye varies by region in Australia. The proportion of the herd with pinkeye in 2018 was significantly greater on farms located in southern Australia compared to those in northern Australia.
- Pinkeye prevalence was greater in herds with more cattle two years of age or younger compared to herds with older cattle. Those categorised as mixed ages had the highest proportion of pinkeye in their herds.
- There was an increased risk of high prevalence of pinkeye on farms of smaller grazing area.
- Herd proportion with pinkeye was greater in herds with no zebu content, in agreement with reports that pinkeye is more common in sub-species *Bos taurus* (*Bos taurus taurus*, British and European breeds) whilst *Bos indicus* (*Bos taurus indicus*, Brahman, zebu) appear resistant to natural disease (17, 63, 64).

Farmer attitudes to treatment

- Farmers with smaller farm sizes were more likely to treat their cattle for pinkeye, as were those who yarded their cattle more often, and those that rate pinkeye as highly painful, suggesting the approach to treating pinkeye may reflect animal husbandry and farm management practices.
- Farmers were more likely to treat pinkeye if they ranked the pain induced by pinkeye higher.
- The most used treatments for pinkeye were pinkeye ointment followed by eye patches, then pinkeye spray, fly control, and pinkeye powder. Current preferred treatments appear to be

based on availability, personal opinion, and previous practices, rather than any scientific research.

- Our study confirmed that pinkeye ointments are the most popular pinkeye treatment in Australia. Furthermore, this treatment was rated by 66% of users as highly effective.
- Pinkeye spray and powder are still used by Australian farmers (n = 623 and n = 408, respectively) but are rated by many as having low effectiveness (40% and 45%, respectively).
- Subconjunctival injection was one of the lesser used treatments in our survey yet had the highest proportion (68%) of those that used it ranking it as highly effective.
- Some non-antimicrobial treatments are popular for treatment of pinkeye in Australia. The second most used treatment in our survey was eye patches (n = 637) and they were ranked by 64% of those who had used them as highly effective.
- The most common reasons given for not treating pinkeye related to the futility or ineffectiveness of treatments. The most common was “too difficult to treat individuals” followed by “cattle recover without treatment”, “pinkeye does not cause enough problem to warrant treatment”, “treatments do not change outcome”, “treatments do not work” and least chosen was “treatment is too costly”.

Impact on farm productivity

- Australian farmers perceive the impact of pinkeye on productivity as higher if they farm younger cattle, treat for pinkeye more frequently, and have a larger herd size.
- Farmers that treat pinkeye-affected cattle are more likely to rank pinkeye as having high impact on productivity
- When asked to rank a list of other consequences of pinkeye on their farms, the most selected were economic concerns of ‘significant treatment expense’, ‘decreased sale value of cattle and farm profits’, and ‘decreased weight gain of affected cattle’, then farm management issues of ‘restricts movement, disrupts farm routine’ and ‘workplace health and safety issue’. The least selected were ‘animal welfare issue’, ‘mismothering of calves’ and ‘affects bull fertility’.
- Money spent on pinkeye increased with increased frequency of disease, if farmers believe the disease impacts are more severe, and if they treat more frequently.
- Farmers with herds containing Angus cattle reported spending more money on pinkeye compared to those without Angus
- The median amount reportedly spent on pinkeye in 2018 was $250 per farm.

Antibiotic concentrations

- After topical administration of cloxacillin (approximately 1 gm) into the lower conjunctival sac, cloxacillin reached peak concentration (834.24 µg/mL) at 1.42 hours, maintained a concentration > 1 µg/mL* up to 17.33 hours, and no cloxacillin was detected after 25.25 hours (lowest level of quantification [LLOQ] > 0.4 µg/mL).
- No penicillin could be detected in any tear sample

Vaccine effectiveness

- The vaccine was not protective against naturally occurring pinkeye under the Australian farm conditions.

