Infectious Bovine Keratoconjunctivitis in Angus cattle

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Infectious Bovine Keratoconjunctivitis in Angus cattle

by

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A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Genetics

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Ames, Iowa

2006
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ABSTRACT

Infectious Bovine Keratoconjunctivitis is a contagious ocular disease that affects cattle worldwide. It is considered the most significant ocular disease in cattle. The economic impact of the disease has been well documented. The available treatments have yet to show their effectiveness in the prevention and/or control of the disease. The objectives of this study were twofold, to evaluate the effects of IBK incidence and severity on production traits such as weaning weight, yearling weight, and carcass traits and to estimate variance components associated with IBK. Our results support previous findings on the effects of IBK on weaning weight. The results of our study also indicate a decrease in the yearling weight of affected cattle. Furthermore, the decrease in weaning and yearling weights became more pronounced as the severity of infections increased. The heritability estimates obtained from our analyses ranged from .05 to 0.11 ± .04. Although these estimates are low and similar to other disease and reproductive traits, genetic improvement could be achieved through the implementation of selection programs, designed to increase resistance to IBK.

Most of the research has been focused on the development of treatments for the disease, with debatable success. Additional research is needed on immunological factors present on the ocular surface and their effects in preventing the onset of IBK. In the absence of lysozyme in bovine tears, secretory IgA and lactoferrin are likely candidates implicated in ocular defense against bacterial infections. The first aim of our second investigation was to quantify these two factors in bovine tears with ELISA’s. Statistical analyses indicated significant differences between affected and unaffected for SIgA, with lower levels on
affected calves. No differences were found in the levels of lactoferrin between unaffected and affected calves. The levels of IgA appear to decrease with increased severity of IBK.

The second aim of this study was to estimate genetic parameters of IgA and carry out correlation studies with incidence of IBK. The estimated heritability was moderate, however the correlation between the two traits although moderate was not in the desired direction.
GENERAL INTRODUCTION

Introduction

Expected progeny differences (EPD) are widely used by beef producers, to improve the genetic merit of cattle. Most of the EPD’s available are for production traits, with a limited number of EPD’s available for disease traits. Some of the most common diseases affecting cattle are economically important and need to be addressed from a breeding standpoint, as the current management schemes are not highly successful. One such example is infectious bovine keratoconjunctivitis (IBK). Differences in the genetic merit of IBK have been documented, which can be utilized to increase profitability. Due to the low heritability of most disease traits, and the lack of uniform guidelines to score phenotypic information, less research has been focused on genetic analyses to select for less susceptible or more tolerant animals. The estimation of genetic parameters and EPD’s for IBK, along with correlation studies of IBK incidence and immunological factors implicated in ocular defense, will enable producers to select for cattle less susceptible to IBK.

Thesis Organization

There are two separate analyses that make up this Thesis. The first paper is an analysis of the effects of Infectious Bovine Keratoconjunctivitis (IBK) on performance traits in cattle, and estimation of variance components for IBK susceptibility. The field data utilized for this study was a combination of data collected by the first author and data sent by producers. The organization, editing, analysis of the data and interpretation of the results was
the responsibility of the first author. A. Hassen and R.J. Tait, assisted with model
development and software expertise. J.M. Reecy assisted with access to field data, discussion
of the results, and oversight of the project.

The second paper is an analysis of immunological factors associated with ocular
infections. Two such factors were chosen as candidates to study their potential relationship
with IBK: SIgA and Lactoferrin. Estimation of variance components was also carried out in
this study. The data utilized for this study was collected by the first author. The organization,
editing, analysis of the data and interpretation of the results was the responsibility of the first
author. A. Hassen and R.J. Tait assisted with model development and software expertise.
J.M. Reecy assisted with access to field data, discussion of the results, and oversight of the
project.

Literature review

*Breeding for disease resistance*

Breeding for disease resistance or tolerance, has not received the attention it merits in
selection programs as a production trait, unlike others such as carcass and growth traits
(Cundiff, 1982). In the 20th century, most of the research in the area of disease control
focused on the development of antibiotics and vaccines. Originally, the development of
antibiotics and other drugs although expensive, was perceived as the final solution to the
diseases that affect production. The outcome of such a reliance on chemicals led to an
increased selection pressure on bacterial agents and the subsequent evolution of resistant
strains to the chemicals utilized (Nicholas, 1987; Axford et al. 2000).
The awareness in the scientific community about the development of pathogen resistance to chemical agents, has led to increased pressure on breeders from governments and consumers to find alternative ways to control diseases. One approach is to breed for disease resistant livestock, which could enhance current strategies of disease control, or replace existing approaches (Bishop, 2003). Alternative approaches for disease control are crucial, since it will lead to a reduction in the use of antibiotics and thus diminish the risk of between species transfer of bacterial resistance to antibiotics and other drugs (Axford et al. 2000).

The first step towards applying disease resistance in selection programs is to exploit genetic variation, between and within breeds. Among the approaches utilized in quantitative genetics for improvement of production traits are the identification and utilization of additive genetic, as well as non-additive genetic variation (Cundiff, 1982). There are several examples of disease traits in cattle, for which additive genetic variation has been documented. Among the disease traits studied include: trypanosomosis, tick-borne diseases (Seifert, 1971), bovine ocular squamous carcinoma (Anderson, Lush and Chambers, 1957), Infectious Bovine Keratoconjunctivitis and mastitis (Kadarmideen; Thompson and Simm, 2000). Additional examples of genetic variation associated with disease traits have been published (Wakelin, 1978; Nicholas, 1987).

From the diseases referred to above, trypanomosis and tick resistance provide evidence of sustainability and stability of breeding for disease resistance or tolerance (Stear et al., 2001). In both cases, cattle that have lived the longest in their respective environments where the diseases occur, display higher resistance and tolerance to disease (Nicholas, 1987; Murray et al., 1991).
The estimation of heritabilities for disease traits is essential to make predictions of the response to selection for disease resistant or tolerant animals. The results of a study, which compared the ranges of heritability estimates between production traits and disease traits, concluded that the ranges of the estimates were similar (Morris, 1998). Some of the diseases with significant heritabilities include, tick infestations in cattle (Stear, 1990), mastitis (Heringstad et al., 2000) and trypanosomosis (Murray et al., 1991). The examples listed thus far are studies for the selection of resistant animals for single disease traits. Recently, studies suggested significant heritability estimates for general immune response, which could be utilized to predict the response in animals for resistance to different diseases, or broad based disease resistance (Mallard et al., 1998; Wilkie and Mallard, 2000). These findings indicate that selection for resistant or tolerant animals is feasible (Stear et al., 2001).

In addition to evaluating the sustainability and feasibility, an additional concern needs to be addressed prior to incorporating disease traits in selection programs, desirability (Stears et al., 2001). Desirability of breeding for disease resistance or tolerance has two components. The first one is related to the effects that selecting for disease resistance might have on production or other disease traits. The second component is related to the frequency of diseases (Stears et al., 2001). In order to evaluate the effects of including disease traits as selection criteria, more studies are needed to evaluate the correlation of the disease traits with production traits. Correlations between these two types of traits should be negative. However, even if there are undesired relationships between disease and production traits, index methodology is available to incorporate traits with unfavorable correlations in selection programs (Cameron, 1997).
Breeding for disease resistance is an alternative to current disease control methods. It can be a cost effective way to improve the health and production of livestock. Furthermore, this approach will help ease the public concerns regarding chemical residues in consumption products. Current methodology for incorporation of disease traits as selection criteria exist, and could be utilized to breed for overall health or resistance to specific pathogens. In addition, classical breeding strategies for selection of resistant and tolerant livestock could be enhanced through the discovery of major genes or genetic markers associated with Quantitative Trait Loci (QTL) associated with the traits of interest.

*Infectious Bovine Keratoconjunctivitis*

Infectious Bovine Keratoconjunctivitis (IBK) commonly referred to, as “Pinkeye” is a contagious ocular disease that occurs in both beef and dairy cattle. It is characterized by inflammation of the conjunctiva and ulceration of the cornea. Infections occur throughout the year, with higher incidence observed during the summer. Infectious Bovine Keratoconjunctivitis is considered the most important ocular disease in cattle, due to the decreased performance of infected individuals and its subsequent economic effects.

*Clinical signs*

The clinical signs of IBK are seen after the resistance of the eye is overcome by the invading organism, primarily *Moraxella Bovis (M. bovis)* (Kopecky and Pugh 1986). The first signs of pinkeye are characterized by excessive tearing, blinking, photophobia and swelling of the eyelids and conjunctiva. As the disease progresses, the ocular discharge becomes purulent. Anywhere from two to five days after the initial signs are observed,
cloudiness develops in the center of the cornea. This opacity extends centrifugally across the whole cornea, which leads to ulceration of the cornea and impaired vision of infected cattle (Bedford, 1976). Based on results from in-vitro assays, it’s been suggested that the ulceration of the cornea is caused by direct cytotoxicity of \textit{M. bovis}, through the release of necrotizing factors, which damage the epithelial cells of the eye (Kagonyera and George, 1988). Another study demonstrated that the changes in the cornea after the onset of infection are associated with collagenase release from fibroblasts, epithelial cells and neutrophils (Frank and Gerber, 1981).

In the most severe cases, rupture of the cornea may occur, which could lead to permanent blindness. The process of healing begins when blood vessels invade the cornea and grow toward the ulcer. As the blood vessels approach the center of the cornea, the opacity of the eye begins to clear. Animals that exhibit mild cases of pinkeye can recover, with total restoration of corneal transparency, within five weeks after the initial signs are observed. However, in the most severe cases, corneal scarring might persist for months or even years. In general, all descriptions of the disease are similar, but variations in the clinical features of pinkeye between individuals have been reported. Also, additional field studies have indicated that differences in symptoms provide evidence for the presence of other ocular diseases, which have erroneously been characterized as pinkeye (Baptista, 1979).

\textit{Risk Factors associated to IBK}

As invasion by \textit{M. bovis} occurs, it is followed by irritation of the eye, which can be caused by a number of factors such as: tall grass, weeds, dust, face flies and ultraviolet radiation and other stress factors (Wilcox 1968; Baptista 1979; Gleeson et.al. 1965; and
Hughes 1971). The influence of tall grass, weeds and dust have been suspected, but not proven. All of the outlined factors, except UV radiation can be controlled to some extent by proper management practices. The effects of flies and UV radiation have been associated with outbreaks in several studies (Gerhardt 1982; Hubbert 1970; Lepper, 1987 and Kopecky 1986). Transport of cattle has been associated with the IBK carrier status of an animal, as higher numbers of the causative agents such as *M. bovis*, were isolated after shipment of the cattle compared with number of *M. bovis* prior to shipment (Pugh and McDonald, 1986).

*Musca autumnalis* (face fly)

*M. bovis* is transmitted by direct contact with infected individuals or by mechanical vectors. The principal vector of this bacterium is the face fly, although other flies might also be able to act as vectors (Kopecky and Pugh, 1986). Kopecky and Pugh (1986) demonstrated that contact transmission was not as influential in the development of IBK in the absence of flies. In this study the calves were placed in a controlled environment in the absence of face flies and UV radiation. Only 5% of the calves developed IBK in the absence of other aggravating factors. These findings indicate that contact transmission, in the absence of other factors such as face flies, is not significantly involved in the development of IBK. Another research study also demonstrated the importance of the face fly in the development of IBK. In this study, two groups of cattle were studied, one treated with insecticides and a second untreated group. There were significant differences between the numbers of IBK cases between the two groups. Furthermore, a regression of the number of observed cases against the number of flies present was positive and significant (Gerhardt et al., 1982). The increased incidence of IBK during the summer has a strong association with the life cycle of
Musca autumnalis. Face flies hibernate during the winter and emerge in the spring (Moon et al., 1991). Once flies emerge mating occurs and the females lay their first eggs in April (Krasfur et al., 1985). The highest population density of face flies occurs during the months of June to early August, when a proportional increase in IBK cases is observed (Cheng, 1967; Gerhardt, 1982). These and other studies points toward the control of face flies as a way of reducing the incidence of pinkeye in cattle.

Face flies, predispose the cattle for infection by irritating their eye with their prestomal spines, which cause lesions and increase the flow rate of tear fluid. Flies carry *M. bovis* in their crops and deliver the bacterium to the eye while they feed on the tear secretions of cattle (Krafsur and Moon, 1997).

**Ultraviolet radiation**

Not only does the increased incidence in IBK coincide with an increase in face fly populations, it also seems to be related to increased ultraviolet radiation observed during the summer months. This increased radiation predisposes the eye to infection by bacterial pathogens (Hughes and Pugh, 1970).

Ultraviolet radiation from the sun has been implicated in a number of ocular diseases in different species. At least two such diseases have been studied in cattle: IBK and bovine ocular squamous cell carcinoma, also known as cancer eye. Studies in rabbits and human corneas indicated that UV radiation could have deleterious effects on the epithelial surface of the cornea. Kopecky et al. (1980), studied the effects of UV radiation on the enhancement of IBK incidence and severity. They used a control group in which, the UV radiation was not filtered and the spectrum of radiation in this case included wavelength of
less than 270nm; therefore the subjects were exposed to a higher amount of radiation. In the experimental group, UV radiation was filtered in order to resemble the wavelength that normally penetrates the atmosphere, which is higher than 285 nm. After submitting the calves to radiation, they challenged calf’s eyes with *M. bovis*. Both groups showed signs of IBK however, the group exposed to unfiltered UV exhibited a higher incidence and severity of infection. These results indicate that the threshold for enhancement of disease was about 270nm. It is conceivable that a change in the ozone layer may result in a widespread spectrum of UV, which could cause an increase in the severity of pinkeye outbreaks, but not necessarily an increase in outbreaks (Kopecky et al., 1980).

