

BACTERIOLOGICAL AND PATHOLOGICAL STUDIES  
OF BOVINE KERATITIS

by

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## INTRODUCTION

Infectious keratitis, often referred to as pinkeye, infectious conjunctivitis, and infectious ophthalmia is an acute infectious disease which affects the eyes of cattle and sheep, and is characterized by a marked inflammation of the conjunctiva and cornea. This disease has been recognized in the United States for more than 70 years and is widespread among range and feedlot cattle. Workers have reported the disease as also occurring in Europe, Africa, Asia, and South America.

Symptoms frequently observed in keratitis are photophobia, lacrimation, pain, and clouding of the cornea with loss of sight in the affected eye. Very few fatalities occur in animals suffering with the disease but the economic aspect is enormous. Animals affected, suffer a loss of body weight, and blindness in some of the more severe cases. Infectious keratitis is probably responsible for greater monetary loss to the stockman than other infectious diseases that respond more readily to therapeutic treatment.

Several workers in this country have implicated the bacterial agent *Moraxella bovis*<sup>1</sup> as the causative organism of the disease. Some foreign investigators have reported the disease caused by a rickettsial-like organism. Many therapeutic agents have been used in the treatment of infectious keratitis but an effective treatment has not been found. Research workers have attempted for several years to produce an effective immunizing agent for keratitis in cattle. Recently a commercial company reported a special

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<sup>1</sup> Hereafter referred to as Mor. bovis.

bacterin that was effective against the disease in cattle in Texas.

The intent of this investigation was to evaluate the ability of the keratitis bacterin to produce immunity in susceptible calves, and to conduct a histopathological study of the bovine eye affected with infectious keratitis.

#### REVIEW OF LITERATURE

The first report on bovine keratitis was that by Billings (5) in 1881 who described a condition of contagious inflammation of the cornea occurring in a herd of dairy cattle on a farm in the vicinity of Lincoln, Nebraska. He stated that this was not a new disease in the United States. He found symptoms of lachrimation, photophobia, and inflammation of the eye. Microorganisms appearing to be bacillus-like, with a belt around the middle, were isolated from sections of the cornea removed from a diseased eye. The writer found that placing moist bandages over the affected eye hastened recovery. Failure resulted when attempts were made to transmit the disease.

Mitter (19) in 1915 reported on his investigation of an outbreak of contagious ophthalmia occurring in cattle in India. He characterized the disease as having symptoms of conjunctivitis and an ocular discharge consisting of serum, mucus, epithelial cells, and leucocytes. In addition, observations were made that the disease generally occurred in an epidemic but occasionally it was found as a sporadic outbreak. He described the course of the disease and the incubation period as three to seven days. Corneal opacity with erosions of the cornea, which in some instances led

to perforation of the eyeball, were observed. His bacteriological findings suggested two causative organisms, Micrococcus lanceolatus and the bacillus of Morax-axenfeld.

Kappeyney and Ward (17) in 1917 translated an article by Poels who reported Bacillus pyogenes as the causative organism of keratitis infectosa of cattle in Holland. Poels described the pathology of the condition as a diffuse infiltration in the sub-epithelial tissue of the cornea. He found the fluid of the anterior chamber replaced with tissue, and adhesions were formed between the cornea and the iris. In severe cases a marked vascularization of tissue was found around the periphery of the cornea. The disease was reproduced by injecting isolated organisms between the layers of the cornea.

Allen (1) of Canada in 1919 reported that an abrasion of the cornea was not the primary factor in the production of infectious keratitis. He isolated a short, thick gram negative diplobacillus from the infected eyes. Transmission attempts were unsuccessful when he used cultures of the isolated organism grown on loefflers blood agar. The writer suggested that failure to reproduce the disease might be due to an attenuation of the organism growing on artificial media. One calf, inoculated with eye secretions collected from a number of affected animals, produced typical symptoms of infectious keratitis. Attempts to infect rabbits and guinea pigs were unsuccessful. Allen suspected that flies might transmit the disease as the occurrence of infectious keratitis was greatest during the seasons when flies were most prevalent.

Jones and Little (14) described their observations of 24 cases of acute ophthalmia in cattle. They were able to isolate a diplobacillus organism from each of the 24 cattle. Morphologically, the organism resembled the diplobacillus of Morax and Axenfield. Cattle inoculated experimentally with the diplobacillus developed the disease in two to five days. The course of the disease was found to be from 15 to 19 days. They were able to isolate the diplobacillus from the eye of an animal four months after clinical symptoms had subsided. The disease did not spread to cattle in adjacent pastures.

Jones and Little (15) reported on transmission experiments using the fly as a natural carrier of the Hemophilis bovis organism. They found that the organism did not remain viable for more than 30 minutes in the digestive tract of the fly, and would live only three hours on the body of the fly.

Farley (7) stated that keratitis is primarily a disease of young cattle but also may be found in adult cattle. He reported the disease to be more prevalent in the summer and early fall months and that it presented a greater problem in cattle located in the midwest area of the United States. Escherichia coli, Corynebacterium pyogenes, streptococci, staphylococci, and Pasteurella bovisseptica were isolated from the eyes of calves affected with keratitis. These organisms did not reproduce the disease when inoculated into experimental calves. Filtrates of eye secretions, when inoculated into susceptible calves, produced symptoms of keratitis. Antiserums prepared from calves recovered from acute keratitis failed to protect susceptible calves. Animals recovered



from the disease proved to be a source of infection for susceptible cattle for seven months. Farley described the disease as occurring in three forms: the mild, the acute, and the chronic.