10.2 Benefits to industry

The overall objective of this project was to improve pinkeye outcomes for Australian cattle. The motivation came from the observations of an experienced veterinarian (MK) that despite 130 years of scientific effort (1) there appeared to be little improvement in clinical outcomes for cattle with pinkeye at the farm level. Much of the previous effort to understand and control pinkeye was done through the discipline of microbiology (10, 38). We undertook to re-examine this ubiquitous, costly, and distressing disease of domestic cattle by combining robust epidemiologic method and clinical veterinary skills. Our findings are directly benefiting the Australian cattle industry studies and have renewed interest in pinkeye by the international research community (10, 11, 65).

Definition of pinkeye

A review of the available literature resulted in the publication of an article in an international journal on how to define and diagnose the disease (62). This supplied cattle industry stakeholders a first-ever definition of infectious bovine keratoconjunctivitis. This was required to ensure that all stakeholders are describing the same disease and thus avoid classification bias.

Estimation of disease frequency and impact

We updated knowledge on the frequency of occurrence and economic significance of pinkeye (66). Using novel methodology, we found pinkeye affects 2.8M cattle or 10.25 % of the entire Australian cattle herd per annum. Cattle farmers spend almost $10M per annum on three pinkeye medications alone (66) and reported spending a median $250.00 per farm on pinkeye in 2018 (67). The findings of these separate studies are noteworthy because they indicate that the cost of pinkeye to Australian industry is higher than previously estimated (17, 19). Our another study found that there is a shift in the attitudes of Australian farmers to the equipoise between the economic impact of pinkeye and its animal welfare implications (15). Taking all these findings into consideration, industry should re-evaluate the cost-benefits of allocating constrained resources to this disease.

Identification of risk factors

Responses to the largest-ever survey of Australian farmers on pinkeye disease were used to develop statistical models to identify risk factors for the disease and explore farmers' perceptions about its treatment and impact at the farm level (15, 43, 67). This was needed because the only previous comprehensive survey of the on-farm impacts anywhere in the world occurred in Australia 40 years prior (8, 9). Although dated, that survey was still being heavily relied on to inform industry about pinkeye, both in Australia (17, 19) and internationally (11). The excellent response rate to our online studies allowed for robust statistical modelling to identify risk factors for pinkeye occurrence (43), reasons why different farmers spend more, or less, on pinkeye (67), and what their attitudes to treatment of pinkeye are (15).

Vaccine and treatment effectiveness

Today's Australian cattle farmers are looking for evidence-based advice on management of pinkeye in their herds to reduce the economic burden and improve welfare outcomes (15, 67). To this end, we undertook the first-ever field trial of the only commercial pinkeye vaccine available in Australia over two pinkeye seasons (68) (unpublished) and the world's largest comparison trial of pinkeye treatments against naturally occurring pinkeye (69) (unpublished). The results of our field trials on vaccination and treatments for pinkeye supply much-needed evidence-based direction to industry on the value of different management approaches for pinkeye. Whilst more studies are required on both antibiotic and importantly non-antibiotic alternatives for treatment and prevention of pinkeye, our vaccine trial results (68) and the conclusions of other studies (65, 70), indicate that pinkeye vaccines are not effective in controlling disease.

We also undertook a pilot pharmacokinetic study of drugs in tears of a calf following treatment for pinkeye (71). We believe this to be the first study of its kind in Australia and one of the few undertaken worldwide (34, 72, 73). The results were unexpected, and a larger study is needed to supply the evidence base for continued use of different pinkeye treatments.

Development of eye scores

To facilitate data collection and recording, during the field trials on vaccination and treatments for pinkeye, various measures of eye characteristics (so-called “eye scores”) were devised from clinical experience (MK) and a review of literature. The “p-Score” was used for to measure the amount of periocular pigmentation, “t-Score” for the extent of tear overflow (epiphora), and “i-Score” to measure the stage and severity of eye lesions. These scores will be valuable to industry, not only for pinkeye but other ocular diseases. For example, the i-Score included not only pinkeye, but a scale to score the other major bovine ocular disease, “cancer eye” (bovine ocular squamous cell carcinoma, OSCC). Ultimately these scores and the > 5000 digital images of eyes recorded during the trials were the basis for another project that promises to be very valuable to the cattle industry as it will develop an artificial intelligence based smart phone app to allow classification of the eye lesions that will ultimately enable point-of-care scoring of all eye diseases of cattle. This should be a great benefit to the whole cattle industry.

