EPIDEMIOLOGICAL ASPECTS OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS IN NEW ZEALAND

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ABSTRACT

Infectious bovine keratoconjunctivitis (IBK) has been recognised in New Zealand only in the past 7 years and there is little information available concerning epidemiological features of the disease under local conditions. There was an urgent need for rational control measures to be formulated and for a preliminary investigation which might indicate particular aspects of the disease deserving more detailed study.

The first of two projects was a postal survey involving 400 beef farmers in the Hunterville and Gisborne districts. A questionnaire was sent out to determine management practices which might influence the occurrence of IBK and to obtain an impression of the impact of the disease on the farming industry. The survey results (72% response rate) showed that IBK is widespread in both areas, appears to have been noticed only within recent years and the annual incidence is still rising (1% in 1975 to 28% in 1980 in the Gisborne area).

Outbreaks follow a seasonal pattern with most occurring in late summer. All age groups of cattle may be affected, but morbidity rates differ (young stock 18%; adults 10%). A greater susceptibility of the Hereford breed to the disease was observed only in the Gisborne district. Difficulties with stock management and lower sale prices were two major consequences of IBK reported and concern was expressed over control measures. Only 11% of farmers routinely treated cattle and yet they reported that treatment improved the recovery rate.

The other project involved a study of haemolytic strains of *Moraxella bovis* isolated from cattle eyes and the relationship of infection rates to clinical IBK cases.

Three local beef farming properties were chosen because of their previous history of IBK and over a 1 year period identified animals were observed for clinical signs and conjunctival sac samples were collected for culture. *Moraxella bovis* was identified by its β-haemolysis, colony and organism morphology, and Gram negative staining characteristics, and confirmed biochemically using five tests including alkaline peptonization of litmus milk.

Only one farm experienced an outbreak of IBK. The latter property operated a feedlot system, and early results implicated "carrier animals" in the initiation of a new outbreak. The prevalence of *M.bovis* isolations increased approximately one month before any clinical signs were apparent and infections remained at a higher level throughout the outbreak. A large number of animals which became infected never developed signs of the disease. (Peak figures in one pen 87% infected and 55% IBK). Seventy-eight percent of those cattle were infected with *M.bovis* in both eyes at some time, whereas only 23% of the cases of IBK were bilateral. *Moraxella bovis* infections decreased slowly after the peak, but many cattle remained infected for at least 5 months.

The widespread nature of *M.bovis* infections indicated the desirability for treatment of both eyes of all animals in a group if control of an IBK outbreak is to be attempted, but such treatment is sometimes impracticable.

More detailed investigation is needed into the local factors that are responsible for converting a latent infection of *M.bovis* into IBK.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Infectious bovine keratoconjunctivitis (IBK) or Pinkeye, is an established world wide animal health problem in cattle (Billings[12] 1889), but in New Zealand it has become apparent as a matter of concern only in recent years. Various reports suggest that the disease first became evident about 1973-1974 (Cooper, pers.comm.), and subsequently there has been a rapid rise in the yearly incidence (Corrin[24] 1980; Harris[41] 1980).

The investigation into the IBK problem in New Zealand upon which the following report is based, was aimed at improving the existing knowledge of the disease as it pertains to this country and to identify areas where further investigations could most usefully be directed.

To achieve the broad objective of the investigation, this project was divided into two main sections. Firstly, a postal survey of farmers was undertaken to obtain information on the extent of the IBK problem and to identify management factors associated with the occurrence of outbreaks of the disease. Secondly, a more detailed study of naturally occurring field outbreaks of IBK was conducted and special attention was paid to the prevalence of *Moraxella bovis* infections as they related to the appearance of clinical disease.

It was anticipated that from a fuller understanding of the nature of the disease in New Zealand, the formulation of more rational methods of control would be possible, and avenues for further research would be determined.

CHAPTER 1

REVIEW OF THE LITERATURE

INTRODUCTION

THE CAUSE OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

ISOLATION OF *MORAXELLA BOVIS*

IDENTIFICATION OF *MORAXELLA BOVIS*

EPIDEMIOLOGY OF *MORAXELLA BOVIS*

OCCURRENCE OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

SURVEYS OF CATTLE FARMING POPULATIONS

TREATMENT OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

IMMUNOLOGICAL ASPECTS OF *MORAXELLA BOVIS* INFECTIONS

PRODUCTION LOSSES DUE TO INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

INTRODUCTION

Infectious bovine keratoconjunctivitis (IBK) is the most important ocular disease of cattle because of its world wide distribution, high morbidity rate and the economic losses consequent upon pain and blindness. The syndrome is known colloquially as infectious ophthalmia, infectious keratitis, New Forest disease, blight and pinkeye (Jones and Little,[59] 1923; Brown,[16] 1934; Formston,[32] 1954; Giles,[38] 1975).

Characteristics of IBK are inflammatory changes of the conjunctiva and cornea, in either one or both eyes. Such changes vary in their extent depending on the severity of the infection. The involvement of the conjunctiva and cornea give rise to the initial clinical signs associated with pain, namely, blepharospasm, photophobia and copious lachrymation. The corneal lesions generally develop in the next 48 hours, initially as an opacity progressing in some instances into an ulcer of the corneal epithelium: a defect which may be more readily detected by staining with fluorescein. The extent of the corneal lesion largely determines the severity of the disease in any individual.

Temporary blindness is a common feature because of the corneal oedema and vascularization. The invasion of the cornea by capillaries is a striking feature of the healing process in all but the very minor lesions, and slowly disappears as healing is completed but in cases where the ulceration has been extensive, some scarring and corneal opacity may remain. In very severe cases the outcome can involve permanent blindness as perforation of a descemetocele can lead to the prolapse of the iris and/or lens which results in irreparable damage to the eye.

THE CAUSE OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

Infectious bovine keratoconjunctivitis was first recorded by Billings[12] in 1889, and although no definite cause had been identified, it was known that the disease could be transmitted directly from one animal to another by the instillation of eye secretions.

The bacterial nature of the infection was established by Farley[29] (1950) who was able to show that the causal agent could not pass through a fine or medium density filter. Subsequently Watt[103] (1951) isolated a Gram negative rod identical to *Haemophilus bovis* which had been isolated from cattle eyes nearly thirty years previously (Jones and Little,[60] 1924) but he was unable to transmit the disease using pure cultures of the organism.

In 1953, an improved method for isolating *H.bovis* was described and pure cultures of the organism, obtained from naturally occurring cases of IBK were used to reproduce the signs and lesions of the disease in susceptible cattle (Jackson,[58] 1953; Faull,[30] 1954; Gallagher,[36] 1954; Henson,[43] 1960). *Haemophilus bovis* was re-classified and named *Moraxella bovis* (Breed,[15] 1948).

Moraxella bovis is generally accepted as the definitive aetiological agent of IBK. Although *M.bovis* is virulent in gnotobiotic calves, inoculation of the organisms into conventionally reared calves produces the clinical syndrome more readily. This evidence supports the view that additional microbial pathogens may play some role in the production of IBK lesions (Chandler,[20] 1980).

ISOLATION OF *MORAXELLA BOVIS*

Most investigators have used sterile cotton wool tipped applicators to soak up conjunctival fluids and have transferred this material directly on to a growth medium for incubation. Variable degrees of success have been reported in attempts to isolate *M.bovis* from diseased stock, successful isolations ranging from 66% to 90% (Baldwin[6] 1945; Faull[30] 1954; Henson[43] 1960; Wilcox[111] 1970; Bryan[18] 1973; Lepper[65] 1980). Although *M.bovis* could sometimes be isolated from the clinically normal eye in cattle with unilateral IBK (Faull[30] 1954), recovery of the organism from the eyes of cattle believed to be free of IBK was considered to be unusual (Faull[30] 1954; Gallagher[36] 1954; Cooper[22] 1960). However, following naturally occurring and experimental infections of IBK, cattle can harbour *M.bovis* in their eyes for periods of up to eight months without the organism causing any visible effects (Baldwin[6] 1945; Farley[29] 1950; Jackson[58] 1953; Gallagher[36] 1954; Pugh[78] 1975).

IDENTIFICATION OF *MORAXELLA BOVIS*

The characteristics of *M.bovis* have been widely studied using gross and ultra-morphology, Gram staining, and biochemical analysis and these features are used as means of identification (Pugh[82] 1966; Pedersen[71] 1970; Wilcox[109] 1970; Bovre[14] 1974; Arora[3] 1976; Fraser[33] 1979).

Moraxella bovis is a Gram negative, short, capsulated diplobacillus which may appear singly, but more commonly in pairs, and sometimes in the form of short chains. Aged cultures show marked pleomorphism. The organism is aerobic and growth is optimal at 37°C (Pugh[82] 1966; Wilcox[109] 1970). On primary culture 1mm to 2mm round, translucent, greyish-white friable colonies appear after 24 hours and the colony size increases to 3mm to 4mm after 48 hours incubation. Certain strains of *M.bovis* produce a clear zone of β-haemolysis on sheep blood agar. However, the proportion of haemolytic strains isolated from surveyed groups varies considerably according to the circumstances of the population under study (Table I.I).

TABLE I.I PROPORTION OF HAEMOLYTIC *MORAXELLA BOVIS* STRAINS ISOLATED

TOTAL NUMBER OF ISOLATIONS	NUMBER OF HAEMOLYTIC STRAINS	%	AUTHOR
101	97	96	Pugh,[82] 1966
264	83	31.4	Arora,[4] 1976
7	7	100	Fraser,[33] 1979

Biochemical properties of *Moraxella bovis*

Biochemical identification of *M.bovis* has been based on the non-fermentation of all carbohydrates, complete proteolysis of litmus milk (alkaline peptonization) and Loeffler's serum, no growth on McConkey agar and a positive oxidase reaction. These reactions are repeatable and are the most commonly used basis of specific identification. Most strains of *M.bovis* do not reduce nitrate, and about half produce catalase (Pugh,[82] 1966; Wilcox,[109] 1970; Pedersen,[71] 1970; Bovre,[14] 1974; Arora,[3] 1976).

Other methods of identification of *Moraxella bovis*

Identification of *M.bovis* by immunofluorescence is a quick and reliable method (Nayar,[68] 1973; Pugh,[92] 1976; McDonald,[66] 1979), and elongation of the organism under the influence of penicillin has been used to distinguish it from the coccal *Neiserria ovis* (Fraser,[33] 1979).

Variation in colony type

Dissociation of colony type from "rough" to "smooth" is associated with the loss of fimbriae from the bacteria and occurs following repeated subculture of *M.bovis*. Studies using electron microscopy have indicated that the large, flat, smooth, non-corroding colonies are not as strongly fimbriated as the smaller, umbonate, rough, corroding colonies (Bovre,[13] 1972; Sandhu,[96] 1974; Simpson,[97] 1976).

Virulence of *Moraxella bovis*

Virulent strains of *M.bovis* are usually derived from friable colonies which on being lifted, leave small depressions in the solid medium (Jackson,[58] 1953). There is also an association between the ability of the organism to colonize the bovine conjunctiva and the occurrence of fimbriae and corroding colonies of *M.bovis* (Pedersen,[74] 1972). However, the presence of fimbriae alone does not seem to be a sufficient reason for the organism to be virulent. It is the corroding characteristic of the friable colonies on primary culture that is likely to be the ultimate indicator of virulence (Pedersen,[74] 1972).

Non-haemolytic strains of *M.bovis* do not reproduce the symptoms of IBK when instilled into calves eyes, and both Pugh[78] (1968) and Sandhu[95] (1977) have concluded that the haemolytic characteristic of the organism contributes to its pathogenicity.

EPIDEMIOLOGY OF *MORAXELLA BOVIS* INFECTIONS

During an outbreak of IBK, the Gram positive cocci normally present in eye secretions are replaced by *M.bovis* and *Neiserria catarrhalis* (Wilcox,[111] 1970), and strains of both these species are found more commonly in cattle affected with IBK than in in-contact stock (Wilcox,[111] 1970). More recently however, during IBK outbreaks, *M.bovis* has been isolated from 72% to 100% of clinically normal eyes (Bryan,[18] 1973; Lepper,[65] 1980). Hence during an outbreak of IBK there are many more animals infected by *M.bovis* than are affected clinically. The proportion of animals infected by *M.bovis* as well as being affected by IBK, appears to depend on the extent of the outbreak of IBK and the age group of cattle involved (Table I.II) (Hughes and Pugh,[47] 1970; Bryan,[18] 1973; Arora[4], 1976; Lepper,[65] 1980).

TABLE I.II THE INFLUENCE OF AGE ON THE PEAK PREVALENCES OF *MORAXELLA BOVIS* INFECTIONS AND CLINICAL INFECTIOUS BOVINE KERATOCONJUNCTIVITIS DURING OUTBREAKS OF THE DISEASE

AGE GROUP OF CATTLE	% *M.BOVIS* ISOLATIONS	% IBK	AUTHOR
Calves	75	58	Hughes and Pugh[47] (1970)
Calves	99	89	Bryan[18] (1973)
Calves	68	26	Arora[4] (1976)
Heifers	66	33	Lepper[65] (1980)
Cows	63	16	Hughes and Pugh[47] (1970)
Cows	21	4	Lepper[65] (1980)

There is a seasonal increase in the prevalence of *M.bovis* isolations in the summer and autumn (Hughes and Pugh,[47] 1970; Wilcox,[111] 1970; Bryan,[18] 1973; Arora[4], 1976), although this is not necessarily associated with the development of the disease (Wilcox,[111] 1970). The incidence of infection varies from year to year but the highest disease rates correspond to the years in which the highest incidence of *M.bovis* occurs (Hughes and Pugh[47] 1970).

In a field epizootic, the peak occurrence of IBK corresponds to the peak prevalence of *M.bovis* infection in the herd. This peak of infections is characterised by a change from exclusively non-haemolytic *M.bovis* isolations prior to the epizootic to predominantly haemolytic strains. Once the epizootic has passed, *M.bovis* strains tend to revert to the original non-haemolytic form (Bryan[18] 1973; Arora[4] 1976).

Carrier state of *Moraxella bovis*

In cattle, the carrier state of *M.bovis* enables the dissemination of the disease to be continued by apparently unaffected animals as well as by cattle having clinical IBK. Therefore, the visual appraisal of eyes for lesions is not a reliable method of identifying animals carrying *M.bovis* (Baldwin[6] 1945; Farley[29] 1950; Jackson[58] 1953; Gallagher[36] 1954; Adinarayanan,[1] 1961; Lepper[65] 1980).

Calves are most prone to the disease, and the concurrent *M.bovis* infection of these young animals persists for longer periods than it does in mature cattle (Wilcox[111] 1970; Bryan[18] 1973; Pugh[78] 1975). The exact mechanism that may account for the apparent increased resistance with age is not known, but both immunoglobulin-G in the tears (Pedersen[73] 1973) and a systemic serological response (Pugh[83] 1971) are two of the possible factors involved. Furthermore, calves with severe clinical symptoms have *M.bovis* infections of shorter duration than those with a milder form of the disease (Kopecky[64] 1980).

OCCURRENCE OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

Infectious bovine keratoconjunctivitis has a seasonal occurrence coinciding with maximum sunlight in the summer months (Hughes and Pugh,[47] 1970; Wilcox,[111] 1970; Baptista,[7] 1979). It can, however, occur in any season of the year, depending on the presence of other predisposing factors such as high density stocking and eye damage, which can be caused by such factors as ultra-violet radiation or dust from the feeding of hay (Wilcox,[108] 1968; Hubbert,[46] 1970; Pugh,[77] 1972; Thrift,[102] 1974; Dodt,[28] 1977; Kopecky,[64] 1980).

Predisposing factors

The possible importance of ultra-violet radiation as a predisposing factor in the initiation of IBK outbreaks is indicated by meteorological records which show that the highest incidence of *M.bovis* infections follow closely the annual peak of solar ultra-violet radiation (Hughes and Pugh,[53,47] 1965, 1970). The optimal ultra-violet radiation for reproduction of the disease is known to be between 2700 and 3200 Å (Hughes,[55] 1968), and radiation of calves' eyes before artificial infection advances the time of onset of the disease and increases the severity of the corneal damage (Kopecky,[63] 1980).

Moraxella bovis can survive on the legs, body and wings of the face fly (*Musca autumnalis*) for up to 3 days, and it is considered possible that the fly acts as a mechanical carrier of *M.bovis* (Steve,[100] 1965). There is a direct relationship between the number of face flies per cow and the percentage of animals in a herd affected with IBK (Tien Hsi Cheng,[21] 1967). The peak fly populations in the summer could be a factor associated with the increased prevalence of the disease at this time (Brown,[17] 1972), although the proportion of flies carrying *M.bovis* is not particularly high (Berkebile,[10] 1981). Therefore, in the U.S.A., the control of fly populations in order to minimise their influence as mechanical carriers of *M.bovis* is an important adjunct to treatment (Beug,[11] 1976; McNutt,[67] 1976).

Eyelid pigmentation of cattle influences the incidence of IBK. Both the prevalence and severity of IBK decreases as the amount of pigmentation increases (Frisch,[25] 1976; Caspari,[19] 1979; Ward,[104] 1979; Pugh,[93] 1982). But despite this decreased susceptibility, in one study 68% of the animals with fully pigmented eyelids still succumbed to IBK by 15 months of age (Frisch,[35] 1976).

Several other factors have been implicated as predisposing causes in IBK, namely: tall grasses, dust and wind, any of which may cause the initial eye trauma that allows *M.bovis* to become established as an infection of the cornea (Baldwin,[6] 1945; Jackson,[58] 1953; Wilcox,[108] 1968; Arora,[4] 1976). In Queensland, high rainfall is associated with an increased IBK incidence (Dodt,[28] 1977). European breeds of cattle (*Bos taurus*) are more susceptible to IBK and have suffered more severe infections than the Zebu breeds of cattle (*Bos indicus*) (Frisch,[34] 1975).

SURVEYS OF CATTLE FARMING POPULATIONS

Surveys of cattle farmers in North America, Australia and New Zealand have been conducted in an attempt to identify the extent and nature of the IBK problem in these countries.

Between the years 1975 and 1979 the annual prevalence of IBK in New Zealand Hereford beef herds increased from 1% to 18% (Harris,[41] 1980): whereas a wider survey of New Zealand beef and dairy farmers over the same period showed an IBK prevalence increasing from 1% to 9% (Corrin,[24] 1980). In both surveys the occurrence of IBK was less in the South Island than in the North Island (Table I.III).

TABLE I.III INCIDENCE* OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS IN NEW ZEALAND

NORTH ISLAND (%)	SOUTH ISLAND (%)	NEW ZEALAND (%)	AUTHOR
6.75	0.7	10.7	Corrin[24] (1980)
33.12	12.28	24.0	Harris[41] (1980)

* farms experiencing at least one episode of IBK during the period 1975-1979 inclusive

In contrast, a recent Australian survey has shown that 81.3% of the national cattle herds have been affected by IBK at least once in the five year period from 1975 to 1979 (Slatter,[98] 1982). The IBK problem is also widespread in the United States of America, where it is estimated that nearly 50% of beef herds are affected annually (Webber,[106] 1981).

