THE *IN VIVO* AND *IN VITRO* SUSCEPTIBILITY OF *MORAXELLA BOVIS* TO SELECTED ANTIBIOTICS AND SULFONAMIDES

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CHAPTER I

INTRODUCTION

Bovine infectious keratitis is an important cause of economic cattle losses in the United States. It is especially prevalent in the Southwest. The greatest economic loss is due to loss of weight in beef animals and a decrease in milk production in dairy animals. The incidence of the disease appears to be greatest during the summer and fall months. Sunlight, flies, and dust are more prevalent during this period of the year and these factors seem to contribute to the incidence and severity of the disease. In beef herds the calves are often weaned during the fall and at this time the animals are crowded together which facilitates the spreading of the disease by flies and contact. The nutritional status of the animal is also probably lowered at this time.

The livestock producer is interested in reducing the incidence of the disease and finding some method of treatment that will shorten the duration of infection so that both weight loss and lowered milk production would be minimized.

At the present time there is no sure method of immunizing animals against the disease. There are a number of different treatments that have been tried with varying degrees of success.

This infectious disease in cattle in the United States is reputed to be caused by a pathogenic bacterium, Moraxella bovis. There has been some doubt among previous workers as to the exact classification of M. bovis and its exact etiological role in the disease. At present M. bovis is incriminated as being the causative agent in cases of bovine infectious

keratitis.

Antibiotics are now being used as therapeutic agents. There have been many studies made on the growth inhibiting properties of the well-known antibiotics and sulfonamides but, to my knowledge there has been no critical study made of the susceptibility of M. bovis to these antibiotics and the sulfonamides.

This study was designed to determine which antibiotics and sulfonamides have the greatest growth inhibitory effect on M. bovis, to determine if this effect is constant for a specific isolate or for all isolates of this species.

CHAPTER II

LITERATURE REVIEW

Bovine infectious keratitis, more commonly called "pinkeye," is an infectious disease which attacks the eyes of cattle. The "pinkeye" appearance may occasionally be due to mechanical injury and such cases are not infectious and do not spread to other animals in the herd. The infection can appear in only one eye, sometimes followed by an infection in the other eye, or it may occur simultaneously in both eyes. The lesions of infectious keratitis are confined to the cornea and conjunctiva.

Not all workers are in complete agreement that one organism is the only causative agent of the disease. There is also some disagreement as to whether the organism causing the disease should be called Moraxella bovis or Hemophilus bovis. That there is an organism present during the disease has been well established. Most authors are in agreement on the morphology and biochemical reactions of this organism. In this paper the organism studied will be referred to as Moraxella bovis which is the terminology used in Bergey's Manual of Determinative Bacteriology, 7th Ed.

This is not a new disease, in 1889 Billings gave a good description of the disease as he observed it in cattle in Nebraska. He described a bacillus that he observed in stained sections of the cornea.

For treatment he states, "All the treatment necessary is a dark place, and cloths constantly hanging over the eyes, kept wet with cold

water all the time."

Another early worker, Poels (1917), conducted an investigation of keratitis among cattle in Holland. He excised a portion of the infected cornea and recovered Bacillus pyogenes. By injecting this organism between the layers of a normal cornea he was able to reproduce the disease. Poels concluded that B. pyogenes was the true cause of keratitis infections of cattle in Holland. Another foreign worker, Mitter (1915), investigated an epidemic of contagious ophthalmia in cattle in India. "It appeared that this epidemic was mainly caused by Micrococcus lanceolatus (pneumococcus of Fränkel), and partly by the bacillus of Morax-Axenfeld. They were found in the superficial epithelial cells of the conjunctiva."

Allen (1919) isolated a short, thick diplobacillus from eye secretions of typical cases of keratitis but could not reproduce the disease by inoculating this culture into the eyes of a calf. Jones and Little (1923) isolated a characteristic paired bacillus, and were able to reproduce the disease by inoculating eyes with pure cultures of this organism. They (1924) treated the eyes of seven cases of "pinkeye" with 20 percent argyrol, one of these animals harbored the diplobacilli in the eye ten days after treatment. Six animals whose eyes were treated with 2.5 percent zinc sulfate remained negative ten days after treatment. Farley (1940) was able to transmit the disease by dropping eye secretion from an active case of "pinkeye" onto the cornea of a susceptible calf but filtrates prepared from active cases of the disease would not reproduce keratitis in susceptible animals. He also proved that a calf apparently recovered from a chronic type of infection was still a source of keratitis infection seven months later. Farley et al, (1950) found that H. bovis could be readily isolated from active cases of "pinkeye." The eye secre-

tions from these active cases could reproduce the disease quite readily, but the freshly isolated organisms would not reproduce the disease. He found that the organisms were difficult to isolate for three to five days after the eyes had been treated with sulfonamides.

Baldwin (1945) reported finding H. bovis in 93 of 112 infected eyes of cattle. This organism was identical to that found by Jones and Little and he was able to induce a keratitis in 12 of 15 animals with these organisms.

Barner (1952) was able to isolate M. bovis from 92 of 95 cattle acutely infected with keratitis. He was able to reproduce an inflammatory eye condition typical of field cases of "pinkeye" in four experimental calves using a pure culture of M. bovis. The incubation period of experimental keratitis in cattle caused by M. bovis varied from fifteen to twenty-one days. Six cattle known to have had an acute attack of keratitis were not susceptible to keratitis when inoculated with M. bovis one year following experimental infection. He also showed that cattle which had been affected with keratitis caused by M. bovis may harbor the organism for a year or longer. He found that cultures of M. bovis could be effectively preserved for eight months or longer by desiccation. He tested the sensitivity of four cultures of this organism to five antibiotics using antibiotic discs placed on an inoculated seed layer surface. Under these conditions chloramphenicol and penicillin gave the largest zones of growth inhibition. The five antibiotics tested were bacitracin, chloramphenicol, penicillin, dihydrostreptomycin, and terramycin. Three of the cultures used were smooth variants and one a rough variant. He states:

> Only smooth colonies were noted on initial isolations of Moraxella bovis from acute cases of bovine keratitis and the colonies upon transfer remained smooth for three or four months; then, intermediate, rough, and dwarf colonies appeared.

Watts (1951) described symptoms of an outbreak of keratitis in a dairy herd in England. The organism he isolated appeared to be identical with the one isolated by American workers from the condition known as infectious keratitis. This organism does not appear to have been previously described in Britain. Attempts to transmit the disease with cultures which had become rather pleomorphic, from being grown on artificial media, failed. Treatment consisted of the local application of sulfonamide preparations, but the results were not very satisfactory. In accordance with the treatment recommended by Jones and Little, zinc sulphate, 1:40 solution, was tried, which resulted in a rapid clearing up of the symptoms, and when used early in a case prevented the development of the opacity.

During an enzootic of infectious keratitis Rastegaeva et al. (1947) found the lesions yielded a penicillin sensitive hemolytic diplococcus morphologically resembling the pneumococcus. To treat these animals 4 drops of penicillin, diluted in distilled water at the rate of 150 units per ml, were poured into the conjunctival sac twice daily. When alterations of the cornea had not become grave, the described penicillin therapy used for 2 to 4 days brought about a cure.

Freelan (1951) obtained good results by treating infectious keratitis with bacitracin ointment containing 500 units per gram. The infected eyes were treated each day for 2 days.

Gallagher (1954) was able to obtain good results by treating early stages of the disease with 1 percent chloromycetin opthalmic ointment in the eye once daily. These cases were treated 3 to 9 days, during treatment, the animals were kept in a darkened place.

Riley et al. (1953) observed that chloromycetin in a base ointment was beneficial in treating ovine keratitis. Faull et al. (1954) using

sensitivity tablets found the organism sensitive *in vitro* to penicillin using 0.5 units, streptomycin using 20 ug, and chloromycetin at 40 ug. Another worker, Anonymous (1948), used trisulfanol, a liquid preparation of sulfanilamide, sulfathiazole, azosulfamide, acriflavine, urea and benzl alcohol in propylene glycol and considered it to be valuable in the treatment of infectious keratitis in dairy herds.

Hawley (1954) used terramycin eye pellets to treat infectious keratitis. These eye pellets contained 5 mg oxytetracycline hydrochloride in crystalline form, 10,000 units of polymyxin B sulfate and 1 mg of tetracaine hydrochloride as an anesthetic. Antibiotic activity in tear samples of treated animals was measured using a plate assay method. He was able to demonstrate a concentration of 0.67 ug per ml of eye secretion 31 hours after the pellet was put in the eye.

The following article by Chiapella (1956) is in answer to the question "What antibiotics penetrate the eye?". Although written to apply to human medicine it could be applied to the treatment of animal medicine especially in treatment of bovine infectious keratitis.

The penetration of antibiotics into the eye depends on the condition of the eye and the route of administration. In general, an inflamed eye will absorb the drugs more readily than a noninflamed eye. Many drugs will not penetrate an intact cornea; however, if the corneal epithelium has been abraded, almost all of the antibiotics will penetrate it well. Subconjunctival injections will usually penetrate better than topical applications. Penetration into the posterior segment of the eye is best accomplished with systemic administration. Chloramphenicol (chloromycetin) penetrates the non-inflamed eye better than any other antibiotic, regardless of route of administration. It will even penetrate fairly well topically. Oxytetracycline, chlortetracycline, and tetracycline penetrate poorly. Topically they will enter the eye in effective doses only if the corneal epithelim is not intact. Even then they do not penetrate much more than the cornea itself. They are slightly more effective in subconjunctival injections but are fairly irritating. There is evidence that systemic administration of 3 gm. per day will give therapeutic intraocular levels. Tetracycline is said to give higher intraocular levels for a given dose than the oxytetracycline of chlortetracycline derivatives. The sulfonamides fall between the tetracycline drugs and chloramphenicol in their ability to penetrate the eye. The pyrimidine

derivatives of sulfanilamide are most effective in this respect. Gantrisin and sulfacetamide are not as effective but are less likely to cause untoward reactions. Penicillin is moderately effective. It does not penetrate the posterior segment well. Therapeutic doses of 4 million units intramuscularly per day give therapeutic intraocular levels. Streptomycin requires high doses to be effective. Therefore, subconjunctival injections of 200 mg. four times a day have been suggested. This gives high levels if administered with epinephrine. However, untoward reactions are fairly common, and its use for anything but tuberculosis is not recommended. Antibiotics that are not commonly used systemically such as polymyxin, bacitracin and neomycin are advised for topical use.