In summary, this project has produced the world-first case definition of the disease, updated the economic cost of disease in Australia using novel methodology, conducted the largest ever survey of Australian farmers on pinkeye to identify risk factors, updated understanding about farmers’ experience with the disease on-farm, conducted a randomised controlled trial of the only commercial pinkeye vaccine in Australia, conducted a large treatment comparison trial on naturally occurring pinkeye and studied pharmacokinetics of drug levels in tears post-treatment. All this will provide an evidence-base to the cattle industry for developing a robust disease control policy.

11. Future research and recommendations

We conducted several studies in this project to better understand the disease epidemiology and to identify approaches for better management and control of the disease. Besides directly benefiting the Australian cattle industry, our studies have renewed interest in pinkeye by the international research community (10, 11, 65). Further international co-operations between research groups offer the opportunity to markedly improve outcomes for cattle with pinkeye.

11.1 Recommendations for the cattle industry

Update economic models of disease impact based on project findings: Our research found that the impact of the disease on the cattle industry is more than previously anticipated as it can affect about 10% of the entire Australian cattle herd and as a result the cattle industry spends about $10 million per annum just on the cost of three medications. Our analysis confirms that pinkeye remains a persistent and serious cattle industry problem in Australia. Therefore, key assumptions in the modelling of the impact of pinkeye in Australia need adjusting. We recommend that the economic models previously developed to estimate the disease impact should be updated.

Increase funding for pinkeye control in southern Australia: Our results confirmed the risk of pinkeye varies by region in Australia. The proportion of the herd with pinkeye was significantly greater on farms located in southern Australia compared to those in northern Australia. Although further work is required investigate the reasons for this difference, this should be taken into consideration while making decisions for allocating funding for disease control.

Control fly levels to manage pinkeye: Farmer-reported fly levels were associated with pinkeye occurrence in our research, indicating that efforts to control flies could help with the control of the disease. Insects, particularly flies, are long suspected of having a role or even directly causing pinkeye as ocular damage by face fly may predispose to pathogen invasion. Therefore, producers should be encouraged to control fly levels to better manage pinkeye.

Monitor younger cattle more often: Pinkeye prevalence was greater in herds with a greater proportion of cattle of two years of age or younger compared to herds with older cattle. This suggests that younger cattle are at a greater risk and should be monitored to protect them from extremes of weather, if possible.

Further work should be done to understand the influence of dust levels on pinkeye: The relationship between dust levels and pinkeye prevalence was ambiguous in our studies. Producers who ranked their farms moderate for dust levels had less pinkeye than those that ranked high, but also less than those that ranked dust as low/none. This latter result is difficult to explain biologically, may involve cryptic interactions, for example with other environmental factors such as wind levels and fly levels, and requires further investigation.

Increase zebu content if it aligns with producers' breeding plan: Pinkeye prevalence was lower in herds with a zebu content confirming that pinkeye is more common in British and European breeds whilst *Bos indicus* (*Bos taurus indicus*, Brahman, zebu) appear to be resistant to natural disease. Therefore, producers at a high risk of the disease can increase zebu content in their herds if it aligns with their breeding plans.

Develop guidelines for pinkeye treatment: Current preferred treatments for pinkeye appear to be based on availability, opinion, and previous practices, rather than any scientific research. The most used treatments for pinkeye were pinkeye ointment followed by eye patches, then pinkeye spray, fly control, and pinkeye powder. Therefore, a workshop involving field veterinarians, animal health policy makers and veterinary academics should be organised to develop guidelines for pinkeye treatment.