The disease in all three countries follows a seasonal pattern with a peak in prevalence during the summer. Forty-seven per cent of the respondents' herds in Australia had been affected by IBK during January 1979, and 45% of cattle herds in Missouri during the summer of 1978 (Webber,[106] 1981; Slatter,[98] 1982). Both the Australian and American surveys indicated a higher prevalence of IBK in Hereford and Hereford-cross cattle than in other breeds, and a lower prevalence in *Bos indicus* breeds than in *Bos taurus*.
Young stock were more frequently affected and the range of clinical signs was closely similar.

The mean morbidity rates were similar in all three countries with approximately 5% of adult cattle and 10% of calves affected. Since 1976 there has been a rise in the number of severe outbreaks in New Zealand involving up to 50% of the cattle on the properties (Harris,[41] 1980). Yet despite this, only 54% of New Zealand farmers routinely treated cattle affected by IBK, whereas up to 97.5% of dairy farmers in Australia employed some method of therapy (Slatter,[99] 1982).

The majority of Australian farmers (75%) and many in New Zealand considered that the disease caused a reduction in liveweight gain in affected cattle and was associated with significant financial losses. The conclusion derived from all three surveys is that more investigational work is needed before effective control methods against IBK can be formulated.

TREATMENT OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

Early treatments of IBK relied on the local instillation of antiseptic solutions on to the structures of the eye, and the results of specific treatments were not recorded until 1951.

Effective therapies included chloromycetin eye ointment (Hall-Patch,[40] 1951), a 2% zinc sulphate solution (Faull,[30] 1954) and sulphur preparations (Watt,[103] 1951).

Antibiotic sensitivity of *M.bovis*

In vitro antibiotic sensitivity testing of *M.bovis* has shown that the organism is susceptible to a wide range of antibacterial agents (Barner,[8] 1952; Faull,[30] 1954; Gallagher,[36] 1954; Pugh,[80] 1977; Webber,[107] 1981) namely; bacitracin, chloramphenicol, penicillin, dihydrostreptomycin and tetracyclines, many of which are already found in commercial ocular preparations. Recent studies in America on *M.bovis* strains recovered from field epizootics, have shown all isolates to be susceptible to penicillin but resistant to cloxacillin, and a high frequency of resistance to streptomycin occurred amongst the haemolytic strains (Webber,[107] 1981). In many parts of England the chemotherapeutic agent ethidium bromide proved to be equally as effective as many antibiotics, when applied locally twice daily for 4 to 5 days (Cooper,[23] 1960).

Methods of treatment

Treatment using the placement of oxytetracycline pellets into the lower conjunctival sac was found to be effective, and minimum inhibitory concentrations (M.I.C.) of the drug against *M.bovis* were maintained in the tears for up to 31 hours (Hawley,[42] 1954).

The intravenous administration of sulphadimidine at a dose rate of 100mg/kg bodyweight maintained the M.I.C. against *M.bovis* in conjunctival fluids for 24 hours and was considered the treatment of choice by Pedersen[72] (1973).

The successful technique of using subconjunctival injections of antibiotics has been adopted in many clinical practices (Bedford,[9] 1976; Cryer,[25] 1976; Dalton,[26] 1976; Giles,[39] 1976). It has been suggested that the use of steroids in conjunction with an antibiotic cover gives significantly better results in cases showing panophthalmia (Pugh,[80] 1977).

A further development has been the use of a polyvinyl tubing ring device that once placed over the globe of the eye can be retained in position by the eyelids. After loading with an antibiotic substance, these rings deliver a prolonged and continuous release of antibiotics over the conjunctiva and cornea for up to 19 days (Hughes and Pugh,[51] 1975).

A surgical approach to treatment using a modified third eyelid flap technique, seems particularly suitable for cases where ulceration of the cornea is extensive and there is a high risk of rupturing the cornea. This method physically protects the cornea from further damage while aiding the healing of the corneal defect (Andersen,[2] 1976).

The commencement of treatment early, when eye damage is still superficial, is essential to appreciably shorten the course of the disease (Barner,[8] 1952; Gallagher,[36] 1954; Cooper,[23] 1960; Wilcox,[108] 1968; Pugh,[80] 1977). However, any evaluation of the effectiveness of a treatment regime is difficult because IBK shows a marked propensity to abate spontaneously (Pugh,[75] 1979).

Antibacterial treatment as a method of control of infectious bovine keratoconjunctivitis

An attempt to avoid outbreaks of IBK by eliminating the carrier state of *M.bovis* in large groups of cattle with a single administration of an antibiotic, met with little success (Pugh,[93] 1982). But further trials using an adequate treatment schedule to ensure removal of *M.bovis* might possibly be successful.

IMMUNOLOGICAL ASPECTS OF *MORAXELLA BOVIS* INFECTIONS

It has been known for approximately 30 years (Jackson,[58] 1953) that antibodies are produced by spontaneous or artificial infections of *M.bovis* and that cattle rarely succumb to a second outbreak of the disease (Barner,[8] 1952). The first investigations into the production of a vaccine against IBK using a systemically administered inactivated bacterial product were reported in 1960 (Henson,[44] 1960). Insufficient immunity resulted from the vaccination to protect against an artificial or a natural challenge, but once the calves had recovered from the initial disease they did not become infected a second time.

The standard gel diffusion precipitin test (Ouchterlony,[70] 1968) has been modified to detect antibodies against *M.bovis* in the sera of cattle affected by IBK, thus demonstrating that a systemic serological response does occur at the time of infection (Pugh,[83] 1971). Studies have also illustrated that there is a selective transfer of immunoglobulin-G-1 from the blood to tears (Pedersen,[73] 1973) during

M.bovis infection, and more importantly, an increase in locally synthesised immunoglobulin-A (Nayar,[69] 1975). The presence of these immunoglobulins may be responsible for the age associated acquired resistance to re-infection with *M.bovis*.

Vaccines used against *Moraxella bovis* infections

Initial studies using a viable vaccine showed a measurable increase in serum antibodies directly related to the number of inoculations, and the resistance amongst the vaccinated cattle compared favourably with that of a group recently recovered from the disease (Hughes and Pugh,[48] 1971). A formalin-killed vaccine proved to be equally as effective (Hughes and Pugh,[49] 1972) and the immunity provoked by the vaccine could be further enhanced by administering the inoculations at 14 day intervals (Hughes and Pugh,[50] 1975).

Field trials using the formalin-killed vaccine produced some equivocal results at a time when other studies (Pugh,[89] 1975) were indicating that vaccination of cattle using a vaccine made from one strain of *M.bovis* may not prove to be very effective against other strains (Hughes and Pugh,[57] 1976). Although very little was known at this time of the nature of the strain differences, significant differences in the extracellular antigens of organisms from rough and smooth colony types of one strain of *M.bovis* had been demonstrated (Sandhu,[96] 1974).

Agglutination tests applied to a number of *M.bovis* strains isolated from Brazil and America have indicated distinct antigenic differences and that the strains could be classified into 6 groups (Gil-Turnes,[37] 1982).

Despite these strain differences, a vaccine produced from one non-haemolytic *M.bovis* strain proved to be as effective in terms of protection afforded against challenge as previous vaccines derived from haemolytic *M.bovis* strains (Pugh,[87] 1982).

In attempts to improve the immunogenicity of a vaccine, various fractions of the *M.bovis* bacterium have been used, but to date vaccines comprised of either pili fractions or ribosomes have been unsuccessful (Pugh,[79 81 91] 1976, 1977, 1981).

Adjuvants have been used in successful attempts to improve the immunogenicity of a formalin killed vaccine (Hughes and Pugh,[52] 1977), but potentiation of an experimental *M.bovis* pilus vaccine using *Mycobacterium paratuberculosis* was not successful (Pugh,[88] 1978).

The immunomodulator levamisole has been shown to enhance the immune response to vaccines when administered simultaneously (Hogarth-Scott,[45] 1980). However, it did not enhance the immune response to a *M.bovis* vaccine (Pugh,[86] 1981).

Site of vaccination

Improvements in the response to vaccination have been attempted by varying the site of inoculation. The localised nature of IBK and the possible role of immunoglobulins in the tears have encouraged investigations into locally administered vaccines and the potential for local immunity. Following the administration of a commercially prepared autogenous vaccine into the third eyelid, fewer cattle were infected by *M.bovis* and vaccination afforded some protection against clinical signs, but the incidence of the disease was not sufficiently reduced for this vaccine to be considered a practical means of control (Arora,[4] 1976).

Vaccination by injection into the subconjunctiva appears to have no advantage over the subcutaneous route of administration (Webber,[105] 1981).

Effect of age on vaccination

The age of calves at the time of vaccination is not a determinant in the development of an immune response, as measured by the humoral antibody response, but very young calves show more severe clinical signs than either older calves or adults, regardless of their vaccination status (Pugh,[84] 1979). Despite this apparent susceptibility of the very young, some protection against IBK can be achieved in new born calves by the feeding of colostrum from vaccinated cows (Pugh,[90] 1980).

Vaccination in its present state does not provide adequate protection against infection with *M.bovis* or against the development of clinical IBK. However, vaccination does bring about a reduction in the number of animals showing clinical signs (Arora,[4] 1976; Webber,[105] 1981; Pugh,[93] 1982), a decrease in the severity of disease (Webber,[105] 1981), and a reduction in the peak infection rates (Arora,[4] 1976). There is no effect on the duration of the *M.bovis* infection and therefore vaccination does not remove the risk of the "carrier" state (Pugh,[85] 1980).

One comparison of vaccination and treatment in controlling naturally occurring IBK indicated that weekly treatments appeared to be more effective in reducing the incidence of disease (Hughes and Pugh,[56] 1979).

PRODUCTION LOSSES DUE TO INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

Infectious bovine keratoconjunctivitis has been recognised for as long as 40 years (Baldwin,[6] 1945) as a cause of depressed profitability in animal production by decreasing milk production and growth rates.

Calves which have contracted IBK prior to weaning can have significantly reduced liveweight gains (Table I.IV). The effect can

TABLE I.IV THE INFLUENCE OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS BEFORE WEANING ON THE LIVEWEIGHT OF CALVES

AGE OF CALVES AT WEIGHING	MEAN REDUCTION IN LIVEWEIGHT OF IBK CALVES	AUTHOR
3 months	-3 kg	Frisch[35] (1976)
7 months	-17 kg	Thrift[102] (1974)
7 months	-5 kg (one eye affected)	Killinger[61] (1977)
7 months	-16 kg (both eyes affected)	Killinger[61] (1977)

continue past weaning and has been still evident at 15 months of age (Thrift,[102] 1974; Frisch,[35] 1976; Hughes and Pugh,[57] 1976; Killinger,[61] 1977; Thomas,[101] 1978; Ward,[104] 1979). The reduction in liveweight gain is influenced by the severity of the clinical syndrome and is dependent on whether one or both eyes are affected (Thrift,[102] 1974; Killinger,[61] 1977).

Although the presence of IBK is firmly believed to be a cause of reduced milk yields, there is no published data to confirm these losses in dairy production.

Affected bulls may have reduced fertility which has been attributed to reduced libido resulting from the discomfort of IBK (Thrift,[102] 1974).

CHAPTER 2

EPIDEMIOLOGICAL ASPECTS OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS IN THE HUNTERVILLE AND GISBORNE DISTRICTS AS REPORTED BY FARMERS IN A POSTAL SURVEY

INTRODUCTION

A postal survey of New Zealand farmers was considered to be an important extension to any practical field work undertaken with IBK in New Zealand. The aim of the survey was to identify the epidemiological factors which might be influenced by management procedures and which may be significant in determining the occurrence of IBK. To achieve this aim, two farming districts were chosen, namely, Hunterville and Gisborne, for the distribution of a questionnaire to enable comparison of management procedures which may influence IBK. The districts were selected because they were similar hill country sheep and beef farming areas. Both were known to have IBK as a problem and lists of Veterinary Club members in each locality were readily available. It was envisaged that such an intensive comparative approach might provide some lead in determining key management factors that predispose towards IBK outbreaks.

MATERIALS AND METHODS

The survey questionnaire form was designed to be easily understood, straightforward to complete and structured so that answers could be coded and readily transferred for computer analysis (Appendix I).

The Hunterville Veterinary Club has a membership of approximately 200 farmers and the Gisborne Veterinary Club has 800 members. To select a similarly sized population in Gisborne, a preliminary card (Appendix II) was sent to all members with an explanatory letter. From the replies to this card, 200 farms were selected to receive the full questionnaire form. An equal number of forms were sent to farmers reporting they had or had not experienced outbreaks of IBK amongst their cattle during the period 1977 to 1981.

Two hundred farmers in each region received the questionnaire form by post and were informed of the objectives of the survey in an explanatory letter. Farmers who had not replied with a completed questionnaire form within six weeks were sent a reminder letter.

All the survey questionnaire data were transferred to a computer and analysed using the Minitab Statistical computing system (Ryan,[94] 1976), and standard statistical methods (Fischer,[31] 1928; Bailey,[5] 1959) (Chapter 3, pg 62).

RESULTS

Response rate

A response rate of 35% (283/800) was achieved from the preliminary card sent to Gisborne farmers. However, there was a reply response of 83.5% from the farmers receiving the full survey form. Hunterville farmers returned 61% of the questionnaires, giving an overall response rate of 72% for the 400 forms sent out.

General Farm Information

Farm size and stock numbers

The mean farm size in Gisborne was approximately twice the size of farms in Hunterville (Table II.I).

There was a significant ($p < 0.05$) difference in the mean farm size between the farms on which IBK did or did not occur during the period 1977 to 1981.

In keeping with the difference in farm size between the two regions, it was found that mean stock numbers per farm differed (Table II.II). The figures demonstrate a lower sheep to cattle ratio in Gisborne. However, in each region the stock ratios remained the same whether the farm had IBK in its cattle or not (Table II.III).

Age structure of the cattle populations

Each district has a distinct difference in the age structure of the cattle populations and this reflects the different emphasis in the region's farm production. Ninety-one per cent of the farms in Gisborne had a breeding herd of cattle as compared with 64% in Hunterville; consequently there is more fattening of stock in Hunterville and therefore a predominance of male cattle over two years of age (Table II.IV).

Calving and weaning data

Calving dates in the two regions were similar: the months of August, September and October being the main period, but with a slightly longer calving spread in Gisborne. Calves were weaned earlier in Gisborne: 87% of farms wean by the end of April as compared with 65% of farms in Hunterville.

Breed distribution of cattle

There was a similar proportion of farms with Aberdeen Angus and Aberdeen Angus-Hereford cross cattle in the two districts, but there was a higher proportion of farms running purebred Hereford in Gisborne ($p < 0.05$) (Table II.V).

Purchase of cattle

Although the same proportion of farmers in each region buy-in cattle, Gisborne farmers are mainly concerned with breeding enterprises, hence most of their purchases (61%) are bulls only (Table II.VI). These purchases average 2.6 bulls per annum. This difference in farming style is also reflected in mean stock numbers purchased: the figure is substantially lower in Gisborne than Hunterville.

Sixty-one per cent of the farmers in Hunterville and 86% in Gisborne purchase their cattle from within their home district. The remaining farmers buy their cattle from a variety of areas with no one district being predominant.

Information Relating to Infectious Bovine Keratoconjunctivitis

Incidence of infectious bovine keratoconjunctivitis

The yearly incidence of IBK showed a marked rise in both districts during the period from 1977 to 1981 (Table II.VII), and consequently a high proportion of farms have been affected by IBK at least once during this period: 38% in Hunterville and 52% in Gisborne.

During the same period, other unspecified eye problems in cattle as reported by farmers, have remained constant at a comparatively low level (Table II.VIII).

Occurrence of outbreaks of infectious bovine keratoconjunctivitis

A definite seasonal pattern was observed in the occurrence of outbreaks of IBK in both districts during 1980 (Fig.II.1).

Influence of breed on the occurrence of infectious bovine keratoconjunctivitis

A breed difference in the occurrence of IBK was evident in the Gisborne district but not in Hunterville. In the former, a higher proportion of IBK outbreaks occurred on farms that carried Aberdeen Angus-Hereford cross or purebred Hereford cattle than on farms that carried Aberdeen Angus cattle ($p < 0.05$) (Table II.IX).

Age groups affected by infectious bovine keratoconjunctivitis

In both regions, all age groups of cattle were affected by IBK, but in Hunterville fattening stock had the highest occurrence of the disease (Table II.X). Most replies were not detailed enough to estimate an accurate morbidity rate for each age group, but there was a trend towards a higher rate in younger cattle: 18% of calves and 10% of adult cattle were estimated to be affected during outbreaks of IBK.

Clinical signs first noticed by farmers

"Watery eyes" and "whiteness of the eyeball" were the two most commonly noticed initial signs of an IBK outbreak. Misadventure was common because of the difficult terrain in both districts (Table II.XI).

Veterinary diagnosis

The number of farmers currently seeking veterinary diagnosis of IBK in their cattle is low: 26% in Hunterville and 38% in Gisborne.

Treatment of infectious bovine keratoconjunctivitis

Treatment of IBK was generally regarded by farmers to be too difficult and time-consuming for the debatable benefits they felt it might have (Table II.XII). Despite the doubt expressed about the benefits of treating cases of IBK, results demonstrate that on farms that regularly treat the cattle there were fewer animals whose eyes took longer than 4 weeks to heal and a greater number of cattle healing in 1 to 2 weeks ($p < 0.01$) (Fig. II.2).

Types of treatment used by farmers

Of the farmers that regularly treated their cattle, 63% used antibiotic powder puffers, 23% used subconjunctival injections and 14% used ointments or aerosols.

Effects on management of infectious bovine keratoconjunctivitis

Fifty-four per cent of farmers in both districts felt that cattle affected by IBK were difficult to move around the farm because of the associated blindness. Specific comments from many farmers included the statement that it was prudent to leave affected stock alone and isolation from other cattle was a prime consideration. However, despite this inconvenience, only 17% of farmers in Gisborne and 4% in Hunterville felt that IBK interfered with the mating management of their cattle.

A majority of farmers in Hunterville considered that an outbreak of IBK on their farms was associated with the introduction of new cattle (Table II.XIII), and herd data subsequently confirmed that a greater proportion of farms which did not buy-in cattle remained free of IBK (Table II.XIV). The situation did not arise in Gisborne where, on average, farmers buy-in fewer cattle than in Hunterville.

However, many farmers from that area considered that the practice of droving cattle past their properties was a significant factor in the introduction of IBK on to their farms. In both regions more cattle are bought on to IBK-positive farms (Table II.XV).