Due to the yearly economic loss in cattle caused by "pinkeye" a study is indicated to determine the susceptibility of M. bovis to selected antibiotics. By using a number of isolates of the organism, it can be determined whether different isolates show varying degrees of susceptibility to specific antibiotics. If it is found that some isolates of M. bovis react differently to different antibiotics, one might be able to show that a combination of antibiotics is a better therapeutic agent than a single antibiotic.

This study is being undertaken to determine the in vivo and in vitro susceptibility of M. bovis to selected antibiotics and sulfonamides in the hope that this information will aid in the treatment of the disease called "pinkeye."

CHAPTER III

EXPERIMENTAL OBJECTIVE

The experimental objective is to determine the *in vivo* and *in vitro* susceptibility of *M. bovis* to selected antibiotics and sulfonamides.

The susceptibility of the organism *M. bovis*, *in vitro* will be determined by measuring the zones of growth inhibition induced by the test drugs. A number of isolates will be used to determine whether or not there is a wide range of antibiotic susceptibility among different isolates of the organism. The zones of growth inhibition will be produced by using assay cups containing 0.1 ml solution of a known concentration of antibiotic.

Antibiotics which show good growth inhibitory effects *in vitro* will be tested *in vivo*. The effectiveness of an antibiotic *in vivo* will be measured by culturing from the eye following treatment to determine whether the eye becomes negative for *M. bovis* and, if so, the length of time the treated eye remains negative.

CHAPTER IV

MATERIAL AND METHODS

It was decided that the best approach to a study of the sensitivity of the organism would be to collect a number of isolates of the organism from as many different sources as possible and see if there was any noticeable difference in their reaction to the test drugs.

Standardizing cultures used: Twenty-one of the cultures used were collected locally over a period of several months. It was not feasible to test each culture as it was isolated, so each culture was lyophilized when isolated. This procedure standardized the length of time that each culture had grown on artificial media prior to being used. This was accomplished in the following manner:

Then a clinical case of keratitis was observed, the eye was swabbed with sterilized cotton which was then streaked on tryptose blood agar containing 10 percent citrated bovine blood. After 24 hours incubation a typical colony was selected and transferred to a blood agar slant. This slant was then incubated for 24 hours, to obtain good growth. The slant was then washed with sterile bovine serum and a small portion of the serum suspension was then lyophilized. The lyophilized samples were stored in a deep freeze at -10^{0}F until opened for use. The twenty-one isolates of the organism obtained locally were from observed clinical cases of "pinkeye." These isolates were obtained from small calves, yearling steers, and older brood cows. The eyes from which the isolates were

obtained all showed clinical symptoms of keratitis although none had progressed to the ruptured corneal ulcer stage. Three lyophilized cultures were obtained from other institutions.

Determining concentration of antibiotic to use: The assay cup method was used for testing antibiotic sensitivity. This procedure allowed a more flexible means of varying the strength of the antibiotic. By using this method the exact amount of antibiotic in each 0.1 ml that was used in the assay cups could be controlled.

In order to find the effective strength of the antibiotics, five cultures of M. bovis were isolated and tested immediately, without lyophilization. Thirteen antibiotics and five sulfonamides were run at different concentrations. Curves were then charted for each antibiotic and sulfonamide and the level at which they were to be tested was determined. A concentration was used for each antibiotic to give a zone of growth inhibition on all isolates, but low enough so that it would not be nearing the upper levels of the growth inhibition zone. The greatest activity per microgram, in most cases, was a concentration below 5 ug per 0.1 ml; therefore, from 0 to 5 ug was the range of greatest activity. As the concentration rose above 5 ug the curve of effectiveness tended to level off.

The sulfonamides showed more variability than the antibiotics in their growth inhibition effectiveness on different cultures. In addition partial inhibition also showed up frequently in the sulfonamides, so they were run at several different concentrations to make sure a zone of complete inhibition was established. The zones of partial inhibition produced by the sulfonamides could easily be overlooked. Colonies were present in the media, in the partial zones of inhibition, but were so small that if not carefully observed the zones would appear to be zones

of complete inhibition.

Pouring and inoculating the culture plates: During preliminary testing standard petri dishes were used. To speed up and facilitate the pouring and inoculating of the culture plates, pyrex pans that measured 13.5 x 8.75 inches were used. The pans were covered with sheets of glass to which absorbent felt had been attached. It was determined that by using 200 ml of base medium and 50 ml of blood agar base plus 5 ml of citrated blood for the seed layer, the depths of the media were virtually the same as the petri dish method using 20 ml of base medium and 5 ml of blood agar base plus 0.5 ml of citrated blood for the seed layer. Test runs established that using the same culture of organism and the same concentration of antibiotic, the zone of inhibition produced was the same for the petri dishes and the large pyrex pans. This procedure also permitted the inoculation of 50 ml of seed medium with a standardized dilution of inoculum. The seed layer could then be poured immediately, thus avoiding long exposures of the test organism to the 50°C temperature of the melted agar.

The base layer was poured at least an hour prior to pouring the seed layer. The base layer was an enriched agar consisting of yeast extract (3 gms), Bacto-peptone (10 gms), beef extract (3 gms), sodium chloride (5 gms), agar (17 gms), and distilled water (1000 ml). The blood agar used for seeding was a tryptose blood agar base (Difco) plus yeast extract (.3 percent). When the seed layer agar had been melted and cooled to 50°C the citrated blood and 1 ml of a suspension of inoculum were then added to the 50 ml of seed agar. The inoculum was standarized to a 60 percent transmittance through a 13 mm tube using a Central Scientific Photolometer set at a wave length of 525 mμ. As soon as the

blood and inoculum had been added, the seeded agar was poured immediately over the base layer and the pan tilted so that it was evenly distributed over the base layer.

To facilitate placing the assay cups in their proper spacing the following procedure was used: The outside bottom of the pans was marked off evenly so three rows with seven cups to the row could be placed on each pan and the assay cups would then be approximately 5 cms apart. Glass ink was used to mark the spot where each assay cup was to be placed. After the media were poured and had hardened, the pyrex pan was set on top of an illuminated Bangs testing box, the marked dots were then clearly visible through the media and the assay cups could be placed directly over the marked spots.

Dilution of the antibiotic: To obtain the required dilution of antibiotic, 100 mg was weighed out on an analytical balance and enough sterile double distilled water was added to make the proper dilution. A small amount of methyl alcohol (0.5 ml per 100 mg) was added to erythromycin and carbomycin to facilitate getting them into solution since they are not readily soluble in water. This quantity of methyl alcohol was just enough to get them into solution. These antibiotics with and without methyl alcohol were then tested on several isolates of M. bovis and no variation in the size of the zones was detected. One ml pipettes were used to put the 0.1 ml solution of antibiotic into the assay cups. These pipettes were marked and a separate pipette was used for each dilution of each antibiotic on every test run. The assay cups used for all tests were stainless steel cups 8 mm in outside diameter. The cups seated themselves on the agar so that there was no leakage of antibiotic solution under their edges.

After 24 hours, all the antibiotic solution has been absorbed by the media so there was none left in the assay cup. After the 24 hours incubation there was a definite zone of growth inhibition so the plates were read at that time.

Method used to read the zone of growth inhibition: To read the zone of inhibition, a low power (10X) microscope was used. It was deemed necessary to use the microscope since the zone, as it appeared to the eye, was not always the true zone. This is especially important when using a blood agar and a hemolytic organism because the zone of hemolysis sometimes extends into the zone of inhibition and obscures the true zone of inhibition. To secure an accurate measurement of the inhibition zone a caliper with sharpened ends was used. The two points were put on the opposite edges of the inhibition zone and the distance between the two caliper points measured with a millimeter rule.

Methods used for reconstituting lyophilized cultures and preparing seed agar layer: The culture tubes were opened and the lyophilized material in each tube was reconstituted with sterile double distilled water to the same volume as the serum which was originally used to suspend the organism prior to lyophilization. A loopful of this material was then streaked on a blood agar plate and incubated for 24 hours. A typical colony was then transferred to a blood agar slant and incubated 24 hours. The growth was then harvested from the slant with double distilled water and put in a screw-top tube containing sterile glass beads. After the tube was agitated and standardized as described, the suspension was used to inoculate the seed layer.

Sugar fermentations and gram stains were run on all the isolates. All test isolates used were gram negative diplobacillus which produced

no acid or gas in the following sugars: Arabinose, dextrin, dextrose, galactose, maltose, mannitol, salicin, sucrose, and xylose.

Preliminary trials designed to test for reproducible results: Numerous preliminary trials were run to determine if there was any great variation in the size of inhibition zones for a certain isolate using a specific concentration of an antibiotic. The diameters of the zones of growth inhibition when so tested all fell within a narrow limit. All runs in the test proper were then made in duplicate which seemed sufficient as there was no great variance noted for any of the isolates used in the preliminary trials. Some of the antibiotics, polymyxin B sulfate, bacitracin, magnamycin, and the sulfonamides required rather large concentrations to give readable zones. These drugs were run at several concentrations so any less resistant isolates would be observed. Achromycin, aureomycin, and penicillin were used at such low concentrations that two concentration levels were used in case any of the isolates were more resistant.

Testing antibiotics in vivo: A culture of M. bovis (ATCC) was grown on a blood agar slant for 24 hours. A suspension of this culture was then used to inoculate the right eye of 9 one-year-old steers. These 9 steers all had "pinkeye" when they were small calves. Swab cultures were taken daily for two consecutive days to check whether the organisms remained in the inoculated eyes or whether the animals had enough residual immunity to quickly eliminate the organism. After the organism had been recovered from the eye for two consecutive days each of 5 animals was treated with a separate antibiotic. The antibiotics used were: penicillin (50,000 units), aureomycin (10 mgs), erythromycin (10 mgs), chloromycetin (10 mgs), and terramycin (10 mgs). The other 4 animals were untreated controls. Swabs were taken daily thereafter and streaked on blood agar plates. The data on these 9 steers are compiled in Table VII

in the appendix.