*Incidence and Severity*

A higher incidence of pinkeye is observed during the summer months, although outbreaks have been reported throughout the year. Overall incidence, during an IBK outbreak can affect up to 90% of a herd (Baptista, 1979). The warmer and humid conditions of summer coincide with the reproductive cycle of *M. autumnalis* and with increased ultraviolet radiation. IBK outbreaks have also been documented during the winter after heavy snowfall (Pugh and Hughes, 1972). It’s been well documented that higher incidence occurs in preweaned calves (Wilcox, 1968, Bedford, 1976). However, adult cattle not previously exposed to pinkeye can be severely infected (Baptista, 1979). Several studies indicate variations between breeds in susceptibility for pinkeye. Among the most susceptible breeds are Herefords, Jerseys and Friesian (Wilcox, 1968, Webber and Selby, 1981, Snowder et al., 2005). *Bos indicus* and their crosses have been found to suffer lower incidence of IBK (Webber and Selby, 1981, Snowder et al., 2005). Besides a difference in susceptibility
between breeds, an age effect on susceptibility to IBK has been suggested. In a clinical trial, comparing young calves to older cows, the younger cows showed a higher incidence of infections although the recovery rates of *Moraxella Bovis* did not differ (Ward and Nielsen, 1979). A recent quantitative analysis of field data indicated higher infection rates in younger animals. In this study, high infections rates were first noted around 45 days of age. Incidence remained high through 130 days of age; at which point a decrease in the number of infected animals was observed (Snowder et al., 2005). Furthermore, calves born from younger dams have shown higher prevalence of infection than calves born from older dams (Ward, 1979). A recent study in which incidence rates were compared between the offspring of Angus*Herford and Herford*Angus calves, suggest a maternal effect on the incidence of pinkeye. In this study, the offspring from Hereford dams exhibited higher incidence of infection than the offspring from Angus dams (Snowder et al., 2005). There is no evidence that supports gender affinity for prevalence of IBK (Powe et al., 1992).

**Eyelid Pigmentation**

Differences between breeds, regarding incidence and severity of IBK have been reported, with Herefords exhibiting a higher incidence and persistency of infection. Hereford cattle exhibit lower eyelid pigmentation than other breeds. The higher incidence of pinkeye among Herefords has been associated to decreased eyelid pigmentation (Frisch, 1975). The incidence and severity of IBK and their association to eyelid pigmentation were analyzed utilizing Hereford and Brahma cattle. Significant differences were found between breeds, with Hereford suffering from a higher incidence rate and severity of IBK. The inverse relationship between pigmentation, incidence, and severity might be associated with the
effects of ultraviolet radiation light radiation. These differences in incidence and severity between breeds, support earlier findings by Gleeson and Griffin (1965), and demonstrates a genetic basis for IBK resistance (Frisch, 1975). The research on eyelid pigmentation and its relationship to pinkeye indicated a protective role against infection. The potential for the selection based on eyelid pigmentation to improve resistance, lead to the estimation of heritabilities for eyelid pigmentation (Caspari et al. 1980). In that study, heritability was estimated by utilizing a half-sib correlation study and verified by an offspring-sire regression analysis. The estimated narrow sense heritability was .30 ± 0.13. A correlation between the two eyes of an individual of .59 ± .03 was estimated, which indicates a similarity in the amount of pigmentation for both eyes. This moderate heritability and correlation between eyes indicates that improvement for IBK resistance can be achieved by crossbreeding and by within breed selection. Selection of sires and dams with a higher amount of pigmentation could also be beneficial in preventing the onset of bovine ocular squamus carcinoma, also known as “cancer eye”. Furthermore, the correlation between eyelid and corneoscleral pigmentation was estimated (Vogt et. al., 1963) by using a ratio of the sire component of covariance to the square root of the sire components of variance. The correlation between the additive gene effects for the two traits ranged from 0.55 to 0.62.

Causative Agents

Moraxella bovis (M. bovis) is the most common etiologic agent, associated with pinkeye outbreaks (Baptista, 1979). M. bovis is gram-negative bacillus (George and Wilson, 1984), for which seven strains have been identified thus far. These strains differ in their ability to cause clinical signs characteristic of pinkeye and are classified as virulent and
avirulent (Ruejl et al., 1993; Vandergaast and Rosenbusch, 1989). The virulence of the *M. bovis* is characterized, based on morphology (rough vs. smooth) and by crystal violet staining. The stained colonies represent the rough morphology, which associated with virulence (Schurig and Lightfoot, 1984). It is a bacterium that is found in the eyes and nose of cattle. Depending on the strain of *M. bovis* and immunological response of the animal, it may not show any clinical signs upon isolation of the agent.

*Mechanisms of M. bovis colonization*

As of today, the necessary circumstances for the bacterium to colonize the eye are not completely understood. The bacterium attaches to the epithelial surface of the conjunctiva. At this point, the bacterium needs to acclimate to changes in the ocular environment. These changes include the ability of *M. bovis* to obtain iron from the host, in order to carry out DNA synthesis and protein production (Weinberg, 1978). These changes are necessary, in order for *M. bovis* to overcome the resistance of the host’s eye and cause infection. The predisposing stressors discussed above and the virulence factors of the particular strains enhance the ability of *M. bovis* to overcome the resistance of the eye. The ability of *M. bovis* to acquire iron from the host environment is crucial for its survival. One of the mechanisms, developed by the bacterium is the expression of binding ligands that resemble bacterial siderophores (Fenwick et al., 1996), which have been correlated with increased virulence (Brown, 1995).
**Virulence factors associated with *M. bovis***

Thus far, a variety of virulence factors have been characterized for *M. bovis*. These include: leukotoxins, proteases, hemolysin and pili. Of these factors, pili and hemolysin appear to be the most significant for the development of IBK (Ruehl et al., 1988). Both pili and hemolysin have been associated to the outer membrane of the bacterium (Ostle and Rosenbusch, 1984; Jackman and Rosenbusch, 1984). Although the mechanism of action is not completely understood, Ruehl et al. (1993) hypothesized that the function of the pili is to aid in the attachment of the bacterium to the epithelial surface and that another type of pili is responsible for colonization of the cornea and for maintenance of infection, thereby enhancing the ability of *M. bovis* to overcome the defense mechanisms of the eye (Jayappa and Lehr, 1986). The pilated form of *M. bovis* is the only strain shown to cause pinkeye after inoculation of cattle eyes (Pedersen et al., 1972). Hemolysin is a toxin produced by the bacterium, which attacks the cornea and conjunctiva and erodes the surface, which results in severe inflammation and might play a role in the ulceration of the cornea (Frank, and Gerber 1981). Furthermore, a study by Arora and Killinger (1976) indicated a positive correlation between the frequencies of isolated hemolytic strains with prevalence of pinkeye. In the same study, it was shown that hemolytic strains were able to kill corneal epithelial cells in vitro.

**Other Causative Agents**

Although *M. bovis* is considered the primary agent, many attempts to artificially produce IBK with *M. bovi* have not been successful. This demonstrates that besides the environmental factors associated with pinkeye infection, other pathogens isolated in IBK
outbreaks such as: *Brahamella ovis* (Arora, 1973), Infectious Bovine Rhinotracheitis (IBR), *Mycoplasma bovoculi* (Nicolet et. al., 1976) and *Thelazia* species might also be associated with pink eye outbreaks. Of these pathogens, IBR and Mycoplasma species have been studied the most as potential causative agents for pinkeye infection (Timoney, 1971). Infectious Bovine Rhinotracheitis infected cattle exhibit similar clinical signs to cases of pinkeye, where *M. bovis* has been isolated. However, no corneal ulceration is noticed in IBR infected animals and most of the time the individuals suffer from respiratory infections. Due to the similarity of the clinical signs, producers vaccinate the animals with a modified live vaccine against IBR. It’s been suggested that erroneous diagnostic and subsequent vaccination for IBR, might aggravate a pinkeye outbreak by invading the eye cells and making them more susceptible to *M. bovis* (Whittier, 2000).

A study about the relationship between IBR vaccination and higher prevalence of IBK, found a higher prevalence of the disease in cattle vaccinated against IBR. They indicated that the relationship is most likely due to a secondary association, rather than a caused directly by vaccination against IBR (Webber and Selby 1981). Another microorganism isolated in pinkeye outbreaks is Mycoplasma bovoculi (*M. bovoculi*). An artificial inoculation of *M. bovoculi* to healthy calves, resulted in mild conjunctivitis infection, however the clinical signs were not the same as those observed with IBK, caused by *M. bovis*. These results indicate that *M. bovoculi* by itself was not capable of causing pinkeye (Rosenbusch and Kudtson, 1980). Further studies showed an enhancement of *M. bovis* pathogenicity can be caused by prior inoculation of *M. bovoculi* (Rosenbusch, 1983). The role of *M. bovoculi* and its potential interaction with *M. bovis* is not clearly understood, but it’s been hypothesized that the enhancement of *M. bovis* pathogenicity by *Mycoplasma*
spp. is dependent on the strain of *M. bovis* (Barber et al., 1986). Furthermore, it’s been suggested that the strong cell association of *Mycoplasma spp.* with the corneal epithelium, might facilitate invasion by *M. bovis* and increase the duration and frequency of infections (Rosenbusch and Knudtson, 1980; Rosenbusch, 1983; Rosenbusch and Ostle, 1986).

**Treatments**

The preferred treatment for pinkeye infections is antimicrobial therapy. However, due to the variety of agents capable of causing infection and the number of different *M. bovis* strains, this method of treatment has not proven to be an effective one, as it may not eliminate the carrier state (George et al. 1988; Punch et al., 1985). The selected treatment needs to completely remove the gram-negative bacterium from the eye, as the disease can recur after healing of the cornea (George and Wilson, 1984). In order to obtain increased efficacy of the selected treatment, it is necessary to identify the particular strain of the bacterium responsible for a given outbreak.

**Administration of parenteral drugs**

One of the most challenging aspects related to efficacy of a drug is to maintain a consistent concentration in the ocular tissues. The distribution of parenteral drugs, which are the treatment of choice of beef producers, is dependent on its lipophilicity and the pKa of the molecule. The first characteristic is of importance because lipophilic drugs are more efficient at filtering into the blood: tear barrier. These drugs (i.e. erythromycin) are able to fixate in the tears, following parenteral injection. However, these drugs are too expensive and do not have strong activity against *M. bovis*. Oxytetracycline is a long acting parenteral drug,
whose distribution is limited, but it is effective in inhibiting *M. bovis* attachment to the ocular tissues due to the high level of the drug in the tear film after parenteral injection. Another effective antibiotic is kanamycin, nevertheless it is not an efficient treatment, because repetitive applications are needed for desirable results (George et al., 1985).

*Administration of parenteral antibiotics*

Subconjunctival administration of penicillin or aminoglycosides is capable of delivering high concentrations of the antibiotics to the tear film at a lower cost compared to other treatments. Effective concentrations are maintained in the eye for as long as 48 hours, which is short-lived compared to parenteral administration, which is effective for 72 hours (Abeynayake and Cooper, 1985). Furthermore, subconjunctival administration may cause necrosis at the injection site (George et al., 1988).

Intramuscular administration of long acting tetracyclines is widely used today, due to the ease of administration and its effectiveness in reducing the carrier stage of the disease (George, 1990). Also, intramuscular injections of long acting oxytetracyclines have been shown to reduce the healing times and progression of corneal lesions (George, 1985).

*Administration of topical antibiotics*

Topical antibiotics such as: neomycin, benzathine cloxacinil and furazolidone are available for pinkeye treatment. These are not as widely used, due to the lacrimation induced by these treatments and subsequent requirement for multiple applications, in order to achieve appropriate concentrations in the eye. Furthermore, field studies demonstrated that
topical administration was less effective than parenteral or subconjunctival antibiotic administration (George et al., 1984).

Ultimately, the method of antibiotic delivery is dependent upon the intended use of cattle. More specifically, if the animal is in a purebred breeding herd the major concern is cosmetic healing. If the cattle are for commercial purposes, the main concern will be production, which includes treatment costs, labor and decreased weaning weight. In addition to a treatment regime, it is recommended to isolate the infected animals and house them in a sheltered area to limit exposure to sunlight. Alternatively, the use of eye patches is also suggested.

Prevention

Eradication of *M. Bovis* is not possible due to its ubiquitous presence in the environment. As a result, prevention is the most effective way of minimizing incidence of pinkeye. First, as previously discussed, it is essential to control the environment in which the cattle are housed; by implementing an effective fly control program, providing adequate nutrition, shade and good pasture management. Another critical aspect of prevention is meticulous observation to detect infected individuals early on during an outbreak and separate them from the rest of the herd. In addition to controlling the environment, a vaccination program could be implemented. Most of the vaccines in the market consist of inactivated bacterins with pili antigens. However, the effectiveness of vaccination in relation to prevalence and severity of pinkeye is debatable (Jayappa and Hehr, 1986). Field studies showed a decreased in the incidence and severity of infections in calves that were fed colostrum, whose dams were vaccinated during pregnancy (Pugh et. al., 1982). IBK can be
caused by a number of different *M. Bovis* strains; therefore the most effective vaccines are those that contain pili from multiple isolates, which will be expected to provide a broad-spectrum protection. Among the risk factors associated with prevalence of pinkeye is the increased risk of pinkeye in cattle that have been vaccinated against IBR virus. Clinical trials indicated that the severity of infection is increased in cattle vaccinated previously against IBR (Webber and Selby, 1981). As a result, vaccination against IBR virus should be avoided during pinkeye season (George et al., 1988).