Effects on production of infectious bovine keratoconjunctivitis

Many farmers were under the impression that IBK caused a check in the growth of their young stock (53%). However, opinion was almost equally divided as to whether IBK caused a delay in the finishing of fattening stock (Table II.XVI).

DISCUSSION

The excellent reply response (72%) to the survey enabled new information regarding IBK in New Zealand to be collated and increased the reliability of conclusions drawn from the replies. The response rate compares more than favourably with the rates of other surveys on the same subject; 44% (Harris,[41] 1980), 43.4% (Webber,[106] 1981) and 29.8% (Slatter,[98] 1982). The excellent response to the survey is probably a reflection of farmer concern with the recent increase in this disease which was all but unknown a few years ago. Other factors contributing to the good response were the personal approach to the survey, with adequate explanation of the objectives, and follow-up reminders to farmers who had not returned the forms.

The survey demonstrated that IBK is still an increasing cattle health problem in both districts, whereas other eye problems have remained constant at a much lower prevalence over the same period. If the rate of increase does not abate, then New Zealand could well experience prevalences of IBK similar to that found in parts of America where an estimated 50% of all beef herds are affected annually (Webber,[106] 1981).

The results confirmed the seasonal nature of outbreaks of IBK in New Zealand, the peak occurrence being observed in February, during late summer, which follows the peak in sunshine hours in January (DSIR Meteorological service, 1981). The same seasonal association has been demonstrated by Hughes and Pugh[47] (1970) over a five year period, and they showed a positive correlation between peak sunshine (UV radiation) and onset of outbreaks.

In contrast to the findings of Harris[41] (1980), there was a significant ($p < 0.05$) difference in the mean farm area according to the farm history of IBK: farms on which outbreaks of IBK occurred were approximately twice the area of the farms that remained unaffected. It therefore follows that IBK affected farms which are larger and carry more stock (Table II.III) would provide a greater reservoir of infection. However, the higher cattle numbers are not at the expense of sheep as shown by similar sheep to cattle ratios on both categories of farm.

The association between an outbreak of IBK and the purchase of new stock was apparent only in Hunterville, and it appears that it may well be closely related to the number of animals bought. This in turn would be determined by the type of farming enterprise; larger properties are less likely to run a breeding herd and are therefore more dependent on buying-in stock. The class of stock purchased, namely weaners, bulls or store steers, would present a variable risk of introducing infection. As well as there being an increased chance of exposure to infection amongst a large number of cattle, clinically affected cattle are less likely to be noticed amongst large groups of animals.

A higher proportion of farmers in Gisborne with Hereford or Aberdeen Angus-Hereford cross cattle have experienced IBK in their stock than farmers who carry Aberdeen Angus cattle. This apparent breed susceptibility would tend to support overseas observations of the greater susceptibility of Hereford cattle (Webber,[106] 1981; Slatter,[98] 1982) which has been suggested to be associated with a lack of eyelid pigmentation (Frisch,[35] 1976; Caspari,[19] 1979; Ward,[104] 1979; Pugh,[93] 1982). A possible explanation for the breed difference not being evident in Hunterville is the much lower number of purebred Herefords in the region. There is a higher level of UV radiation in Gisborne which could probably predispose to a greater susceptibility of the Hereford breed in that region (Hughes and Pugh,[53] 1966).

The age structure of the cattle population in each region reflects the type of farming enterprises being undertaken; mainly breeding in Gisborne, and fattening in Hunterville. It also offers a possible explanation for the high proportion of outbreaks involving fattening cattle in Hunterville (Table II.X), as they form the largest single age group in that region.

In contrast to overseas outbreaks in which it is mainly the youngest cattle that become affected (Webber,[106] 1981), New Zealand weaners ("young cattle") appear not to be substantially more susceptible than other age groups. This pattern of disease tends to support the concept that IBK has been introduced into New Zealand quite recently and that beforehand, the cattle population was completely susceptible. As IBK becomes more widespread throughout New Zealand, the overall resistance to infection could be expected to rise, in which case it could result in a change in the pattern of the disease. Thereafter, the annual crop of calves would become the largest susceptible group within the population.

Insufficient data were available to calculate an accurate morbidity rate for each age group. However, observations indicate that the morbidity rates in New Zealand are approximately twice those observed overseas in both young and adult stock (Webber,[106] 1981; Slatter,[98] 1982).

Despite the fact that only 11% of farmers regularly treated their cattle for IBK, there was a general feeling that treatment resulted in a slight speeding up of recovery (Fig. II.2). The shorter resolution time might be attributable to treatment at an early stage, at which time healing would proceed without any need for the lengthy process of corneal vascularization. In such cases superficial ulceration would commence to resolve immediately *M.bovis* infection was overcome. According to farmer opinion, treatment did not appear to influence the prevalence of blindness. When corneal damage is advanced, treatment will only remove *M.bovis* and healing will still have to occur by vascularization, and any extensive eye damage will remain. Improved results from treatment may yet be achieved because of the growing farmer acceptance of subconjunctival antibiotic injections as a more satisfactory form of treatment. In particular, the concept of mass treatment can be applied to large groups of cattle. In such a treatment regime the aim would be to treat all cases of IBK at the earliest possible stage, and to eliminate *M.bovis*, thereby removing the reservoir for re-infection.

Management of cattle with IBK continues to be a concern to farmers in both districts, mainly because of the difficulty encountered in moving stock on hilly terrain, the need to isolate affected animals and the desire to diminish the spread of the disease by reducing the stocking rates. However, as IBK tends to occur at a time of year when grazing needs to be managed with some care, the conflicting farming objectives make such management decisions difficult.

The peak prevalence of IBK occurs outside the main mating period and therefore few farmers have reported any disruption to mating management on their farms. Decreased libido in bulls affected by IBK has been demonstrated (Thrift,[102] 1974) and if this was to happen with a free-range mating system as employed on the majority of New Zealand beef farms, combined with the difficult terrain, calving percentages could easily suffer if the disease was undetected and the bull not replaced.

The production limiting effects of IBK were widely recognised by farmers. Many farmers commented that in addition to losses from decreased growth rates, some mobs of animals yielded less than their expected monetary value because of the need to be slaughtered rather than disposed of privately or through sale yards. Culling of stock which would otherwise have been retained, and death of stock by misadventure were other aspects cited as major concerns by farmers.

It has been impossible to gauge whether farmers consider IBK causes a significant financial loss as there has been no consensus of opinion on the acceptable level of losses in the normal running of a farm. However, a majority of farmers consider that the occurrence of IBK is a sufficiently important problem for some far more effective control measure to be investigated and developed.

TABLE II.I MEAN SIZE OF FARMS IN HUNTERVILLE AND GISBORNE

	MEAN FARM SIZE (HECTARES)		
	ALL FARMS	FARMS WITH IBK	FARMS WITHOUT IBK
Hunterville	410	884	316
Gisborne	921	1400	738

TABLE II.II MEAN STOCK NUMBERS AND STOCK RATIOS IN HUNTERVILLE AND GISBORNE

	MEAN STOCK NUMBERS PER FARM		SHEEP TO CATTLE RATIO
	SHEEP	CATTLE	
Hunterville	3262	314	10.4 : 1
Gisborne	4330	718	6.0 : 1

TABLE II.III MEAN STOCK NUMBERS AND STOCK RATIOS RELATIVE TO THE OCCURRENCE OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

	FARMS WITH IBK MEAN STOCK NUMBERS			FARMS WITHOUT IBK MEAN STOCK NUMBERS		
	SHEEP	CATTLE	RATIO	SHEEP	CATTLE	RATIO
Hunterville	5142	506	10.2 : 1	2124	201	10.5 : 1
Gisborne	5539	921	6.0 : 1	2992	483	6.2 : 1

TABLE II.IV AGE STRUCTURE OF THE CATTLE POPULATIONS IN HUNTERVILLE AND GISBORNE

AGE GROUP OF CATTLE	% OF TOTAL CATTLE POPULATION	
	HUNTERVILLE	GISBORNE
Male/Female under 1 Year	17	32
Male 1 to 2 Years	20	10
Female 1 to 2 Years	7	12
Male over 2 Years	34	7
Female over 2 Years	23	39
TOTAL	100	100

TABLE II.V BREED DISTRIBUTION OF CATTLE IN HUNTERVILLE AND GISBORNE

BREED	HUNTERVILLE % FARMS	HUNTERVILLE % CATTLE	GISBORNE % FARMS	GISBORNE % CATTLE
Aberdeen Angus	26	17	29	25
Aberdeen Angus x Hereford	47	59	45	50
Hereford	7	8	20	20
Other	20	16	6	5

TABLE II.VI CATTLE PURCHASES PER ANNUM BY FARMERS IN HUNTERVILLE AND GISBORNE

	% FARMERS BUYING CATTLE	MEAN NUMBER CATTLE PURCHASED	% FARMERS BUYING ONLY BULLS
Hunterville	71	118	22
Gisborne	74	45	61

TABLE II.VII YEARLY INCIDENCE OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS OVER THE PAST FIVE YEARS

	% FARMS WITH IBK				
	1977	1978	1979	1980	1981*
Hunterville	11	14	22	23	(19)
Gisborne+	10	14	25	28	(24)

+ results compiled from preliminary card
* six month period, January to June, only

TABLE II.VIII YEARLY INCIDENCE OF EYE PROBLEMS IN CATTLE: OTHER THAN INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

	% FARMS WITH EYE PROBLEMS				
	1977	1978	1979	1980	1981*
Hunterville	2	2	3	6	(4)
Gisborne	8	7	6	7	(4)

* six month period, January to June, only

TABLE II.IX OCCURRENCE OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS ACCORDING TO BREED OF CATTLE INVOLVED

BREED	HUNTERVILLE		GISBORNE	
	NUMBER FARMS WITH IBK (%)	NUMBER FARMS WITH NO IBK (%)	NUMBER FARMS WITH IBK (%)	NUMBER FARMS WITH NO IBK (%)
Aberdeen Angus	12 (37)	20 (63)	20 (42)	28 (58)
Aberdeen Angus x Hereford	23 (40)	34 (60)	40 (53)	36 (47)
Hereford	2 (22)	7 (78)	22 (67)	11 (33)
Other	6 (32)	13 (68)	2 (33)	4 (67)

TABLE II.X AGE GROUPS OF CATTLE AFFECTED BY INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

AGE GROUP	% OF REPLIES	
	HUNTERVILLE	GISBORNE
Calves	8	12
Weaners	21	23
Fat Stock	41	17
Heifers	2	12
Cows	15	19
Bulls	13	17

TABLE II.XI CLINICAL SIGNS FIRST NOTICED BY FARMERS DURING AN OUTBREAK OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

SIGN FIRST NOTICED	% REPLIES
Watery eyes	40
Whiteness of eyeball	41
Seeking shade	2
Loss of condition	7
Misadventure	9

TABLE II.XII TREATMENT OF CASES OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS BY FARMERS

	% REPLIES		
	DID TREAT	DID NOT TREAT	TREATED SOMETIMES
Hunterville	7	64	29
Gisborne	13	39	48

TABLE II.XIII FARMER'S ASSOCIATION OF AN OUTBREAK OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS WITH THE INTRODUCTION OF NEW CATTLE ON TO THEIR FARMS

	% REPLIES		
	YES	NO	SOMETIMES
Hunterville	49	33	18
Gisborne	26	67	7

TABLE II.XIV CATTLE PURCHASES BY FARMERS IN HUNTERVILLE AND GISBORNE RELATIVE TO THE OCCURRENCE OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

	IBK-POSITIVE* FARMS		IBK-NEGATIVE+ FARMS	
	NUMBER FARMS BUYING CATTLE (%)	NUMBER FARMS NOT BUYING CATTLE (%)	NUMBER FARMS BUYING CATTLE (%)	NUMBER FARMS NOT BUYING CATTLE (%)
Hunterville	38 (31)	7 (6)	48 (39)	29 (24)
Gisborne	66 (39)	21 (13)	57 (34)	23 (14)

* IBK has occurred at least once during 1977 to 1981

\+ no IBK during 1977 to 1981

TABLE II.XV MEAN NUMBER OF CATTLE PURCHASED IN HUNTERVILLE AND GISBORNE RELATIVE TO OCCURRENCE OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

	MEAN NUMBER OF CATTLE PURCHASED PER ANNUM	
	IBK-POSITIVE* FARMS	IBK-NEGATIVE+ FARMS
Hunterville	184	69
Gisborne	71	17

* IBK has occurred at least once during 1977 to 1981
\+ no IBK during 1977 to 1981

TABLE II.XVI FARMER OPINION ON THE EFFECT OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS ON PRODUCTION

	% REPLIES		
	YES	NO	SOMETIMES
Decreased growth of young stock	53	38	9
Delayed finishing of fat stock	46	42	12

FIGURE II.1 SEASONAL OCCURRENCE OF OUTBREAKS OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

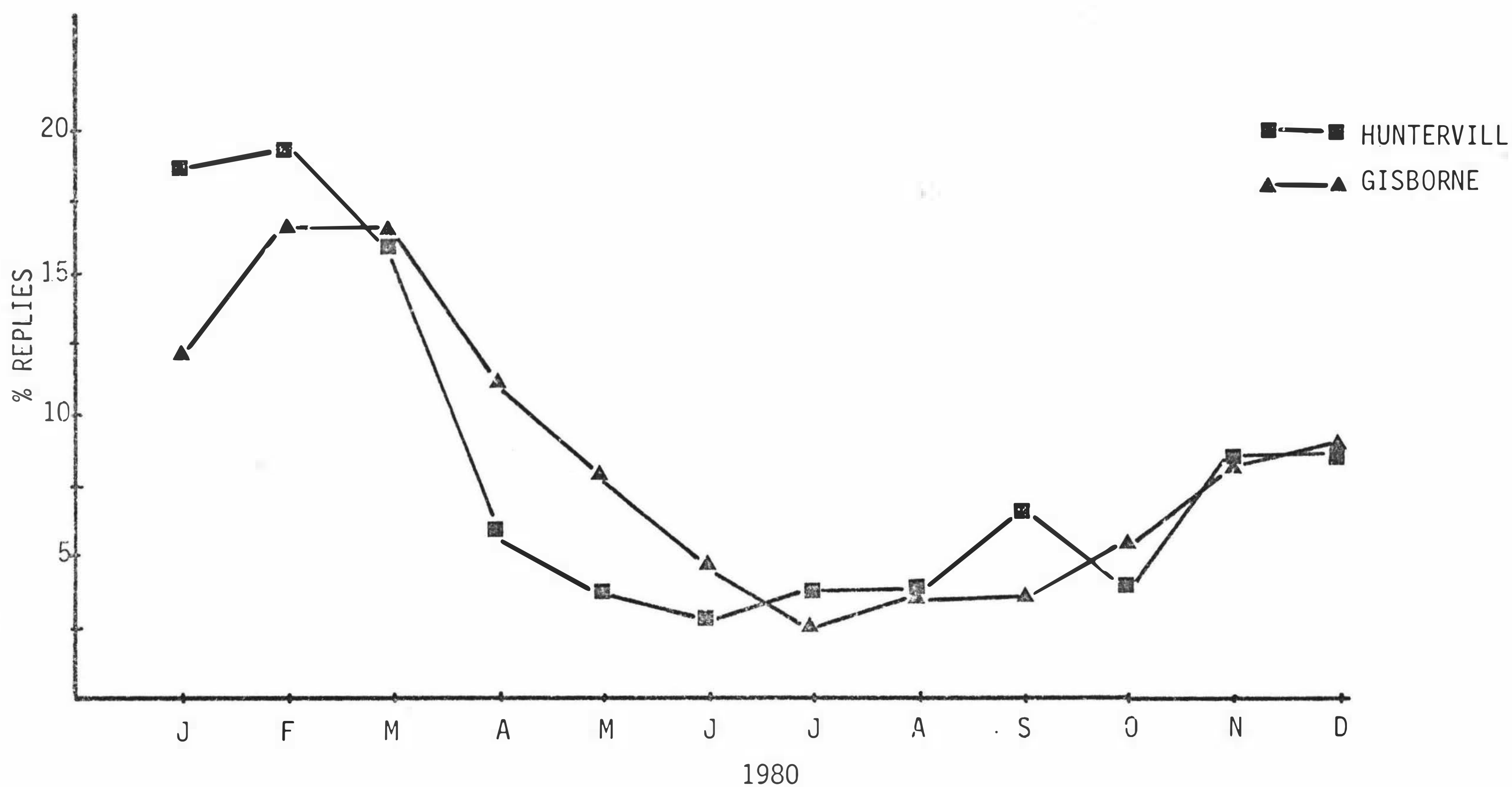
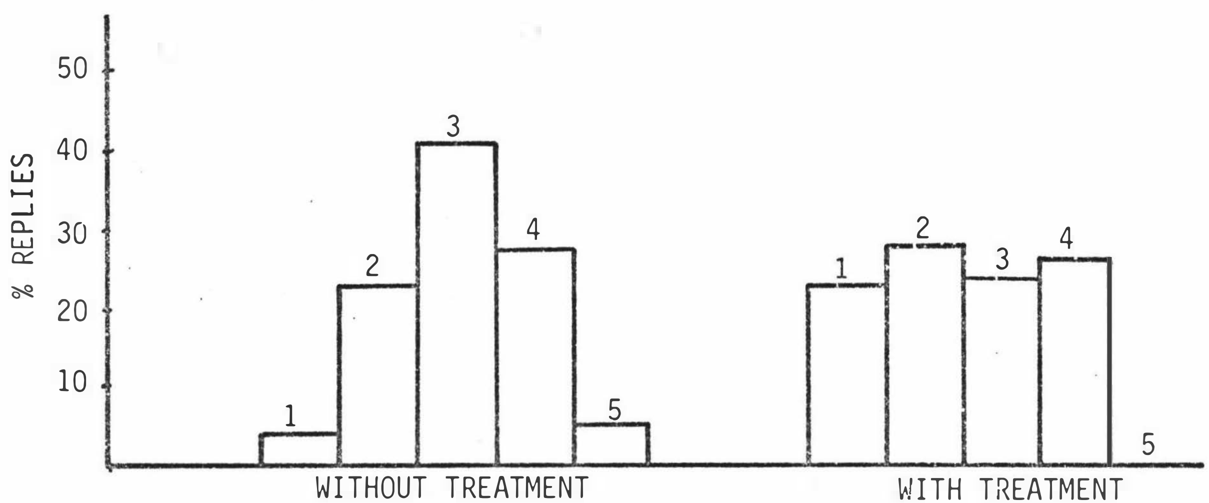


FIGURE II.2 OUTCOME OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS WITH REGARD TO ADMINISTRATION OF TREATMENT



KEY:

1. Eyes healed in 1-2 weeks
2. Eyes healed in 2-4 weeks
3. Eyes healed in > 4 weeks
4. Animals remained blind
5. Other - culled, died

CHAPTER 3

GENERAL MATERIALS AND METHODS

INTRODUCTION

PROCEDURE USED FOR THE COLLECTION OF SAMPLES AND IDENTIFICATION OF *MORAXELLA BOVIS*

EVALUATION OF EYE LESIONS

TREATMENT OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

STATISTICAL METHODS

INTRODUCTION

As the field study section of the investigation (Chapters 4 and 5) was concerned with the prevalence of *M.bovis* infections in relation to the occurrence of IBK, standardized sampling, processing and identification procedures had to be developed. Their description follows in this chapter. Eye lesions were classified into two categories to simplify the recording scheme, and a standard schedule of treatment was administered according to the method described. Statistical analysis of the information collected was undertaken using standard methods.