Rose (21) described three types of keratitis: allergic keratitis, caused by plants; nutritional keratitis, due to a deficiency of vitamin A; and infectious keratitis, caused by bacterial organisms. He reported treating 3,662 cattle with varying doses of infectious keratitis bacterin that produced a protective immunity through the summer months. The bacterin used consisted of Pasteurella bovisseptica, 40 percent; Cornybacteria pyogenes, Streptococcus, and Staphylococcus albus, 20 percent each; and Staphylococcus aureus, 10 percent.

Reid and Anigstein (22) investigated infectious kerato conjunctivitis in cattle located on the Gulf Coast of Texas. They were able to isolate Escherichia coli, Bacillus pyogenes, Bacillus subtilis, Pasteurella (sp.), and Staphylococcus (sp.) in bacteriological studies of infected eyes. Experimental inoculations of these organisms into the conjunctiva of normal cattle failed to produce the disease. The disease was reproduced in susceptible calves with isolated organisms of Hemophilus bovis. Reid and Anigstein concluded that H. bovis was the specific agent of infectious kerato conjunctivitis in cattle.

Baldwin (3) reported on a bacteriological study of 112 eyes infected with infectious keratitis. He was able to isolate Hemophilus bovis from 83.5 percent of the eyes examined. The disease was reproduced in 12 of the 15 animals when inoculated with Hemophilus bovis organisms into the conjunctival sac.

Scarification of the cornea was not necessary to induce the infection. His experimental data showed that one test animal presented symptoms in one eye in 15 days, while the other eye became infected 35 days after inoculation. Efforts to demonstrate systemic antibodies in the serums of calves that had recovered from infectious keratitis were negative. Baldwin used both a precipitin and a complement fixation test for his serology studies. Sheep failed to show ocular lesions when inoculated with either virulent eye secretions of calves or cultures of Hemophilus bovis. Mice, guinea pigs, and rabbits were not found susceptible to H. bovis.

Farley, et al. (8) reported on his attempts to produce keratitis with cultures of H. bovis. The cultures used in his experiment were obtained from Baldwin. When 34 calves were exposed to H. bovis organisms, not a single animal developed the disease. He examined the H. bovis cultures by morphological and cultural methods and found the organism to meet the descriptions of the bacterium described by Jones and Little (14) and by Baldwin (3). He stated that H. bovis is a secondary invader in keratitis along with other microorganisms present in the eye secretions of affected animals.

Blakemor (6) reported on his investigation of conjunctivitis and keratitis of cattle in England. He was able to reproduce the disease by instilling lacrimal secretions into the eyes of calves. Blakemor found cell inclusion bodies in conjunctival epithelial cells. The inclusions were noted to appear only in the very early stages of the disease. Pook (20) also described the presence of



inclusion bodies in cases of conjunctivitis of cattle.

Alvarez (2) described a vitamin A deficiency of cattle in Columbia, South America. He found clinical symptoms of photophobia, reddened conjunctiva, ulcerated cornea, and in some cases a prolapse of Descemet's membrane with destruction of the cornea.

Barner (4) reported on a study of Mor. bovis and its relation to bovine keratitis. He was the first to report the isolation of Mor. bovis from the eyes of cattle in Kansas. Barner isolated the organism from the eyes of 92 out of 95 cattle affected with keratitis. The eye secretions of 36 normal cattle were cultured, and revealed that the organism was not present. The disease was reproduced in four calves when inoculated with a pure culture of Mor. bovis, and the incubation period varied from 15 to 21 days. In his trials, six cattle were found not to be susceptible to the organism one year after the cattle had recovered from the disease. Isolation of the organism from the blood stream of cattle acutely affected with keratitis was unsuccessful.

Barner, after a study of a number of artificial media, found that proteose peptone agar adjusted to a pH of 7.2 enriched with 5 percent defibrinated blood, was a medium of choice for the growth of Mor. bovis. The bacteria he described was a short, plump, diplo-bacillus with rounded ends measuring 0.5 to 1.0 microns in width and varying in length from 1.5 to 2 microns. The organism was uniform in 24- and 48-hour cultures but showed pleomorphism in older cultures. Surface colonies were smooth and measured 3 to 5 microns in 48 hours. Rough, intermediate, and dwarf colonies were observed after prolonged culturing. Lyophilized cultures were

found viable after being stored eight months. Barner was able to isolate the bacteria Mor. bovis from cattle one year after the animals had been affected with infectious keratitis. He stated that keratitis was of an infectious nature, as encountered in cattle in the State of Kansas, and was caused by Mor. bovis. "Infectious Bovine Keratitis" appeared to him to be the appropriate nomenclature for the disease.

Farley, et al. (9) classified infectious keratitis of cattle. His pathological classification listed three forms of the disease. He observed, in the mild form: lacrimation, conjunctivitis, a slight cloudiness of cornea, and a distention of the circumcorneal vessels. The acute form was characterized by a sudden onset and rapid progress of the disease. Lacrimation, conjunctivitis, photophobia, and cloudiness of the cornea in which the opacity in some cases might cover the cornea and result in blindness to the animal. The chronic form differs from the acute in the extensive changes that occur in the cornea. The cornea thickens, ulceration develops, and sometimes hypopyon forms. Leucocytes infiltrate the layers of the cornea and impairment of vision results. The cornea, in a few cases, may rupture with the escape of the lens.

Kanawyer, et al. (16) described a bacteriophage active against Moraxella bovis. Kanawyer and co-workers were able to demonstrate clear zones on Moraxella bovis seeded plates. The reported phage was obtained from filtered eye secretions of two calves suffering from infectious keratitis. They suggested the presence of a bacteriophage in eye secretions might explain some of the inconsistency and variable results in transmission of bovine infectious

keratitis.