Discontinue outdated treatments: Some outdated treatments such as kerosene or turpentine are still used to treat pinkeye. Measures should be taken to encourage producers to discontinue outdated treatment practices.

Avoid spending on pinkeye vaccine: A key finding of the project was that the only pinkeye vaccine used in Australia was not found to be effective against preventing the occurrence of pinkeye. Therefore, the money spent on vaccination does not appear to be worth it. Although further work is required to understand the microbiome affecting pinkeye cases, our findings indicate that there is no benefit of vaccination under the Australian field conditions.

Educate producers about the study findings: Many of the study findings would be useful for producers. Flyers and other educational materials should be created for educating producers about the study findings, particularly those that can be used to improve pinkeye management. Articles should be included in farmer newsletters and magazines highlighting key study findings.

11.2 Recommendations for future research

Further analysis of survey data: We have collected a large dataset that should be useful for many years to come. For example, from the 1644 useful responses to our online survey on pinkeye we already updated much about the on-farm experience of Australian farmers with pinkeye by publishing two articles in an international peer-reviewed journal (15, 43), and are about to submit a third (15). However, this dataset may be used for further articles. In particular, we would like to directly compare and contrast our demographic findings about pinkeye and Australian cattle farms with those of the seminal survey conducted 40 years earlier and which informed a lot of our questionnaire (8, 9). Since that article was published in the Australian Veterinary Journal in 1982, as a tribute we would ideally aim to publish our article in the same journal in 2022.

Finalisation of eye-scores: During the field trials, to facilitate data collection and recording, three measures of eye characteristics (so-called “eye scores”) were devised following a review of literature and using veterinary clinical experience (MK). These were the “p-Score” for the amount of periocular pigmentation, “t-Score” for the extent of tear overflow (epiphora), and “i-Score” for the stage and severity of eye lesions, both for pinkeye and for “cancer eye” (bovine ocular squamous cell carcinoma, OSCC). Further field studies to confirm the value and repeatability of these scores are warranted. A study on risk factors and management of OSCC has been discussed and some interest in funding expressed.

Development of an app for diagnosing pinkeye: Approximately 9000 images of cattle eyes were collected during the field trials. Because of the remoteness and geographical location of the trial region (on the border of Queensland and NSW) it was not always possible to revisit farms during the trial and farmers were instructed to take photos on their smart phones. The images were then scored remotely by MK. From this, the idea of a farmer-friendly smart phone app to allow classification of the eye lesions of cattle was conceived. We proposed to use artificial intelligence (AI) to develop a cutting-edge classification tool for cattle eye diseases that aimed to enabling the point-of-care classification and scoring of all eye diseases of cattle. This project has already been funded by MLA. The app will provide an objective eye disease score and keep track of the disease progression over time and should be a great benefit to industry.

Further investigation of the effect of flies and dust on pinkeye: Further work is required to better understand the effect of flies and dust as the findings in this study were based on an online survey. Future studies could involve measuring fly and dust levels in the environment of various farms and comparing the incidence of pinkeye between them after adjusting for other confounders.

Impact of pinkeye on farm productivity: Like dust and flies, our estimates about the impact of farm productivity were farmer reported. Further studies should be conducted by conducting economic analyses based on farm records to refine the estimates obtained in this study.

Investigate pharmacokinetics of treatments: Our investigation of cloxacillin and penicillin concentrations in bovine tears has developed sensitive assays to measure cloxacillin and penicillin concentrations in bovine tears. This pilot study indicated levels of cloxacillin ointment may exceed MIC for *M bovis*, however the frequency of dosing required to be effective for pinkeye appears to be more than the 48 hourly repeat dose currently recommended. SJ pinkeye injection of procaine penicillin in a water base are not likely to exist in a sufficient concentration in tears to be efficacious. Consequently, this team is keen to undertake further studies to investigate the efficacy of pharmacological agents to improve bovine keratitis. A brief approach to conduct this investigation is presented in the Appendix (Section 13.2).