PROCEDURE USED FOR THE COLLECTION OF SAMPLES AND IDENTIFICATION OF *MORAXELLA BOVIS*

Collection of samples

1. Sterile cotton-wool tipped applicators (swabs) were used to obtain conjunctival sac secretions from the cattle under study. The swabs were immersed in sterile normal saline and excess fluid squeezed out before use.
2. With the cattle beast immobilised in a head bail, pressure was applied with the fingers, on to the upper eyelid and eyeball. This resulted in extrusion of the nictitating membrane and exposure of the ventral conjunctival sac. The swab was then placed over the nictitating membrane at the medial canthus, passed firmly along the lower border of the conjunctival sac and then returned to the medial canthus before removal.
3. Care was taken at all times to avoid contamination of the swab from any external surfaces of the animal.

4. The swab was applied directly to a 2% sheep blood agar plate and by gentle pressure and rotation a pool of fluid was left on the surface.
5. The procedure was then repeated for the other eye.
6. In the laboratory, the fluid sample on the blood agar plate was streaked out in the conventional manner using a bacterial loop.
7. Blood agar plates were incubated at 37°C for 18-24 hours (primary plates).

Examination of blood agar plates for *Moraxella bovis*

Most freshly isolated strains of *M.bovis* are β-haemolytic (Fig. III.1). Non-haemolytic colonies of *M.bovis* have also been recorded but usually after repeated subculture or following isolation from clinically normal eyes rather than associated with field outbreaks of IBK (Pugh,[82] 1966; Arora,[3] 1976; Fraser,[33] 1979). Because of the above factors and the very large number of plates having to be processed single handed, the investigation was confined to β-haemolytic isolates only.

In this thesis any reference to *M.bovis* refers to β-haemolytic strains unless otherwise qualified.

1. All β-haemolytic colonies on the primary plates were examined and colony morphology recorded.
2. A sample of each colony was emulsified with normal saline, smeared on a slide, Gram-stained and examined microscopically.
3. All the colonies showing a small, Gram negative diplobacillus characterstic of *M.bovis* were subcultured for 24 hours.
4. The primary plates were incubated for a further 24 hours and re-examined for any further β-haemolytic colonies.

Colony type

Cultures of *M.bovis* sometimes yielded two distinctly different colony types (Fig. III.2). The first type was rough; small (1mm at 24 hours), umbonate, firm, dry and autoagglutinating. The other was a smooth type; larger, flatter and of a mucoid character. The small colonies occasionally differentiated into the larger form on subculture.

Electron microscopy

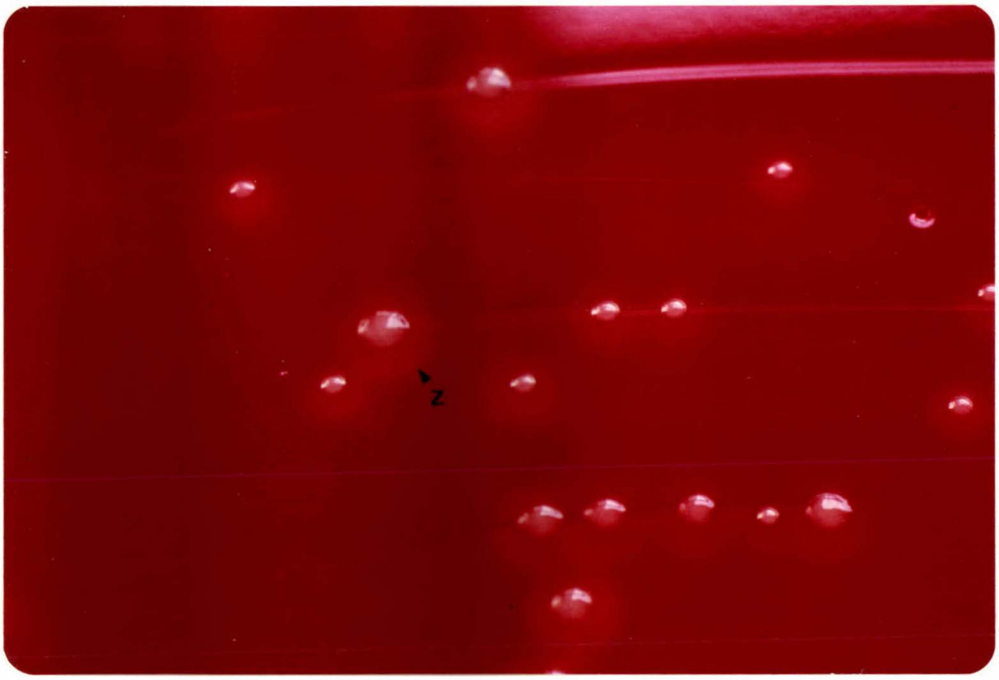
Two methods of electron microscopy were employed to examine bacteria from the different colony types for the presence of pili. Firstly, negative staining (Appendix III) was undertaken as a quick and relatively simple screening procedure using the Philips EM200 electron microscope, enabling numerous samples to be examined (Fig. III.3; Fig. III.4). Selected samples were then examined using a double fixation resin block sectioning method (Appendix IV) (Fig. III.5; Fig. III.6).

FIGURE III.1 β-haemolytic colonies of *Moraxella bovis* grown on 2% sheep blood agar at $37^{O}C$ for 24 hours

z = zone of β-haemolysis

FIGURE III.2 Rough and smooth colony types of *Moraxella bovis* grown on 2% sheep blood agar at $37^{O}C$ for 24 hours

r = rough
s = smooth


z

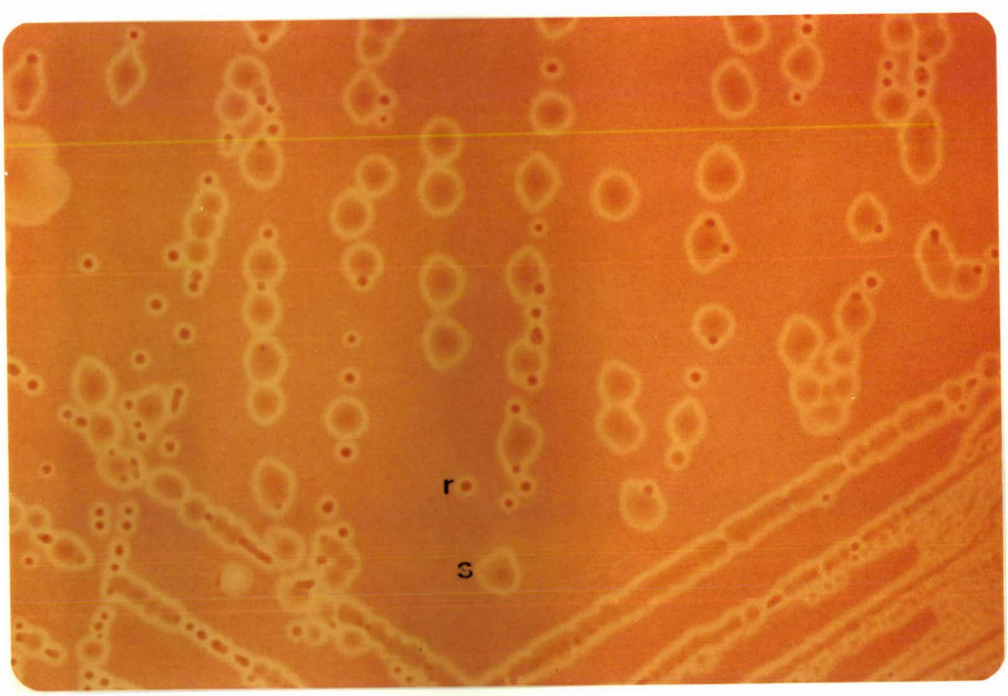

r
s

FIGURE III.3 *Moraxella bovis* from a rough colony using negative staining

p = pili x 87,500

FIGURE III.4 *Moraxella bovis* from a smooth colony using negative staining

x 17,500

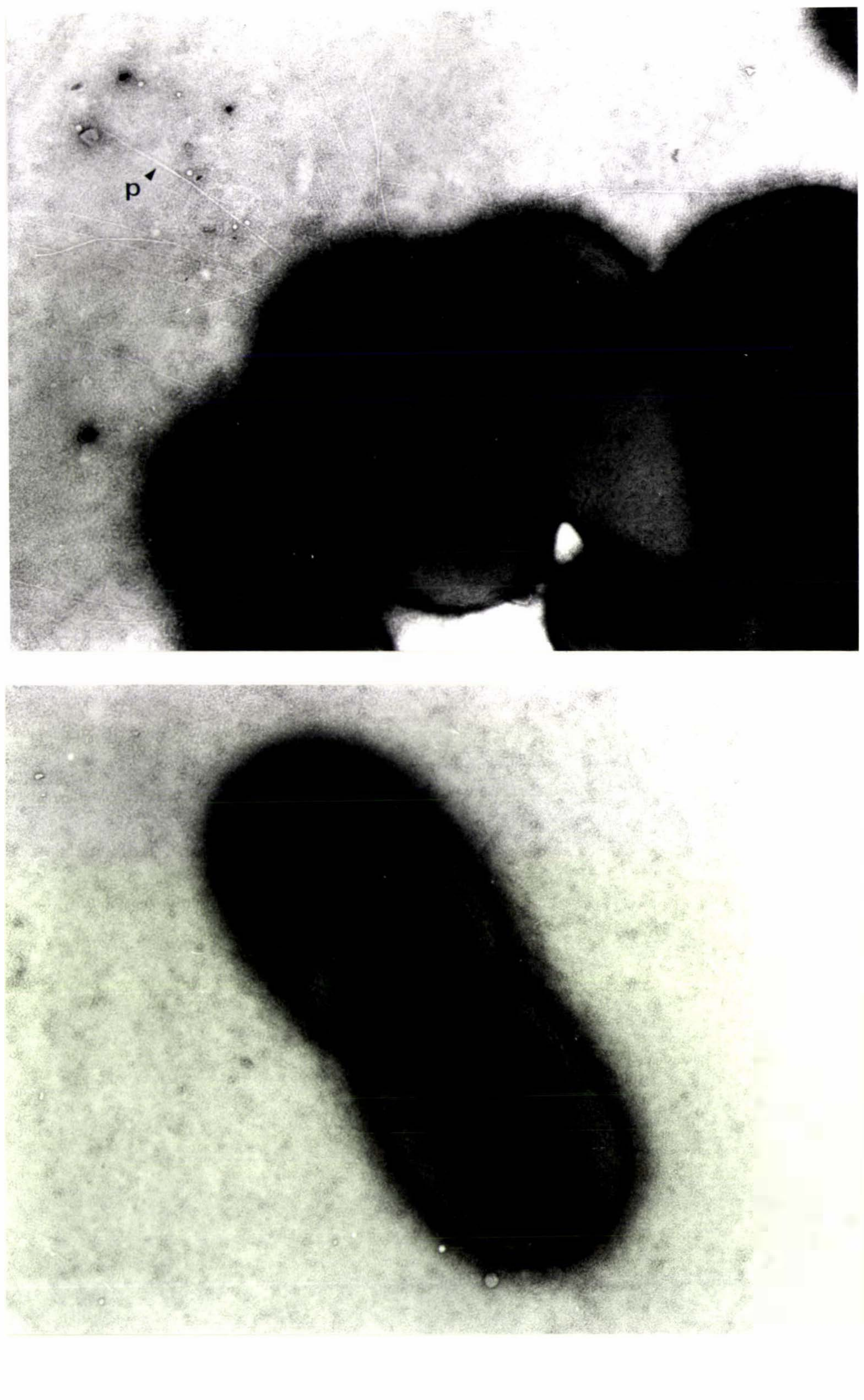

p

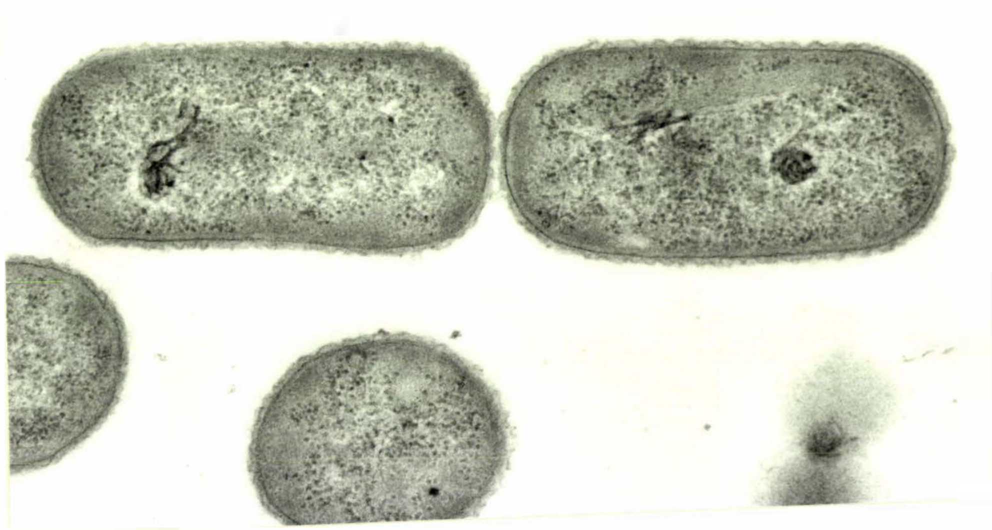
FIGURE III.5 *Moraxella bovis* from a rough colony using a block sectioning method

p = pili x 35,200

FIGURE III.6 *Moraxella bovis* from a smooth colony using a block sectioning method

x 159,820


p



Confirmation of *Moraxella bovis*

Once provisional identification of *M.bovis* had been made by colony and cell morphology, subcultures were subjected to a series of biochemical tests for confirmation.

Following an early period when all suspect *M.bovis* cultures were confirmed biochemically, it became impracticable to deal with every isolate, and thereafter approximately one third of suspect cultures, randomly chosen, were subjected to further investigation.

Every isolate subjected to biochemical testing proved to be *M.bovis* and so it was confidently assumed that all like isolates were *M.bovis*.

Biochemical reactions

Appropriate biochemical tests were selected from the 'CDC system' (King,[62] 1978) and on the basis of recommendations given by Pugh[82] (1966), Pedersen[71] (1970), Wilcox[107] (1970), Bovre[14] (1974), Arora[3] (1976) and Fraser[33] (1979).

Utilizing three tests, namely failure to ferment carbohydrate, failure to grow on McConkey agar and production of oxidase, it was possible to reduce the possibilities to 13 species of Gram negative bacilli. The remaining 12 were eliminated by two further tests, namely litmus milk digestion and nitrate reduction.

Biochemical media were inoculated with *M.bovis* suspended in heart infusion broth and were incubated at 37^{o}C for up to 10 days.

<u>Positive reactions brought about by *M.bovis* were as follows:</u>

1. <u>Carbohydrate fermentation</u> - *M.bovis* does not ferment carbohydrates. The carbohydrate chosen was dextrose, and incubation with *M.bovis* causes a colour change from green to blue, indicating that growth had produced an alkaline pH but that the dextrose remains unfermented (Fig. III.7).
2. <u>McConkey agar</u> - *M.bovis* does not grow on McConkey agar.
3. <u>Oxidase reaction</u> - *M.bovis* produces oxidase on growth. Colonies grown on blood agar are flooded with a 0.5% solution of tetramethyl-p-phenylene-diamine dihydrochloride and the purple staining of the colonies denotes a positive reaction (Fig. III.8).
4. <u>Litmus milk</u> - *M.bovis* causes a very characteristic alkaline reaction forming distinct clear bands at the top of the litmus milk. This change is followed by complete peptonization of the milk resulting in a clear homogenous purple coloured fluid after 5 to 10 days incubation (Fig. III.9 (a) and (b)).
5. <u>Nitrate reduction</u> - *M.bovis* does not reduce nitrate to nitrite, and growth of the organism in the indole nitrate solution is encouraged by the addition of 0.2ml cattle serum. Bacterial growth is visually assessed to avoid false negative results. The presence of persisting nitrate is demonstrated by the addition of zinc powder which turns red after 1 to 5 minutes (Fig. III.10).