Workers have reported therapeutic agents to be effective in the treatment for keratitis. Formston (10) of England found that early treatment, irrespective of medicament employed, tends to shorten the course of the disease. Klussendorf (18) reported the use of stock bacterins, in varying dosages of 5, 10, or 15 ml, as a means of preventing the disease. Jackson (12) worked on the serology of Mor. bovis infected calves and reported that antibodies were produced in calves infected with keratitis. Jackson (13) of Kansas found chloromycetin ophthalmic ointment, 1 percent, instilled into the conjunctiva once daily for three to nine days, was an effective treatment for early cases of keratitis. Barner (4) found in experiments designed to determine the sensitivity of Mor. bovis to various antibiotics, that chloramphenicol and penicillin produced the larger zones of inhibition on cultural plates.

Scott (23) reported the use of cortisone in the treatment of infectious keratoconjunctivitis in cattle. He found cortisone acetate injected intramuscularly in conjunction with antibiotic ophthalmic powder used locally to be effective in treating perforated corneal ulcers.

Recent literature presented by research workers (5, 3, 9, and 12) indicate Mor. bovis to be the causative organism of infectious keratitis. A Mor. bovis bacterin produced by a commercial company has created a great interest among investigators.

## EXPERIMENTAL PROCEDURE

## Experiment I

Lyophilized cultures of Mor. bovis were obtained from a commercial firm for use in these vaccine evaluation tests. Calves, used as test animals, were purchased from the local auction sale. The bacterin used was a commercially-prepared bacterin.

Twelve calves ranging in age from 8 to 12 weeks were identified with ear tag numbers. These calves were divided into two equal groups. Each calf in the vaccinate group was inoculated subcutaneously with two cubic milliliters of the experimental test bacterin. This group received a second inoculation of three cubic milliliters of the test bacterin after seven days. A four-week interval was allowed for the vaccinated calves to develop immunity.

The eyes of all calves in the experimental group were examined by means of a dry, sterile cotton swab which was placed on the conjunctiva in the region of the membrana nictatana and the mucosa of the eyelids. The swab was rotated several times to facilitate absorption of the eye secretions. The tip of the swab was used to inoculate a previously-prepared sterile plate of Bordet Gengou agar enriched with 5 percent defibrinated bovine blood. The cultures were incubated for 48 hours at 37° C. and examined for the presence of Mor. bovis colonies. This procedure was followed for each of the 12 calves assigned to this experiment. Each animal used in the experiment was found to be free of harboring the organism Mor. bovis.

Proteose peptone broth was adjusted to a pH of 7.2 and inoculated with lyophilized cultures of Mor. bovis. These cultures were allowed to incubate four hours at 37° C. and were then streaked onto Bordet Gengou agar plates enriched with 5 percent bovine blood and incubated 48 hours at 37° C. Four agar plates were selected, having colonies demonstrating the morphological characteristics of Mor. bovis. The growth was washed from the plates with four milliliters of double distilled water. The pooled washings from four agar plates were used as challenge material for one eye. Sterile swabs saturated in the bacterial suspension were rotated over the nictana membrane and the conjunctiva. The remaining suspension of bacteria was poured into the eye; the lids closed and massaged externally for one minute. This procedure was followed for each eye of all calves included in the experiment. The calves were placed in a corral where they were exposed to direct sunlight. The Mor. bovis organism was isolated from each inoculated eye 48 hours later.

Each calf was examined twice daily for clinical symptoms of infectious keratitis, and if symptoms were present, the affected eye was swabbed with a sterile swab and plated on Bordet Gengou plates. The plate was examined for colonies of Mor. bovis after 48 hours incubation at 37° C. A positive diagnosis of infectious keratitis was made only if clinical symptoms were found and Mor. bovis organisms were isolated. This procedure was followed for a period of four weeks after inoculation.



A positive diagnosis of infectious keratitis was made in one calf on the third day after inoculation. Three more positive cases were diagnosed by the 7th day. All calves except five were affected by the 14th day and these succumbed by the 29th day.

A comparison of vaccinated and control calves challenged with cultures of Mor. bovis is shown in Table 1.

Table 1. Comparison of vaccinated\* (bacterin) and control (non-vaccinated) calves challenged\*\* with cultures of Mor. bovis.

Calf No.	Date of lacrimation	Date of isolation of <u>Mor. bovis</u>	Diagnosis
<u>Vaccinates</u>			
358	7/ 4/55	7/ 5/55	Positive
359	7/ 8/55	7/ 5/55	"
363	6/23/55	6/24/55	"
365	6/15/55	6/18/55	"
361	6/29/55	6/24/55	"
369	6/13/55	6/13/55	"
<u>Controls</u>			
360	6/25/55	6/25/55	Positive
364	6/20/55	6/20/55	"
367	6/27/55	6/27/55	"
362	6/25/55	6/25/55	"
366	6/18/55	6/18/55	"
370	6/15/55	6/27/55	"

\* 2 ml bacterin administered 3/5/55; 3 ml bacterin administered 5/12/55.

\*\* Challenged 6/11/55 with culture of Mor. bovis.

One eye in each of two calves in the vaccinated group remained normal throughout the test period. One eye in one calf in the control group was not affected. Experimental evidence in



this test was conclusive that the bacterin failed to immunize susceptible calves against Mor. bovis infection. Animals in both test groups were equally susceptible to the organism Mor. bovis.