**Prevalence of Bacterial Infection**

In general, there are two routes by which ocular infection can occur: exogenous (accidental and surgical trauma) and endogenous (Bron and Seal, 1986). Currently, only two types of bacteria (corynebacteria and pathogenic neisseria) are known to disrupt an intact mucous membrane, thereby colonizing the ocular surface through the exogenous route (Jones et. al 1981), this kind of bacterial invasion is known as traumatic breach. However, it is important to point out that most microorganisms do not depend on a traumatic breach as described above to colonize the ocular surface. Most bacteria colonize the ocular surface through epithelial breaks caused by punctuate erosions on the conjunctiva and cornea. The punctuate erosions are known to occur frequently in diseases such as keratoconjunctivitis sicca. After colonization of the ocular surface, infection of the surface such as conjunctivitis may occur (Bron and Seal, 1986). In order to protect the integrity of the eye upon bacterial colonization, the mammalian eye contains a number of specific and non-specific defense mechanisms.
Defense Mechanisms

The outer eye is protected from physical and bacterial/viral caused damage by mechanical barriers, as well as soluble substances. The two mechanical barriers are the eyelid and the blink reflex. The eyelid is responsible for preventing the absorption of macromolecules, whereas the blink reflex prevents the attachment of foreign objects to the eye. Tears employ a variety of both specific and non-specific antimicrobial substances, which protects the ocular surface. This line of defense is supported by other defense mechanisms such as tear flow, blinking and mucus trapping.

Defenses of the Ocular Surface: Tear Film

The tear film consists of three layers each of which will be discussed in detail. The three layers are: lipid, aqueous and mucin (Holly, 1987, Whitcher, 1987). These three layers work in conjunction to exert the protective functions of the tear film on the ocular surface. Among the protective functions of the tear films, identified thus far are: lubrication of the conjunctiva and cornea, transportation of by-products from the surface of the cornea (Prydal, 1992), lubrication of the eyelids (Wolff, 1951), removal of damaging materials and defense against invading pathogens. The defense mechanisms of the tear film are exerted via non-specific, as well as specific immunologic activities (Bron and Seal, 1986; Smolin, 1987). There has been renewed interest in the study of the tear film, specifically on the mucin genes present in the mucus layer. These studies have been conducted utilizing the dry eye syndrome in humans and keratoconjunctivitis sicca (KCS) in dogs as disease models. These two syndromes are characterized by decreased tear production, which predispose the ocular
surface to infection. The next three sections describe the lipid, aqueous and mucin layers of
the tear film.

**Lipid layer**

The lipid layer is composed mostly of meibomian lipid, secreted from the meibomian
gland, which unloads its entire contents during secretion (Wolf, 1954). It contains two lipid
reservoirs, one in the upper lid and second one in the lower lid. The lower lid reservoir
appears to be the primary source of spreading lipid. The meibomian oil is spread throughout
the aqueous layer during blinking. The lipid layer aids in the stability of the tear film,
slowing the evaporation of tears (Mathers, 1993), acts as a barrier against pathogen invasion
(Shimazaki et al., 1995), provides antimicrobial activity (Gotto et al., 2003) and increases the
tear film thickness (Mathers et al., 1993). The regulation of tear film thickness by the lipid
layer is of particular importance. As the lipids spread through the ocular surface the tension
of the tear film is decreased. As a result, water is taken up by the tear film, thus causing an
increase in thickness. The importance of the meibomian gland in the lipid layer was further
stated when it was removed from a rabbit and it led to increased osmolarity, which is
hypothesized to be crucial in the pathogenesis of dry eye conditions (Driver et al., 1996;
Gilbard et al., 1986).

**Aqueous layer**

The second layer in the tear film is a vital component for the protection of the ocular
surface, as well as its lubrication. It is composed mostly of water and a small amount of
solids, which are mostly proteins. The lacrimal gland and the gland of the third eyelid in
cattle produce the aqueous portion of the tear film. The secretion of lacrimal gland fluid is stimulated by the cornea, conjunctiva, optic nerve and brain via sympathetic and parasympathetic efferent pathways (Dartt, 1994). It contains various antibacterial factors, soluble mucins, which allows the spreading of the aqueous layer through the surface and is important in controlling tear film stability (Kaura, et al., 1986). The aqueous layer is also involved in supplying nutrients such as oxygen, inorganic salts and proteins (Iwata, 1983). Among the factors involved in the protection of the ocular surface, present in the aqueous layer are: lysozyme, lactoferrin, secretory immunoglobulin A (SIgA), which will be described in detail and immunoglobulin G (IgG), immunoglobulin M (IgM), transferrin, tear specific prealbumin and glycoproteins (Iwata 1983, German et al., 1998). Detailed information about lysozyme, lactoferrin and SIgA is presented in the next three sections.

*Lysozyme*

This protein is found in mammals, birds, bacteria, fungi and plants. However, the presence of lysozyme in bovine tears is controversial, with most of the experimental evidence indicating that is present in insignificant amounts at best. In human tears, lysozyme comprises 20-40 per cent of total protein (Selinger et al., 1979). This concentration is higher than that found in nasal, gastro-intestinal, milk, urine, serum and salivary secretions. It’s been suggested that the higher concentration of lysozyme in external secretions, is related to its role as the first line of defense against microbial invasion (Jolles and Jolles, 1986). Studies have shown that Lysozyme is a heat stable protein that exerts a direct effect on micrococci and has a molecular weight of 14,000 (Phillips, 1966). From a phylogenetic standpoint, it is thought to exert an older defense role than the more specific lymphocyte cell dependent
defense system (Osserman, 1976). It is secreted by the lacrimal acini and has been localized, through immunohistochemistry assays, to the apex of the acinus (Franklin et al, 1973).

The primary biological function of lysozyme is catalysis of enzymatic hydrolysis of peptidoglycan, an essential component of bacterial cell wall (Lehninger, 1975). The direct lytic role of lysozyme on bacteria is limited to gram-positive bacteria. Gram-negative bacteria are resistant to the effects of lysozyme, as a result of a liposaccharide coat which protects the cell wall from enzymatic attack. It's been demonstrated, that the effect of this lytic protein is enhanced by its interactions with other proteins active in the ocular surface, such as Lactoferrin and IgA (Adinolfi, 1981; Repaske, 1956). Another biological function of Lysozyme is the enhancement of phagocytosis of certain microorganisms by macrophages (Biggar and Sturgess, 1977; Thiace, and Willet, 1966), neutrophils (Klockars and Roberts, 1976) and monocytes (Thiace and Willet 1966 and Kokoshis et al., 1978).

**Secretory IgA (SIgA)**

Specific immunity in the tear film is mediated primarily by secretory IgA antibodies. These antibodies are the most abundant type of immunoglobulin’s present in the ocular system of humans and experimental animals (Josephson and Weiner, 1968; Sullivan et al., 1994). IgA is also the predominant immunoglobulin in external secretions including saliva, intestinal fluids, colostrum and tracheobronchial secretions (Lamn, 1976; Waldman and Ganguly, 1974). Thus, secretory IgA (SIgA) appears to be the most important factor in the secretory immune system, which protects the ocular surface against bacterial and virus challenges (Sullivan, 1999). The IgA produced in the tear film is produced locally by plasma cells in the lacrimal gland interstitium, as shown by immunohistochemistry experiments.
(Peppard and Montgomery, 1987). These plasma cells also synthesize both J chain and polymeric IgA (pigA; Brandtzaeg, 1983). The IgA antibodies secreted by the lacrimal gland are a mixture of monospecific and polyspecific antibodies (Gregory and Filler, 1987). Ocular IgA is bound and transported by a secretory component (SC), which is produced and secreted by lacrimal epithelial cells (Sullivan et al., 1988). As a result, the IgA in the tears is referred to as secretory IgA (SIgA).

Secretory IgA differs from serum IgA in structure, as a result of its polymeric form (Coyle et al., 1989). It has two IgA molecules linked by a polypeptide J chain, and a second polypeptide known as the secretory component. The secretory component attachment to IgA through a disulfide bridge protects SIgA from enzymatic degradation (Fallgreen et al., 1993). SIgA is produced by plasma cells in the secretory surface underneath the epithelial cells. It then combines with the secretory component, when it is transported through the epithelial layer and subsequently released, in a process referred to as endothelial trancytosis (Selinger et al., 1979).

Factors involved in SIgA response

Research studies have shown that production of secretory antibody can be elicited by antigens administered at a local site or at a distant site of secretion. In those studies, SIgA was detected in saliva, tears and mammary secretions, following oral administration of antigens (Mestecky et al., 1978). The detection of SIgA at distant sites indicates a migration of SIgA producing cells from the site of antigen stimulation to distant secretory glands. Evidence from analyses on gut commensals from human and mice, which are covered with SIgA (Bos et al., 1996), suggests that the commensal population selects for B cells with
affinity for antigens (Meek et al., 2003). Additional evidence to support the selection of B cells theory comes from immunoblots and ELISAs that detected increased staining and signal in bacterial extracts (Macpherson et al., 2000; Cebra, 1999).

It is commonly accepted that the main factor driving secretory IgA production is bacterial colonization of the mucosa. However, SlgA has been detected in mice tear samples under germfree conditions. The detected SlgA did not appear to be directed to bacterial antigens (Macpherson et al., 2001), these findings points toward the presence of additional factors, with a role in the secretion of SlgA antibodies. The majority of the experimental evidence, related to SlgA responses has been obtained from SlgA secretion in the intestines. The antibody responses of other mucosal systems including the eye are likely to have a different organization. In order to obtain a better understanding of the SlgA responses in the ocular system, a comparative phenotypic analysis of B cells between the intestinal mucosal surface and the ocular surface should be carried out (Meek et al. 2003).

**Biological Functions of SlgA**

Secretory IgA antibodies have been shown to play a defensive role in the eye by neutralizing viruses, agglutinating bacteria, binding toxins, preventing bacterial adherence, colonization and activity in mucosal surfaces, interference with parasitic infestation and reduction of antigen induced damage to the cornea and other mucosal sites (Tomasi, 1993; Mestecky and McGhee, 1987; Lamn, 1998). The viruses neutralized by SlgA are: cold viruses, Influenza, parainfluenza and polio viruses (Selinger et al., 1979). The main mechanism of viral neutralization is through the prevention of viral attachment and penetration as shown by *in vitro* studies with influenza virus (Taylor and Dimmock, 1985).
SIgA does not appear to be involved in complement dependent lysis of bacteria. This inability to activate the complement system is believed to be important in preserving the integrity of mucosal surfaces (Corthesy and Spertiny, 1999). Furthermore, it has not been demonstrated to enhance phagocytosis. As a result, SIgA is not thought to mediate the direct lysis of bacteria. SIgA acts on bacteria by inhibiting their attachment to mucosal surfaces. This inhibition results in a decreased bacterial colonization of the ocular surface. In addition, the SIgA enhances the ability of tear fluid to wash away unattached bacteria (Williams and Gibbons, 1972). Additional evidence to support the actions of SIgA on mucosal surfaces comes from a study in which shigella induced keratoconjunctivitis in guinea pigs was prevented by precoating the bacteria with SIgA (Reed and Cushing, 1975).

In addition to serving as an immunological barrier against pathogens adherence and absorption, SIgA have been shown to mediate intracellular neutralization and antigen excretion (Mazanec et al., 1993). These additional functions were attributed to SIgA from evidence obtained from studies that utilized polarized epithelial monolayers as models. The intracellular neutralization of viruses may occur during the passage of IgA antibodies through the lining epithelial cells of the mucosal membranes (Fujoka et al., 1998). Similar studies indicated that antigen excretion occurs upon binding of IgA antibodies to antigens in the lamina propria, and excreted through the epithelium and into the lumen (Kaetzel et al., 1991). The excretion of antigens by SIgA could facilitate the elimination of toxic material, which could be absorbed through the mucosal epithelium (Corthesy and Spertiny, 1999).
Lactoferrin

Lactoferrin (Lf) is an iron binding glycoprotein, member of the transferring (Tf) family with molecular weight of 82KD (Selinger, 1979; Van Haeringen, 1981). This protein is abundantly present in tears. It plays an essential role in the non-specific defense of the ocular surface to pathogen infection, by binding iron and preventing proliferation of invading microorganisms. Lactoferrin has also been detected in other mucosal secretions and in specific granules of mature neutrophils (Baggiolini et al., 1970). Recent research, demonstrates important roles for Lf in the adaptive immune response. Lf is produced in neutrophils, and stored in an iron depleted state (Iyer and Lonnerdal 1993), secreted from the lacrimal gland upon pathogen invasion and has been identified in many species (Bron and Seal, 1986; Selinger et al., 1979). In general the importance of Lf can be attributed to its strong binding of iron cations and its contribution to anti-microbial, anti-fungal, anti-viral, antitumour, inflammatory and immunomodulating properties. The following sections present an overview of the structure-function relationships, the mechanisms of action and biological functions of this multifunctional protein.