Heritability of pinkeye: We did not study heritability of pinkeye in this project. A study could be conducted by comparing DNA profile to phenotypic traits although the value to industry of any such study should not be overstated because pinkeye is clearly a multifactorial disease impacted by the environment (43, 74).

Diagnostic study of Australian pinkeye using PCR assay: A diagnostic study of pinkeye in Australian cattle using new molecular biology-based techniques such as polymerase chain reaction (PCR) assay for detecting DNA has not been done and would be ideal. Such tests have been described and validated for pinkeye. Initially these tests targeted Moraxella species, specifically *M. bovis, M. bovoculi* and *M. ovis* (75). They have been used to differentiate between these species (76, 77) and to confirm diagnosis of *M. bovis* (78). These PCR tests and other molecular studies such as next generation sequencing (NGS) like 16S rRNA to characterise the entire bovine ocular microbiome with relationship to pinkeye (79, 80) raised fresh doubts about the role of *M. bovis* in pinkeye. Pinkeye researchers are rightly casting a wider diagnostic net. Combined PCR assays testing for Moraxella and Mycoplasma species were described from pinkeye cases in Germany (20) and USA (81). A report was published on development and use of real-time multiplex PCR for five pathogens associated with bovine pinkeye, Moraxella and Mycoplasma species and bovine herpesvirus (BHV1) (81). Using these assays under the Australian conditions would provide evidence-base about the pathogens associated with the disease.

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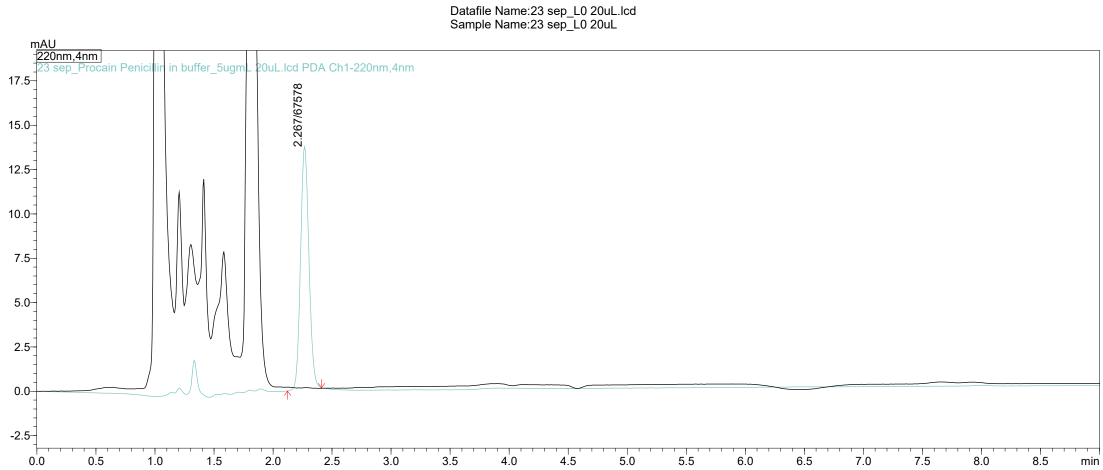
13. Appendix