## Experiment II

The second keratitis bacterin test was conducted during the months of October and November 1955. Calves used for the second bacterin trial were obtained from three sources. Seven calves of mixed breed were purchased at the local auction sale; four calves were procured from the Kansas State College Dairy herd and four calves were raised from cows on the Veterinary Research farm. The latter group of four calves were from cows that had previously been affected with infectious keratitis. The calves were divided into two groups; eight in the vaccinated group and seven in the control group. Members of each group were identified with ear tag numbers. One calf in the control group and two calves in the vaccinated group were allowed to nurse cows for eight weeks prior to challenge with the culture of Mor. bovis. The remaining calves in the two groups were bucket fed on a commercially-prepared milk substitute.

The lyophilized culture of Mor. bovis was of the same strain that was used in Experiment I. Bacterin used for immunization was supplied by a commercial company and was identical to the bacterin used in Experiment I except that a 5 ml dose was used for each injection. Calves in the vaccinated group received 5 ml of vaccine subcutaneously. This injection was followed in seven days by a second dose of 5 ml of vaccine. Thirty days were allowed for

immunity to develop in the calves. Eyes from calves of both groups were examined for the presence of Mor. bovis organisms. The same procedure was followed for this examination as was followed for Experiment I. Challenge cultures were prepared of Mor. bovis organisms as was described in Experiment I. Daily examinations were made and the findings recorded. When lacrimations occurred, the secretions were inoculated on Bordet Gengou agar plates and incubated 48 hours at 37° C. These plates were examined for Mor. bovis colonies. A positive diagnosis was established as described in Experiment I.

One calf became infected three days after inoculation. Two more calves were showing severe ulceration of the cornea and Mor. bovis was isolated from the lesion at the end of the first week. Fourteen days after inoculation, seven positive cases of infectious keratitis were under observation. Three additional calves had become infected within a period of 25 days, post inoculation. Calf number 8 of the control group presented a severe ulceration of the cornea 78 days after inoculation. Calf number 4 of the vaccinated group demonstrated photophobia and lacrimation 12 days after inoculation but Mor. bovis was not isolated from the eye secretions. Two calves of the vaccinated group and two calves of the control group remained normal throughout the test period.

Isolations failed on two occasions despite symptoms of lacrimation, photophobia, and conjunctivitis in calf number 8 of the control group. An interval of 66 days occurred before an isolation of Mor. bovis was made. Three of the four calves, raised on the research farm and born from cows that had previously been

affected with infectious keratitis, developed the disease, while one calf remained normal throughout the test period.

Table 2 gives a comparison of vaccinated and control calves challenged by conjunctival inoculation with Mor. bovis.

Table 2. Comparison of vaccinated\* (bacterin) and control (non-vaccinated) calves challenged\*\* by conjunctival inoculation with Mor. bovis.

Calf No.	Date of lacrimation	Date of isolation of <u>Mor. bovis</u>	Diagnosis
<u>Vaccinates</u>			
1	10/12/55	10/18/55	Positive
2	10/14/55	10/18/55	"
3	10/14/55	10/19/55	"
4	10/18/55	Negative	Negative
5	10/17/55	10/18/55	Positive
7	10/11/55	11/ 2/55	"
272	10/23/55	10/24/55	"
273	Negative	Negative	Negative
<u>Controls</u>			
26	Negative	Negative	Negative
6	"	"	"
8	10/24/55	12/30/55	Positive
9	10/10/55	10/10/55	"
10	10/10/55	10/13/55	"
11	10/12/55	10/12/55	"
12	10/14/55	10/17/55	"

\* 5 ml bacterin administered 9/1/55; 5 ml bacterin administered 9/7/55.

\*\* Challenged 10/7/55 with culture of Mor. bovis.

Six of the eight calves in the vaccinated group developed positive cases of infectious keratitis while five of the seven animals in the control group developed the disease. The results

of this second test gave further conclusive evidence that the animals injected with bacterin had not been immunized against the organism Mor. bovis.

### Experiment III

This experiment was conducted during the months of January and February of 1956. Ten calves were used in conducting this test. Seven calves were purchased at the local auction sale; three calves were raised at the Pathology Research farm. Animals subjected to the test were identified with ear tag numbers. Both the control group and the vaccinated group contained five animals.

The bacterin used in Experiment III was also a commercially-prepared bacterin with twice the number of organisms per milliliter as the bacterin used in Experiments I and II. The lyophilized culture of Mor. bovis was also the same strain as that used in the previous experiments. Test animals were placed in a lot with access to an open shed for protection against the winter weather. Two subcutaneous injections of 3 ml each of the vaccine were administered at weekly intervals to all members of the vaccinated group. Thirty days were allowed to develop immunity in the calves. Eye secretions of each calf of both groups were cultured and found negative for Mor. bovis.

The suspension used for inoculation was a pooled washing from four petri plates inoculated with Mor. bovis and incubated 48 hours. Dry, sterile swabs were saturated in this suspension of Mor. bovis organisms, suspended in saline, and placed on the conjunctiva and rolled under the nictitating membrane. This procedure

was followed for each eye of all animals in both experimental groups. The calves were observed twice daily for symptoms of infectious keratitis. Both clinical symptoms and bacteriological findings were necessary for a positive diagnosis of the disease.

Seven of the 10 calves included in this experiment were found to be susceptible to the Mor. bovis organism. A comparison of vaccinated and control calves challenged with cultures of Mor. bovis is shown in Table 3.

Table 3. Comparison of vaccinated\* (bacterin) and control (non-vaccinated) calves challenged\*\* with cultures of Mor. bovis.