This study was also conducted on an isolated group of small calves having no previous history of "pinkeye." The eyes of these calves were inoculated with the same culture of M. bovis (ATCC) as used on the 9 steers. After swab cultures demonstrated the presence of M. bovis the same 5 antibiotics were used to treat the infected eyes. The treatment consisted of one concentration for all five antibiotics and two concentrations with chloromycetin, erythromycin, and terramycin. This was to determine if the amount of antibiotic influenced the elimination of the organism from the eye.

After the eyes were treated with an antibiotic, swabs were taken and streaked on blood agar plates every 3 hours for 12 hours following treatment. At the same time the swabs were taken 0.1 ml of eye secretion was obtained aseptically. The eye secretions were then diluted in 50 mls of sterile distilled water and filtered through a millipore filter. The filter leaf was then incubated on an absorbent pad containing enriched nutrient broth. With neither the swab nor filter technique was it possible to demonstrate viable organisms of M. bovis in a treated eye sooner than 2 days after treatment. The 3 hour schedule was then discontinued and swabs were taken daily thereafter. The millipore filter technique was also discontinued as the number of M. bovis organisms recovered was comparable to that obtained using the sterile swab method and the contamination rate was much higher on the millipore filters.

The organism used in the in vivo studies had been growing on blood agar for some time and had lost most of its virulence. When used on the 9 steers with a previous history of "pinkeye" no clinical symptoms were observed even when the eye harbored the organism. When used on

the small calves with no previous history of "pinkeye" many of the eyes showed profuse lacrimation but no corneal ulcers developed.

To terminate the study all animals in the isolation pen were treated in both eyes with an antibiotic.

CHAPTER V

RESULTS

Zones of growth inhibition induced by the antibiotics: The susceptibility of M. bovis to an antibiotic in vitro was measured by the zone of growth inhibition produced by a known concentration of antibiotic in solution. The quantity of solution used was 0.1 ml in stainless steel assay cups which had an outside diameter of .80 cms. The average diameter of the zone of growth inhibition for a given antibiotic, is the average for the 24 isolates of M. bovis used in this study. These data are compiled in Table I. The 24 isolates used are listed in Table V in the appendix.

In this study thirteen different antibiotics were used. The concentration of antibiotic used in the test varied from .25 units (.15 ug) for penicillin to 90 ug for magnamycin.

Due to the wide variance of susceptibility of M. bovis to the selected antibiotics, each antibiotic was tested over a wide enough concentration range to insure obtaining a readable zone of inhibition.

Penicillin showed a great inhibitory effect on M. bovis producing large zones of inhibition which ranged in diameter from 4.85 cms to 3.03 cms when using a concentration of .5 units. Magnamycin had very little inhibitory effect on the organism; therefore, the diameters of its zones were very small, ranging in diameter from 1.30 cms to .80 cms when using a concentration of 90 ug.

TABLE I

THE AVERAGE ZONE OF INHIBITION AND THE RANGE OF ZONES FOR THE TWENTY-FOUR CULTURES OF MORAXELLA BOVIS

Antibiotic	Micrograms per 0.1 ml. solution	Average diameter * for zone of inhibition (cms)	Range in diameter of zones (cms)
Achromycin	0.5	1.188	1.60 to 0.91 or 0.69
Achromycin	1.0	1.553	2.15 to 1.25 or 0.90
Albamycin	1.0	1.255	1.68 to 0.875 or 0.805
Aureomycin	0.5	1.246	1.60 to 0.94 or 0.56
Aureomycin	1.0	1.576	2.10 to 1.21 or 0.89
Bacitracin	10.0	0.945	1.25 to 0.80 or 0.45
Bacitracin	20.0	1.22	1.55 to 0.875 or 0.675
Bacitracin	40.0	1.43	1.75 to 1.13 or 0.62
Carbomycin	5.0	1.61	2.40 to 1.05 or 1.35
Chloromycetin	1.0	1.425	2.55 to 0.95 or 1.60
Erythromycin	10.0	1.5877	2.05 to 1.10 or 0.95
Magnamycin	30.0	0.80	0.80 to 0.80 or 0
Magnamycin	60.0	0.832	1.05 to 0.80 or 0.25
Magnamycin	90.0	0.982	1.30 to 0.80 or 0.50
Neomycin	10.0	1.352	2.07 to 1.00 or 1.07
Penicillin	0.25 units	2.852	3.60 to 1.55 or 2.05
Penicillin	0.50 units	3.776	4.85 to 3.03 or 1.82
Polymyxin B Sulfate	20.0	0.95	1.26 to 0.80 or 0.46
Polymyxin B Sulfate	40.0	1.145	1.63 to 0.80 or 0.83
Polymyxin B Sulfate	60.0	1.335	2.47 to 0.875 or 1.60
Streptomycin	5.0	1.650	2.21 to 0.925 or 1.285
Terramycin	1.0	0.97	1.35 to 0.80 or 0.55
Terramycin	2.0	1.281	1.97 to 0.80 or 1.17

* The diameter of the zone includes the width of the assay cup which is 0.80 cms in diameter.

The data in Table I show that achromycin, albamycin, aureomycin, chloromycetin, erythromycin, and penicillin all had average inhibition zones greater than 1 cm in diameter when using a concentration of 1 ug or less. Terramycin was the only other antibiotic used at the 1 ug level and it produced an average inhibition zone of .97 cms. Appendix Table V lists the diameters of the zones of inhibition for each of the 24 cultures used.

Zones of growth inhibition induced by the sulfonamides: The susceptibility of M. bovis to the sulfonamides is listed in Table II. The range in the diameters of the zones of inhibition for the 24 cultures is also listed. Appendix Table VI lists the zones of inhibition for each of the 24 cultures.

The five sulfonamides used in this study were sulfanilamide, sulfadiazine, sulfapyridine, sulfamerazine, and sulfathiazole. It was observed that the phenomenon of partial inhibition was produced with some of the isolates. In the zones of partial inhibition colonies were present but were so small they could be easily overlooked if not carefully observed. The average diameter of the zones as listed in Table II represents the zone of complete inhibition. The zones of partial inhibition, encountered with the sulfonamides, are recorded in Table VI in the appendix.

Activity curve for the antibiotics and sulfonamides: To obtain a more complete activity curve for the antibiotics and sulfonamides they were tested at the following concentrations: Antibiotics; .0625, .125, .25, .50, 1.0, 2.0, 4.0, 10.0, 20.0, 30.0, 40.0, and 50.0 ug. Sulfonamides; 25, 50, 100, 150, 200, and 250 ug. The concentrations of penicillin are expressed in units (1 unit = 0.6 ug). M. bovis (ATCC) was used as the test organism.

TABLE II

THE AVERAGE ZONE OF INHIBITION AND THE RANGE OF ZONES FOR THE TWENTY-FOUR CULTURES OF MORAXELLA BOVIS

Sulfonamide	Micrograms per 0.1 ml solution	Average diameter * for zone of inhibition (cms)	Range in diameter of zones (cms)
Sulfanilamide	30	0.81	1.05 to 0.80 or 0.25
Sulfanilamide	60	0.82	1.31 to 0.80 or 0.51
Sulfanilamide	90	0.86	1.50 to 0.80 or 0.70
Sulfanilamide	120	1.03	1.70 to 0.80 or 0.90
Sulfanilamide	250	1.301	2.00 to 0.975 or 1.125
Sulfadiazine	30	0.83	1.20 to 0.80 or 0.40
Sulfadiazine	60	0.898	1.60 to 0.80 or 0.80
Sulfadiazine	90	1.091	1.82 to 0.80 or 1.02
Sulfadiazine	250	1.417	2.20 to 1.00 or 1.20
Sulfamerazine	30	0.8375	1.10 to 0.80 or 0.30
Sulfamerazine	60	0.918	1.40 to 0.80 or 0.60
Sulfamerazine	90	1.094	1.70 to 0.80 or 0.90
Sulfamerazine	250	1.412	1.97 to 0.975 or 1.015
Sulfapyridine	30	0.84	1.20 to 0.80 or 0.40
Sulfapyridine	60	0.885	1.52 to 0.80 or 0.72
Sulfapyridine	90	1.146	1.61 to 0.80 or 0.81
Sulfapyridine	250	1.567	2.55 to 0.94 or 1.61
Sulfathiazole	30	0.867	1.30 to 0.80 or 0.50
Sulfathiazole	60	0.956	1.67 to 0.80 or 0.87
Sulfathiazole	90	1.362	2.03 to 0.985 or 1.145
Sulfathiazole	250	1.816	2.27 to 1.35 or 0.92

* The diameter of the zone includes the width of the assay cup which is 0.80 cms in diameter

Figure 1 shows the activity curves of penicillin, erythromycin, achromycin, streptomycin, albamycin, magnamycin, and bacitracin. From the graph it is evident that penicillin at very low concentrations had a marked inhibitory effect on the organism. Magnamycin showed no inhibition at 50 ug; so it was tested at a concentration of 60 ug. At this concentration magnamycin showed only slight growth inhibition.

Figure 2 shows the activity curves of chloromycetin, aureomycin, terramycin, carbomycin, neomycin, and polymyxin B sulfate. It was interesting to note that chloromycetin showed very little activity at the low concentrations, but from concentrations of 0.5 ug and up its activity increased rapidly. Polymyxin B sulfate showed very little activity at concentrations below 10 ug.

Figure 3 shows the activity curves of sulfadiazine, sulfamerazine, sulfapyridine, sulfanilamide, and sulfathiazole. The five sulfonamides used fell in two groups. Sulfathiazole and sulfapyridine showed greater inhibition than sulfanilamide, sulfadiazine, and sulfamerazine.

A study of the antibiotics in vivo: The antibiotics used to test in vivo effectiveness were aureomycin, chloromycetin, erythromycin, penicillin, and terramycin. These antibiotics were used because of their in vitro effectiveness against M. bovis. They also belong in different chemical groups with the exception of aureomycin and terramycin whose structural formulae are quite similar.