13.1 Quantification of antibiotic concentrations in bovine tears

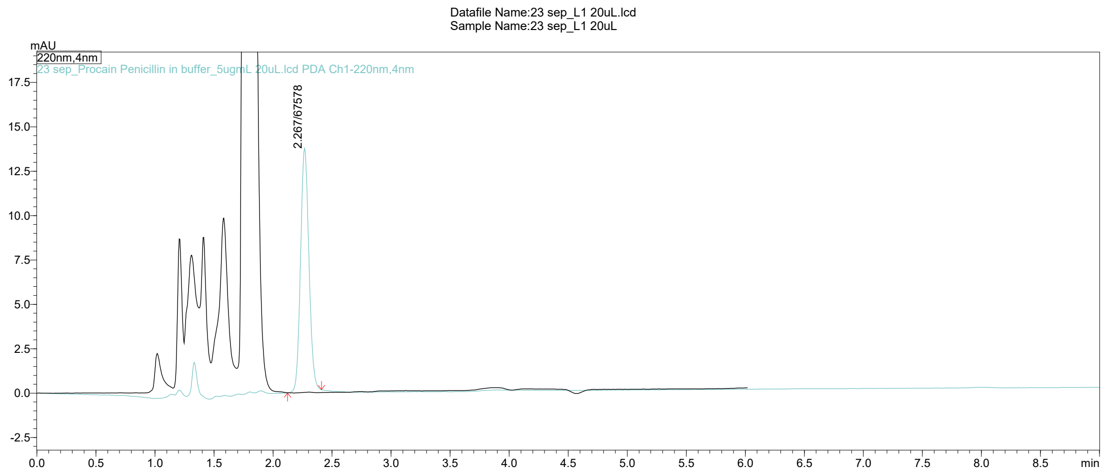
Figure A1. Chromatogram demonstrating the analytical sample of penicillin (large green peak just after two minutes) when added to bovine tears.



Black: Baseline (LO) sample – prior to drug administration (no penicillin at retention time 2.26 min).

Analytical standard 5ug/mL procaine benzylpenicillin (MW 516) (Green)

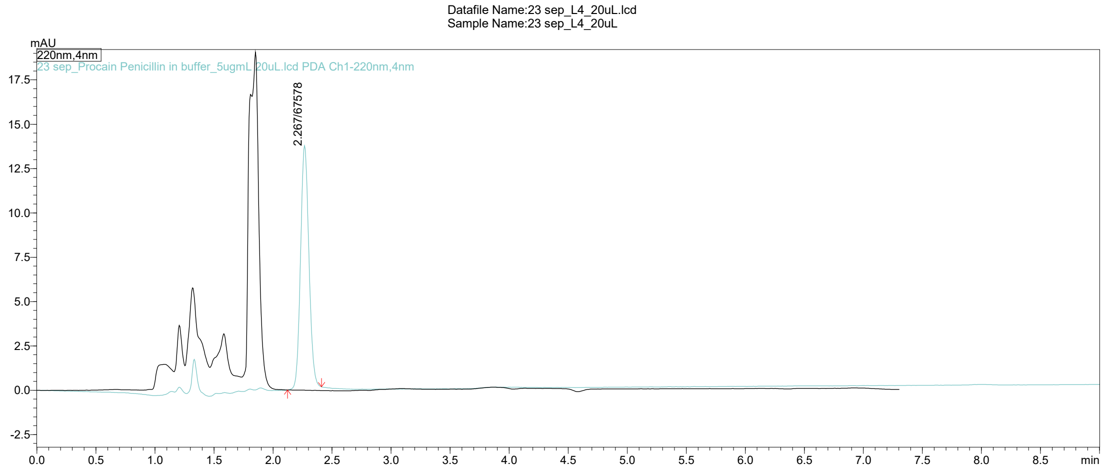
Figure A2. Chromatogram demonstrating the analytical sample of penicillin (large green peak just after two minutes) when added to bovine tears, 1 hr after the penicillin injection into the conjunctiva, demonstrating no penicillin peak identifiable in the tears.



Black: L1 (1 hr 25 mins after drug administration) sample (no visible peak at retention time 2.26 min).