Calf No.	Date of lacrimation	Date of isolation of <u>Mor. bovis</u>	Diagnosis
<u>Vaccinates</u>			
38	2/12/56	2/12/56	Positive
40	Negative	Negative	Negative
42	1/28/56	2/ 2/56	Positive
44	2/ 8/56	Negative	Negative
45	1/30/56	1/31/56	Positive
<u>Controls</u>			
39	2/ 7/56	2/ 8/56	Positive
41	2/ 2/56	2/ 2/56	"
43	1/30/56	Negative	Negative
33	1/25/56	1/25/56	Positive
78	1/25/56	1/25/56	"

\* 3 ml bacterin administered 12/14/56; 3 ml bacterin administered 12/22/56.

\*\* Challenged 1/23/56.

Active cases of infectious keratitis were slower to develop and the infections were not as acute in Experiment III as was observed in the previous experiments. Sixty percent of the vaccinated animals were susceptible to the challenge of live Mor. bovis organisms and 80 percent of the control group were susceptible. This bacterin was considered to be inadequate in protecting calves against infectious keratitis, considering the high count of organisms in the bacterin and the percentage of vaccinated animals affected.

While conducting the third experiment, a survey was made to determine how long the Mor. bovis organism could be isolated after the eye had been inoculated with the organisms. Minute amounts of secretions were pipetted from the eye of each calf of the two groups of animals. The secretions were inoculated onto Bordet Gengou agar plates enriched with 5 percent blood. The plates were designated as right or left eye and numbered according to ear tag number of the calf. After incubating the plates 48 hours, they were examined for the presence of Mor. bovis colonies. Eye secretions were collected on the 7th and 14th days after inoculation. It was observed that all secretions contained viable Mor. bovis organisms on the 7th day, and on the 14th day organisms were found in the secretions of all except two calves. These two calves did not develop an active case of infectious keratitis during the test period. The secretions of calf number 45 contained viable organisms on the 14th day but did not become infected with the disease. Moraxella bovis was isolated from the secretions of three calves 35 days after inoculation with a live culture of the organism.



## Experiment IV

Histopathological studies were made on bovine eyes affected with infectious keratitis. Selections were made so a study could be made on eyes that were in various stages of the disease. Specimens studied were from eyes infected from three days to two months.

The surgical procedure employed for enucleating the bovine eye was that described by Frank (11). Immediately upon removal, the eye was placed in a solution of 10 percent buffered formalin. Two small incisions were made near the junction of the cornea and the sclera (Plate I, Fig. 1) to facilitate the passage of fixing solution into the chambers of the eye globe (Plate I, Figs. 2 and 3). Later in the experiment, fixation was observed to be adequately established throughout the eye without the opening being made into the inner chambers. The minimum time allowed for fixation was 48 hours. After fixation, the eye was incised through the vertex of the cornea (Plate I, Fig. 4) into two equal halves. These were embedded in paraffin and sectioned according to usual laboratory procedures. Sections of each eye were stained with azure-eosinate and Giemsa's stains. The Giemsa's stain was prepared according to Walback's (24) method.

The histopathology study was limited to the cornea because the primary pathology of keratitis is found in the cornea of the eye.

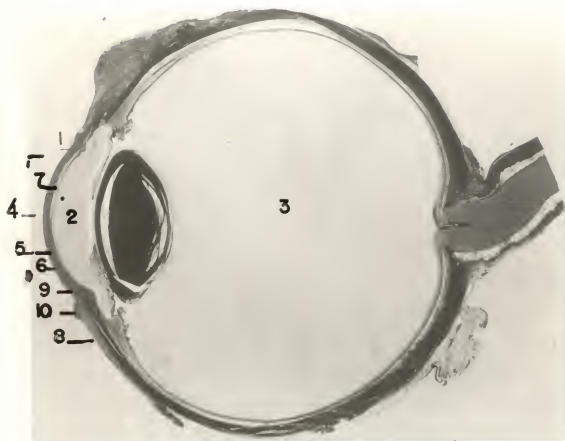
The cornea (Plate I, Fig. 5) is a transparent, curved layer of tissue forming the anterior one-fifth of the globe of the eye.

EXPLANATION OF PLATE I

Horizontal meridional section of the eye.

1. Junction of cornea and sclera.
2. Anterior chamber.
3. Posterior chamber.
4. Vertex of cornea.
5. Cornea.
6. Bowman's membrane.
7. Descemet's membrane.
8. Conjunctiva.
9. Limbus.
10. Iris angle.

PLATE I



It is composed of five layers of tissue: the epithelium, Bowman's membrane, substansia propria, Descemet's membrane, and endothelium. The epithelium of the cornea in the ox contains eight to ten layers of squamous corneal epithelial cells; the outer layer is not cornified. The basal layer of cells are in close apposition with Bowman's membrane and some descriptions note minute serrations between the cells and the membrane. Mitosis occurs in the basal cell layer. Mitotic figures are readily found when examining injured corneal tissue.

Bowman's membrane (Plate I, Fig. 6) is a structureless non-elastic layer found between the epithelium and the substansia propria. It is avascular and does not have the ability of regeneration. Small corneal nerves pass through Bowman's membrane into the epithelia of the cornea. Bowman's membrane is found to be soluble to inflammatory products.

The third layer or substansia propria is composed of ground substance and of cells. The ground substance consists of minute fibers of connective tissue united by a cement substance in flat bundles. These flat bundles are adhered, one to another, so that layers are built up, giving the cornea its thickness. The corneal cells found in the substansia propria are flat, protoplasmic cells with numerous branched processes; these processes connect with the processes of neighboring cells. Leucocytes may be found wandering throughout the substansia propria.