FIGURE III.7 Non-fermentation of carbohydrates by *Moraxella bovis* as demonstrated by inoculation and incubation of dextrose medium

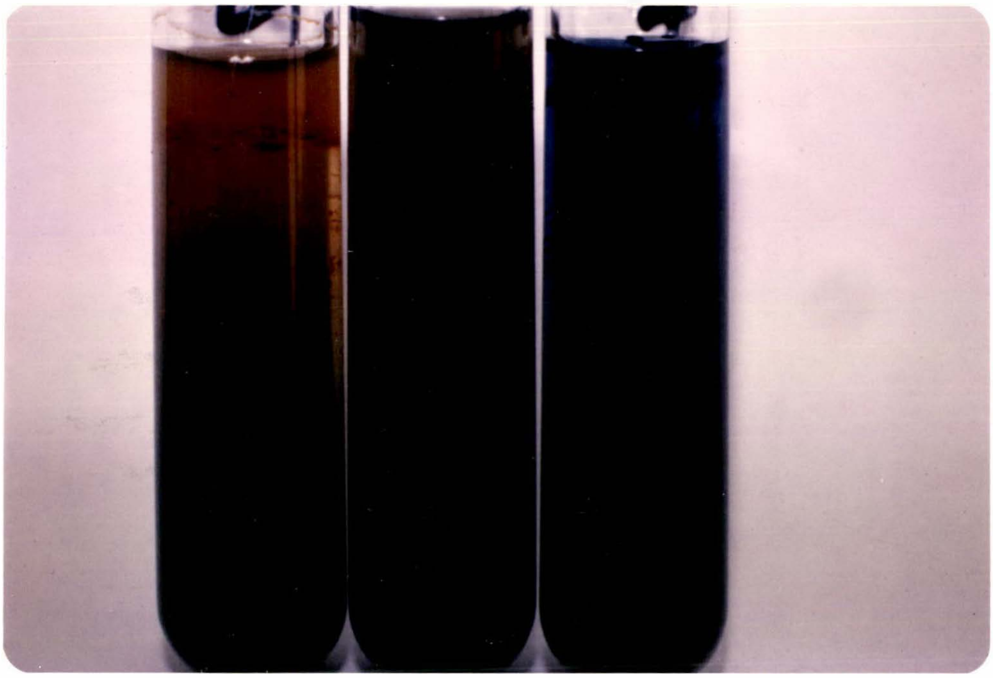
tubes: 1 = dextrose fermentation by a contaminant indicated by yellow colouration

2 = original colour of dextrose media

3 = growth of *M.bovis* causes an alkaline pH as indicated by blue colouration

FIGURE III.8 Oxidase production by *Moraxella bovis* colonies indicated by the purple colouration after the addition of the oxidase reagent

1 2 3



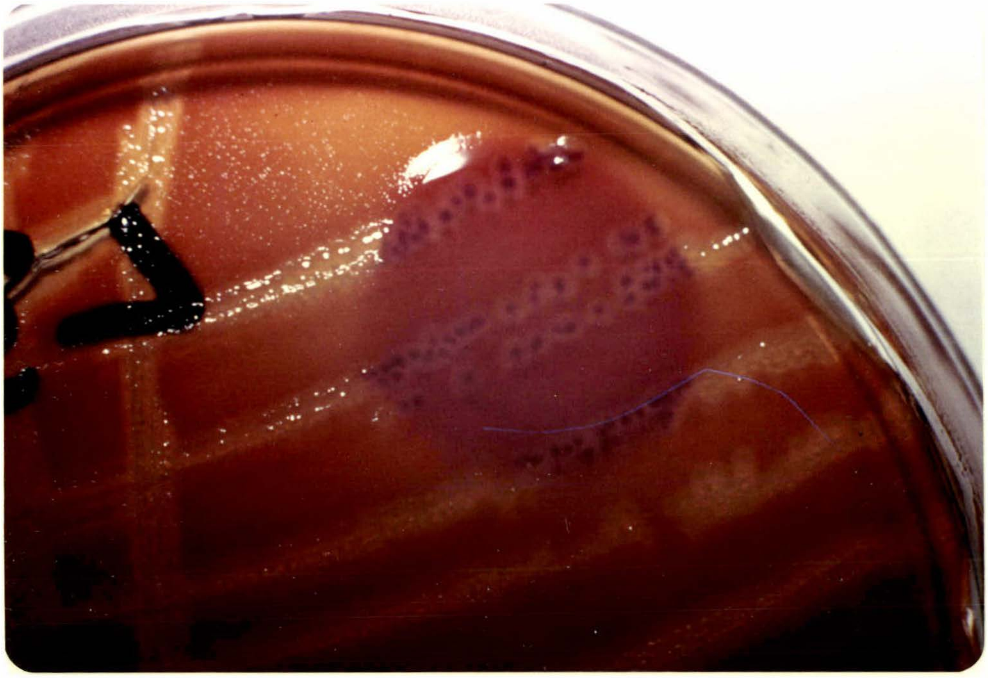


FIGURE III.9 Digestion of litmus milk by *Moraxella bovis*

(a) 24 hours incubation at $37^{o}C$

zones 1 = alkaline peptonization

2 = original colour of litmus milk

3 = formation of clot

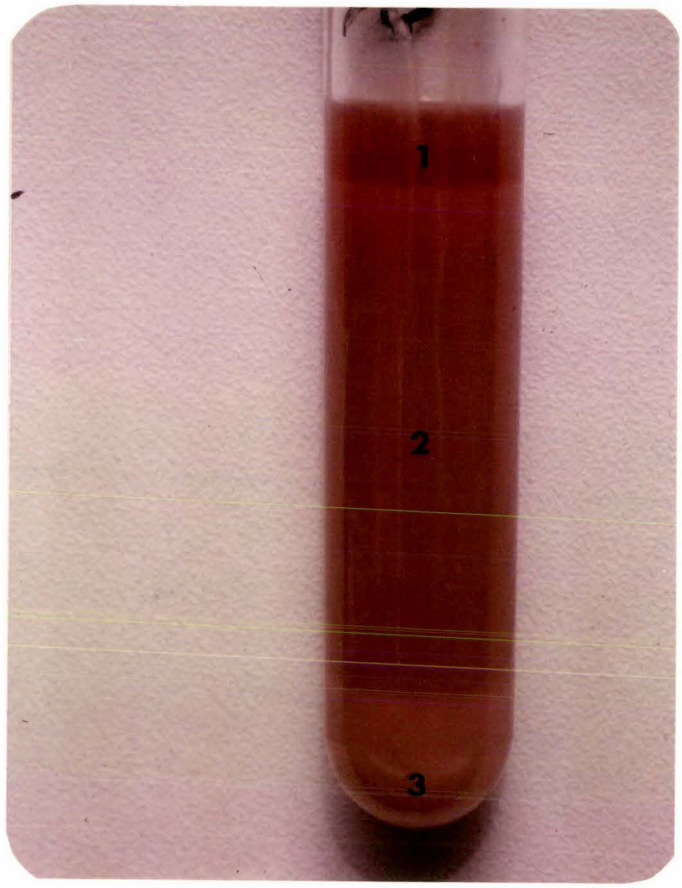
FIGURE III.9 Digestion of litmus milk by *Moraxella bovis*

(b) 120 hours incubation at $37^{o}C$

tube 1 = original litmus milk

tube 2 zones 1 = complete peptonization

2 = clot



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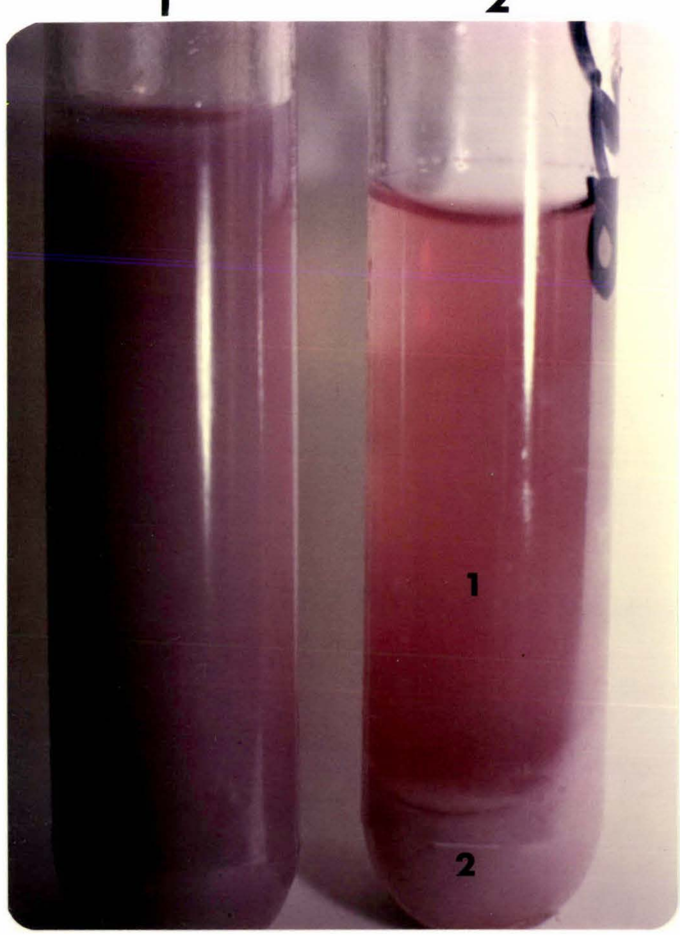
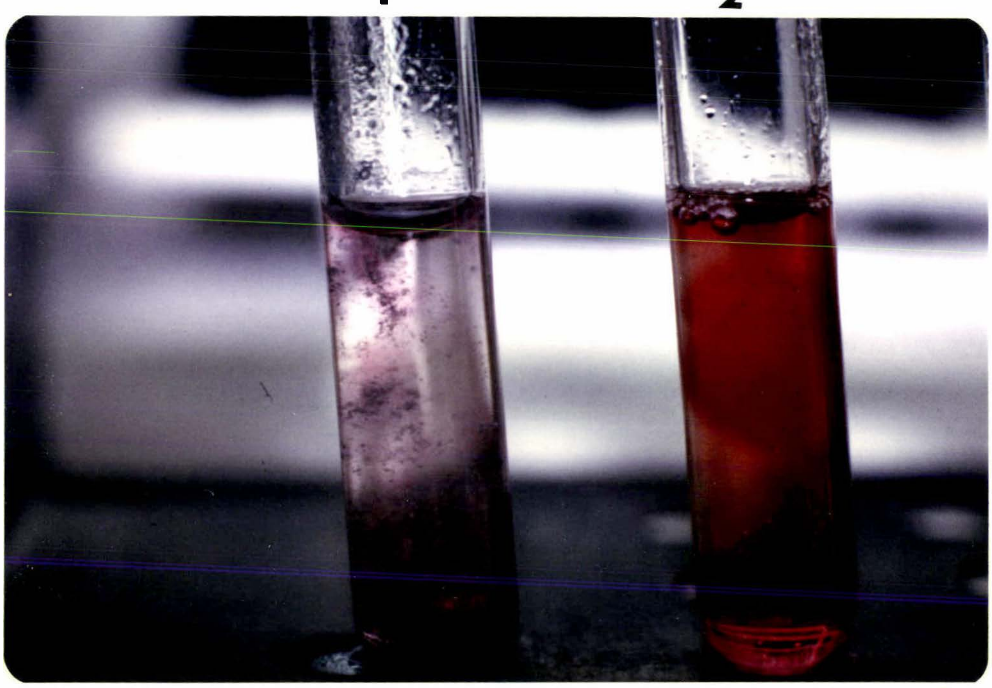


FIGURE III.10 The non-utilization of nitrate by *Moraxella bovis*

tube 1 = addition of zinc powder to media to demonstrate persistence of nitrates 5 days after inoculation with *Moraxella bovis*

tube 2 - red colouration indicates the reduction of the nitrate to nitrite by an unknown organism

1 2



EVALUATION OF EYE LESIONS

For the purposes of the study, the clinical signs observed were recorded as either mild or severe, according to the following criteria.

Eyes designated mild showed one or more of the following signs:

1. corneal ulcer(s) under 5mm in diameter (Fig. III.12);
2. corneal opacities without ulceration under 5mm in diameter (Fig. III.13);
3. no temporary blindness; and
4. lesions healed with no vascularization of the cornea.

Eyes designated severe showed one or more of the following signs:

1. corneal ulcers over 5mm in diameter (Fig. III.14; Fig. III.15).
2. complete corneal opacity (Fig. III.16);
3. temporary blindness;
4. resolution of lesions involving vascularization of the cornea (Fig. III.17);
5. formation of a descemetocele;
6. occasional prolapse of the iris and/or lens with permanent blindness consequent upon these changes; and
7. residual scarring of the cornea (Fig. III.18).

Irrespective of the final severity of the corneal lesion, most eyes showed conjunctivitis, blepharospasm and excessive lachrymation at some stage during the course of the disease.

<u>FIGURE III.11</u> A mild lesion of infectious bovine keratoconjunctivitis involving a small ulcer of the cornea

u = ulcer of cornea < 5mm in diameter

<u>FIGURE III.12</u> The use of fluorescein to demonstrate a small, shallow corneal ulcer


u

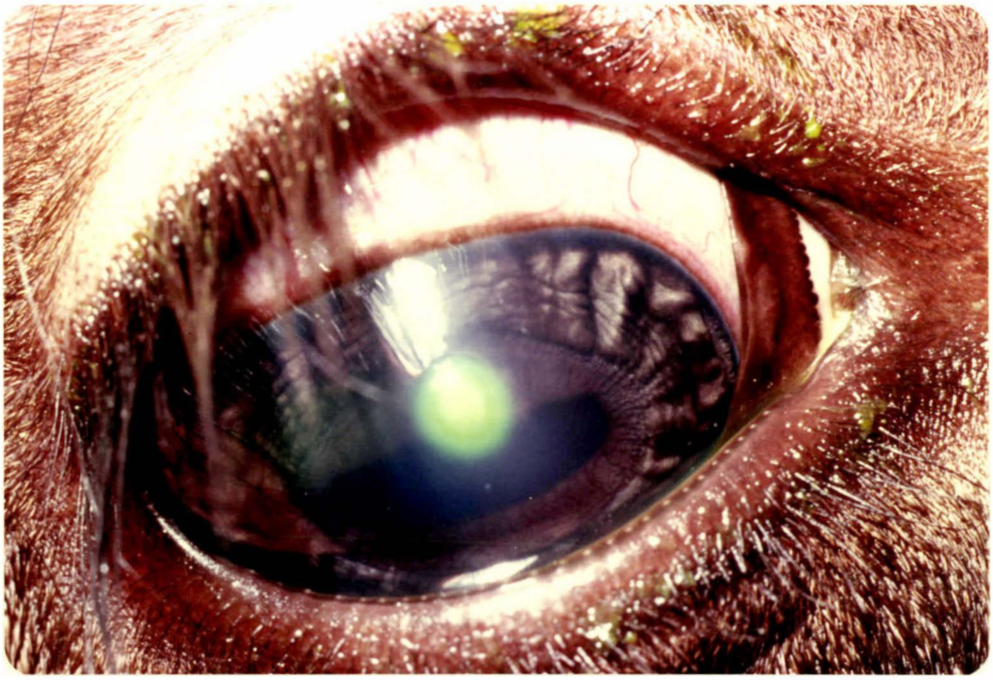
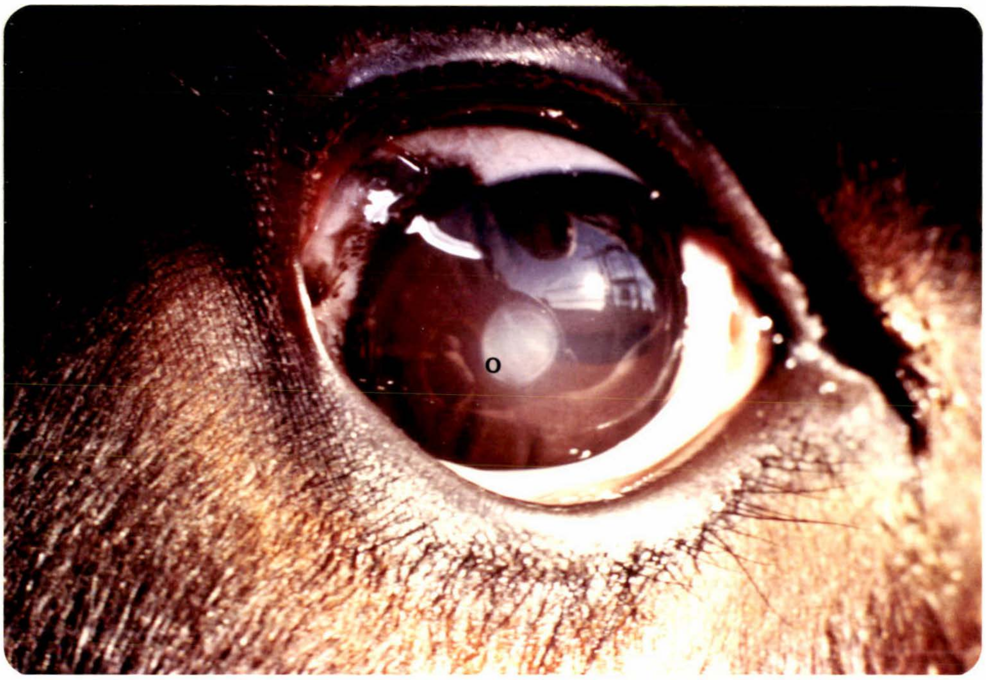


FIGURE III.13 A mild lesion of infectious bovine keratoconjunctivitis involving a small area of opacity in the cornea

o = opacity of the cornea < 5mm in diameter

FIGURE III.14 The progression of a mild lesion of infectious bovine keratoconjunctivitis to a severe form with increasing corneal opacity

O = developing corneal opacity


o


O

FIGURE III.15 A severe case of infectious bovine keratoconjunctivitis

o = complete corneal opacity

u = corneal ulcer > 5mm diameter

c = conjunctivitis

p = pannus formation

l = lachrymation

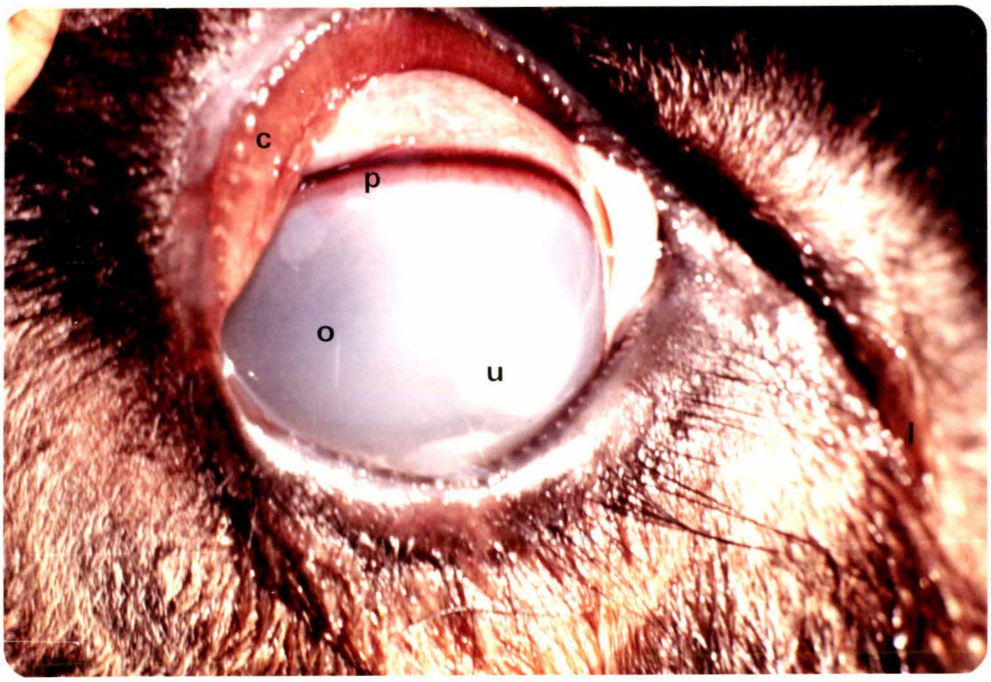
FIGURE III.16 A severe case of infectious bovine keratoconjunctivitis

o = complete corneal opacity

u = large deep corneal ulcer

p = well developed vascularization

l = lachrymation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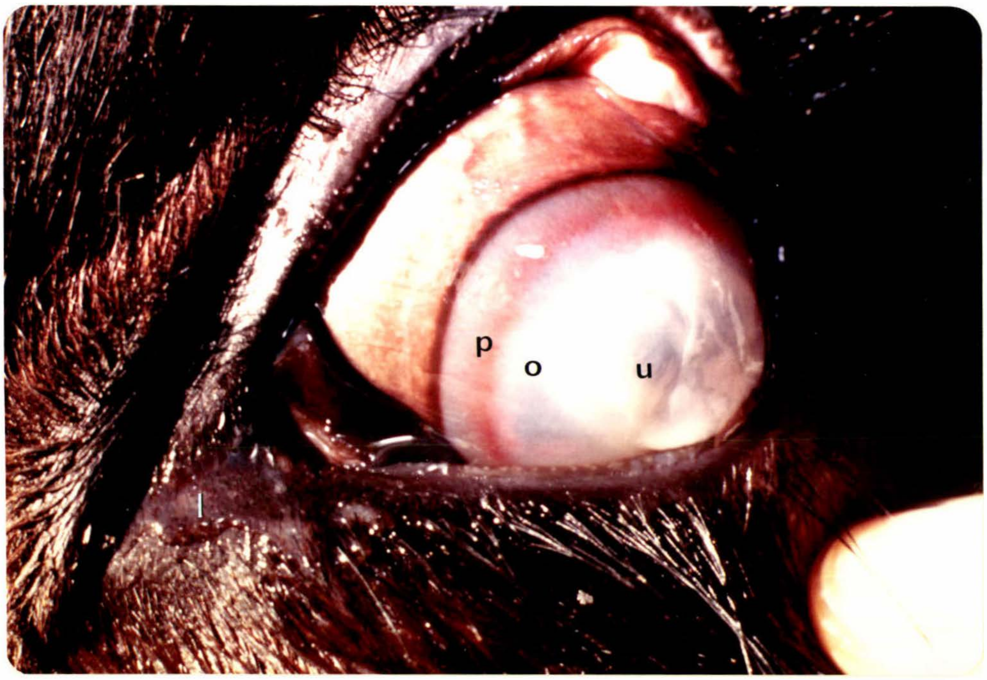
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l

<u>FIGURE III.17</u> A severe case of infectious bovine keratoconjunctivitis in the process of healing

o = opacity beginning to clear

v = well developed vascularization

h = healing ulcer

<u>FIGURE III.18</u> A residual corneal scar from a severe case of infectious bovine keratoconjunctivitis

s = scar tissue


o
v
h


s

TREATMENT OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

Throughout this project, the following method of treating cases of IBK was employed.