The eyes of the 9 one-year-old steers with a previous history of having had "pinkeye" as small calves that were treated with antibiotics gave negative swab cultures for M. bovis within 24 hours. Two of the untreated eyes used for controls also became negative after a period of a week or more. No conclusive evidence as to the effectiveness of these

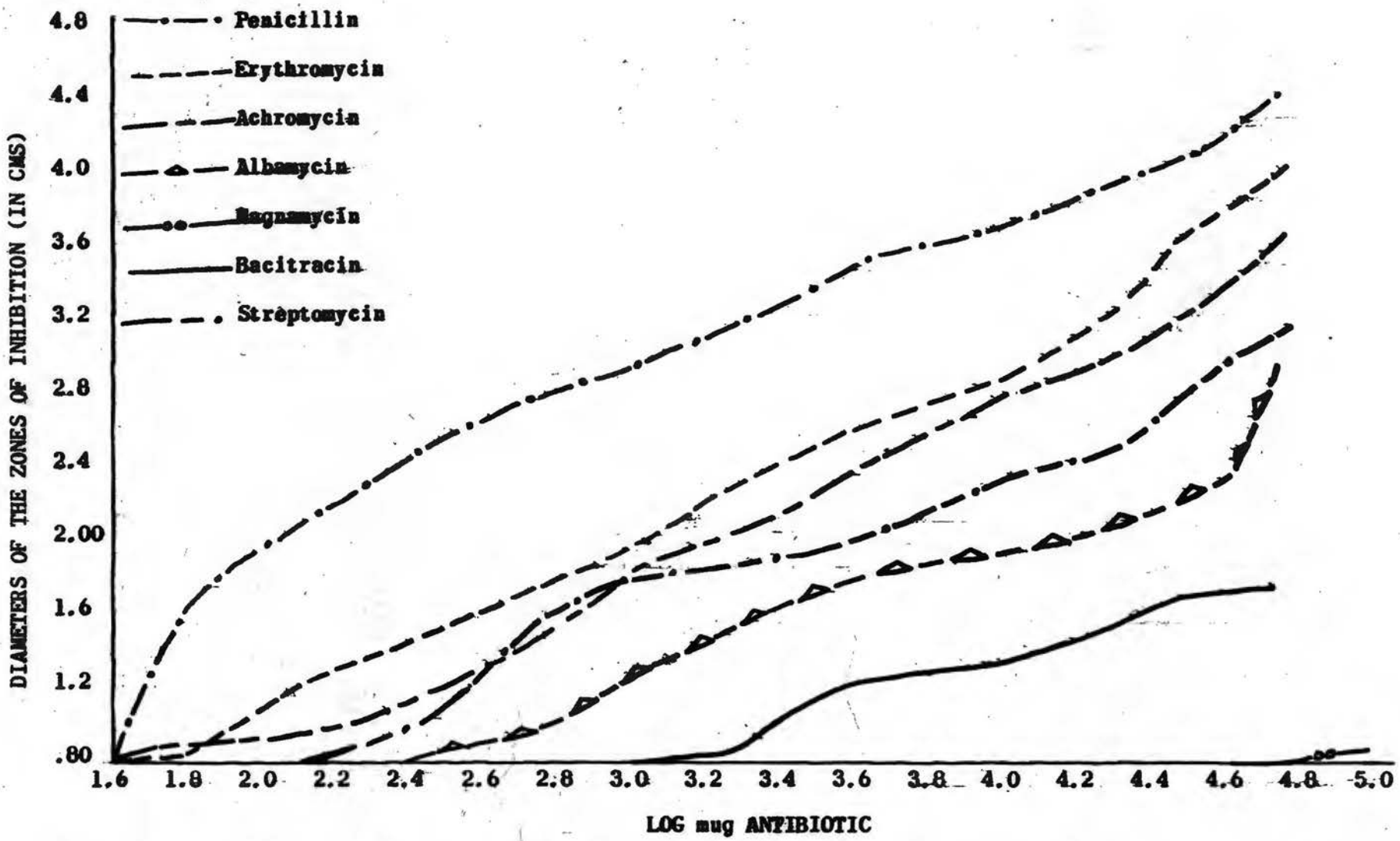

Fig. 1. Growth Inhibition curves for M. bovis (ATCC) using Penicillin, Erythromycin, Achromycin, Streptomycin, Albamycin, Magnamycin, and Bacitracin.

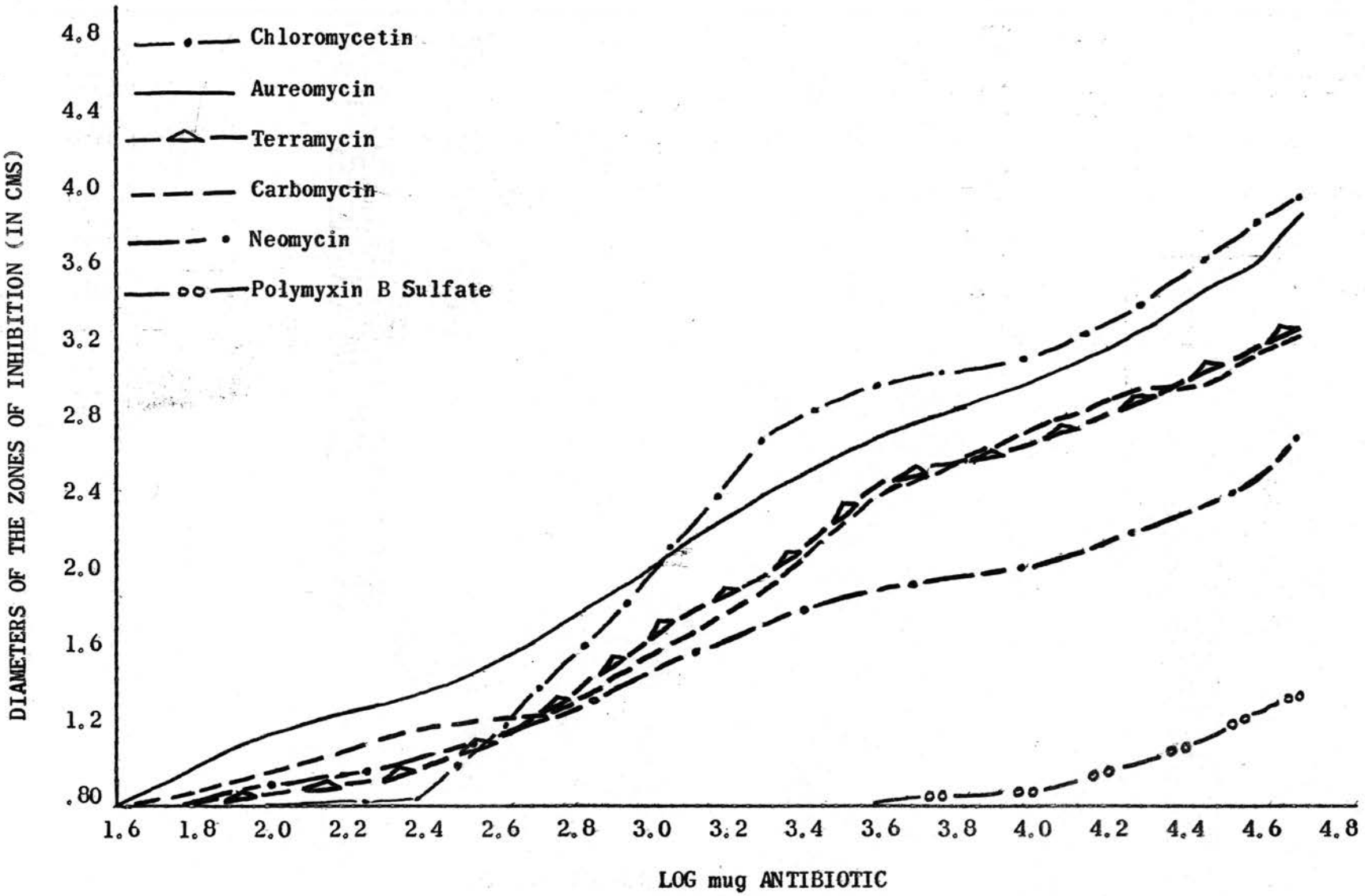

Fig. 2. Growth Inhibition curves for M. bovis (ATCC) using Chloromycetin, Aureomycin, Terramycin, Carbomycin, Neomycin, and Polymyxin B. Sulfate.