Descemet's membrane (Plate I, Fig. 7) is a homogenous, elastic membrane which forms the posterior border of the cornea. This membrane is unlike Bowman's membrane in that it is resistant to

inflammatory conditions. Descemet's membrane has the ability of regeneration when injured.

The endothelial layer is a single layer of flat, polygonal cells. The only connection of the cornea to the uvea tract is by way of the endothelial layer.

Histopathology Findings. This study was made on tissue sections prepared from a normal eye as well as infected eyes.

Eight to ten cellular layers were visible in the epithelial layer of the cornea. Mitotic figures were present in the basal layer of cells. Bowman's membrane was visible and intact. The corneal corpuscles of the substantia propria contained a large, flattened, irregularly-shaped nucleus, staining blue in color with azure eosinate stain. The substantia propria was avascular except for the region near the ciliary body. A few wandering leucocytes were present in the tissue. Descemet's membrane, staining bright pink, appeared to be a homogenous band of tissue. The endothelium was a single layer of cuboidal-like cells.

Tissue sections were prepared from the eye of calf number 272, which had been positively diagnosed as having infectious keratitis three days previous to the enucleation of the eye.

Sections from this calf showed slight swelling of the substantia propria of the cornea. The epithelial layer and Bowman's membrane were absent in the region of the ulcer. In the periphery of the lesion, the basal cells of the epithelium appeared to have undergone degenerative changes, while the superficial layer appeared normal. Numerous fibroblast and polymorphonuclear cells had infiltrated the substantia propria. There was a marked absence

of blood vessels in the immediate area of the lesion. Vessels in both the conjunctiva (Plate I, Fig. 8) and in the area of the limbus (Plate I, Fig. 9) were greatly distended. Many polymorphonuclear cells and lymphocytes had infiltrated the area of the iris angle (Plate I, Fig. 10). Descemet's membrane and the endothelium were normal.

The right eye of calf number 10 was surgically removed on the sixth day after infection. Microscopic study revealed an ulcer, extending deep into the cornea. Thin bands of necrosis lined the ulcerated area in the substantia propria. Polymorphonuclear cells, mononuclear phagocytes, and fibroblasts were numerous. The epithelium and Bowman's membrane had eroded away in the area of the ulcer. A marked regeneration of the epithelial cells was observed. Descemet's membrane appeared unchanged. Numerous bacteria resembling Mor. bovis organisms were found to have invaded the substantia propria (Plate I). The bacteria were noticed to be proximal to Descemet's membrane in one area of the section and many phagocytic cells were present in the area. Distended blood vessels had invaded the area of the cornea near the lesion.

The left eye was removed from calf number 9 on the tenth day after infection. The cornea was greatly thickened and many lymphocytes and polymorphonuclear cells were observed in the area of the lesion. New blood vessels were present in the substantia propria. Mononuclear phagocytes were numerous throughout the cornea tissue. Many fibroblasts were surrounding the ulcerated area and new fibrous connective tissue was observed. Blood vessels in the iris angle were engorged with blood, and extravascular cells



were found in great numbers. The epithelium was destroyed in the area of the ulcer. The endothelium was intact and Descemet's membrane appeared normal throughout the area. Sections were prepared from the left eye of calf number 10 on the 18th day after infection.

The epithelium consisted of 10 to 12 layers of cells near the area of ulceration. Mitotic figures were numerous in the basal cells in the epithelium. Both the epithelium and Bowman's membrane were destroyed in the area of the primary lesion (Plate II). Fibroblast, lymphocytes, and mononuclear phagocytes infiltrated the area around the lesion, and some necrosis was evident. Dense layers of fibrous connective tissue filled in around the area of the lesion. Numerous distended vessels had invaded the area of the lesion. Many polymorphonuclear cells were observed near the corneal surface. Inflammatory changes were pronounced in the iris angle. Descemet's membrane was unchanged and the endothelial layer contained a few polymorphonuclear cells.

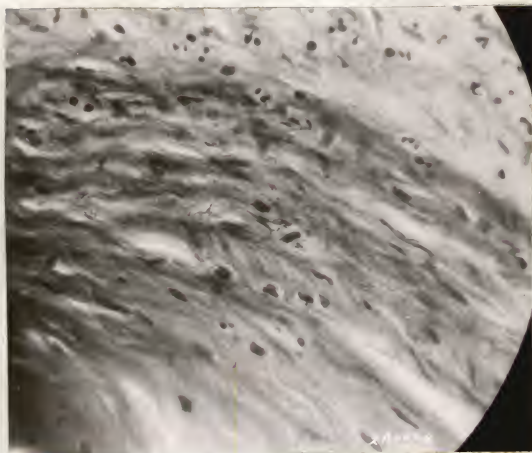
Sections were prepared from the eye of a calf that had suffered ulcerative keratitis for a period of eight weeks. This calf was submitted from the field for diagnosis. The calf presented a noticeable opacity of the cornea at the time of surgery.

The epithelium near the border of the corneal scar was thickened with as many as 15 layers of epithelial cells. The epithelium and Bowman's membrane were destroyed in the immediate area of the scar. Dense fibrous connective tissue had replaced most of the normal corneal lamella of the substantia propria (Plate III) and only a few wandering leucocytes were present.

EXPLANATION OF PLATE II

Section through substansia propria of eye  
showing many bacteria resembling Moraxella bovis  
(X980).