1. Subconjunctival injection of 2ml of a penicillin-streptomycin* mixture beneath the upper eyelid using a 2.5cm 20 gauge hypodermic needle.
2. The injection was administered by rolling the upper eyelid over the finger and injecting through and beneath the exposed palpebral conjunctiva. This technique left a visible bleb of the antibiotic product in the upper eyelid.
3. Both eyes of the animal were always treated.
4. Unless otherwise stated, only one treatment was administered.

STATISTICAL METHODS

Students t-test was used to compare means (Fischer,[31] 1928), and a 2 x 2 contingency table and Chi-squared test (Dawkins,[27] 1968) were used to examine the differences in the observed and expected frequency of events. Where expected values were five or less, Fischer's Exact method was used (Bailey,[5] 1959).

* procaine penicillin 250mg) per ml - Streptopen
and dihydrostreptomycin sulphate 250mg)
Glaxo New Zealand Limited, Palmerston North.

CHAPTER 4

PREVALENCE OF *MORAXELLA BOVIS* INFECTIONS AND INFECTIOUS BOVINE KERATOCONJUNCTIVITIS ON TWO MANAWATU BEEF FARMS (FARMS I AND II)

PREFACE

Moraxella bovis has been associated with outbreaks of IBK for a considerable period (Watt,[103] 1951) and at the present time it is generally accepted as the sole causative agent (Jackson,[58] 1953; Faull,[30] 1954; Gallagher,[36] 1954; Henson,[43] 1960). The organism can be readily isolated from cattle eyes during outbreaks of the disease, provided appropriate sampling procedures are adopted.

It was therefore planned that a major portion of the investigation into IBK should be devoted to a field study into the dynamics of *M.bovis* infections during naturally occurring outbreaks of IBK. Particular reference was to be paid to the relationship between the prevalence of *M.bovis* infections and the occurrence of eye lesions.

To achieve this aim, three farms in the Palmerston North area were chosen. These farms had a history of previous problems involving IBK, and if outbreaks were to continue it was hoped that they would provide an opportunity of studying any changes in the prevalence of *M.bovis* infections in relation to the number of animals showing overt signs of IBK. It was planned that on each farm a group of individually identified animals would be studied at regular intervals over a period of 12 months.

The investigations on two of the farms (Farm I and Farm II) were limited in their success, because of the absence of IBK for the duration of the study. Results from these two farms are recorded and discussed in this chapter. However, the outbreak of IBK on Farm III was typically serious for that property and it provided a great deal of important information which is reported separately in Chapter 5.

FARM I

Farm Profile

1. Location: 1.5 km along Kapikopiko Road, 20 km SW of Pahiatua.
2. Property size: 688 hectares.
3. Soil type: Matamou silt loam with clay subsoil.
4. Altitude: 61m - 242m asl.
5. Annual rainfall: 1770mm - 2020mm.
6. Pasture: ryegrass and clover on lower hills with brown top and native species on higher hills.
7. Stock numbers: 5620 stock units (3500 Romney breeding ewes, 1200 hoggets and 350 Aberdeen Angus cattle).
8. Management: This property operates a sheep and beef breeding and fattening enterprise. All replacement stock are bred on the farm, which functions as a closed unit.

History

Since 1979, IBK had been a continuing problem in the cows, 2 year olds and yearlings. During the winter of 1980 approximately 40% of a mob of 90 yearlings and the in-calf heifers were affected by IBK and treatment using subconjunctival injections of oxytetracycline* was given at this time. Confirmation of *M.bovis* infection was made on 25/11/80 by veterinarians from Massey University.

* Oxytetracycline - Terramycin LA, Pfizer Laboratories,
(200mg/ml) Auckland, New Zealand.

Investigation Procedure

It was planned to observe and sample at monthly intervals over a period of 12 months, a group of rising 2 year old heifers that had experienced IBK during the winter of 1980. However, the programme proved to be impracticable because of adverse weather conditions over the winter months and a reduced schedule had to be employed. Specimens of conjunctival sac fluids were collected and sampled according to the method described in Chapter 3, pg 46.

Results

Moraxella bovis was not isolated at any time during the five month period of study and the only eye lesions observed were in two heifers which had persistent corneal scars from a previous episode of IBK (Table IV.I).

The owner of the cattle, a competent stockman, reported that there were no signs of IBK in any of his other cattle over the same period, even though in previous years IBK had been common at that time.

Discussion

The rising 2 year old heifers had experienced IBK in June 1980 and confirmation of *M.bovis* infection was made in November 1980. It was expected that this infection would have been passed on to the next crop of calves.

As shown by the results of the first sampling (Table IV.I) infection was not passed on, possibly because of specific immunity transferred in the colostrum (Pugh,[90] 1980). The heifers had apparently shed their *M.bovis* infections by the time sampling commenced and were therefore no longer a source of infection for the calves.

Until early 1982 the farm was being managed as a closed unit and therefore it was assumed that the stock would remain free of *M.bovis*. However, there was a change in management practice which involved the purchase of a mob of affected steers and this led to the reintroduction of IBK on to the property.

FARM II

Farm Profile

1. Location: 3.2 km south of Shannon on State Highway 57.
2. Property size: 729 hectares.
3. Soil type: Grey wacke
4. Altitude: 73m - 545m asl.
5. Annual rainfall: 1136mm.
6. Pasture: ryegrass and clover on flat land with native grasses and clover on hill country.
7. Stock numbers: 6600 stock units (5000 Perendale ewes and 400 Hereford and Aberdeen Angus (cross) cattle).
8. Management: This property operates Perendale and Hereford studs with surplus stock being fattened. All replacement stock are bred on the farm and therefore it functions as a closed unit.

History

Farm II experienced its first outbreak of IBK in September 1978 amongst the stud Hereford cattle. Because of the serious nature of the outbreak, veterinary consultation was sought and confirmation of widespread *M.bovis* infection was made by veterinarians from Massey University on 3/9/78. Treatment of affected stock with subconjunctival infections of a penicillin-streptomycin mixture (Chapter 3, pg 62) was undertaken at this time.

Investigation Procedure

Thirty Hereford stud cows and their calves were available for the study period. Sampling was undertaken at approximately monthly intervals from January to June 1981 until calving was imminent, at which time it became undesirable to muster the stock on the hills. During the period of calving, namely August to October, observation by the farmer was relied upon to detect any IBK. A further sampling of the cows and their new-season calves was undertaken in November 1981.

Results

Six of the 30 cows observed showed residual corneal scars from previous IBK infections and the lesions persisted for the entire length of the study. At no time over the 11 month period were there any further lesions of IBK recorded (Table IV.II).

The only visit that yielded *M.bovis* isolates coincided with the appearance of excessive lachrymation amongst several animals but no conjunctival or corneal lesions appeared then or later. One of the cows from which *M.bovis* was isolated, exhibited signs of severe photosensitization at the time of sampling on 25/2/82.

Discussion

At the time of the first examination there was no active clinical IBK and sampling did not yield *M.bovis*. The second sampling, however, revealed two cows that were infected with *M.bovis* although there were still no lesions indicative of IBK. From this low level of infection amongst the cows it was anticipated that infection might well spread to the calves and initiate an outbreak of IBK. This possibility was considered likely because of the highly contagious nature of *M.bovis* to stock of this age. They were approximately 6 months old by which time any maternally derived immunity would have waned (Pugh,[90] 1978).

One possible explanation for the spontaneous and temporary nature of the *M.bovis* infections is that the organism may have reverted to a non-haemolytic state and thereafter would not have been identified by the standard methods adopted (Chapter 3, pg 47). Alternatively, some form of stress on the cows may have provoked an ascending infection from remote sites in the nares. Either the non-haemolytic form and therefore non virulent status of the organism (Arora,[4] 1976) or residual immunity from previous exposure to *M.bovis* appeared to prevent further dissemination of the disease. At the completion of the study, no active IBK had been observed and the stock were apparently clear of *M.bovis* infection (Table IV.II).

SUBSEQUENT HISTORY ON FARM II

Three months after the completion of the planned study (7/3/82), the owner reported a suspected outbreak of IBK on this farm. He had observed one animal amongst a mob of nine bulls showing signs which could be attributed to IBK, namely; excessive lachrymation, blepharospasm and corneal opacity with resulting blindness.

The affected 18 month old bull had been sampled five times from 28/1/81 to 14/5/81 and on every occasion was clear of *M.bovis* infection as well as never having shown signs of IBK. This single mob of nine animals had been run as an isolated unit since June 1981 without any further introductions or contact with other stock.

Investigation Procedure

On the 8/3/82, all nine animals were examined closely for eye lesions and sampled for *M.bovis*. Mass treatment was commenced immediately using a penicillin-streptomycin mixture administered subconjunctivally (Chapter 3, pg 62) and the same schedule was repeated 96 and 144 hours later.

The cattle were maintained in isolation and re-sampled on the 17/3/82 (i.e. 3 days after the last treatment).

Results

Clinical examination confirmed the one obvious case of severe IBK (definition Chapter 3, pg 57): this was the bull first noticed by the farmer. Four other bulls showed degrees of minor corneal involvement. Of the nine animals, six had *M.bovis* infections in one or both eyes (Table IV.III).

By 17/3/82, and following treatment, no *M.bovis* could be isolated, one eye lesion had healed completely and the remainder of the lesions had not developed further.

The farmer has subsequently reported that there has been no further IBK in this group of cattle or amongst any other cattle on his farm.

Discussion

The source of the outbreak of IBK affecting the nine bulls was not determined. The farmer runs a closed unit and there had been no new cattle introduced on to the farm. In spite of regular sampling of cattle on the farm over the study period, *M.bovis* had not been isolated since 25/2/81, i.e. some 13 months previously. Therefore, the most likely source of this infection may have been affected cattle on neighbouring properties. Although neighbouring farmers reported their stock to be unaffected by IBK, some animals might have been acting as clinically normal carriers of the disease. It was not feasible to eliminate such a possibility within the scope of this study.

Mass treatment introduced at an early stage of the outbreak appeared to be most successful. Handling facilities were good and the owner was an enthusiastic, knowledgeable farmer who was keen to attempt eradication of *M.bovis* infection. To be sure of success, sampling should be continued for some weeks, include other more remote sites in which *M.bovis* might lodge, and the non-haemolytic strains of *M.bovis* should be accorded the same priority as the more easily identifiable forms.

TABLE IV.I PREVALENCE OF *MORAXELLA BOVIS* AND INFECTIOUS BOVINE KERATOCONJUNCTIVITIS ON FARM I DURING THE WINTER AND SPRING OF 1981

DATE SAMPLED	CATTLE SAMPLED	TOTAL NUMBER OF CATTLE SAMPLED	NUMBER OF *M. BOVIS* ISOLATIONS	NUMBER OF IBK LESIONS
8/4/81	17 rising 2 year old heifers & 17 calves	34	0	0*
29/4/81	same 34 animals as 8/4/81	34	0	0*
5/8/81	previous 17 calves plus 20 additional calves	37	0	0*
23/9/81	same 37 animals as 5/8/81	37	0	0*

* two heifers showed corneal scars resulting from IBK during the previous winter

TABLE IV.II PREVALENCE OF *MORAXELLA BOVIS* AND INFECTIOUS BOVINE KERATOCONJUNCTIVITIS ON FARM II DURING 1981

DATE SAMPLED	CATTLE SAMPLED	TOTAL NUMBER OF CATTLE SAMPLED	NUMBER OF *M. BOVIS* ISOLATIONS	IBK LESIONS
28/1/81	30 cows & 30 calves	60	0	0*
25/2/81	30 cows & 30 calves	60	2 (cows)	0*
25/3/81	30 cows & 30 calves	60	0	0*
22/4/81	30 cows & 30 calves	60	0	0*
14/5/81	30 cows & 30 calves	60	0	0*
25/11/81	the same 30 cows but 30 new-season calves	60	0	0*

* six cows had corneal scars from previous IBK

TABLE IV.III PREVALENCE OF *MORAXELLA BOVIS* AND INFECTIOUS BOVINE KERATOCONJUNCTIVITIS AMONGST YOUNG BULLS ON FARM II DURING A SPONTANEOUS OUTBREAK AND FOLLOWING TREATMENT

	DATE OF EXAMINATION 8/3/82	17/3/82
Total number of animals	9	9
Number showing IBK	5	4
Number of *M.bovis* isolations	6	0*

* all animals had been treated in both eyes on three occasions

CHAPTER 5

INVESTIGATIONS INTO THE EPIDEMIOLOGICAL FACTORS OF AN INFECTIOUS BOVINE KERATOCONJUNCTIVITIS OUTBREAK UNDER FEEDLOT CONDITIONS (FARM III)

INTRODUCTION

FARM PROFILE

HISTORY OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS ON THE PROPERTY

OBJECTIVES OF THE INVESTIGATION

MATERIALS AND METHODS

RESULTS

DISCUSSION

TABLES AND FIGURES

INTRODUCTION

The farm chosen for this section of the investigation offered the best opportunity for study as there were 400 cattle available, and they were readily accessible in a feedlot close to cattle yards. The farmer offered every cooperation for the study because IBK had been a recurrent problem amongst cattle on this property for a number of years. The likelihood of another outbreak occurring under these same conditions was considered to be high.

FARM PROFILE

1. Location: Green Road, 2.4 km from Awahuri, 10 km north of Palmerston North.
2. Property size: 173 hectares of effective farm land divided into 2 blocks: home farm - 95 hectares and run off - 78 hectares.
3. Soil type: home block - Karapei silt loam
   run off - Kairanga silt loam.
4. Altitude: 45m asl.
5. Annual rainfall: 940mm.
6. Pasture: ryegrass and white clover.
7. Stock numbers: 6518 stock units (5000 wether lambs and 1530 cattle).
8. Management: since about 1966, this farm had employed a feedlot system over the winter months in order to increase the carrying capacity at that time of year. During April and May between 1200 and 1600, 6 month old male cattle were purchased and in June, 800 of these were placed in the feedlot, the remainder being left on pasture. Whilst in the feedlot the stock were fed once daily with hay, at a rate of 2.72kg per animal, and chopped chou mollier at a rate of 13.6kg per animal. All cattle were drenched with an anthelmintic at 3 weekly intervals during

the period April to August. From August onwards the better conditioned animals were sold in batches and by October the remaining cattle had been put back on to pasture.

HISTORY OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS ON THE PROPERTY

For the past seven years (i.e. since 1974), IBK had been a serious problem in the cattle on this farm, especially amongst the cattle in the feedlot. Great care was always exercised by the farmer when buying the weaners, to avoid mobs showing any signs of IBK. Despite this precaution, during each winter there had been serious outbreaks of IBK. Various forms of treatment, which included topical antibacterial agents as well as antibiotic subconjunctival injections, had been employed at different times but with variable success, and IBK remains as this farm's largest single animal health problem.

OBJECTIVES OF THE INVESTIGATION

The overall aim was to study the epidemiological features of an outbreak of IBK and from the results obtained to determine suitable control measures that might be introduced. In general, the farmer wished to avoid having to make any change in their current farming practices. Accordingly the more specific objectives of the investigation were to determine the extent of infection at various times relative to the number of cattle exhibiting overt IBK, with a view to establishing a practical control programme.

MATERIALS AND METHODS

Preliminary study

The six-month old weaner calves were purchased over a period of approximately 2 months from 1/4/81 to 28/5/81. A random selection of animals were sampled according to the procedure described in Chapter 3, pg 46, to give some indication of the prevalence of *M.bovis* infection amongst the stock on arrival.

Selection of feedlot animals on 8/6/81

1. All 1600 weaners were run through the yards and by a visual appraisal of body condition were selected into three groups; good (400), average (800) and poor (400).
2. The 800 weaners of average condition were returned to pasture for wintering.
3. The remaining 800 were divided into four mobs of 200 animals (2 x 200 of good condition and 2 x 200 of poor body condition). Each group of 200 was then allocated to a pen in the feedlot where they would remain for the next 2 to 4 months (Fig. V.1).

Identification for observation and sampling on 10/6/81

1. Two pens closest to the yards, each containing 200 weaners, were chosen for subsequent investigation (Pen A and Pen B).
2. Cattle were identified with numbered blue eartags apart from every fourth animal which was identified by a yellow eartag.
3. All 100 yellow tagged animals had their body weights recorded.
4. All 400 tagged animals had their breed recorded.
5. The weaners in Pen A were of good body condition and those in Pen B were of poor body condition.

Procedure for observation and sampling

Every second week, cattle in Pen A and Pen B were mustered separately and all animals put into the head bail where their eyes were examined thoroughly for any lesions. The yellow tagged animals had their eyes sampled for *M.bovis* (Chapter 3, pg 46) and any cases of IBK were treated as they arose, according to the method described (Chapter 3, pg 62).