Structure

Lf is a protein that is basic in nature with a high isoelectric point. The basic nature and high isoelectric point enables it to bind in a non-specific manner to a variety of targets (Baker and Lindely, 1993). The three dimensional structure of both transferrin and Lf, elucidated with X-ray crystallography, demonstrates two homologous iron binding lobes. These lobes are capable of binding to one ferric ion with the help of a carbonate anion, this structure suggests that this binding is a reversible one (Mazurier and Spik, 1980). This
particular binding is essential for the uptake of iron by lactoferrin. Lactoferrin differs from transferrin in its expression at different time points of development (Iyer and Lonnerdal, 1993). Another difference is the presence of an N-terminal domain in Lf, which mediates many of the functions of Lf.

Lactoferrin undergoes a conformational change upon binding with iron as shown by X-ray crystallography and different molecular forms of Lf have been detected (Baker and Lindely, 1993). The implication of the different molecular forms or apoforms lies in the different affinities for lactoferrin receptors that each apoform exhibit (Davidson and Lonnerdal, 1989). Lactoferrin binding receptors have been identified in the gastrointestinal tract, macrophages, leukocytes, bacteria, neutrophils, monocytes and platelets (Iyer and Lonnerdal 1993; Davidson and Lonnerdal, 1993; Kawakami, 1991). Three isoforms have been defined: lactoferrin-beta and gamma, which contain RNase activity and lactoferrin alpha, with no RNase activity. The isoform without RNase activity does not bind to iron, whereas the isoforms, presenting RNase activity do. These findings may play a role in the wide array of functions attributed to Lf (Baker and Lindely, 1993).

**Biological Functions**

Lactoferrin has been referred to as a polyfunctional protein. When Lf was first studied, it was believed that only its iron binding capabilities mediated the bactericidal effects. The importance of this sequestering ability was demonstrated in patients having granulocytes without specific granules and also by the altered function of the granulocytes, in patients exhibiting Lf deficiency (Raphael et al., 1989; Villde et al., 1982). However, in the last two decades it has been shown that lactoferrin is responsible for others intrinsic
bactericidal activities that are not related to its role in iron scavenging (Arnold et al. 1980). These additional functions are further supported by the discovery of various receptors, found on leukocytes for lactoferrin previously discussed. As stated, Lf plays a critical role in the innate immune and adaptive immune responses, demonstrated by its widespread presence in mucosal secretions and its antimicrobial effects, which will be described here.

*Antimicrobial Qualities of Lactoferrin*

Lactoferrin exhibits both bacteriostatic (inhibition of growth) and bactericidal effects (direct antimicrobial effect). The bacteriostatic effect of Lf is attributed to its iron binding capability. It exerts a broad-spectrum bacteriostatic effect against bacteria, fungi and viruses; this activity has been demonstrated both in vivo and in vitro. This property is considered temporary and some microorganisms have developed mechanisms to evade it, through the use of siderophores, iron chelators.

The second effect (bactericidal) results in direct killing of bacteria and recent studies suggest that this property is associated with the N-terminal domain of lactoferrin. This amino acid domain is known as lactoferritin. This region also has been linked to antifungal, antitumour and antiviral properties, as well as to its anti-inflammatory and immunomodulating properties (Vogel et al., 2002). Studies have shown that Lf is capable of binding to the membrane of gram-negative bacteria, which leads to the release of lipopolysaccharides (LPS), resulting in an increase of bacterial cell permeability (Ellison et al., 1991). An additional antibacterial property of Lf is the protection of epithelial cells against pathogen invasion by preventing intracellular invasion by foreign agents (Longhi et al., 1993). In this section we have seen the variety of ways in which Lf protects the ocular
surface from bacterial infection. In the next section, the role of Lf in regulating the immune system and its contributions to the anti-inflammatory response are presented.

Regulation of immune and anti-inflammatory response

During the last decade, a significant amount of research has been directed towards the understanding of the mechanisms by which Lf regulates immune and inflammatory responses. To this date, the mechanisms are not clearly understood. The release of LPS that occurs after lactoferrin binds to bacteria, results in inflammation. The anti-inflammatory response to this event has been associated with lactoferricin. It has been suggested that the N-terminal region of Lactoferrin (Lactoferritin), might be responsible for modulating the immune response (Vogel et al., 2002). Another mechanism by which lactoferrin provides anti-inflammatory response is by preventing the release of TNF-α, secreted from the macrophages (Brock 1995; Crouch et al., 1992). Studies suggest that Lf mediates the production of cytokines such as TNF-α and interleukins, by direct binding to receptors present in monocytes (Crouch et al., 1992). To conclude the discussion of Lf, two additional biological functions need to be addressed briefly: effects of Lf on cell fate and allergic inflammation. Lf exerts an effect on proliferation and differentiation of immune cells. Lf was shown to increase and control lymphocyte proliferation (Bi et al., 1997; Esaguy et al., 1993). Evidence to support the role of Lf in cell differentiation was provided by Zimecki et al., (1995). The results suggested a role for Lf in differentiation of immature B-lymphocytes. Other studies also show an influence of Lf in T lymphocyte differentiation.

This summary of structure and biological functions of Lf provides an insight to the many functions of Lf in the protection of the ocular surface. However, more research is needed to
elucidate the molecular mechanisms by which Lf exerts its functions, specifically the characterization of Lf receptors. A discussion of the third component of the tear film, the mucus layer and its mucins follows.

*Mucus Layer*

The third layer of the trilaminar tear film is the mucus layer. This layer works in conjunction with the aqueous and lipid layers previously discussed, to protect the ocular surface. One example of this interaction can be seen in patients that suffer from tear deficiency. The production of tears is important for preventing the attachment of pathogens to the corneal and conjunctival epithelia. This is also one of the functions of the mucus layer. Deficiency of tears can lead to mucus adherence to the epithelium or itself and cause corneal damage and instability in the tear film (Ashutosh, 1993). Other biological functions associated with the mucus layer are: lubrication and hydration of epithelial cells, increasing tear stability, increasing the concentration of IgA, facilitating the spread of the aqueous layer. In this portion of the review, the focus will be centered on the mucin component, although this layer also contains leukocytes, salts, glucose, immunoglobulins as well as enzymes (Nichols et. al., 1985).

*Mucins*

Mucins are glycoproteins expressed by the epithelial tissues. In the tear film, they form a gel like mucus layer, which forms as a result of associations between mucins by disulfide bonds (Strous and Dekker, 1992). Mucins are characterized by a high molecular weight and glycosylated pattern. Another distinctive characteristic of mucins lies in its
structural organization. Most of them characterized at the molecular level contain Tandem Repeat Sequences (TR). As of today, 15 mucins have been identified, of which 6 have been associated to the tear film. In recent years, there has been renewed interest on these proteins and their effects in ocular disease. Through the use of molecular genetics, classical genetics, comparative genomics and immunobiology techniques (RT-PCR, knockouts, ELISA), their structure and functions have been partially characterized. A more comprehensive understanding is needed to elucidate the exact mechanisms by which they contribute to immune response in diseases such as dry eye and keratoconjunctivitis. Ocular mucins are classified as transmembrane and secretory. The next two sections will address each of these two classes of mucins.

**Transmembrane Mucins**

The discussion on transmembrane mucins will be based, mostly on mucin 1 (MUC1), because it is the most widely studied. The corneal and conjunctiva epithelia have been demonstrated to express MUC1 through the use of Northern blot and immunoblot analysis (Inatomi et al., 1995). MUC1 is composed of a heavily glycosylated transmembrane cytoplasmic domain. The TR is located in the extracellular domain and extends about 500nm on top of the apical cell membrane and therefore on top of the glycocalyx (Inatomi et al., 1995). This organization suggests that it may be essential for MUC1 mediated functions. As mentioned, Mucins are expressed in other mucosal surfaces such as the intestine. Research in these surfaces, can help in the elucidation of functions for MUC1 in the ocular surface. Specifically, Braga and Gendler (1993), demonstrated down regulation of MUC1 in the endometrium by hormone progesterone in the secretory phase for implantation. This
evidence is suggestive of inhibition of cell-to-cell adhesion (Inatomi. et al., 1995). This property might be essential for another proposed function of MUC1, facilitation of the spread of secreted mucins through the epithelium (cornea and conjunctiva) (Gipson, and Inatomi 1997). Another suggested biological function of MUC1 in the ocular surface is the prevention of attachment of pathogen. Evidence supporting this function was provided by Fleiszig et al., (1994) and Chen et al.,(1993), where mucins were shown to inhibit the attachment of *Pseudomonas aeruginosa* and rotavirus. Effective protection from pathogens by the ocular surface would not be as effective with membrane bound mucins alone. Other kinds of mucins must be present for effective protection, the secretory mucins.

**Secretory Mucins**

Currently three secretory mucins have been associated with the tear film: MUC5AC, MUC5B and MUC2. All three have been linked to the globet cells (McKenzie et. al., 2000). These mucins are further subdivided into gel forming or soluble (Watanabe, 2002). The gel forming mucins are the ones responsible for lubrication and protection of the cells. An example of a soluble mucin is MUC7. These mucins are distinguished from its transmembrane counterparts, due to the lack of a transmembrane domain and longer cDNA sequences. However, they are similar to the membrane-spanning domain in that they possess a TR (Gipson and Inatomi 1997). These mucins are not as well characterized as the transmembrane mucins, therefore their functions are only speculative at this point. MUC5AC has been implicated in Sjogren’s disease as described by Argueso et al., (2002), where a decrease in MUC5AC was reported in patients of this syndrome as compared to normal individuals. The transmembrane mucins are responsible for spreading the gel
forming mucins through the tear film. The spread of these gel-forming mucins may facilitate the spreading of the two other layers of the tear film. In conclusion, both types of mucins work in synteny to protect the ocular surface and to maintain the stability of the tear film.

Analysis of Categorical Traits

There are two classes of phenotypes, which are routinely measured and analyzed in animal breeding for economically important traits. The two phenotypes are continuous and discrete. Threshold traits fall into the second classification, and are defined as polygenic traits in which the phenotypes are expressed in a categorical or discrete manner (Lynch and Walsh, 1998; Bourdon, 2000). Some of the best known examples of these traits are dystocia, cow fertility, susceptibility to disease and litter size. The phenotypes of the first three examples are expressed in an all or none fashion (infected, non-infected); thus, they are referred to as dichotomies. Traits such as litter size are referred to as polychotomous, because more than two categories can be observed (Lynch and Walsh, 1998).

The concept of threshold, originated from studies on the number of digits in guinea pigs. The conclusions of this study were that the genetics dictating this trait were not simple genetics. It was suggested that the trait was discontinuous in an observable scale. However, the analysis indicated that the trait was continuous in an unobservable scale and that it would not be expressed until the contribution of genes and the environment reaches a threshold (Wright, 1934). The underlying continuous distribution was referred to as the liability scale and treated as the combination of genotypic values and environmental deviation, assuming a normal distribution of the environmental effects (Falconer, 1965). Liability scale is the term
commonly used today to refer to the underlying continuous distribution of threshold characters.

As a result of the non-continuous distribution on the observed scale, the standard linear model methodology utilized for continuous traits cannot be directly applied. This creates additional challenges for the analysis of binary traits, making discrete traits more difficult to analyze than continuous traits. One of the most important reasons as to why linear model methodology is not suitable to analysis of binary traits is that linear models assume a normal distribution. The discontinuous distribution of threshold traits violates this assumption (Gianola, 1980; Gianola, 1982).

Statistical methods such as logistical regression can be used for the analysis of binary traits. This is used to estimate the probability of infection, the useful parameter obtained with this approach is the odds ratio (Bishop, 2003). In addition, transformations such as the probit link function can be used to analyze binary traits and applied in general linear mixed model (GLMM) methodology (Kadarmideen et. al., 2000). The probit link function is an inverse probability transformation (Gianola, 1980).

Problems with analysis of binary traits

There are several challenges presented by the analysis of categorical traits, which are not encountered in analysis of continuous traits. These problems need to be overcome if linear methodology is to be applied for genetic evaluation. Two publications which discuss these problems to great extent were published by Gianola, (1980) and (1982). The scores assigned to categorical traits are arbitrarily assigned in most cases and could lead to an overestimation of heritability in some traits. Another problem presented by binary traits to
the application of linear methodology, is that mixed model methodology impose no restriction on the sum of probabilities which should be no larger than 1. The third shortfall of applying linear model techniques for the analysis of binary traits is that the variance in the observable scale varies and depends on the genotypic values of the animals in question. In addition, the incidence in the population influences the additive genetic variance, and significant nonadditive genetic variance is present in heritability estimates from an observable scale (Dempster and Lemer, 1950). Lastly, the assumption of independence between genetic and environmental effects is not valid when analyzing the outward scale of threshold traits (Gianola, 1982).

Estimation of genetic parameters

For the reasons outlined above and because linear model methodology assumes a normal distribution, linear models are not suited for the genetic analysis of binary traits. As a result, non-linear threshold models are utilized for the estimation of genetic parameters, because they account for the probabilistic nature of binary or categorical data (Gianola, 1980 and Gianola, 1982). As alluded to before, the application of threshold model methodology is based on the assumption of an unobservable continuous variable in the underlying scale or liability scale. It has been shown through several simulation studies that if threshold models are applied for analysis of binary traits, fewer parameters need to be estimated. This is one of the advantages of utilizing threshold models over linear models (Kadarmideen et. al, 2000). For specific formulas and additional information please refer to: Lynch and Walsh, (1998); Gianola (1980); Gianola, (1982).