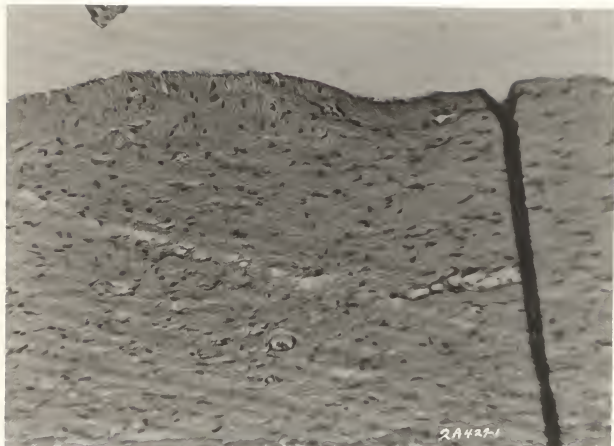
## PLATE II



EXPLANATION OF PLATE III

Section of substansia propria in area of  
18-day lesion. The Bowman's membrane and the  
epithelium are absent over this area (X430).

## PLATE III



Many large blood vessels had invaded the area, and fibroblasts were noted to be active throughout the area. Inflammatory changes were not found in the iris angle. Many plasma cells were observed near the limbus. Descemet's membrane appeared normal across the posterior border of the cornea.

#### DISCUSSION

Bacterin evaluation experiments were conducted on three groups of calves. These bacterins of Moraxella bovis were prepared by a commercial company according to a special process. Barner (4) and Baldwin (3) in their investigations concluded that Mor. bovis was the causative organism of infectious keratitis. Jackson (12) demonstrated that antibodies were produced in calves infected with keratitis. Antiserum was prepared from three calves that had recovered from keratitis. He injected calves with this antiserum and later was unable to produce keratitis in the calves by inoculating virulent eye secretions onto their conjunctiva. Baldwin (3) was unable to detect antibodies in the serums of calves either before or after inoculations with cultures of Mor. bovis. Rose (21) and Klussendorf (18) reported successful results when using stock bacterins to protect calves against keratitis.

Experiment I was conducted in June and July of 1955, using 12 calves as experimental animals. Six animals were injected with the experimental bacterin and the remaining animals were designated as controls for the experiment. Thirty days were allowed for immunity to develop in the bacterin-treated animals. Bacteriological examinations of the conjunctiva of the calves in



both groups were found to be negative for the presence of Mor. bovis organisms. Baldwin (3) was unable to isolate the Mor. bovis organism from the eyes of 20 normal cattle. All calves in this experiment were challenged by inoculating a suspension of living Mor. bovis organisms onto the conjunctiva of each eye. The calves were observed twice daily for symptoms of infectious keratitis. A positive diagnosis of infectious keratitis was made only when clinical symptoms were present and the Mor. bovis organisms were isolated from the eye secretions. All animals in both the vaccinated and the control groups were found to be susceptible to Mor. bovis organisms when challenged. The results of this experiment gave conclusive evidence that the vaccinated animals had failed to develop immunity to Mor. bovis organisms.

The second bacterin evaluation experiment was conducted in the fall of 1955. Fifteen calves were used in this experiment. Eight of the animals were injected with the identical bacterin used in Experiment I, while seven calves were used in the control group. A time interval of 30 days was allowed for immunity to develop in the vaccinated animals. All calves were challenged with a live culture of Mor. bovis organisms by instilling a suspension of the organisms onto the conjunctiva. Careful observations were made twice each day for symptoms of infectious keratitis.

The results of the second experiment were similar to those of the first experiment. Six of the injected animals and five of the control animals were found susceptible when challenged by the eye route with a live culture of Mor. bovis. Four of the calves used

in this test were born from cows which were previously infected with infectious keratitis. Three of these calves were found to be susceptible when challenged. The increase in quantity of the bacterin, from 3 to 5 ml, did not provide any immunity in this experiment.

The third bacterin evaluation experiment was conducted during the winter months of 1956. Ten calves were divided into two equal groups. Experimental bacterin used in this experiment contained twice the number of organisms per milliliter as the bacterin used for the first and second experiments. The five calves of the vaccinated group received a subcutaneous injection of 3 ml of bacterin. Seven days later the animals were injected with a second dose of 3 ml of bacterin. The time interval allowed for the vaccinated animals to develop immunity was 30 days.

Prior to challenge, the conjunctiva of the eyes of all calves examined bacteriologically were found to be negative for the presence of Mor. bovis organisms. A suspension of Mor. bovis was inoculated onto the conjunctiva of all animals to challenge them. Observations were made twice each day to detect symptoms of infectious keratitis following challenge. A positive diagnosis of infectious keratitis was made only upon isolation of Mor. bovis organisms from the eye secretions and the presence of clinical symptoms.

Three of the vaccinated animals and four of the control group were susceptible to Mor. bovis when challenged. This bacterin, too, was found to be of little value in protecting susceptible calves against Mor. bovis infection. Keratitis lesions produced in this

experiment were not as severe nor extensive as in Experiments I and II. Dust and sunlight apparently are agents that aggravate this infection.

Bovine eyes affected with the disease, infectious keratitis, were surgically removed and prepared for tissue sectioning. Eyes selected had been infected from three days to eight weeks. Sections were stained with azure eosinate for the histology study and Giemsa's stain for the bacteriology study.

The epithelium of the cornea was found to be very susceptible to Mor. bovis infections. The layers of epithelial cells of the cornea were eroded and destroyed in the area of ulceration in all of the infected eyes studied. An interesting observation made in these studies was the ability of the outer corneal membrane to repair and replace the damaged tissue. Sections studied from a 16-day-old lesion showed the epithelium around the immediate necrotic area to have a thickness of only two or three new cell layers. These layers gradually increased in number of cells until reaching the area of normal tissue. Bowman's membrane was absent in the area of infection in all studies that were made, and failed to demonstrate the ability of repair in any of the older lesions.