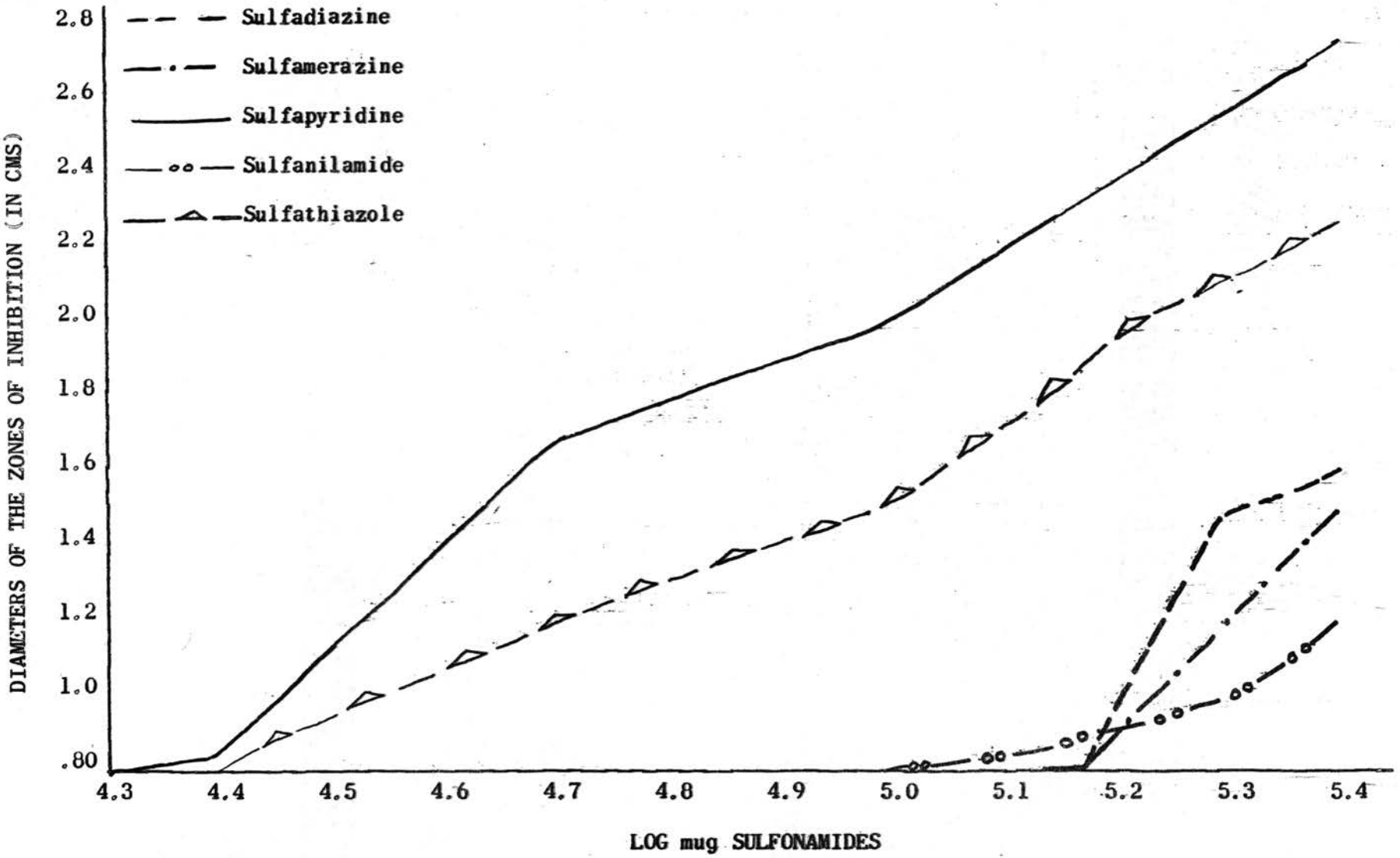

Fig. 3. Growth Inhibition curves for M. bovis (ATCC) using Sulfadiazine, Sulfamerazine, Sulfapyridine, Sulfanilamide, and Sulfathiazole.

antibiotics was established with these animals. The complete information on these 9 animals can be found in Table VII in the appendix.

Table III is a summary of the results obtained in the study conducted on the group of small calves with no previous history of "pinkeye." Under ideal circumstances many more animals should have been used to obtain statistically valid observations; therefore, the results shown in Table III can only indicate a trend.

In most cases it was observed that on the day following treatment the majority of the eyes were negative for M. bovis. In the case of erythromycin when 10 mgs was used only 50 percent were negative the next day, but when 20 mgs was used 100 percent were negative the next day. The other antibiotics gave substantially the same results at both the 10 mg and 20 mg level.

To try to determine whether the organism remains in the eye and reappears or whether the eye is reinfected, the following treatment was instigated: All calves which remained in the isolation pen were treated with an antibiotic regardless of whether they harbored the organism or not. In this study both eyes of one calf were treated with one of the following antibiotics: aureomycin, chloromycetin, terramycin, or erythromycin. The rest of the calves were treated in both eyes with penicillin. All of the eyes treated, with the exception of one eye which received penicillin, harbored the organism at the time of treatment. Penicillin was used most frequently because it produced the largest zones of growth inhibition in the in vitro tests and it is fairly economical and easy to obtain. The penicillin gave some rather unpredictable results. The day following treatment with 50,000 units 9 of 10 eyes treated were negative. The second day following treatment 8 of the 10 eyes harbored organisms.

TABLE III

SUMMARY OF RESULTS OBTAINED USING FIVE ANTIBIOTICS IN VIVO

Antibiotic	Mgs used	No. of eyes treated	Results of swabs taken the following days after treatment*											
			One day		Two days		Three days		Four days		Five days		Six days	
			No. Pos.	No. Neg.	No. Pos.	No. Neg.	No. Pos.	No. Neg.	No. Pos.	No. Neg.	No. Pos.	No. Neg.	No. Pos.	No. Neg.
Aureomycin	10	4	0	4	1	3	1	2	1	1	3	1	3	1
Chloromycetin	10	4	2	2	1	3	1	1	2	1	3	0	1	2
Chloromycetin	20	2	0	0	1	1	2	0	1	1	1	1	1	1
Erythromycin	10	4	2	2	2	2	1	0	1	1	2	0	2	0
Erythromycin	20	2	0	1	0	2	1	1	1	1	1	1	0	1
Terramycin	10	4	1	3	2	2	1	2	1	1	1	1	1	2
Terramycin	20	2	0	0	0	2	0	2	0	2	0	2	0	2
Penicillin	25,000 units	4	0	4	2	2	0	2	2	1	3	0	3	0

* Some of the eyes were not cultured on six consecutive days after treatment; therefore, the totals under days following treatment are not always equal to the totals under number of eyes treated.

On the 3rd, 4th, 5th, and 6th days some of the eyes which were positive on the 2nd day following treatment became negative and remained that way. This was also noted with the other antibiotics but not as frequently as with penicillin. The results of this study are tabulated in Table IV. Appendix Table VIII lists all the calves used and the treatment each received.

TABLE IV

SUMMARY OF *IN VIVO* RESULTS WHEN ALL EYES WERE TREATED SIMULTANEOUSLY WITH ANTIBIOTICS

Antibiotic	Mgs used	No. of* eves treated	Results of swabs taken the following days after treatment									
			One day		Two days		Three days		Four days		Five days	
			No. Pos.	No. Neg.	No. Pos.	No. Neg.	No. Pos.	No. Neg.	No. Pos.	No. Neg.	No. Pos.	No. Neg.
Aureomycin	20	2	0	2	0	2	0	2	1	1	1	1
Chloromycetin	20	2	0	2	0	2	0	2	1	1	1	1
Erythromycin	20	2	0	2	1	1	0	2	0	2	0	2
Terramycin	20	2	0	2	0	2	0	2	1	1	1	1
Penicillin	50,000 units	10	1	9	8	2	3	7	4	6	4	6

* All eyes that were treated but one were positive for *Moraxella bovis* at the time they were treated.

CHAPTER VI

DISCUSSION AND CONCLUSIONS

In this study there was a significant difference among the selected antibiotics in their growth inhibitory effect on the organism M. bovis when tested in vitro.

The antibiotics tested fell into three main groups when their effectiveness was measured by the zone of inhibition they induced. Penicillin, erythromycin, achromycin, chloromycetin, and aureomycin gave the largest zones of inhibition. They all produced zones with a diameter of 3.6 cms or greater when tested at the 50 ug level.

The next group, consisting of carbomycin, terramycin, neomycin, streptomycin, and albamycin had inhibition zones which ranged from approximately 2.7 cms to slightly over 3.2 cms in diameter when tested at the same level.

The third or lowest group ranged from practically no zone for magnamycin to zones with a diameter of 1.28 cms for polymyxin B sulfate and 1.8 cms for bacitracin.

The sulfonamides, when tested in vitro, did not inhibit M. bovis as effectively as did most of the antibiotics. Two of the sulfonamides, sulfapyridine and sulfathiazole, showed markedly greater inhibitory action than the other 3 sulfonamides. Sulfapyridine and sulfathiazole had zones of inhibition with diameters of 2.75 cms and 2.25 cms respectively as compared with zones of 1.6 cms, 1.5 cms. and 1.37 cms for sulfadiazine, sulfamerazine, and sulfanilamide respectively. These zones

were all based on the highest concentration used which was 250 ug. The phenomenon of partial inhibition occurred with some of the isolates when using the sulfonamides. This phenomenon was not observed with any of the antibiotics tested.

Twenty-four different isolates of the organism M. bovis were used to test the antibiotics and sulfonamides in vitro. Twenty-one of these isolates were obtained from clinical cases of "pinkeye," in the locality of the Veterinary Research Station at Pawhuska, Oklahoma. The other three isolates were obtained from the American Type Culture Collection, Lederle Laboratories, and Kansas State College.

Of the data obtained from these 24 isolates it appeared there was no great difference in susceptibility to the antibiotics and sulfonamides among different isolates of the organism. At a given concentration of a specific antibiotic all the isolates exhibited about the same susceptibility. In addition, all isolates tested were highly susceptible to penicillin and at the same time were very resistant to magnamycin. This same characteristic to a lesser degree held true for the sulfonamides.

In the in vivo tests there were not enough animals used to reach any statistically valid conclusions. In most cases the eyes that were treated would be negative to swab cultures for several days following treatment, then many would become positive. To eliminate the possibility that the eyes were being reinfected, all eyes whether positive or not were treated with antibiotic. Even when treated in this manner many of the eyes became positive again after a period of several days. This suggests the possibility that the organisms are harbored in the inner portion of the eye or in the tear ducts and nasal passages. The organism has been cultured from nasal exudate, but is not capable of surviving on

the mucosa of nasal passages. It is also possible that the antibiotic does not kill the organism and in time the antibiotic is diluted enough by eye fluid to permit growth. From the data obtained it would seem advisable to treat the infected eye more than once.

The eyes were treated by putting the antibiotic powder under the lower lid of the eye. The antibiotics used were in the pure powder form. The antibiotics did not seem to cause the animal any undue distress. Better and more lasting results might be obtained by using the antibiotic in a small pellet form which would be slow in dissolving. This would tend to maintain a high concentration of the antibiotic in the eye over a longer period of time. Since some eyes that are in the incubation stage or the carrier state may harbor the organism without clinical symptoms, it would seem logical to treat all the eyes of any given group of animals if treatment of any of the animals in this group is instigated.

SUMMARY

A study was made to determine the susceptibility of Moraxella bovis to selected antibiotics and sulfonamides.