On the alternate weeks, all 400 animals were observed from the central race as they approached the feeding troughs, and the presence of any eye lesions were recorded. Ample trough space enabled all animals to feed simultaneously and provided they were observed from a distance of approximately one metre, they remained quiet for about 1 hour, enabling adequate inspection.

This procedure for observation and sampling continued for 8 weeks until 6/8/81, by which time most of the cattle in Pen A had been sold. Thereafter the cattle in Pen B continued to be studied for a further 15 weeks, until 2/11/81, but without observations being made from the central race (Fig. V.2).

Group treatment

On completion of the main study (6/8/81), the cattle in Pen B having even numbers, were treated (Chapter 3, pg 62) in both eyes irrespective of whether they exhibited IBK.

RESULTS

Preliminary study

There was a high prevalence of *M.bovis* amongst certain groups of cattle arriving on the farm (Table V.I), whereas other consignments were free both of signs of IBK and *M.bovis* infection.

Main study

The prevalence of *M.bovis* infections in the cattle of both mobs (Pen A and Pen B) increased markedly over the 8 weeks of the study period (Table V.II (a) and Table V.II (b)). The level of *M.bovis* infection in Pen A rose immediately, whereas in Pen B there was no infection initially, but after 4 weeks the outbreak followed a similar pattern as in Pen A (Table V.II (a); Table V.II (b); Fig. V.3). A marked drop in *M.bovis* infections occurred following treatment of half of the cattle in Pen B, but this reduction was only temporary with a return to the former level within one month.

Many animals yielded *M.bovis*, some of them repeatedly, but the same animals at no time showed any signs of IBK (Table V.III). The prevalence of IBK rose, about a month after the rise in *M.bovis* isolations, but was always at a much lower level than the prevalence of infection (Fig. V.3). There was no difference in the proportion of each breed that did not show signs of IBK; there was also no significant difference in the proportion of each breed exhibiting severe* as opposed to a milder* form of IBK (Table V.IV). Over the whole period of investigation and including both pens, there was almost an equal number of either mild or severe cases of IBK; 19% and 20% respectively.

* mild and severe forms of IBK are defined in Chapter 3, pg 57.

Of all cattle that exhibited *signs* of IBK, the greatest number were unilaterally affected (Table V.V), yet 68% of *M.bovis* infections of cattle in Pen A were bilateral.

Every yellow tagged animal was infected with *M.bovis* in either one or both eyes at some stage of the study period (up to 2/12/81).

At least 6% of cattle eyes in Pen A were positive for *M.bovis* on each of the five sampling occasions (Table V.VI), whereas only 12% of eyes were negative on every occasion. Pen B could not be analysed in the same way because of the probable influence of treating half of the animals on 6/8/81 (Fig. V.3).

Based on the proportion of each breed represented in the yellow tagged animals, they were a truly representative sample of the total population.

Weight losses by the feedlot cattle

Over a period of 9 weeks the cattle in Pen A lost an average weight of 20kg and those in Pen B, 10kg. Animals affected with IBK lost more weight, but the mean differences were not significant.

DISCUSSION

The results of the preliminary study demonstrated that *M.bovis* infection was introduced on to the farm with the weaners purchased for the feedlot. The introduction of *M.bovis* occurred despite a conscientious effort by the stockman to avoid buying animals that exhibited eye lesions. The possibility of *M.bovis* infection being carried by clinically normal animals had not been considered at the time of purchase, but was subsequently shown to be a significant factor in the development of the disease on the property.

When the cattle had been allocated to the feedlot pens and the investigation proper had commenced, the prevalence of *M.bovis* infection increased rapidly. The animals were in close contact for much of the time because of the high density stocking and trough feeding system in the feedlot, and this provided an excellent opportunity for the spread of infection.

The different times of onset of the IBK outbreaks in the two pens could be attributed to the apparently lower initial level of *M.bovis* infection amongst the stock in Pen B. *Moraxella bovis* was not isolated from the yellow tagged animals in Pen B in the first two samplings, indicating that either the whole mob was clear of *M.bovis* infection or the prevalence was very low, or more probably *M.bovis* was present but in a non-haemolytic form and thus passed undetected. The latter would be the most likely explanation (Arora,[4] 1976), but it was not within the scope of this project to identify non-haemolytic *M.bovis* (Chapter 3, pg 47). If the entire mob of cattle in Pen B was clear of *M.bovis*, then the most probable source of infection would have been the cattle in the other pens. This spread of infection would most probably have occurred by mechanical transmission in the yards or by the stockman, as there was no direct contact between the groups of animals.

Despite the difference in time of onset of the outbreaks in each pen, the dynamics of the *M.bovis* infections and the rate of development of clinical cases of IBK were very similar. The peak prevalences recorded for both *M.bovis* and IBK were comparable to results obtained by researchers overseas (Hughes and Pugh,[47] 1970; Bryan,[18] 1973; Arora,[4] 1976). The results suggest that under feedlot conditions, outbreaks of IBK occurring in New Zealand may be following much the same pattern as those observed in other countries.

The prevalence of *M.bovis* infection was always higher than the prevalence of IBK, many animals having *M.bovis* infections without showing signs of IBK (Bryan,[18] 1973; Lepper,[65] 1980). Some of the infected animals subsequently developed IBK, from a few days to many weeks after infection was first noted, whereas others never developed the disease. Such differences in response to *M.bovis* infection suggest that some precipitating factor may enable *M.bovis* to become pathogenic, possibly through the release of unknown factors causing corneal damage. This very complex aspect of the disease might involve the influence of factors derived from the host, the organisms, or a combination of the two. Host immunity both local and systemic, effects of eye trauma of varying types, stress on the animal and modifications in the virulence of the organism are but a few of the predisposing factors that might determine the eventual outcome of an *M.bovis* infection. The interaction of the host and the infective organism is an aspect of IBK which needs far more attention and would be a worthy study area.

The notion that some initiating trauma to the cornea is required before *M.bovis* becomes fully virulent could provide an explanation for the low number of bilateral cases of IBK accompanied by the high prevalence of bilateral *M.bovis* infections. In Pen A, only 26% of the cases of IBK were bilateral but 68% of the *M.bovis* infections were bilateral. The reason for the marked difference between the occurrence of signs and *M.bovis* infection demands further attention because such a study could reveal the initiating factor(s) for IBK.

From the extended study on cattle in Pen B, it was clear that the animals that had recovered from IBK either spontaneously or following treatment, might still carry *M.bovis* in their eyes. For the time of the study, i.e. 20 weeks, infection persisted, and it could be argued therefore that such animals might continue to have *M.bovis* infection for an indefinite period during which time they might well be a source of infection to other groups of susceptible animals (Farley,[29] 1944; Baldwin,[6] 1945; Jackson,[58] 1953; Gallagher,[36] 1954). Such a situation had occurred amongst cattle purchased for the feedlot on this farm and it confirms that the 'carrier' state of *M.bovis* in cattle is an important method by which the disease might be spread (Adinarayanan,[1] 1961; Lepper,[65] 1980).

Treatment of half of the animals in Pen B resulted in an immediate and substantial reduction in the prevalence of *M.bovis* (Fig. V.3), but within a month the overall infection rate had returned to its former level. Results from this study show that a large number of animals are likely to be infected with *M.bovis* at a time when only a few individuals are exhibiting IBK. Therefore, the re-infection of treated animals in Pen B was most probably from contact with the non-treated infected animals remaining in the group. As *M.bovis* is known to be susceptible to a wide range of antibiotics (Barner,[8] 1952; Faull,[30] 1954; Gallagher,[36] 1954; Pugh,[80] 1977; Webber,[107] 1981), it is tempting to speculate that had all cattle been treated using a proper treatment schedule, then the organism might have been eliminated from this group of animals. Such mass treatment of all animals at the initial stage of an attack should avoid a serious outbreak of IBK if treatment is wholly successful. A further advantage of such tactics would be the removal of the 'carrier' status of some animals, thereby removing the risk of further spread of the disease.

Mass treatment of all individuals in a mob was carried out by Pugh[93] in 1982, but the outcome was not completely successful. In view of the treatment schedule adopted, it was possibly inadequate to totally remove *M.bovis*. If treatment is carried out thoroughly on all animals it offers the most suitable control measure currently available to farmers in New Zealand.

Several overseas researchers (Frisch,[35] 1976; Caspari,[19] 1979; Ward,[104] 1979; Pugh,[93] 1982) have referred to a decrease in prevalence and severity of IBK with increasing eyelid pigmentation. Results from this study however, do not demonstrate any difference in prevalence or severity of disease between the breeds involved (Table V.IV). Any subtle influence that breed might have played, could well have been masked by the serious nature of the outbreak. These observations do not necessarily refute the findings of the survey questionnaire in which there appeared to be some breed influence on the occurrence of IBK (see survey results Chapter 2, pg 25).

A production loss associated with IBK could not be demonstrated because of the very large between-animal differences and the poor condition of all the animals involved. However, from simply observing the animals, it was obvious that the severe pain caused by IBK stopped many animals foraging for a few days. Enforced starvation becomes particularly important in the feedlot situation where food is only available for a few hours each day.

The temporary blindness associated with the IBK caused some stock handling problems. Complete blindness persisted in some of the animals and meant that they had to be farmed with particular consideration for their welfare.

When the feedlot cattle were sold, they were drafted into mobs according to body size and whether or not they showed eye lesions. Hence, there were several mobs that showed no visible signs of any previous IBK but they may have been carrying *M.bovis* infections. The apparently disease-free animals may therefore have been a potential source of infection for a new outbreak of IBK.

Footnote

Following yet another serious outbreak of IBK in the winter feedlot during 1981 (the period of the study), the farmer decided to change to an all-grass wintering system and a reduction in cattle numbers for 1981. The change in management practice coincided with the first winter free of IBK and produced stock in a much improved condition at the time of sale in the spring.

TABLE V.I POINT PREVALENCE OF *MORAXELLA BOVIS* INFECTION AND OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS IN CATTLE ARRIVING ON FARM III

	DATE OF EXAMINATION 1/4/81	15/4/81	27/5/81
Number of animals examined/total	64/64	70/345	71/500
Number of animals showing IBK (%)	14 (22)	0 (0)	10 (14)
Number of animals *Positive/number sampled (%)	5/5 (100)	0/70 (0)	49/71 (69)

* *M.bovis* isolated from one or both eyes

TABLE V.II(a) ISOLATIONS OF *MORAXELLA BOVIS* FROM CATTLE IN THE FEEDLOT PEN A

	DATE OF EXAMINATION 10/6/81	25/6/81	9/7/81	22/7/81	6/8/81
Number of animals *Positive/Number Sampled (%)	13/50 (26)	27/48 (56)	35/47 (74)	42/48 (87)	31/45 (69)
Bilateral *M.bovis* infections %	62	42	49	48	64

TABLE V.II(b) ISOLATIONS OF *MORAXELLA BOVIS* FROM CATTLE IN THE FEEDLOT PEN B

	DATE OF EXAMINATION 10/6/81	25/6/81	9/7/81	22/7/81	6/8/81	19/8/81	23/9/82
Number of animals *Positive/Number Sampled (%)	0/49	0/46	4/48 (8)	13/47 (28)	29/47 (62)	14/38 (37)	23/38 (61)
Bilateral *M.bovis* infections %	0	0	0	8	17**	43	39

* *M.bovis* isolated from one or both eyes

** alternate cattle treated

TABLE V.III ISOLATIONS OF *MORAXELLA BOVIS* FROM CATTLE NOT EXHIBITING SIGNS OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

	PEN A	PEN B
Number of animals showing no IBK but positive*/total number animals (%)	23/50 (46)	21/48 (44)

* *M.bovis* isolated from either one or both eyes at least once during the study period

TABLE V.IV PREVALENCE AND SEVERITY OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS ACCORDING TO BREED

PEN A				
BREED	NO IBK (%)	IBK MILD*(%)	IBK SEVERE*(%)	TOTAL NUMBER OF ANIMALS (%)
Aberdeen Angus	58 (45)	33 (26)	38 (29)	129 (65)
White Face	25 (45)	14 (25)	17 (30)	56 (28)
Hereford	6 (50)	3 (25)	3 (25)	12 (6)
TOTAL	89 (45)	50 (25)	58 (30)	197

PEN B				
BREED	NO IBK (%)	IBK MILD*(%)	IBK SEVERE*(%)	TOTAL NUMBER OF ANIMALS (%)
Aberdeen Angus	86 (76)	15 (13)	12 (11)	113 (59)
White Face	32 (74)	7 (16)	4 (9)	43 (22)
Hereford	19 (83)	1 (4)	3 (13)	23 (12)
TOTAL	137 (76)	23 (13)	19 (11)	179

* Mild or Severe IBK were defined in Chapter III, pg 57.

TABLE V.V OCCURRENCE OF UNILATERAL CASES OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

	PEN A	PEN B	TOTAL
Number animals with unilateral IBK/total number animals with IBK (%)	86/109 (79)	40/43 (93)	126/152 (83)

TABLE V.VI FREQUENCY OF *MORAXELLA BOVIS* ISOLATIONS FROM CATTLE EYES DURING THE OUTBREAK OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS IN PEN A

	NUMBER OF TIMES EYE POSITIVE FOR *M. BOVIS**				
	1	2	3	4	5
Proportion of cattle eyes positive %	18	24	25	15	6

* all cattle were sampled five times

FIGURE V.1 DISTRIBUTION OF WEANER CATTLE INTO FEEDLOT PENS SHOWING ARRANGEMENTS FOR FEEDING, OBSERVATION AND SAMPLING.

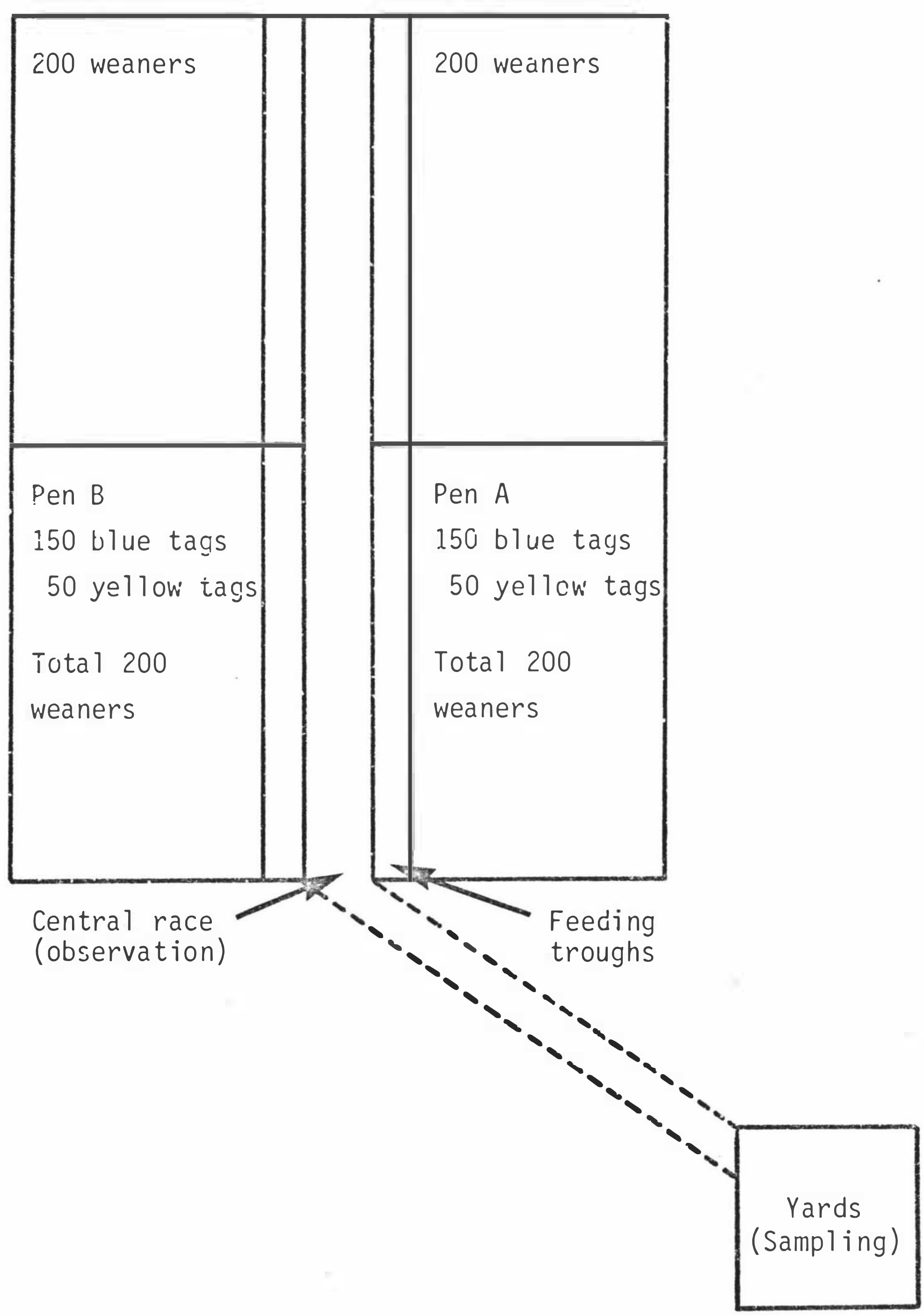
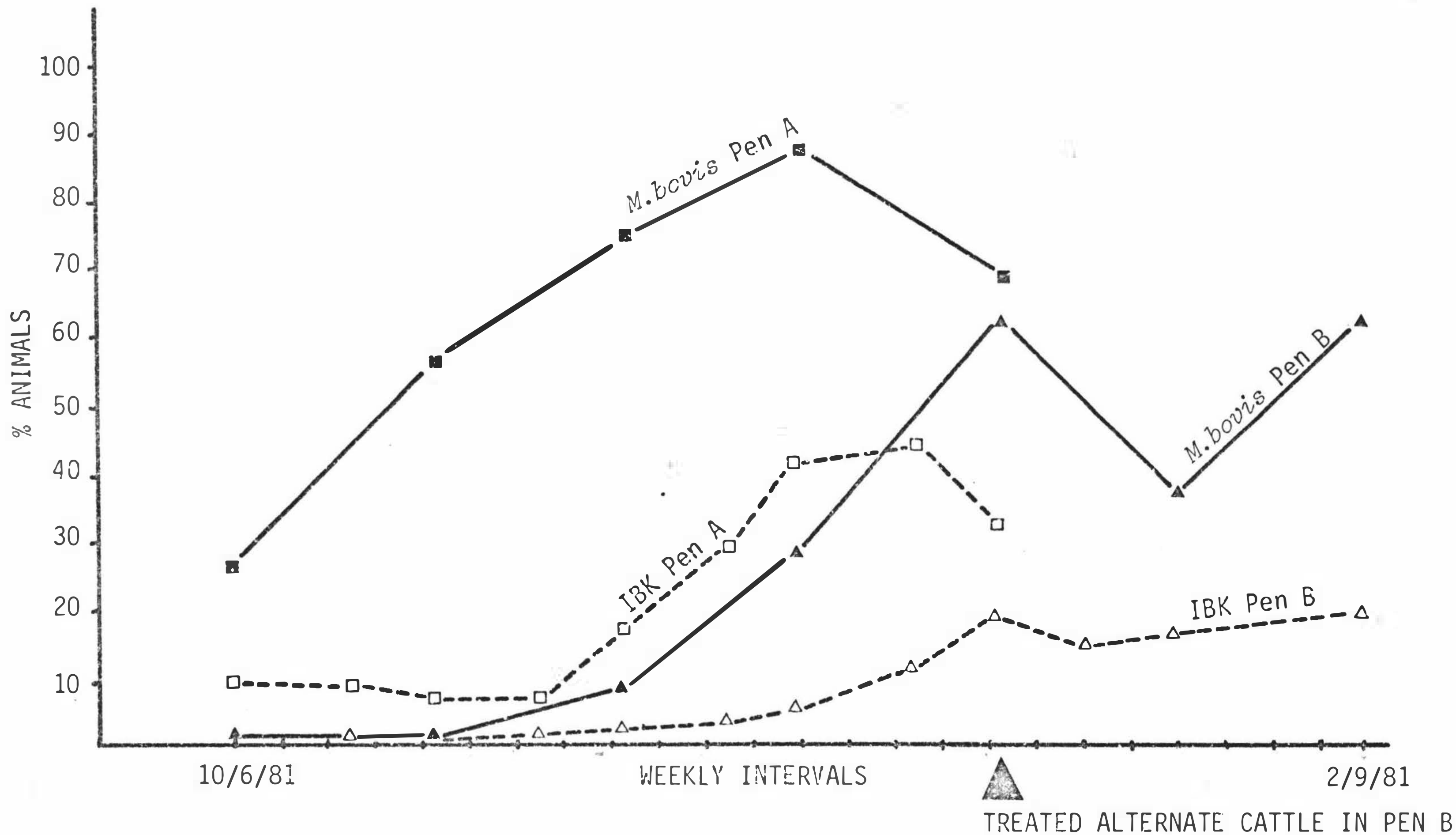