Erosion of the epithelium and disappearance of Bowman's membrane in ulcerative cases exposes the substantia propria to invading bacteria. The substantia propria showed many responses to the Mor. bovis infection; as lymphocytes, phagocytes, and fibroblast respond to the tissue damage and collect in the area. The substantia propria thickens and new connective tissue infiltrates into the lesion. The lesions of prolonged cases showed dense

areas of fibrous connective tissue. Many vascular vessels invade the area of necrosis in the cornea. Moraxella bovis organisms were observed in great numbers in the corneal lamellae. Descemet's membrane and the endothelium of the cornea were noted to be resistant to the invasion of Mor. bovis.

The anterior portion of the sclera, the limbus, and conjunctiva showed acute inflammatory reaction in the early stages of keratitis. The blood vessels were engorged and many phagocytic cells observed in the surrounding tissue.

#### CONCLUSIONS

Infectious keratitis was readily transmitted to calves by inoculating Moraxella bovis organisms onto the conjunctiva.

Commercial bacterins, specially prepared, did not produce effective immunity in experimental calves to Moraxella bovis organisms.

Incubation period varied from 3 to 78 days in experimental calves inoculated with Moraxella bovis organisms.

Keratitis, in experimental calves, was noted to be more severe in summer months than during the winter months.

Moraxella bovis organisms had the ability to invade the cornea through the epithelium.

The corneal epithelium has the ability to regenerate.

Bowman's membrane was destroyed in all lesions studied, and failed to show the ability of regeneration.

Moraxella bovis organisms were found to invade the deeper layers of the substantia propria.

Sections of keratitis lesions of the cornea revealed many young capillaries and vascular vessels extending into the substantia propria around the lesion.

Phagocytic cells and fibroblast invade the cornea in keratitis infections.

Descemet's membrane is not affected by Moraxella bovis infection but the membrane may protrude outwards through the substantia propria which is weakened by the infection.

In ulcerated lesions of keratitis, fibrous connective tissue replaces the diseased tissue in the substantia propria of the cornea.

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BACTERIOLOGICAL AND PATHOLOGICAL STUDIES  
OF BOVINE KERATITIS

by

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This study was made to evaluate the ability of experimental bacterins of Moraxella bovis to produce immunity in calves; and to study the histopathological changes that occur in the bovine eye affected with infectious keratitis.

Keratitis, an infectious disease caused by Moraxella bovis, which attacks the eyes of cattle, has been studied in this country and throughout the world. The disease, for many years, has been an important economic problem in range and feedlot cattle in this country. The disease affects all ages of cattle and sheep but is confined principally to young cattle. The outstanding symptoms observed in this disease are photophobia, lacrimation, cloudiness of the cornea, emaciation, and conjunctivitis. In many severe cases, total blindness may result in the affected eye.

The economic loss is especially great when blindness occurs in cattle. Many investigators of keratitis have concluded that Moraxella bovis was the causative organism of the disease. The organism was readily isolated from field cases of keratitis. Cultures of Moraxella bovis inoculated onto the conjunctiva of experimental calves produced typical clinical symptoms of keratitis. Some workers were able to demonstrate antibodies produced in calves affected with keratitis while other investigators reported negative results in their immunity tests. Experimental animals that have recovered from keratitis failed to develop symptoms when challenged with cultures of Moraxella bovis organisms.

Three experiments were conducted using specially-prepared commercial experimental bacterins to immunize calves against

infectious keratitis. The first experiment was conducted during the early summer months of 1955, in which six calves were injected with bacterin and six were control calves. All calves were challenged with conjunctival inoculations of live Moraxella bovis organisms. Positive cases of keratitis were diagnosed in all calves of both the vaccinated and the control groups. Diagnostic procedures consisted of observations of typical symptoms and isolation of Moraxella bovis organisms from the secretions of the affected eye.

The second experiment was conducted in the late fall and it also failed to demonstrate resistance or immunity in the vaccinated animals. The dosage was increased from 3 ml to 5 ml of bacterin. Six of the eight vaccinated animals developed keratitis when challenged with Moraxella bovis organisms. A similar percentage of the control animals developed the disease when they were challenged.

In the third experiment, the bacterin used contained twice the concentration of organisms per milliliter as in Experiments I and II. The third experiment was conducted during the winter months of 1956. The vaccinated calves were found susceptible to the challenge of Moraxella bovis organisms. The bacterins tested failed to produce sufficient resistance or immunity in the vaccinated calves in these three experiments.

Histopathology studies were made on bovine eyes infected with keratitis. The eyes studied had been infected with the disease from three days to eight weeks. Moraxella bovis organisms were observed in the deep layers of the substantia propria of the

cornea. The epithelium and Bowman's membrane of the cornea were found to be quite vulnerable to the invading Moraxella bovis organisms. The epithelium was found to be capable of repair. Dense layers of fibrous connective tissue replaced the diseased tissue in the substantia propria. Blood vessels, which are absent in the normal cornea, were found to have invaded the areas of ulceration. Descemet's membrane was found to be a protective layer when infectious keratitis occurs.

In these experiments it was concluded that the experimental bacterins used were of little value in protecting calves against keratitis. Histopathology studies revealed that Moraxella bovis organisms gained entrance into the cornea through the epithelial layer, and that fibrous connective tissue replaced the diseased tissue in the ulcerated area. Corneal opacities of the eye may result when these changes occur.