Cultures of M. bovis were obtained from 24 clinical cases of "pink-eye." The susceptibility of these cultures to the test drugs in vitro was determined by testing the following antibiotics and sulfonamides: achromycin, albamycin, aureomycin, carbomycin, chloromycetin, erythromycin, magnamycin, neomycin, penicillin, polymyxin B sulfate, streptomycin, bacitracin, terramycin, sulfadiazine, sulfamerazine, sulfapyridine, sulfanilamide, and sulfathiazole.

The in vitro tests were made by using stainless steel assay cups placed on seeded blood agar plates. One tenth ml of a known concentration of antibiotic solution was used in each assay cup.

The diameter of the zone of growth inhibition induced by each antibiotic at a given concentration was determined for each of the 24 cultures used. The average diameter and the range in the difference of diameters of the zones of growth inhibition for the 24 cultures using a given antibiotic were also determined.

There was a significant difference in the size of the zones of growth inhibition induced by the selected antibiotics. Penicillin, aureomycin, achromycin, chloromycetin, and erythromycin produced the largest zones of growth inhibition while albamycin, carbomycin, neomycin, streptomycin, and terramycin produced medium sized zones of inhibition. Magnamycin, polymyxin B sulfate and bacitracin had rather

small zones of inhibition. The extreme range of antibiotic susceptibility may be illustrated as follows: When using penicillin at .5 units (0.3 ug) the average zone of inhibition for the 24 cultures used was 3.776 cms in diameter while magnamycin produced a zone of only .982 cms in diameter when a concentration of 90 ug was used.

With the five sulfonamides used, sulfapyridine and sulfathiazole produced larger zones of inhibition than did sulfadiazine, sulfamerazine, and sulfanilamide.

In vivo tests were made on 44 eyes using aureomycin, chloromycetin, erythromycin, terramycin, and penicillin. All of the antibiotics tested *in vivo* gave good initial results, in that the eye became negative to *M. bovis* for several days following treatment. These results were not permanent, however, in that the organism could be cultivated from many of the eyes after not being recoverable for several days.

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A P P E N D I X

TABLE V

THE ZONES OF GROWTH INHIBITION (CMS) FOR THE 24 CULTURES OF MORAXELLA BOVIS

USING 13 ANTIBIOTICS*

Antibiotic	Micrograms per 0.1 ml	Culture A.T.C.C.	Culture #192	Culture #125	Culture #526	Culture #136	Culture Lederle
Achromycin	0.5	1.40-1.41	1.20-1.40	1.20-1.20	1.60-1.56	1.60-1.58	1.30-1.30
Achromycin	1.0	1.80-1.81	1.94-1.95	1.89-1.91	2.00-2.05	2.05-2.10	1.72-1.70
Albamycin	1.0	1.30-1.31	1.30-1.30	1.10-1.10	1.50-1.50	1.70-1.67	1.30-1.32
Aureomycin	0.5	1.50-1.70	1.40-1.70	1.30-1.60	1.40-1.45	1.60-1.60	1.47-1.45
Aureomycin	1.0	2.05-2.10	2.10-2.11	2.00-2.02	1.80-1.80	2.05-2.05	1.80-1.80
Bacitracin	10	0.88-0.80	0.99-0.95	0.90-0.89	1.00-1.01	1.11-1.12	1.00-1.02
Bacitracin	20	1.40-1.42	1.13-1.11	1.40-1.40	1.10-1.30	1.31-1.31	1.10-1.12
Bacitracin	40	1.52-1.55	1.61-1.60	1.65-1.70	1.30-1.32	1.40-1.42	1.35-1.40
Carbomycin	5.0	1.93-1.80	1.80-1.92	2.20-2.00	2.10-2.05	2.50-2.30	1.65-1.65
Chloromycetin	1.0	1.90-2.00	1.50-1.30	1.80-2.00	1.30-1.30	2.50-2.60	1.82-1.80
Erythromycin	1.0	1.90-1.70	1.80-1.80	1.80-1.70	2.10-2.00	1.80-1.75	1.72-1.70
Magnamycin	30	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80
Magnamycin	60	0.84-0.85	0.80-0.80	0.80-0.90	1.10-1.00	0.90-0.90	0.90-0.90
Magnamycin	90	1.20-1.17	1.05-1.10	1.15-1.20	1.30-1.30	1.10-1.15	1.20-1.22
Neomycin	10	1.55-1.57	1.15-1.10	1.20-1.20	2.10-2.04	1.30-1.30	1.59-1.60
Penicillin	.25 Units	2.45-2.40	3.00-2.80	2.81-2.80	2.71-2.70	2.90-2.91	2.50-2.51
Penicillin	.50 Units	3.40-3.50	3.90-3.80	3.85-3.84	3.62-3.63	3.60-3.62	3.61-3.60
Polymyxin B Sulfate	20	1.00-1.00	0.80-0.80	1.00-1.00	0.90-0.90	1.00-1.00	1.27-1.25
Polymyxin B Sulfate	40	1.40-1.41	0.96-1.00	1.30-1.35	1.02-1.03	1.12-1.15	1.65-1.60
Polymyxin B Sulfate	60	1.50-1.52	1.42-1.40	1.56-1.55	1.20-1.22	1.40-1.42	2.50-2.45
Streptomycin	5.0	2.00-1.93	1.70-1.90	1.80-1.80	2.00-2.00	1.90-1.85	2.20-2.22
Terramycin	1.0	1.30-1.20	1.10-1.11	1.09-1.10	1.00-1.01	1.10-1.10	0.90-0.91
Terramycin	2.0	1.68-1.70	1.37-1.38	1.48-1.47	1.52-1.51	1.50-1.52	1.21-1.22

TABLE V (Continued)

Antibiotic	Micrograms per 0.1 ml	Culture #129	Culture #277	Culture #148	Culture #430	Culture #443	Culture #419
Achromycin	0.5	1.00-1.05	1.40-1.45	1.00-1.20	0.92-0.90	1.00-1.10	1.05-1.10
Achromycin	1.0	1.40-1.45	1.75-1.70	1.45-1.50	1.20-1.30	1.40-1.35	1.50-1.40
Albamycin	1.0	1.00-1.00	1.50-1.50	1.00-1.10	1.00-1.10	1.10-1.10	1.10-1.20
Aureomycin	0.5	1.10-1.05	1.30-1.25	1.00-1.10	1.00-1.10	1.30-1.30	1.30-1.40
Aureomycin	1.0	1.50-1.48	1.75-1.72	1.45-1.50	1.40-1.50	1.60-1.60	1.70-1.70
Bacitracin	10	0.85-0.87	1.00-1.00	0.80-0.80	0.92-0.91	0.93-0.93	1.00-1.01
Bacitracin	20	0.90-0.95	1.30-1.32	1.20-1.20	1.20-1.21	1.13-1.14	1.30-1.32
Bacitracin	40	1.10-1.15	1.40-1.39	1.40-1.35	1.40-1.50	1.25-1.30	1.60-1.58
Carbomycin	5.0	2.00-1.90	1.80-1.75	1.80-1.90	1.40-1.45	1.50-1.40	1.19-1.20
Chloromycetin	1.0	1.40-1.50	1.20-1.30	1.30-1.40	1.40-1.60	1.00-1.00	1.25-1.30
Erythromycin	1.0	1.90-2.10	1.70-1.75	1.70-1.80	1.20-1.30	1.40-1.10	1.20-1.30
Magnamycin	30	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80
Magnamycin	60	0.80-0.80	0.90-0.90	0.80-0.80	0.80-0.80	0.80-0.80	0.83-0-80
Magnamycin	90	1.00-1.00	1.10-1.12	1.00-1.05	0.90-0.95	1.10-1.20	0.90-0.95
Neomycin	10	1.10-1.05	1.70-1.68	1.00-1.10	1.50-1.40	1.00-1.00	1.30-1.40
Penicillin	.25 Units	2.60-2.61	3.00-2.92	2.80-2.81	3.20-3.21	1.55-1.55	3.60-3.61
Penicillin	.50 Units	3.41-3.42	3.80-3.82	4.00-3.94	3.80-3.81	4.00-4.02	4.10-4.12
Polymyxin B Sulfate	20	1.20-1.10	0.90-0.90	0.80-0.80	1.10-1.20	0.95-0.90	1.00-1.00
Polymyxin B Sulfate	40	1.35-1.40	1.10-1.20	0.95-0.90	1.30-1.30	1.20-1.10	1.20-1.22
Polymyxin B Sulfate	60	1.67-1.60	1.30-1.35	1.10-1.05	1.35-1.40	1.30-1.30	1.29-1.28
Streptomycin	5.0	1.70-1.90	2.10-2.00	1.60-1.70	1.60-1.50	1.60-1.50	1.50-1.57
Terramycin	1.0	1.08-1.09	1.06-1.05	1.03-1.02	0.80-0.80	1.10-1.09	1.40-1.30
Terramycin	2.0	1.32-1.33	1.32-1.33	1.35-1.36	1.22-1.23	1.30-1.32	1.60-1.50

TABLE V (Continued)