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Estimation of genetic components associated with Infectious Bovine Keratoconjunctivitis (IBK), and the impact of IBK on production traits in Angus cattle

A paper to be submitted to The Journal of Animal Science

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Abstract

Infectious Bovine Keratoconjunctivitis is a contagious ocular disease that affects cattle worldwide. It is considered the most significant ocular disease in cattle. The economic impact of the disease has been well documented. The available treatments have yet to show their effectiveness in the prevention and/or control of the disease. The objectives of this study were twofold, to evaluate the effects of IBK incidence and severity on production traits such as weaning weight, yearling weight, and carcass traits and to estimate variance components associated with IBK. Our results support previous findings on the effects of IBK on weaning weight. The results of our study also indicate a decrease in the yearling weight of affected cattle. Furthermore, the decrease in weaning and yearling weights became more pronounced as the severity of infections increased. The heritability estimates obtained from our analyses ranged from .05 to .11 ± .04. Although these estimates are low and similar to other disease and reproductive traits, genetic improvement could be achieved through the implementation of selection programs, designed to increase resistance to IBK.
Introduction

Infectious Bovine Keratoconjunctivitis (IBK), which is commonly referred to as pinkeye, is a contagious bacterial disease. It is considered the most important ocular disease that affects cattle worldwide (Baptista, 1979). Although, not considered a fatal disease, economic losses attributed to pinkeye are significant to the US beef industry and estimated at about $150 million/year without inclusion of treatment costs (Hansen, 2001). The economic losses occur through decreased growth rates, as affected calves display an average of a 17-18 kg decrease in weaning weight (Thrift and Overfield, 1974; Killinger et al., 1977; Ward and Nielson, 1979; Trout and Schurig, 1985). Furthermore, lower performance in post weaning cattle has also been documented, specifically lower average daily gain, 365 day weight, and final weight (Thrift and Overfield, 1974; Ward and Nielson, 1979).

Breed differences in susceptibility of IBK have been documented. In a number of studies, Hereford, Jersey and Holstein breeds appear to be more susceptible to infection than Bos Indicus (Wilcox, 1968; Frish, 1975) breeds. Furthermore, the extent of eyelid pigmentation has been associated with susceptibility to ocular diseases. Cattle with lower amounts of lid pigmentation exhibit higher incidence and severity to IBK (Frish, 1975; Caspair et al., 1980).

At present, there are no effective treatments or prevention methods against IBK. As a result, IBK continues to cause significant economic losses to the beef industry. The objectives of this study were to use field data to evaluate the effects of IBK on production
traits, estimate variance components and heritability of susceptibility to IBK, through the utilization of data from various Angus producers.

Material and Methods

The data utilized for this study was collected from the Iowa State University Angus research herd, and provided by producers from five Angus herds in four midwestern states. The field data contained pedigree information and performance data such as weaning weight, yearling weight, birth weight, and carcass data on 1879 calves. A field recording sheet was developed to record IBK information for left and right eyes, active infections, healed eyes, treatment and vaccination programs. Along with the recording sheet, a scoring system was provided to producers to evaluate the severity of active and healed lesions. The scoring system contains a scale from 0 to 4. In the scoring system, a score of 0 denote corneas with no apparent lesions, a score of 1 represents a cornea with a lesion covering less than 1/3, a score of 2 represents a lesion covering 1/3 to 2/3 of the cornea, a score of 3, represents a lesion covering more than 2/3 of the cornea, and a score of 4 represents perforation of the cornea (figure 1). The severity scores used in this study were collected at weaning time. As we were not able to observe the calves on a daily basis, it is likely that the severity scores collected did not completely assess the severity of infections (i.e. some animals could have begun to heal from their most severe score). Health data was merged with a five-generation pedigree, which was assembled by the American Angus Association. The data was used to estimate the effects of IBK on performance traits, and for the estimation of genetic parameters such as heritability.
For the estimation of the effects of IBK on performance and carcass traits, PROC GLM of SAS was utilized (SAS, 1985). The performance traits analyzed included weaning weight (WWT) and yearling weight (YWT). Incidence of IBK was coded as 0 (unaffected) and 1 (affected) in SAS. The following model was used to estimate the effect of incidence on WWT: \[ Y = \mu + \text{TRT}_i + \text{Sex}_j + \text{Sire}_k + \text{Wage} + e_{ijklm} \] where: \( Y = \) WWT, \( \text{TRT} = 0 \) (unaffected), or 1 (affected), Sex, TRT, Sire and CG were fitted as fixed effects, and weaning age was fitted as a covariate. Contemporary group (CG) was defined as farm, and year. A similar model was utilized to evaluate the effect of IBK on YWT: \[ Y = \mu + \text{TRT}_i + \text{Sex}_j + \text{CG}_k + \text{Yage} + e_{ijkl} \] where: \( Y = \) YWT, \( \text{TRT} = 0 \) (unaffected), or 1 (affected), Sex, TRT and farm were fitted as class variables and yearling age was fitted as a covariate. The same models were utilized to estimate the effects of bilateral infections, and severity of infection, on the traits analyzed. Incidence was scored as 0, 1 and 2 for unaffected, single eye affected and both eyes affected, respectively, to estimate the effects of bilateral infections on the traits studied. For the effects of severity on the traits, the analyses were carried out in two ways. First, the scores of (0-4) were fitted in the model. Second, a new variable was created in which the severity scores of left and right eye were added (0-8), in order to utilize more information. For example, an animal with right eye score of 2 and a left eye score of 1 was now scored as a 3.

The analysis of the effects of IBK on carcass traits was carried out on hot carcass weight (HCW), rib eye area (REA), marbling score (Marb), chemical percent fat, KPH and fat thickness (FT). The model used to analyze the six carcass traits was: \[ Y = \mu + \text{TRT}_i + \text{CG}_j + \text{hdate(CG)}_k + \text{Hage} + e_{ijkl} \] where: \( Y = \) each of the six traits, \( \text{TRT} = 0 \) (unaffected), or 1
(affected), $CG = \text{sex (bull or steers)} + \text{year, date of harvest, and harvest age}$. Incidence, $CG$, harvest date were analyzed as fixed effects, while harvest age was fitted as a covariate.

Infectious Bovine Keratoconjunctivitis data was classified as binary (affected/non-affected). Binary data is not continuous, therefore a normal distribution cannot be assumed. The discrete distribution of binary traits, presents additional challenges for statistical analysis and the estimation of genetic parameters. For the estimation of genetic parameters, the data was converted in SAS (1985), to a binary trait with scores of 0 for non-affected and 1 for affected calves. PROC GENMOD of SAS was used for model selection to determine the model to be fitted in ASREML (Gilmour et al., 1998) and MTDFREML (Boldman et al., 1993). The probit link function of PROC GENMOD was used to transform the binomial data. The model used in ASREML was: $TRT = \mu + CG_i + Age + animal + e_{ik}$ where: $TRT =$ incidence where 0 designated unaffected calves, and 1 denoted calves with corneal lesions, regardless of the severity scores, $Age =$ age of calves at time of observation, and $CG =$ contemporary group (Sex + Farm). Contemporary group was the only fixed effect, while Age was fitted as a covariate and animal as a random effect. A similar model (model 1) was fitted in MTDFREML, with the exception of $TRT$, which was recoded as 100 to denote animals with no corneal lesions and 200 for calves with occlusions, regardless of severity scores. Two additional models were analyzed in MTDFREML to estimate maternal and permanent environmental effects. In the second model (model 2), maternal effects were added as random effects. In the third model (model 3), permanent environmental effects were included. The program (MTDFREML) was run twice in order to achieve the global maximum, after the convergence criteria was met.
Inbreeding coefficients were estimated with PROC INBREED of SAS. For the estimates of the effects of inbreeding on incidence of IBK, PROC LOGISTIC of SAS was utilized. The model analyzed was: Incidence = inbreeding coefficient.

Results

The six midwestern herds represented in the dataset were located in Iowa, Missouri, Indiana and Wisconsin. The distribution of records by herd are represented in Table 1, along with the year and contemporary groups to which each farm was assigned for further analysis. Only herd 4 provided records from more than one year, with records from the 2003, 2004 and 2005 IBK seasons. The rest of the farms provided records from a single season, which ranged from 2003-2005.

The dataset was first used to calculate the incidence of IBK within each herd (figure 2). Each farm was represented by the contemporary groups described in table 1. The incidence rates ranged from 1 to 52 percent, with the higher incidence observed in herd 4. In table 2, the incidence rates per herd are shown for each eye, in addition to the incidence of bilateral infections. The incidence rates recorded for these six herds displayed a large variation in infection rate, in which CG #7 had the lowest rate at 1%, and CG #4 had the highest rate at 52% (see figure 2 and table 2). As shown, a high incidence (above 25%) of bilateral infections was reported for herds #4 and #5. The incidence rates were similar between left and right eyes.
The dataset was also used to evaluate the severity of infection in each of the six herds. Table 3 shows the percentage of severity scores for each eye, and the average severity score per eye. As illustrated the rates for severity of infections were higher in farms 3, 4 and 5.

The analysis of the effects of IBK incidence on WWT revealed significant differences between affected and non-affected calves, as well as differences in weight between single eye and bilateral infections. First, a comparison was made between the weaning weights, in which scores were assigned as 0 or 1 for unaffected and affected calves respectively. In this analysis, the affected individuals weighed 26 pounds less (p<0.005) than unaffected calves. Next, we compared the weight of unaffected calves to the weight of cattle affected in one eye and those affected in both eyes. Calves with a single affected eye weighed an average of 19 pounds less than unaffected individuals. Calves with bilateral infections weighed on average 40 pounds less than unaffected animals. The results are shown in figure 3 and 4, and the P-values for the variables/covariates tested on the model are presented in table 4. The results of the analysis on weaning weight were similar when the incidence was fitted as a class variable or covariate.

The effect of severity of infections on WWT was also evaluated, by incorporation of severity scores into the analysis, instead of assigning scores of 0 and 1. The results indicate differences in weight between affected and unaffected individuals. Calves with higher severity scores had lower weaning weights. Calves with no signs of corneal lesion weighed an average of 506 pounds. Calves with severity scores of 1, 2, 3, and 4 weighed 498, 487, 481 and 469 pounds, respectively (figure 5). The P-Values of the effects tested in the model are presented in table 5. The analysis in which the scores from each eye were added across eyes
indicated increased weight loss when the calves suffered occlusions covering more than one third of the cornea, supporting the results obtained with the 0-4 scale.

The statistical analysis on the effect of IBK on yearling weight, revealed a significant effect of IBK incidence on yearling weight (P-value = .03). The results indicate a decrease in yearling weight in affected calves, compared to calves with no signs of infection. Animals that exhibited bilateral infection exhibited a lower yearling weight than individuals affected in a single eye (figure 6 and 7). The effects of severity of infections on YWT indicate lower weights with increased severity scores. Calves with severity scores of 0, 1, 2, 3, and 4 weighed 924, 917, 906,900 and 896 pounds respectively, when the severity scores were fit as covariates. The P-Values of the effects tested in the model are shown in table 6. The analysis in which the scores from each eye were added (0-8) indicated decreased weight gain when the calves suffered occlusions that covered more than one third of the cornea, which supports the results obtained with the 0-4 scale.

The statistical analysis of the effect of IBK incidence on carcass traits was conducted by fitting the incidence data as class variables and covariates in SAS. The results did not reveal any significant effects of IBK on the carcass traits tested, although it appears that incidence could potentially have an effect on HCW (Tables 7 and 8).

The average inbreeding coefficient in the pedigree utilized for variance component estimation was 0.015. An estimate of .331 was obtained from the logistic procedure. This estimate represents the log of odds for a one-unit increase in inbreeding coefficient. An odds ratio estimate of 1.39 was obtained, which represents the ratio of odds for a one-unit change in the inbreeding coefficient. An additive variance (Va) of 0.076682 and a phenotypic variance (Vp) of 1.077 were estimated with ASREML. The heritability estimate was 0.0712 ±
0.0480 for IBK susceptibility (table 9). The estimates from MTDFREML (model 1) were: $V_A = 107.55$, $V_P = 1657.25$ and a heritability estimate of $0.064 \pm 0.043$. For model 2, the estimates were: $V_A = 144.19$, $V_P = 1681.1$, $V_{mat} = 352.8$, $h^2_{mat} = 0.21 \pm 0.07$, and $h^2 = 0.09 \pm 0.05$. For model 3, the estimates were: $V_A = 93.80$, $V_P = 1639.7$, $V_{mat} = 32.65$, $h^2_{mat} = 0.02 \pm 0.06$, $V_{pe} = 0.15$ and $h^2 = 0.06 \pm 0.04$ (table 10). Breeding values for IBK susceptibility were estimated with MTDFREML (table 11).

Discussion

This study was carried out with pre-weaned Angus calves. Our study utilized data provided by breeders; therefore, it is not confounded to a single population. Thus, the results should be applicable to all Angus populations in the Midwest. In general, most of the literature agrees with the fact that higher incidence rates occur in young calves. The economic impact of IBK is estimated at $150$ million per year (Hansen, 2001) and its classification as one of the most important diseases that affect calves warranted this study. The objectives of this study were: first, to carry out a field study to re-examine the effects of IBK incidence on pre-weaned calves performance, secondly to evaluate the effects of severity of infection on performance and carcass traits, and thirdly to estimate genetic parameters of IBK. The estimation of genetic parameters allowed us to obtain Estimated Breeding Values (EBV’s), which could aid breeders with the selection of cattle less susceptible to IBK. To our knowledge, no study has been carried out on the effects of severity of IBK on performance traits.