FIGURE V.2 FLOW CHART OF EVENTS ON FARM III

DATES 1981

1/4	first consignment of weaners arrived on farm: examined and sampled
15/4	random sampling of weaners
27/5	random sampling of weaners
8/6	selection of stock for feedlot
10/6	identification of stock for the investigation
19/6	examination of eyes only from feedlot
25/6	sampling of Pen A and Pen B
3/7	examination of eyes only from feedlot
9/7	sampling of Pen A and Pen B
17/7	examination of eyes only from feedlot
22/7	sampling of Pen A and Pen B
31/7	examination of eyes only from feedlot
6/8	sampling - cattle in Pen A sold, treated 50% cattle in Pen B
13/8	sampling of eyes only
19/8	sampling of Pen B
2/9	sampling - more cattle added to Pen B
23/9	sampling of Pen B
7/10	sampling - all cattle in feedlot returned to pasture
4/11	sampling of yellow tagged cattle originally in Pen B
2/12	sampling of yellow tagged cattle originally in Pen B

FIGURE V.3 PREVALENCE OF *MORAXELLA BOVIS* AND EYE LESIONS DURING AN OUTBREAK OF INFECTIOUS BOVINE KERATOCONJUNCTIVITIS



6/S

CHAPTER 6

GENERAL DISCUSSION

Prior to this study being undertaken there was little detailed information available on infectious bovine keratoconjunctivitis as it occurred in New Zealand. The results of the field study and the two postal surveys of farmers have helped to define the nature of the IBK problem and a better understanding has been obtained of the *M.bovis* infection dynamics that occur during IBK outbreaks. In addition, the impact of the disease on farming management practices has been recognised and the implications of this as demonstrated in depressed livestock production, clearly indicates the importance of IBK to the cattle farming industry.

Although the data obtained from an outbreak under feedlot conditions on Farm III was from an unusual farming enterprise by New Zealand standards, the pattern of events was similar to that reported in various studies overseas. The major differences appear to be in the magnitude of the peak prevalences of *M.bovis* infection and clinical IBK relative to the severity of the outbreak. Information gained from studying the collated epidemiological data from Farm III and the surveys of farmers has been used as the basis of a rational proposal for controlling IBK on individual farms.

Present evidence suggests that either the disease has been recently introduced into New Zealand or a more virulent form of the causative organism has evolved. The dramatic rise in the prevalence of IBK since 1975 from 1% to 28% in 1980 (Corrin,[24] 1980; Harris,[41] 1980) shows no signs of abating. Therefore there is a distinct possiblity that unless effective preventive measures are undertaken, the national herd prevalence could reach the levels seen overseas; an estimated 5% of cattle in half of North America's beef herds are affected annually (Webber,[106] 1981). The rise in prevalence of IBK in New Zealand has been accompanied by a change in the character of the disease. The number of outbreaks involving up to 50% of cattle in a herd has increased, whereas the usual world-wide morbidity rate is 5%. The rising morbidity rate is in contrast to the static rates presently observed in Australia and North America, where the disease has been known for several decades.

The introduction of new cattle to the herd was associated with an outbreak of IBK by 49% of farmers in the Hunterville district. The sequence of events on Farm III further substantiates this view. Opinions differ in Australia, where only 1% of farmers associate new outbreaks with introduced cattle (Slatter,[98] 1982). All the foregoing aspects of the disease support the suggestion that IBK has been recently introduced into this country and consequently the epidemiological features differ from those seen in countries in which the specific infection has been established for a much longer period.

Farmers in New Zealand consider IBK to be a significant animal health problem, both in terms of financial losses incurred and by virtue of the disruption it causes to normal stock management. The financial losses are derived from several sources; veterinary expenses, inefficient grazing management, reduced weight gains, deaths by misadventure and reduced sale value of stud and export stock. Yet despite the recognition of the consequences of outbreaks, there is reluctance amongst farmers to use any form of treatment. Only 11% of New Zealand farmers treat their cattle. In comparison, 77% of Australian beef farmers routinely treat their cattle for IBK (Slatter,[99] 1982). The reason most frequently given by New Zealand farmers for not treating the disease, is a lack of confidence in the beneficial effects of medication. That attitude has probably been adopted because eyes treated after lesions have become well established do not show an immediate response to treatment and furthermore, provided trauma is avoided, resolution eventually occurs irrespective of therapy. Better promotion of the methods available for effective treatment, together with a clearer explanation to farmers of the rationale for therapy should improve attitudes and hopefully would result in better control of outbreaks.

There are two specific aspects of the dynamics of *M.bovis* infections that need to be considered before deciding upon a treatment schedule. Firstly, many animals are infected with *M.bovis* but show no overt clinical signs; and secondly, the majority of *M.bovis* infections are bilateral even when the clinical signs of the disease are observed in one eye only. Consequently both eyes of all animals in a single group of cattle should be treated if eradication of *M.bovis* is the aim. The advantages of eliminating all *M.bovis* infections are two fold; prevention of the development of new cases of IBK and the removal of the risk of animals remaining subclinically infected. If infected animals remain, then they may become the foci from which disease spreads to other susceptible animals. By treating every eye, there is an additional benefit associated with treatment of cattle having very early lesions that might otherwise be overlooked. The early treatment of IBK is essential if the course of the disease is to be appreciably shortened and the severe form of the disease avoided. Although mass treatment of a herd might be considered ideal, the procedure could become impracticable when large numbers of animals are involved. However, if mass treatment was to be adopted with each new group of cattle being introduced to a herd, then the introduction of the disease on to an otherwise IBK-free property might be avoided.

Treatment methods should be practical as well as effective if the elimination of *M.bovis* from a herd is to be attempted. The practicability of the method would ensure better farmer compliance, and an appropriate schedule would be known to provide an optimum concentration of the antibacterial agent in the tears for an adequate period. Accordingly, subconjunctival antibiotic injections, which are simple to administer and, in the case of penicillin, provide approximately 48 hours of sustained therapeutic effect (Abeynayake, pers. comm.), are gaining favour.

The application of antibacterial products is widely accepted as the most effective form of treatment readily available. Unfortunately the regime is susceptible to breakdowns because treated cattle can become reinfected later. As far as this study is concerned any results of treatment are based on the identification of haemolytic *M.bovis* only, and no attempt was made to exclude the possibility of strains reverting to a non-virulent non-haemolytic form. In addition, specimens were collected from the conjunctival sac only, whereas *M.bovis* might conceivably lodge in some more remote sites. If *M.bovis* did survive in sites such as the nares or lachrymal glands, then local ocular treatment would not necessarily eliminate the infection from the animal. At a later date, the latent infection could possibly spread to the conjunctival sac and provide a potential source for a new outbreak of IBK. Any of these explanations might account for the temporary appearance of *M.bovis* in the two cows on Farm II (pg 73) as well as the apparent disappearance of all infection from cattle on Farm I (pg 72).

Studies, on Farm III in particular, have illustrated the wide range of possible outcomes of *M.bovis* infection in cattle eyes. Such infections can be found in apparently normal eyes, in those with minor lesions of IBK and associated with the most severe cases. Further research is therefore required to determine the factors responsible for this diversity in response to infection.

Studies into the local mechanisms involved in acquired resistance to *M.bovis* infection, might provide some new direction for vaccine development. Prevention of the disease would clearly be preferable to any other form of control but research into vaccine production overseas has been beset with difficulties, many of them associated with incomplete cross-protection. The development of a vaccine for New Zealand might be more easily achieved if *M.bovis* strains have experienced less antigenic drift because of the relatively short history of the disease in this country.

Infectious bovine keratoconjunctivitis has emerged as an important cattle health problem in New Zealand and its detrimental effect on the production of the national herd has probably not yet become fully apparent. Research to find an effective method of prevention must therefore be rated highly if New Zealand agriculture hopes to avoid the serious problems encountered with IBK by the beef and dairy cattle industries overseas.

APPENDIX I

300206

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CATTLE PINKEYE SURVEY

All information given by individual farmers will be treated in the strictest confidence.

(A) FARM INFORMATION

Name:

Farm Address:

Phone No:

Farm Size: ____________acres/or__________hectares

What will your sheep number be on 30/6/81?

____

(B) GENERAL INFORMATION ON CATTLE

1. What is the major breed(s) or cross-breed(s) of cattle on your farm? ____________________

2. Numbers of cattle according to age on 1/2/81

Age Group of Cattle	Number of Cattle	
	Male Steers&Bulls	Female
Birth - one year		
One year - two years		
Over two years		
TOTAL		

3. If you have a breeding herd, over which months of the year did your cows calve in 1980? (Tick one or more boxes)

Jan	Feb	Mar	Apl	May.	June.	Jul.	Aug.	Sep	Oct	Nov	Dec

1 4 8 12 13 19 25 31 37 43

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4. What month do you wean your calves? ____________

44

5. Do you buy cattle?

If yes, then for 1980, what classes of cattle were they, when did you buy them and from what source (i.e. saleyard, privately, etc).

Class of Cattle	Number bought (1980)	Month bought (1980)	Place bought (1980)
Male weaners			
Female weaners			
12 month old steers			
24 month old steers			
In-calf heifers			
In-calf cows			
Bullocks(over 2yr old)			
Bulls			
Other - please specify			

51
57
63
69
75
81
87
93
99

6. From which district(s) do you buy your cattle?

____

105

7. How often did you yard your cattle in 1980?

Place a tick in one or more boxes for each age group of stock. If you yarded more than one in any month place a tick for each time you yarded them.

(Example: If you yarded your under 2 year old cattle in February and twice in March, it should look like this).

	Jan	Feb	March
Under 2 years old		✓	✓✓

Class of Cattle	Jan '80	Feb '80	Mar '80	Apr '80	May '80	Jun '80	Jul '80	Aug '80	Sep '80	Oct '80	Nov '80	Dec '80
Under 2yrs												
Over 2yrs												

106
112
118
124

8. Sale of cattle off your farm for 1980.

Class of Cattle	Freezing Works		Saleyards		Privately	
	Number Sent	Month (1980)	Number Sent	Month (1980)	Number Sent	Month (1980)
Weaners						
Yearlings						
Store Steers						
Fat Steers						
Bullocks-over 2 years						
Cows						
Other						

(C) PREVIOUS EYE PROBLEMS IN CATTLE ON YOUR FARM

1. Have you had cattle eye problems of any kind on your farm in the last five years?

(Tick one or more boxes)

Year	Yes	No
1977		
1978		
1979		
1980		
1981		

Questions about pinkeye in your cattle

Pinkeye is a disease affecting one or both eyes of cattle and it causes a distinct watery discharge from the eye. This is soon followed by a cloudiness (whitening) of the eyeball which may develop into an ulcer. This can heal by itself within a few weeks or months and may leave a permanent scar on the eyeball. Temporary blindness is not uncommon.

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200 205 210 215 220 225 230 235 240 245

249 250 251 252 253

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2. Have you had pinkeye in your cattle at anytime in the past five years?

(Tick one or more boxes)

Year	Yes	No
1977		
1978		
1979		
1980		
1981		

3. Was a specific diagnosis made by a veterinarian?

(Tick box)

Yes	No

259

If you have never had pinkeye on your farm then you need not answer any further questions. Thank you for your co-operation and could you now please return this form in the envelope provided.

If you have had pinkeye in your cattle, please continue this questionaire.

4. How was pinkeye first noticed on your farm?

(Tick one or more boxes).

Signs first noticed	
Watery discharge from eye(s)	
"Whiteness" of eyeball	
Cattle seeking shade	
Cattle loosing condition	
Cattle misadventure (falling in drains)	
Other - please specify	

260

266

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5. When your cattle had pinkeye, was any form of treatment given?

(Tick one box)

Yes	No	Sometimes

6. If no treatment was given what was the most likely outcome of the pinkeye?

(Tick one or more boxes)

Animals eyes healed within 1-2 weeks	
Animals eyes healed within 2-4 weeks	
Animals eyes healed after 4 weeks	
Some animals remained blind	
Other - please specify	

7. If treatment was given what method of treatment was used?

(Tick one or more boxes)

Antibiotic injection into the eyelid	
Antibiotic powder puffed into the eye	
Eye ointment	
Other - please specify	

8. If treatment was given, what was the most likely outcome of the pinkeye?

(Tick one or more boxes)

Animals eyes healed within 1-2 weeks	
Animals eyes healed within 2-4 weeks	
Animals eyes healed after 4 weeks	
Some animals remained blind	
Other - please specify	

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9. What class of cattle have been affected with pinkeye on your farm?
(Please tick box next to class of cattle and if you have the numbers involved in your most recent outbreak, please fill in remainder of the table).

Class of cattle	✓	Total No. in mob.	Number with pinkeye.
Calves while still on cows			
Weaners			
Fattening steers			
First calvers			
Older cows			
Bulls			
Other - please specify			

10. In your most recent outbreak of pinkeye, in which month(s) of the year were most of the cattle affected?

(Tick one or more boxes)

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

330
336

ASSESSMENT OF THE EFFECT OF THE DISEASE ON YOUR FARM.

1. Do you think that pinkeye interfered with the moving of cattle on your farm? (Tick box)

Yes	No	Don't know

337

2. Do you associate the start of a pinkeye outbreak with freshly bought in cattle? (Tick box)

Yes	No	Don't know

338

3. Do you think that pinkeye in your cattle interfered with their mating management? (Tick box).

Yes	No	Don't know

339

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4. Do you think that pinkeye checked the growth of your young stock?

(Tick box)

Yes	No	Don't know

5. Do you think that pinkeye delayed the finishing of your fattening stock?

(Tick box)

Yes	No	Don't know

6. Do you think that costs and/or losses incurred on your farm due to pinkeye are :-

(Tick box)

Negligible	
Moderate	
Considerable	

Could you put a figure on these costs and/or losses? $ ____________

GENERAL PERSONAL COMMENTS ON PINKEYE

THANK YOU VERY MUCH FOR YOUR CO-OPERATION IN FILLING IN THIS FORM.
WOULD YOU PLEASE NOW RETURN IT IN THE ENVELOPE PROVIDED.

THANK YOU :- Jane Sinclair

APPENDIX II PRELIMINARY SURVEY CARD SENT TO 800 GISBORNE FARMERS

NAME:

ADDRESS:

FARM SIZE:

HAVE YOU HAD PINKEYE IN YOUR CATTLE IN THE LAST FIVE YEARS?

YEAR	YES	NO
1977		
1978		
1979		
1980		
1981		

APPENDIX III PREPARATION OF CELLS FOR ELECTRON MICROSCOPY: NEGATIVE STAINING METHOD

(1) place 3 drops of distilled water into tube

(2) place 3-4 colonies of organism in water

(3) mix very gently

(4) place 3 drops 1% phosphotungstic acid (P.T.A.) of pH 7.0 - mix

(5) place 1 drop of bovine serum albumin

(6) final mix

(7) place drop of suspension of organism on grid

(8) dry with blotting paper

(9) place grid in electron microscope

APPENDIX IV PREPARATION OF CELLS FOR ELECTRON MICROSCOPY: METHOD OF DOUBLE FIXATION (after Karnovsky, 1965)

(1) bacterium grown for 24 hours on BA plates

(2) colonies placed in distilled water and cells deposited as a pellet by centrifugation

(3) pellet fixed on half strength Karnovsky fixative consisting of:
 2% formaldehyde
 3% glutaraldehyde
 0.1 M phosphate buffer pH 7.2
for 2 hours at 4°C

(4) washed twice in fresh buffer 15 min each, at 4°C

(5) post-fixation in 1% osmium tetroxide in phosphate buffer pH 7.2 for 1 hour at 4°C

(6) washed twice in fresh buffer 15 minutes each at 4°C; further steps carried out at room temperature

(7) dehydration in graded alcohol series namely,
 25% ethanol 30 min
 50% ethanol 30 min
 75% ethanol overnight
 95% ethanol 30 min
 100% ethanol 30 min
 100% ethanol 30 min

(8) infiltration of dehydrated pellet using propylene oxide 100% 10 min, repeated once

(9) introduction of epoxy resin, "Durcupan - ACM" (Fluka AG, Buchs SG, Switzerland) in propylene oxide

25% 1 hour
50% 1 hour
75% overnight
100% 6 hour

(10) pellet embedded in fresh 100% epoxy resin in size 4 gelatin capsules for 48 hr at 60^{o}C

Sections were cut on the LKB "Ultrotome" and picked up on carbon coated formvar support films on 200 mesh copper grids. The preparations were stained for 10 min with lead citrate and examined in a Philips EM200 electron microscope.

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