Antibiotic	Micrograms per 0.1 ml	Culture #127	Culture #535	Culture #435	Culture #418	Culture #533	Culture #534
Achromycin	0.5	1.05-1.00	1.10-1.15	1.20-1.00	1.00-1.05	1.05-1.00	1.00-0.95
Achromycin	1.0	1.30-1.40	1.40-1.45	1.40-1.40	1.40-1.40	1.30-1.40	1.30-1.25
Albamycin	1.0	0.90-0.90	1.00-1.00	1.10-1.00	1.10-1.20	1.37-1.40	1.00-1.10
Aureomycin	0.5	1.10-1.10	1.05-1.00	1.20-1.30	1.05-1.10	1.10-1.20	0.90-0.98
Aureomycin	1.0	1.40-1.50	1.35-1.40	1.60-1.65	1.40-1.30	1.40-1.60	1.20-1.22
Bacitracin	10	1.11-1.10	0.80-0.80	1.20-1.20	1.25-1.25	1.00-1.00	0.80-0.80
Bacitracin	20	1.20-1.21	1.12-1.10	1.30-1.31	1.35-1.32	1.30-1.32	1.00-1.05
Bacitracin	40	1.50-1.45	1.30-1.34	1.60-1.55	1.60-1.60	1.50-1.50	1.30-1.35
Carbomycin	5.0	1.60-1.50	1.00-1.10	1.20-1.40	1.40-1.30	1.60-1.50	1.10-1.15
Chloromycetin	1.0	1.30-1.30	1.30-1.35	1.30-1.50	1.40-1.40	1.45-1.50	1.10-1.20
Erythromycin	1.0	1.30-1.40	1.10-1.10	1.30-1.40	1.20-1.30	1.19-1.20	1.20-1.20
Magnamycin	30	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80
Magnamycin	60	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80
Magnamycin	90	0.95-0.95	0.80-0.80	0.90-0.90	0.80-0.85	0.80-0.80	0.80-0.80
Neomycin	10	1.40-1.30	1.40-1.45	0.95-1.00	1.30-1.40	1.50-1.40	1.20-1.30
Penicillin	.25 Units	3.10-3.05	3.00-2.89	3.22-3.20	3.30-3.31	3.00-2.98	3.40-3.30
Penicillin	.50 Units	3.82-3.83	3.60-3.61	3.82-3.81	4.00-4.01	3.74-3.72	3.82-3.81
Polymyxin B Sulfate	20	1.10-1.10	0.95-0.90	0.80-0.80	0.90-0.92	1.01-1.00	0.80-0.80
Polymyxin B Sulfate	40	1.15-1.20	1.10-1.15	0.94-0.90	0.95-0.90	1.12-1.20	0.85-0.83
Polymyxin B Sulfate	60	1.30-1.30	1.20-1.20	1.10-1.20	1.10-1.20	1.22-1.25	0.95-0.90
Streptomycin	5.0	1.20-1.22	1.60-1.50	1.40-1.60	1.60-1.40	1.30-1.50	1.70-1.50
Terramycin	1.0	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.98-0.97	0.80-0.80
Terramycin	2.0	1.00-1.02	1.05-1.07	0.80-0.83	0.90-0.92	1.30-1.32	1.05-1.07

TABLE V (Continued)

Antibiotic	Micrograms per 0.1 ml	Culture K.S.C.	Culture #156	Culture #530	Culture #532	Culture #350	Culture #11
Achromycin	0.5	1.07-1.00	1.20-1.30	1.20-1.10	1.20-1.10	1.50-1.60	1.10-1.20
Achromycin	1.0	1.20-1.30	1.50-1.30	1.40-1.50	1.50-1.53	1.89-1.80	1.40-1.50
Albamycin	1.0	0.90-0.85	1.10-1.20	1.20-1.20	1.12-1.15	1.20-1.20	1.50-1.60
Aureomycin	0.5	1.10-1.00	1.22-1.20	1.10-1.10	1.00-1.10	1.60-1.70	1.10-1.15
Aureomycin	1.0	1.30-1.30	1.50-1.40	1.30-1.35	1.40-1.35	2.00-2.10	1.45-1.52
Bacitracin	10	0.80-0.80	0.95-0.96	0.80-0.80	0.80-0.80	1.00-1.00	0.90-0.85
Bacitracin	20	0.80-0.95	1.55-1.55	1.10-1.11	0.91-0.93	1.50-1.45	1.20-1.30
Bacitracin	40	1.12-1.20	1.70-1.80	1.30-1.35	1.20-1.22	1.70-1.70	1.40-1.53
Carbomycin	5.0	1.73-1.60	1.50-1.40	1.10-1.15	1.40-1.45	2.00-1.90	1.70-1.50
Chloromycetin	1.0	1.30-1.40	1.40-1.50	1.10-1.20	1.45-1.30	0.90-1.00	1.30-1.32
Erythromycin	1.0	1.90-1.70	1.25-1.30	1.40-1.30	1.15-1.20	1.90-1.80	1.80-1.60
Magnamycin	30	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80
Magnamycin	60	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.90-0.92
Magnamycin	90	0.98-1.00	0.90-0.85	0.80-0.80	0.80-0.80	0.94-0.90	1.00-1.06
Neomycin	10	1.76-1.60	1.30-1.35	1.40-1.50	1.38-1.40	1.00-1.00	1.32-1.40
Penicillin	.25 Units	2.42-2.43	2.80-2.70	2.80-2.82	2.90-2.91	2.90-3.00	2.85-2.70
Penicillin	.50 Units	3.02-3.05	3.80-3.90	3.85-3.83	3.80-3.82	4.90-4.80	3.31-3.40
Polymyxin B Sulfate	20	1.10-1.00	1.00-1.00	0.80-0.90	0.80-0.80	0.80-0.80	0.80-0.85
Polymyxin B Sulfate	40	1.20-1.30	1.20-1.10	1.00-1.10	0.80-0.80	0.90-0.93	0.94-0.96
Polymyxin B Sulfate	60	1.57-1.60	1.30-1.25	1.20-1.20	1.25-1.20	1.10-1.15	1.10-1.17
Streptomycin	5.0	1.90-1.70	1.30-1.43	1.60-1.40	1.50-1.45	1.40-1.30	1.60-1.57
Terramycin	1.0	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	1.30-1.27	1.00-0.94
Terramycin	2.0	1.30-1.32	1.10-1.13	0.94-0.92	1.15-1.17	2.00-1.95	1.27-1.21

* The average diameter of the zones of inhibition for the 24 cultures used and the range in the diameters of the zones is given in Table I under results.

TABLE VI

THE ZONES OF GROWTH INHIBITION (CMS) FOR THE 24 CULTURES OF MORAXELLA BOVIS

USING 5 SULFONAMIDES**

Sulfonamide	Micrograms per 0.1 ml	Culture A.T.C.C.	Culture #192	Culture #125	Culture #526	Culture #136	Culture Lederle
Sulfanilamide	30	0.80–0.80	0.80–0.80	1.10–1.00	0.80–0.80	0.80–0.80	0.80–0.80
Sulfanilamide	60	0.80–0.80	0.80–0.80	1.30–1.32	0.80–0.80	0.80–0.80	0.80–0.80
Sulfanilamide	90	0.80–0.80	0.80–0.80	1.50–1.50	0.80–0.80	0.80–0.80 1.00–0.90*	0.90–0.90
Sulfanilamide	120	0.80–0.80 1.20–1.10*	0.80–0.80 1.00–0.90*	1.70–1.70	0.80–0.80 0.90–0.95*	0.90–0.92 1.20–1.10*	1.22–1.20
Sulfanilamide	250	1.30–1.40 1.65–1.60*	1.00–1.10 1.40–1.35*	2.00–2.00	1.10–1.12 1.20–1.30*	1.05–1.10 1.45–1.50*	1.38–1.40
Sulfadiazine	30	0.80–0.80	0.80–0.80	1.00–1.00	0.80–0.80	0.80–0.80	0.80–0.80
Sulfadiazine	60	0.80–0.80 1.10–1.00*	0.80–0.80	1.20–1.25	1.10–1.10	0.80–0.80 1.10–1.20*	1.00–1.00
Sulfadiazine	90	0.80–0.80 1.20–1.25*	0.80–0.80 1.40–1.45*	1.35–1.40	1.30–1.32	1.12–1.10 1.40–1.35*	1.40–1.35
Sulfadiazine	250	1.50–1.50 1.70–1.60*	1.32–1.30 1.50–1.45*	1.70–1.70	1.60–1.60	1.40–1.42 1.55–1.60*	1.67–1.65
Sulfamerazine	30	0.80–0.80	0.80–0.80	0.80–1.00	0.80–0.80	0.80–0.80	1.00–1.00
Sulfamerazine	60	0.80–0.80 1.00–0.97*	0.80–0.80	1.10–1.05	1.00–1.00	0.92–0.90	1.30–1.30
Sulfamerazine	90	0.80–0.80 1.30–1.20*	0.80–0.80 1.01–1.00*	1.30–1.30	1.30–1.30	1.22–1.20	1.57–1.55
Sulfamerazine	250	1.20–1.20 1.50–1.65*	1.00–1.05 1.30–1.35*	1.60–1.55	1.60–1.57	1.45–1.50	1.88–1.80
Sulfapyridine	30	0.80–0.80	0.80–0.80	1.10–1.10	0.80–0.80	1.00–1.00	0.80–0.80
Sulfapyridine	60	0.80–0.80	0.80–0.80	1.30–1.30	0.80–0.80	1.22–1.23	0.85–0.85
Sulfapyridine	90	1.50–1.30 1.80–1.70*	1.00–1.07 1.40–1.35*	1.47–1.44	0.80–0.80 1.30–1.35*	1.60–1.62	1.15–1.18

TABLE VI (Continued)

Sulfonamide	Micrograms per 0.1 ml	Culture A.T.C.C.	Culture #192	Culture #125	Culture #526	Culture #136	Culture Lederle
Sulfapyridine	250	2.60–2.50	2.00–2.07	1.70–1.70	1.10–1.12 1.50–1.60*	1.87–1.85	1.45–1.50
Sulfathiazole	30	0.80–0.80	0.80–0.80	0.90–0.90	1.30–1.30	1.30–1.30	0.80–0.80
Sulfathiazole	60	0.80–0.80 1.60–1.50*	0.80–0.80 1.20–1.30*	1.10–1.20	1.70–1.65	1.60–1.60	0.95–0.95
Sulfathiazole	90	1.40–1.46 1.70–1.75*	1.20–1.24 1.40–1.47*	1.34–1.35	2.00–2.05	1.92–1.93	1.40–1.50
Sulfathiazole	250	2.25–2.20	2.01–2.10	1.80–1.85	2.25–2.30	2.10–2.12	1.90–1.95