Outbreaks of IBK have been shown to vary with season. On average, an incidence rate of 5.67% (Slatter et al, 1982)-8.75% (Webber, 1981) has been reported per IBK
outbreak. Nonetheless, incidence rates as high as 90% have been documented (Baptista, 1979). This variation in IBK incidence rates could be explained in part by sporadic outbreaks of the disease with epidemics propagating within each herd. The high incidence rates, reported in herd 4 over the three years of data collection, were the highest rates observed in the herd’s history. Herd #4 was managed in the same way in the years prior to the reported outbreaks. Furthermore, this herd was moved to a different location in 2004, and it still suffered from a high incidence rate, greater than 40%.

As a result of the status of IBK as an economically important disease trait, the effects of incidence and severity of IBK on production traits such as WWT, YWT and carcass traits were estimated. The decrease in WWT and YWT exhibited by affected cattle significantly impact producer profitability due to the increased cost associated with treating and feeding these animals. For example, assuming an 8% incidence of IBK in the state of Iowa, where an estimated 1.4 million calves were born in 2005 (Iowa Beef Industry Council) and assuming a $1.18/lb weaning weight sale price, IBK would translate into a $3.4 million lost in revenue, due to lower weaning weight. If the same data is used to estimate the economic loss as a result of lower yearling weight, this would result in an estimated $1.9 million reduction in potential income. Our results agree with previous findings in which affected calves showed a decrease of 20-40 pounds in weaning weight (Thrift and Overfield, 1974, Thomas et al, 1978, Snowder et al., 2004). Like previous publication, our findings indicated that calves with bilateral infections have a lower weaning weight (Killinger et al., 1977; Snowder et al., 2004). To the best of our knowledge, most of the research on the effects of incidence on performance has been focused on weaning weight. Our results also indicate that affected cattle had lower yearling weights than non-affected cattle, which indicates that IBK has long
lasting effects. As indicated by the results of this study, as severity of infection increases, the associated costs of the disease increase, due to the lower performance exhibited by cattle with higher severity of infections. The economic effect of decreased weight may have a greater impact on purebred producers than on commercial producers, as a result of a decreased value of cattle with ocular scarring (Snowder et al., 2005). Our results showed no effect of IBK incidence or severity on carcass traits. However, a P-value of 0.09 was observed for the effect of IBK on HCW, which may indicate that it might be possible for IBK to have a negative effect on HCW. In order to investigate this matter further, a larger sample is needed.

Genetic susceptibility to disease in cattle has not received the same emphasis as other production traits through selection programs. The reasons for this vary, however one of the most important is that disease traits typically have lower heritability than other production traits, therefore lower response to selection is expected (Cundiff, 1988). Our research was a pilot project in this area to select for cattle less susceptible to IBK. Earlier studies on this topic found differences between breeds to IBK susceptibility. These studies concluded that Herefords were the most susceptible breed to IBK (Wilcox, 1968). Later studies supported these findings, most recently by Snowder et al. (2005). Further studies, found that indigenous breeds, as well as crossbreds are more resistant to IBK than British and European Breeds (Gleeson and Griffin, 1965; Frish, 1975a and Frish, 1975b). In addition to these findings, a study conducted on the effects of eyelid pigmentation on incidence and severity of IBK suggested a negative correlation between lid pigmentation on incidence and severity (Frisch, 1975b; Caspari, 1980). The evidence cited above, provide indication of a genetic component affecting IBK.
The software utilized for the estimation of heritability, is well suited for the estimation of genetic parameters, because incidence of IBK can be fitted as a binary trait. In addition to ASREML, a second software (MTDFREML) was used to estimate genetic parameters, to compare the estimates obtained with ASREML. As shown in tables 9 and 10, the estimates were similar with both software programs. Our estimates are significantly different from the estimates obtained by Snowder et al. (2005), which estimated a heritability of 0.25 for Angus calves when the full model was fitted. However, it was closer to the estimate of 0.10, when the R2 model was fitted in which permanent environmental effect of dam and maternal genetic effect of dam were not included. The differences between these studies could be attributed partly by the differences in the phenotypes measured and/or differences in the models analyzed. Additional discrepancies between these studies were the maternal heritability estimates and the effects of dam on IBK incidence. In our study, the maternal heritability was considerably higher than reported by Snowder et al. (2005). In addition, the estimates of the maternal permanent environmental effects from our results indicate that the effect of dam was important in Angus cattle. In addition to estimating the genetic parameters, utilizing incidence scores (0, 1) as response variables, severity scores (0-4) were also utilized. The estimates obtained with MTDFREML when the severity scores were used were lower than those obtained with incidence scores (results not shown). Because, we are not able to account for the maximum severity of infections in the calves measured at weaning time, the lower heritability estimates observed when severity score was used could be due to this lack of ability to accurately reflect true severity of infection.

In addition to estimating heritability for IBK, the inbreeding coefficients of the calves with phenotypic information were estimated. This analysis was performed in order to
determine if the amount of inbreeding was relevant to the susceptibility of the disease. The results of the logistic regression showed that the incidence of IBK increases with higher levels of inbreeding. The amount of inbreeding needs to be accounted for when selection decisions are made, as selecting cattle with high inbreeding coefficients could lead to higher probabilities of IBK infections.

Implications

The results of this study indicate that the effects of IBK incidence are long lasting as demonstrated by the lighter weaning weights, yearling weights and potentially hot carcass weight. The heritability estimates of IBK were low, similar to most disease traits studied thus far. However, these results demonstrate that it is important to include disease traits in selection programs for genetic improvement of health related traits.

Literature Cited


Table 1. Distribution of records by herd.

<table>
<thead>
<tr>
<th>Herd</th>
<th>Year</th>
<th>n</th>
<th>CG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2003</td>
<td>254</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2003</td>
<td>74</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>2003</td>
<td>104</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>2003</td>
<td>318</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>2004</td>
<td>314</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>2005</td>
<td>285</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>2004</td>
<td>256</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>2005</td>
<td>204</td>
<td>8</td>
</tr>
<tr>
<td>Herd</td>
<td>Left eye</td>
<td>Avg score</td>
<td>Right eye</td>
</tr>
<tr>
<td>------</td>
<td>----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>92.0</td>
<td>0.0</td>
<td>4.8</td>
</tr>
<tr>
<td>2</td>
<td>86.5</td>
<td>12.2</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>80.7</td>
<td>3.8</td>
<td>4.8</td>
</tr>
<tr>
<td>4(1)</td>
<td>69.0</td>
<td>19.2</td>
<td>3.5</td>
</tr>
<tr>
<td>4(2)</td>
<td>66.3</td>
<td>19.9</td>
<td>6.7</td>
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<tr>
<td>4(3)</td>
<td>74.5</td>
<td>20.6</td>
<td>1.9</td>
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<tr>
<td>5</td>
<td>90.5</td>
<td>5.3</td>
<td>3.5</td>
</tr>
<tr>
<td>6</td>
<td>99.6</td>
<td>0.0</td>
<td>.39</td>
</tr>
</tbody>
</table>
Table 3. Incidence of IBK per eye (%).

<table>
<thead>
<tr>
<th>Herd</th>
<th>Left eye Only</th>
<th>Right eye Only</th>
<th>Bilateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.0</td>
<td>7.2</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>13.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>18.4</td>
<td>16.3</td>
<td>8.6</td>
</tr>
<tr>
<td>4 (2003)</td>
<td>17.3</td>
<td>13.8</td>
<td>16.5</td>
</tr>
<tr>
<td>4 (2004)</td>
<td>17.2</td>
<td>17.5</td>
<td>13.5</td>
</tr>
<tr>
<td>4 (2005)</td>
<td>15.7</td>
<td>6.4</td>
<td>3.9</td>
</tr>
<tr>
<td>5</td>
<td>9.10</td>
<td>7.4</td>
<td>9.3</td>
</tr>
<tr>
<td>6</td>
<td>0.39</td>
<td>0.39</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Table 4. P-values of variables/covariates fitted in the model to estimate the effects of IBK incidence on weaning weight.

<table>
<thead>
<tr>
<th>Class vs. continuous</th>
<th>Treatment</th>
<th>Sex</th>
<th>Contemporary group</th>
<th>Sire</th>
<th>Weaning age</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT a</td>
<td>.0013</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>.406</td>
</tr>
<tr>
<td>TRT b</td>
<td>.0013</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>.406</td>
</tr>
</tbody>
</table>

a = Incidence scores of 0, and 1 were analyzed as fixed effects.
b = Incidence scores of 0, and 1 were analyzed as linear covariates.
Table 5. *P*-values of variables/covariates fitted in the model to estimate the effects of severity on weaning weight.

<table>
<thead>
<tr>
<th></th>
<th>Score</th>
<th>Sex</th>
<th>Contemporary Group</th>
<th>Weaning age</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT $^a$</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.187</td>
</tr>
<tr>
<td>TRT $^b$</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.183</td>
</tr>
</tbody>
</table>

$^a$ Severity scores of 0-4 were fitted as class variables.

$^b$ Severity scores of 0-4 were fitted as covariates.
Table 6. P-values of variables/covariates fitted in the model to estimate the effects of severity on yearling weight.

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>Sex</th>
<th>Contemporary Group</th>
<th>Yearling age</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT a</td>
<td>0.1074</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.570</td>
</tr>
<tr>
<td>TRT b</td>
<td>0.0170</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.570</td>
</tr>
</tbody>
</table>

a Severity scores of 0-4 were fitted as class variables.

b Severity scores of 0-4 were fitted as covariates.
Table 7. Effects of IBK incidence on carcass traits.

<table>
<thead>
<tr>
<th>Trait</th>
<th>n</th>
<th>Least Square Means Affected</th>
<th>Least Square Means Unaffected</th>
<th>SE</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCW</td>
<td>299</td>
<td>749.2</td>
<td>758.9</td>
<td>7.0</td>
<td>.31</td>
</tr>
<tr>
<td>REA</td>
<td>299</td>
<td>12.7</td>
<td>12.7</td>
<td>.12</td>
<td>.41</td>
</tr>
<tr>
<td>MARB</td>
<td>299</td>
<td>5.9</td>
<td>5.9</td>
<td>.1</td>
<td>.48</td>
</tr>
<tr>
<td>Ether %</td>
<td>298</td>
<td>5.0</td>
<td>5.0</td>
<td>.19</td>
<td>.42</td>
</tr>
<tr>
<td>KPH</td>
<td>205</td>
<td>2.3</td>
<td>2.2</td>
<td>.06</td>
<td>.31</td>
</tr>
<tr>
<td>FT</td>
<td>300</td>
<td>.34</td>
<td>.34</td>
<td>.01</td>
<td>.32</td>
</tr>
</tbody>
</table>
Table 8. P-values of variables/covariates analyzed in the models.

<table>
<thead>
<tr>
<th>Carcass Trait</th>
<th>Treatment</th>
<th>Contemporary Group</th>
<th>Kill (CG)</th>
<th>Harvest Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCW</td>
<td>0.168</td>
<td>0.003</td>
<td>&lt;0.0001</td>
<td>0.054</td>
</tr>
<tr>
<td>REA</td>
<td>0.780</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.054</td>
</tr>
<tr>
<td>MARB</td>
<td>0.433</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Ether %</td>
<td>0.818</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
<td>0.0005</td>
</tr>
<tr>
<td>KPH</td>
<td>0.243</td>
<td>&lt;0.0001</td>
<td>0.0002</td>
<td>0.829</td>
</tr>
<tr>
<td>FT</td>
<td>0.916</td>
<td>0.200</td>
<td>&lt;0.0001</td>
<td>0.646</td>
</tr>
</tbody>
</table>
Table 9. Additive genetic variance, phenotypic variance and direct heritability ($h^2_d$) ± standard error using ASREML.

<table>
<thead>
<tr>
<th>$V_A$</th>
<th>$V_P$</th>
<th>$h^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0766</td>
<td>1.077</td>
<td>0.071 ± 0.048</td>
</tr>
</tbody>
</table>
Table 10. Models fitted in MTDFREML for the estimation of variance components for IBK and the estimates corresponding to each of the models.

<table>
<thead>
<tr>
<th>Model</th>
<th>( V_A )</th>
<th>( V_P )</th>
<th>( V_{pe} )</th>
<th>( V_{mat} )</th>
<th>( \text{Cov}_{a,\text{mat}} )</th>
<th>( h^2_{\text{mat}} )</th>
<th>( h^2 )</th>
<th>( r_{am} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>193.86</td>
<td>1744.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.11 ± .077</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>144.19</td>
<td>1681.12</td>
<td>-</td>
<td>352.88</td>
<td>-225.57</td>
<td>0.21 ± .07</td>
<td>0.09 ± .053</td>
<td>-1.00 ± .274</td>
</tr>
<tr>
<td>3</td>
<td>93.80</td>
<td>1639.76</td>
<td>0.15</td>
<td>32.65</td>
<td>-55.34</td>
<td>.02 ± .058</td>
<td>.060 ± .046</td>
<td>-1.00 ± .919</td>
</tr>
</tbody>
</table>
Table 11. Estimated Breeding Values (EBV’s) for IBK susceptibility averages, minimums, and maximums using the single trait model.