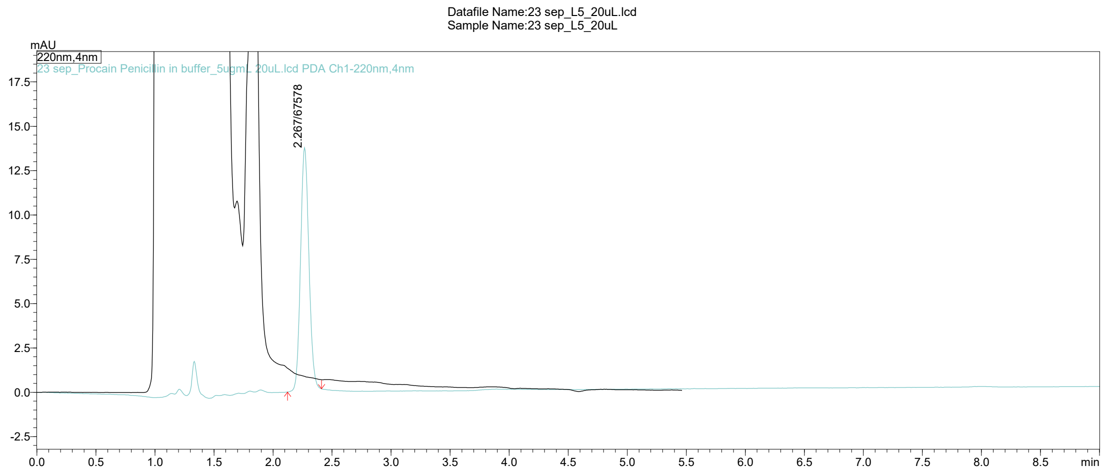
Analytical standard 5ug/mL procaine benzylpenicillin (MW 516) (Green)

Figure A3. Chromatogram demonstrating the analytical sample of penicillin (large green peak just after two minutes) when added to bovine tears, 4.5 hr after the penicillin injection into the conjunctiva, demonstrating no penicillin peak identifiable in the tears.



Black: L4 sample (4 hr and 30 min after drug administration (no visible peak at retention time 2.26 min).
Analytical standard 5ug/mL procaine benzylpenicillin (MW 516) (Green)

Figure 4. Chromatogram demonstrating the analytical sample of penicillin (large green peak just after two minutes) when added to bovine tears, 5.5 hr after the penicillin injection into the conjunctiva, demonstrating no penicillin peak identifiable in the tears.



Black: L5 sample (5 hr and 30 minutes after drug administration) (no visible peak at retention time 2.26 min).
Analytical standard 5ug/mL procaine benzylpenicillin (MW 516) (Green)

13.2 Further investigation

Next steps after this pilot study would investigate the efficacy of current therapeutic treatments for pinkeye in order to make recommendations on the most efficacious treatments, best dosage, and frequency of dosage to improve animal welfare and to minimise treatment costs for the producers. Future studies should include

1. Investigation into the constituents of tears in clinical normal animals versus animals with active corneal infections. Tears are an important defence to protect the cornea and to remove and inhibit the action of pathogens across the surface of the cornea. There is minimal information of the role of tears to protect from pinkeye. It is proposed to
    a. compare the antimicrobial activity of bovine tears in clinically normal cows, versus cows with keratitis
    b. compare the level of endogenous lysosome in tears of clinically normal cows versus cows with keratitis
2. Identify the microorganisms over the cornea in infected animals in the Australian environment. To the best of the authors' knowledge there have been no studies conducted in Australian cattle to identify the identity of pathogenic microorganisms.
    a. Identifying infectious agents on the bovine cornea associated with pink eye by taking swabs of normal and inflamed corneas and identifying the microflora inhabitants by PCR.
    b. Perform antibacterial susceptibility tests to determine cloxacillin, penicillin, tetracycline, florfenicol minimal concentrations to inhibit the bacterial isolates
3. Undertake pharmacokinetic studies to quantify the change in drug concentrations in tears over time. Such drugs would include the currently recommended formulations to treat pinkeye such
    a. Topical cloxacillin ointment – In the study reported in the previous pages we only performed this component in one animal's eye. There is a need to repeat this component in additional subjects
    b. Subconjunctival administration of procaine (or benzathine) penicillin in both an aqueous and oil-based formulation
    c. Intramuscular injection of oxytetracycline
    d. Systemic injection of florfenicol

Once drug concentrations over time have been described, these can be cross-checked that they inhibit ocular pathogens as determined in point 3.d. We would also suggest investigating cortisone topical ointment for the treatment of pinkeye.

13.3 Questionnaire used for the survey