<table>
<thead>
<tr>
<th></th>
<th>Avg.</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV</td>
<td>-0.058</td>
<td>-11.35</td>
<td>11.02</td>
</tr>
</tbody>
</table>
Figure 1. Illustration of the criteria used to assign severity scores to affected eyes.

Score 1 = ocular lesion covering less than 1/3 of the cornea
Score 2 = ocular lesion covering 1/3-2/3 of the cornea
Score 3 = ocular lesion covering more than 2/3 of the cornea
**Figure 2:** Occurrence of IBK by contemporary group.
Figure 3. Differences in weaning weight between affected and unaffected calves.

a. Significantly different from treatment b (p < .0004)
Figure 4. Differences in weaning weight between single eye infections, and bilateral infections.

a. Significantly different from treatment b (p < .005)
Figure 5. Effects of severity of infections on weaning weight.

a. Significantly different from trt b (p< 0.05), c (p< 0.0001), d (p< 0.0001) and e (p <0.0001)
b. Significantly different from trt c (p< 0.005), d (p< 0.0023), e (p < 0.0011)
c. Significantly different from trt b (p< 0.001)
Figure 6. Differences in yearling weight between affected and unaffected calves.

a. Significantly different from trt b (p < 0.024)
Figure 7. Differences in weaning weight between single eye infections, and bilateral infections.

a. Significantly different from treatment b (p< 0.031)
Quantification and evaluation of IgA and Lactoferrin, as candidate factors influencing susceptibility/severity to IBK in Angus calves: Estimation of genetic parameters.

A paper to be submitted to *The Journal of Animal Science*

J.E. Rodríguez, J.R. Tait, A. Hassen and J.M. Reecy

Abstract

Infectious bovine Keratoconjunctivitis (IBK) is considered the most important contagious ocular disease affecting cattle. This disease has a significant economic impact on beef producers, due to decrease performance and treatment expenses. Most of the research has been focused on the development of treatments for the disease, with debatable success. Additional research is needed on immunological factors present on the ocular surface and their effects in preventing the onset of IBK. In the absence of lysozyme in bovine tears, secretory IgA and lactoferrin are likely candidates implicated in ocular defense against bacterial infections. The first aim of our study was to quantify these two proteins in bovine tears with ELISA’s. Statistical analysis indicated significant differences between affected and nonaffected calves for SIgA, with lower levels on affected calves. No differences were found in the levels of lactoferrin, between the two groups studied. The levels appear to decrease with increased severity of IBK. The second aim of this study was to estimate genetic parameters of IgA and carry out correlation studies with incidence of IBK. The
estimated heritability was moderate, however the correlation between the two traits although moderate was not in the desired direction.

Introduction

Infectious Bovine Keratoconjunctivitis (IBK) commonly referred to as pinkeye, is a highly contagious ocular disease that affects both beef and dairy cattle worldwide. Pinkeye is characterized by inflammation of the conjunctiva and ulceration of the cornea. The clinical signs exhibited by infected cattle may vary, with some animals recovering faster than others. In the most severe cases the affected animal might suffer rupture of the cornea, resulting in permanent blindness (Bedford, 1976). Experimental studies have shown that pinkeye is caused by a gram-negative bacterium, Moraxella bovis (Pugh and Hughes, 1975). However, additional pathogens have been isolated in IBK outbreaks (Baptista, 1979). In addition to the causative agents, the pathogenesis is influenced by the environment, season, strains of the causative agent and immune system of cattle (Baptista, 1979).

IBK is considered the most important ocular disease affecting cattle, due its economic impact. The economic losses attributed to pinkeye per year have been estimated at $150 million in the United States (Hansen et al., 2001). This estimate does not include treatment costs, which could be significant if an outbreak occurs. The estimated losses are attributed to decreased performance of infected calves. Several studies have concluded that IBK significantly lowers weaning weight in affected individuals (Thrift and Overfield, 1974; Ward and Nielson, 1979; Snowder et. al, 2005).
Most of the research devoted to IBK in cattle has been focused in the development of treatments and vaccines. Neither of these two approaches has proven to be effective. As a result the cattle industry continues to suffer significant economic losses due to pinkeye. For these reasons, more research is warranted on the immunological factors involved in protecting the eye against bacterial infections. Specifically more research is needed on immunological factors such as: Lactoferrin (Lf), and Secretory IgA (SIgA), which are protective proteins present in the tear film (Davidson and Kuonen, 2004). The relationship between these factors to IBK susceptibility and severity of infection requires additional investigation.

To this end, we selected two candidate immunological factors: SIgA and If to determine if the levels of these proteins differ between infected and non-infected individuals, and whether these levels vary with increased severity of infection. The second objective of this study was to estimate genetic parameters of IgA, and carry out correlation analyses between IBK susceptibility and SIgA levels.

Material and methods

Collection procedures

The data for this study consisted of 546 calves from the ISU beef herd, collected during the IBK seasons of 2004 and 2005, with a total of eleven different collection dates. A scoring system with a scale of 0-4 was developed to evaluate the severity of infection. The scoring system is based on the extent of ocular occlusion. A score of 0 is assumed to be unaffected; a score of 1 was assigned when the eye exhibited less than 1/3 of corneal abrasion, a score of 2 when the eye exhibited at least 1/3 of corneal abrasion, a score of 3
when the ocular lesion showed covered more than 2/3 of the cornea, and a score of 4 when perforation of the cornea occurred.

Tear samples were collected from both eyes. The samples were collected with sterile plain cotton swabs. The swabs were rolled around the mucosa of the third eyelid and the lower lid for 20-30 seconds approximately until the swabs were damp. The tips of the swabs were placed in a 0.5µl microcentrifuge tube and stored in -80°C until analyzed. Finally, the 0.5µl tubes were punctured, placed in a 1.5µl microcentrifuge tube and spun at 13,000 x g for 3 minutes. Approximately, 80-120 µl of tear fluid were obtained from each swab. During the 2005 pinkeye season, blood samples were collected from all calves to perform correlation studies between ocular IgA and serum IgA. The samples were collected in 10 ml BD Vacutainer™ K₃ EDTA tubes and were spun at 5000x g to separate serum from the blood. A total of 205 blood samples were collected and analyzed for the correlation studies during the 2005 IBK season. The correlation analysis was carried out with PROC CORR of SAS. The serum IgA levels were correlated with the IgA levels from both right and left eye.

**ELISA's**

Enzyme-Linked Immunosorbent Assays (ELISA’s) were utilized to quantify tear fluid IgA and lactoferrin protein levels. Bovine IgA and Lf ELISA kits were purchased from Bethyl labs (Montgomery, TX). The following reagents were utilized: 0.05M carbonate-bicarbonate, pH 9.6 buffer (coating buffer), 50mM Tris, .14 M NaCL, 0.05% Tween20, pH 8 (Washing solution), 50mM Tris, .14 M NaCL, 1% BSA, pH 8.0 (Postcoat), 50 mM Tris, 0.14 M NaCl, 1%BSA, 05% Tween 20, pH 8 (sample diluent), tetramethylbenzidine TMB substrate (Enzyme substrate), and 2 M H₂SO₄ (stopping solution).
The bovine IgA quantitation kit contained: a) sheep anti-bovine IgA-affinity purified coating antibody (1mg/ml), which was diluted 1/100 to carry out the assay, b) bovine reference serum (0.18mg/ml) which were used as the standards with a range from 15.6-1000 ng/ml, c) Sheep anti-bovine IgA-HRP conjugate (1mg/ml, detection antibody). The bovine Lf quantitation kit contained: a) goat anti-bovine Lf-affinity purified (1mg/ml, coating antibody, b) bovine Lf calibrator (1mg/ml), which was used as the standard samples c) goat anti-bovine Lf-HRP conjugate (1mg/ml, detection antibody).

The optimum dilutions for the tear samples and HRP conjugate were determined by checkerboard titration. The optimum dilutions for the tear samples were: 0.625ng/μl and 4ng/μl for the detection antibody. All reactions were carried out in 96 well microtiter-plates, with each sample was analyzed in duplicate. The standard samples were used as positive controls, while Tris Buffered Saline (1X TBS) was used as a negative control. The protocols, included with the purchased kits were followed step by step (Bethyl labs, Montgomery TX). The enzyme substrate reaction was stopped after an eight minute incubation period. After completion of the protocol the plates were read in a Thermo Max micro plate reader at 450nm.

Statistical Procedures

Field data was merged with the data obtained from the ELISA’s. Statistical analysis for this investigation was carried out through the use of SAS (SAS, 1985). The following SAS procedures were utilized: PROC GLM, and Logistic. For the preliminary analyses, the severity of infection was not taken into account, and the infections were fit into the models as yes (1) or no (0) variables. Two statistical analyses, using GLM’s were carried out: first an
analysis of the IgA levels for each eye separately, taking into account only the IBK scores for either right or left eye. The second analysis took into account the scores of both eyes. The model was: \( \text{IgA} = \mu + \text{TRT}_i + \text{Sx}_j + \text{AD}_k + \text{Date}_l + \text{Age} + e_{ijklm} \) where: \( \text{IgA} \) is the secretory IgA levels for either left or right eye and both eyes, \( \text{Sx} = \text{sex} \), \( \text{TRT} = \text{IBK score} \), \( \text{AD} = \text{age of dam} \), \( \text{date} = \text{day when phenotype was scored} \) and \( \text{Age} = \text{age of the calves at time of observation} \), which was fit as a covariate. Treatment, sex, age of dam and date were analyzed as fixed effects, while age was analyzed as a linear covariate. Residual plots were used, along with goodness of fit estimates to evaluate the reliability of the models to estimate the effects of the variables tested on IgA levels. To evaluate whether there was a relationship between the levels of IgA prior to IBK season, during IBK season and at weaning time, a generalized linear model was used for each time point. The model used was: \( \text{TRT} = \mu + \text{Date}_i + \text{Sx}_j + \text{PrevTRT}_k + \text{PrevIgA} + \text{IgA} + \text{Age} + e_{ijkl} \) where: \( \text{TRT} = \text{IBK score during active season or weaning time} \), \( \text{Date} = \text{date of observation} \), \( \text{Sx} = \text{sex} \), \( \text{PrevTRT} = \text{IBK score prior to IBK onset, during active season or at weaning time} \), \( \text{PrevIgA} = \text{IgA titers measured previously, for example if dependent variable was the IBK score during active season the PrevIgA were the titers measured prior to IBK season} \), \( \text{IgA} = \text{current IgA levels} \). Additionally, a pearson correlation coefficient analysis was carried out between the IgA levels measured prior to the IBK season, and the IgA levels quantified during the active season.

In order to evaluate the effects of severity on IgA levels, PROC MEANS was utilized to compare the levels of IgA in calves with occlusions scored from 0-4. Due to the relative small number of calves with a severity score of 3 or 4, the scores of 1 and 2 were combined and coded as 1. The severity scores of 3 and 4 were combined and coded as 2. A binary logistic procedure was utilized to evaluate the relationship between incidence of IBK and
IgA levels. The model was: Eye = IgA, were: Eye= severity score and IgA= levels quantified with ELISA’s.

*Estimation of Genetic Parameters*

The data was merged with a five generation pedigree assembled by American Angus Association (AAA) for the estimation of genetic parameters. The model selection for the estimation of genetic parameters was carried out with SAS PROC GLM’s. The software utilized for this analysis was MTDFREML (1995). The following model was used: IgA = \( \mu \) + Date, + Eye score, + Age + Animal + \( e_{ijk} \), where: Date and Eye score were fit as fixed effects, Age was fit as a covariate, and Animal was used as a random effect. The program was run twice, in order to achieve a global maximum estimate after the convergence criterion was met.

*Correlation Analysis between IgA and IBK*

The breeding value estimates obtained from MTDFREML for IgA levels, along with breeding values for IBK incidence from a previous study, were used to carry out correlation analyses. Two correlation analyses were conducted with SAS (1985). In the first analysis, pearson product correlation coefficients were estimated. In the second analysis, spearman correlation coefficients were estimated. A two trait analysis was carried out with MTDFREML, to estimate the genetic correlation between IgA and IBK.
Results

For the sIgA ELISA, the between assay variability was 33.6 ng/ml, while the within assay variability was 29.2 ng/ml. The analysis for the IgA levels in the right eye was carried out with 273 observations. The General Linear Model (GLM) analysis in SAS (1985) of the IgA levels in the right eye indicated that three variables had a significant effect on the levels of IgA. The significant variables were: TRTR (right eye score) ($p < 0.0001$), Age ($p < 0.01$) and date ($p < 0.0001$). Age of dam and sex were not significant with $p$-values of 0.11 and 0.47 respectively. The R-square of the model was 0.46 with a coefficient of variation of 40%. The least square means for the levels of IgA in the right eye were: 382.4 ng/ml ± 15.6 for unaffected calves, and 252.5 ng/ml ± 20.9 for calves with a score of 1 (Figure 1).

The same model was used for the analysis of IgA levels in the left eye tear fluid. The number of observations utilized for this analysis was 272. The results were similar to the analysis of the right eye. The R-square for the model was 0.40 with a coefficient of variation of 42%. The least square means for the IgA levels in the left eye were: 371.2 ng/ml ± 17.4 for unaffected calves, and 253.0 ng/ml ± 20.8 for calves with a score of 1 (Figure 2).

A total of 273 observations were utilized for the analysis of IgA levels in both eyes. The R-square for the analysis on IgA levels on both eyes was 0.46 with a coefficient of variation of 44%. The results were similar to individual eye analysis in that the variables TRT ($p < 0.0002$), Age ($p < 0.01$) and Date ($p < 0.0001$) were significant. The least square means for unaffected calves was 291.45 ng/ml ± 12.25, while the IgA levels for calves with ocular scarring were 230.5 ng/ml ± 12.67 (Figure 3). In order to verify whether the lower amounts exhibited by affected calves where a consequence of infection, or whether sIgA2
was bound to mucus, and consequently washed away during the assay, a sputolysin treatment was applied to the tear samples collected in 2004. After carrying out the ELISA’s with the treated samples, the affected calves still exhibited lower levels of IgA when compared to unaffected calves. The levels of IgA quantified prior to the onset of IBK do not seem to influence the levels of IgA quantified during the IBK season, indicated by the results from the GLM’s prevIgA levels (p-value = 0.175). The results from the correlation analysis support these findings, as the pearson correlation was 0.23 with a p-value of 0.072). To determine whether there was a cause and effect relationship between the levels of IgA measured prior to the IBK season, and IBK incidence during the active season, a logistic regression was carried out. The results indicated no apparent relationship between the two variables (results not shown). This indicates that additional research is needed to determine whether the IgA levels present prior to the onset of IBK, predispose cattle to infection.

The estimates obtained from PROC MEAN indicate that decreasing levels of IgA were associated with increased severity of infection for both eyes. The mean right eye tear fluid IgA values for unaffected calves were 365.2ng /ml ± 11.3. The mean IgA levels for calves with a score of 1 was 345.2 ng/ml ± 19.5 and those with a score of 2 was 273.2 ng/ml ± 34.3. The number of observations for each severity score were: 347, 94 and 24, respectively (Figure 4). Similar estimates were obtained for the left eye; the mean level of unaffected calves was 368.4 ng/ml ± 12.7. Calves with a score of 1 had a mean of 326.7 ng/ml ± 16.7 and those with a score of 2 had a mean of 251.8 ng/ml ± 26.9 (Figure 5).

The logistic regression indicated a higher probability of infection with decreasing level of tear fluid IgA. An odd ratio estimate of 0.895 was estimated with the logistic
procedure. This point estimate demonstrates that for every 100-unit increase in IgA levels (ng/ml), the odds of infection decreases by 0.895 (Figure 6).

The lactoferrin ELISA data was analyzed with the same GLM used for the IgA analysis. The incidence of IBK did not appear to have an effect on the levels of lactoferrin in bovine tear fluid.

The correlation analysis between serum and tear fluid IgA did not reveal a significant relationship between the IgA levels, with a correlation coefficient of .017. The mean IgA concentration in tear fluid was 454.3 ng/ml ± 10.71. The mean IgA concentration in serum was 245.4 ng/ml ± 8.0.

The convergence criterion of 1.E-009 was reached in the estimation of variance components. The estimated variance components were: additive genetic variance (V_A) 8278.8, phenotypic variance (V_p) 24347.1. The estimated heritability was 0.34 ± .192 (table1). The breeding value estimates for IgA levels are shown in table 3.

The pearson correlation coefficient estimated with SAS, was .005 with a p-value of .54. In the second correlation procedure, the spearman correlation coefficient estimated was -.23 with a p-value of < 0.0001. A genetic correlation of .29 ± .399 was obtained from the two trait analysis in MTDFREML, with an environmental correlation of -.25 ± .116 between the IgA levels and IBK incidence.

Discussion

The eye has several barriers through which a pathogen must pass through, before it can cause an infection. Among these barriers are: the eyelids, bony orbit, corneal, and
conjunctival epithelium (Selinger et al., 1979; Lemp and Blackman, 1981). If microorganisms evade these physical barriers, the next line of defense is the tear film. The tear film is made up of three layers; the lipid layer, aqueous layer and a mucus layer respectively (Davidson and Kuonen, 2004).

The aqueous layer is produced by the lacrimal gland, which is considered the most important tissue in ocular defense, the gland of the third eyelid and the Hardenian gland in cattle (Davidson and Kuonen, 2004). The middle layer of the tear film contains both specific and innate defense mechanisms. The innate defense mechanisms include lactoferrin, lysozyme and proteins involved in the complement response (Bron and Seal, 1986). The specific substances present in the aqueous layer are: SIgA, IgM and IgG (Bron and Seal, 1986).

Lactoferrin has been shown to provide non-specific protection against a variety of bacteria (Arnold, 1977). It is an iron binding protein; as a result it deprives invading pathogens from the iron necessary for the colonization of the mucosal surface (Oram and Reiter, 1968). In addition to its iron binding capability, Lf has been shown to have a direct effect on some bacterial strains (Arnold et al., 1982). This bactericidal effect occurs upon binding of Lf to bacterial membranes and it's been shown to cause damage to the membranes of gram-negative bacteria, causing increased cell permeability and susceptibility to antibiotic treatment (Pruitt et al., 1994).

Secretory IgA is the primary immunoglobulin present in external secretions such as tears, saliva, colostrum and intestinal fluid (Selinger et al., 1979). With respect to bacteria, SIgA has been shown to inhibit bacterial adherence of pathogens in two ways: by surrounding microorganism and preventing attachment to the mucosal surface (Russell et al.,
1999) and by agglutinating bacterial pathogens (Korkeilla et al., 1996). In addition, SIgA has been implicated in antigen exclusion through the epithelial cells (Kaetzel et al., 1991). It has been suggested that SIgA acts synergistically with other substances in the tear film to prevent the colonisation of the mucosal surface by bacteria (Russell et al., 1999).

Even though, we did not find any evidence that indicated differences in If levels between unaffected and affected calves, more research is needed about If and its relationship with the causative agents of IBK. Specifically, additional emphasis is warranted on the mechanisms employed by *M. bovis*, which appears to have siderophore-like activity that allows the bacterium to acquire iron from If (Fenwick and Rider, 1996).

The results of the statistical analyses carried out in this investigation with respect to IgA levels demonstrates differences in the levels of IgA between affected and non-affected calves. In our study, calves that suffered from IBK exhibited lower levels of IgA as compared to their contemporaries with no signs of IBK. The second statistical analyses carried out to evaluate the relationship of IgA levels with severity of infection, which indicated that lower levels of IgA were associated with increased severity of infection. The logistic regression analysis supports our previous findings, which demonstrates that the probability of infection decreased with increased levels of IgA. As presented in the results section, the coefficients of variation of the statistical analyses are rather large. The standard errors of our results, suggest that larger number of samples are needed to validate our conclusions.

The outcome of our analysis was contrary to what has been reported in other ocular conditions such as: acute bacterial conjunctivitis, and acute Keratoconjunctivitis among other conditions, where the subjects displayed increased levels of IgA, IgG or IgM. However, our
results are similar to what has been reported in conditions such as: IgA deficiency, Keratoconjunctivitis sicca, where the subjects displayed decreased levels of IgA (Sullivan, 1994; Sullivan, 1999). Therefore it is conceivable, that SIgA is indeed playing an important role on the protection against IBK.

As expected, the correlation between the levels of SIgA and serum IgA was poorly correlated. There are key differences between secretory IgA and serum IgA. Secretory IgA is polymeric in nature while serum IgA is monomeric. Secondly the structure of secretory IgA consists of an IgA dimer and two polypeptide chains, the J chain and the secretory component (SC) (Mestecky et al., 1999). Therefore, these different forms of IgA have distinct functions in the systemic circulation and the ocular environment.

The estimated heritability of IgA was moderate, which suggest that selection of cattle with higher breeding values for IgA levels could aid in production of less susceptible calves to IBK. The pearson correlation analysis did not indicate any correlation between the breeding values of IBK incidence and IgA. However, the spearman correlation analysis showed a low correlation between the breeding values in the desired direction, as the incidence data was coded as 100 for unaffected calves and 200 for affected animals. This correlation was considered to be a better fit for the correlation analysis, because the animals were ranked by their breeding values. Because neither one of the correlation analyses accounted for the environmental effect on the traits studied, we carried out a two trait analysis. The correlation between IBK incidence and IgA levels was moderate, however it was not on the direction obtained with the breeding value spearman correlation analysis. The environmental correlation between the traits, indicates that the environment causing higher incidence of IBK, it is also resulting in lower levels of IgA in susceptible calves. These
findings suggest that IgA might be playing an important role with respect to IBK susceptibility. However, it is likely that the IgA deficiency displayed by affected calves, is compensated by other immunological factors in the eye.

Implications

The lower levels of IgA quantified in affected calves, along with the lower levels of IgA with increased severity of IBK suggest that IgA is playing an important role in the ocular defense mechanisms, against IBK. Furthermore, the heritabilities estimated for IgA and their estimated correlation to IBK, suggest that the incorporation of IgA as an indicator trait in selection programs, would be beneficial for breeding animals less susceptible to IBK.

Literature Cited


Tables and Figures

Table 1. Additive genetic variance, phenotypic variance and direct heritability ($h^2_d$) ± standard error of SIgA.

<table>
<thead>
<tr>
<th>$V_A$</th>
<th>$V_P$</th>
<th>$h^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>8278.8</td>
<td>24347.1</td>
<td>0.34 ± .192</td>
</tr>
</tbody>
</table>
**Table 2.** Variance component estimates from the two trait analysis Phenotypic variance ($V_p$), genetic variance ($V_A$), heritabilities ($h^2$) ± standard error, genetic correlation ($r_{a1a2}$), and environmental correlation ($r_{c1e2}$).

<table>
<thead>
<tr>
<th>$V_{P1}$</th>
<th>$V_{A1}$</th>
<th>$h^2_{a1}$</th>
<th>$V_{P2}$</th>
<th>$V_{A2}$</th>
<th>$h^2_{a2}$</th>
<th>$r_{a1a2}$</th>
<th>$r_{c1e2}$</th>
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<tr>
<td>24137</td>
<td>8930</td>
<td>.37 ± .19</td>
<td>1671</td>
<td>185</td>
<td>.11 ± .05</td>
<td>.29 ± .40</td>
<td>-.25 ± .12</td>
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</tbody>
</table>

*1 = trait #1, IgA  
*2 = trait #2, IBK incidence
Table 3. Estimated Breeding Values (EBV’s) for SIgA levels averages, minimums, and maximums using a single trait model.

<table>
<thead>
<tr>
<th></th>
<th>Avg.</th>
<th>Min.</th>
<th>Max.</th>
</tr>
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<tbody>
<tr>
<td>EBV</td>
<td>0.64</td>
<td>-82.5</td>
<td>129.14</td>
</tr>
</tbody>
</table>
**Figure 1.** Levels of IgA in affected and unaffected calves in left eye.

![IgA Levels Left Eye](image)

- a. Significantly different from affected calves ($p < 0.0001$).

```plaintext
IgA Levels Left Eye

Unaffected  | Affected
---|---
450         | 250
350         | 200
250         | 150
150         | 100
50          | 50
0           | 0
```
Figure 2. Levels of IgA in affected and unaffected calves in right eye.

IgA Levels Right Eye

- a. Significantly different from affected calves ($p < 0.0001$).

```
ng/ml
450
400
350
300
250
200
150
100
50
0

Unaffected
Affected
```
Figure 3. Levels of IgA in affected and unaffected calves.

IgA Levels Both Eyes

a. Significantly different from affected calves (p < 0.0002).
Figure 4. IgA levels and severity of infection in left eye.

a. Significantly different from score 1 (p < 0.05) and score 2 (p < 0.001)

b. Significantly different from score 2 (p < 0.05)
Figure 5. IgA levels and severity of infection in right eye.

a. Significantly different from score 1 (p < 0.0001) and score 2 (p < 0.0001)
b. Significantly different from score 2 (p < 0.003)
Figure 6. IgA Levels and Odds of Infection.
GENERAL CONCLUSIONS

The analysis of the field data revealed significant detrimental effects on performance traits such as weaning and yearling weight, associated with IBK incidence and severity. Our studies indicated that the effects of IBK were long lasting, as cattle affected with IBK exhibited lower weight as yearlings. The heritability estimates for IBK susceptibility are low; nevertheless slow improvement could be made through selection by integrating IBK susceptibility to selection criteria.

The results from our second study indicated that affected calves had lower levels of IgA. Furthermore, the severity of infection appear to influence the IgA levels, as affected calves appear to secrete lower levels of IgA with increased severity of infection. The heritability estimate of IgA is moderate, however the two trait analysis conducted did not reveal a significant correlation between IBK incidence and IgA levels, in the desired direction. These findings suggest that although IgA appears to be playing an important role in the protection of the eye against IBK, other immunological factors of the eye might compensate for the perceived IgA deficiency in affected calves.
This thesis project would not have been possible without the input and assistance of many people. First of all I would like to thank Dr. Reecy for giving me the opportunity of joining his lab group, and working in this project. I would like to thank the National Beef Cattle Evaluation Consortium for the funding of this project.

I will also like to thank Dr. O’connor and Dr. Fernando for serving in my committee. Thank you Dr. Hassen and Dr. Tait for helping me with the statistical analyses, your help was invaluable for the completion of this project.

I would like to thank, the members of the Reecy group for all the support and friendship. Special thanks to James Koltz, Symantha Anderson, and Cari Steelman for acting as mentors and motivating me to succeed.

Lastly, I would like to thank my family for all the support, and unconditional love through all this time, they all deserve credit for my achievements. Dad, thank you for the sacrifice that you have made for this country by protecting our freedom. I am proud of you, your bravery and courage inspired me to complete my work. Thanks to my beautiful wife for her patience, understanding, and unconditional support.