Sulfonamide	Micrograms per 0.1 ml	Culture #129	Culture #277	Culture #148	Culture #430	Culture #443	Culture #419
Sulfanilamide	30	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfanilamide	60	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfanilamide	90	0.85–0.85	0.80–0.80	0.80–0.80	1.00–1.00	0.80–0.80 0.90–0.95*	0.80–0.80
Sulfanilamide	120	1.00–1.05	0.97–0.99 1.10–1.20*	0.89–0.90	1.20–1.25	0.97–0.95 1.20–1.25*	0.95–0.90
Sulfanilamide	250	1.30–1.32	1.20–1.25 1.34–1.43*	1.10–1.15	1.40–1.40	1.30–1.20 1.40–1.45*	1.10–1.20
Sulfadiazine	30	0.80–0.80	1.10–1.10	0.80–0.80	1.10–1.20	0.80–0.80	0.80–0.80
Sulfadiazine	60	0.80–0.80	1.40–1.45	0.80–0.80	1.60–1.60	0.80–0.80 1.10–1.15*	0.80–0.80
Sulfadiazine	90	0.94–0.95 1.20–1.25*	1.70–1.70	0.95–0.98	1.80–1.85	1.00–1.05 1.30–1.25*	1.10–1.20
Sulfadiazine	250	1.00–1.00 1.35–1.40*	1.95–1.90	1.00–1.05	2.21–2.20	1.30–1.30 1.60–1.70*	1.35–1.40
Sulfamerazine	30	0.80–0.80	0.90–0.90	0.80–0.80	1.00–1.10	0.80–0.80	0.80–0.80
Sulfamerazine	60	0–80–0–80	1.20–1.20	0.80–0.80	1.30–1.40	0.80–0.80	0.80–0.80

TABLE VI (Continued)

Sulfonamide	Micrograms per 0.1 ml	Culture #129	Culture #277	Culture #148	Culture #430	Culture #443	Culture #419
Sulfamerazine	90	0.82–0.82 0.95–1.00*	1.40–1.45	0.90–0.92	1.60–1.70	0.80–0.80 0.96–0.94*	0.80–0.85
Sulfamerazine	250	1.10–1.12 1.33–1.40*	1.70–1.75	1.20–1.21	1.90–1.98	1.00–1.00 1.30–1.40*	1.20–1.23
Sulfapyridine	30	0.80–0.80	0.80–0.80	0.80–0.80	1.20–1.20	0.80–0.80	0.80–0.80
Sulfapyridine	60	0.80–0.80	1.00–1.00 1.20–1.25*	0.80–0.80	1.50–1.55	0.80–0.80	0.80–0.80
Sulfapyridine	90	1.20–1.30 1.40–1.45*	1.45–1.50 1.55–1.65*	1.30–1.20	1.70–1.60	0.95–1.00 1.20–1.30*	1.10–1.20
Sulfapyridine	250	1.84–1.89	2.00–1.95	1.90–1.89	1.90–2.00	1.20–1.30 1.40–1.40*	1.40–1.45
Sulfathiazole	30	0.80–0.80	0.90–0.90	0.80–0.80	1.30–1.20	0.80–0.80	0.80–0.80
Sulfathiazole	60	0.80–0.80	1.30–1.25	0.80–0.80	1.50–1.60	0.90–0.90 1.10–1.20*	0.85–0.84
Sulfathiazole	90	1.30–1.40	1.70–1.72	1.30–1.40	1.80–1.90	1.15–1.20 1.30–1.40*	1.30–1.40
Sulfathiazole	250	1.80–1.75	2.00–2.10	2.00–1.95	2.10–2.20	1.40–1.50 1.60–1.55*	1.60–1.80

Sulfonamide	Micrograms per 0.1 ml	Culture #127	Culture #535	Culture #435	Culture #418	Culture #533	Culture #534
Sulfanilamide	30	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfanilamide	60	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80 0.95–0.92	0.80–0.80	0.80–0.80
Sulfanilamide	90	0.90–0.85	0.80–0.80	0.90–1.00	0.98–0.80 1.10–1.20*	0.80–0.80	0.80–0.80
Sulfanilamide	120	1.10–1.05	0.80–0.80	1.30–1.20	1.30–1.30 1.50–1.60*	1.10–1.11	0.80–0.80
Sulfanilamide	250	1.40–1.30	0.95–0.99	1.60–1.60	1.90–1.70 2.10–2.00*	1.60–1.40	1.20–1.30

TABLE VI (Continued)

Sulfonamide	Micrograms per 0.1 ml	Culture #127	Culture #535	Culture #435	Culture #418	Culture #533	Culture #534
Sulfadiazine	30	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfadiazine	60	0.80–0.80 0.87–0.90*	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80 0.90–0.92*	0.80–0.80
Sulfadiazine	90	0.94–0.90 1.20–1.25*	0.89–0.93	0.90–0.90	1.00–0.95 1.30–1.25*	0.90–1.00	0.80–0.85
Sulfadiazine	250	1.40–1.40 1.60–1.55*	1.40–1.30	1.30–1.40	1.50–1.40 1.65–1.70*	1.40–1.30	1.20–1.10
Sulfamerazine	30	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfamerazine	60	0.80–0.80	0.85–0.80	0.80–0.80 0.90–0.85*	0.80–0.80 1.20–1.00*	0.80–0.80	0.80–0.80
Sulfamerazine	90	1.10–1.12	0.95–1.00	0.92–0.98	1.10–1.00 1.30–1.37*	1.20–1.20 1.35–1.40*	0.95–0.90
Sulfamerazine	250	1.60–1.70	1.60–1.50	1.50–1.70	1.50–1.40 1.70–1.90*	1.60–1.70	1.40–1.30
Sulfapyridine	30	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfapyridine	60	0.80–0.80 0.96–0.95*	0.80–0.80	0.80–0.80 0.92–0.96*	0.80–0.80	0.80–0.80	0.80–0.80
Sulfapyridine	90	1.20–1.30	1.30–1.30	1.10–1.20	1.20–1.00 1.40–1.30*	0.90–0.96	0.92–1.00
Sulfapyridine	250	1.70–1.80	1.70–1.75	1.50–1.50	1.50–1.40 1.70–1.65*	1.30–1.20	1.60–1.50
Sulfathiazole	30	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfathiazole	60	0.80–0.80 0.91–0.93*	0.90–0.95	0.80–0.80	0.80–0.80 0.92–0.96*	0.80–0.80	0.80–0.80
Sulfathiazole	90	1.30–1.20	1.30–1.40	1.10–1.20	1.30–1.20 1.40–1.45*	1.10–1.20	1.30–1.40
Sulfathiazole	250	1.60–1.70	1.80–1.90	1.70–1.90	1.80–1.90 2.00–2.10*	1.50–1.60	1.80–1.90

TABLE VI (Continued)

Sulfonamide	Micrograms per 0.1 ml	Culture K.S.C.	Culture #156	Culture #530	Culture #532	Culture #350	Culture #11
Sulfanilamide	30	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfanilamide	60	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfanilamide	90	0.80–0.90	0.93–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfanilamide	120	0.80–0.95	1.10–1.10	0.94–0.90	0.90–0.95	0.89–0.85	0.92–0.96
Sulfanilamide	250	1.10–1.20	1.30–1.40	1.30–1.20	1.20–1.30	1.00–1.07	1.30–1.27
Sulfadiazine	30	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfadiazine	60	0.80–0.80 1.00–1.05*	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfadiazine	90	1.20–1.10 1.30–1.35*	1.10–1.20	1.30–1.40	0.92–0.90	0.88–0.85	0.90–0.93
Sulfadiazine	250	1.60–1.50	1.50–1.40	1.70–1.50	1.10–1.18	1.00–0.98	1.20–1.25
Sulfamerazine	30	0.80–0.80	1.00–1.10	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfamerazine	60	0.80–0.80	1.40–1.40	0.95–1.00	0.80–0.80	0.80–0.80	0.80–0.80
Sulfamerazine	90	1.00–1.10	1.80–1.60	1.30–1.40	0.89–0.92	0.80–0.80	0.86–0.84
Sulfamerazine	250	1.40–1.60	2.00–1.95	1.70–1.50	1.20–1.15	0.97–0.94 1.20–1.15*	1.00–1.02
Sulfapyridine	30	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfapyridine	60	0.80–0.80	0.94–0.97	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfapyridine	90	0.90–0.95 1.20–1.27*	1.00–1.00	0.97–1.00	0.85–0.80	0.90–0.92	0.95–1.00
Sulfapyridine	250	1.20–1.30 1.50–1.45*	1.40–1.50	1.30–1.30	0.96–0.92	1.00–1.06	1.30–1.35
Sulfathiazole	30	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80	0.80–0.80
Sulfathiazole	60	0.80–0.80 1.00–1.10*	0.92–0.95	0.80–0.80 0.97–0.94*	0.80–0.80	0.80–0.80	0.80–0.80
Sulfathiazole	90	1.10–1.20 1.35–1.40*	1.40–1.30	1.20–1.25 1.30–1.35*	1.20–1.15	1.00–0.97	1.10–1.15
Sulfathiazole	250	1.70–1.60	1.80–1.89	1.60–1.65	1.40–1.60	1.30–1.40	1.60–1.50

*Diameter of the zones of partial inhibition.

**The average diameter of the zones of inhibition for the 24 cultures used and the range in the diameters of the zones is given in Table II under results.

TABLE VII

THE RESULTS OF ANTIBIOTIC THERAPY ON ANIMALS WHICH HAD RECOVERED FROM A "PINKEYE" INFECTION

History of animal used	Results of Initial swabs	Inoc. with* 1 ml susp. of A.T.C.C.	Results of swabs after inoculation	Treatment	Results of swabs after treatment	
					Date	Rt. eye / Lt. eye
Animal #530 Animal over 1 year old with history of a mild case of Pinkeye as a small calf	7-15-57 RE -- LE -- 7-16-57 RE -- LE -- 7-17-57 RE -- LE --	7-17-57 RE	7-18-57 RE +++ LE -- 7-19-57 RE ++++ LE --	7-19-57 RE - 10 mgs Aureomycin LE - None	7-20-57	-- / -- Both eyes remained negative through the 30th
Animal #592 Animal over 1 year old with history of a mild case of Pinkeye as a small calf	7-15-57 RE -- LE -- 7-16-57 RE -- LE -- 7-17-57 RE -- LE --	7-17-57 RE	7-18-57 RE ++ LE -- 7-19-57 RE +++ LE --	7-19-57 RE - 10 mgs Chloromycetin LE - None	7-20-57	-- / -- Both eyes remained negative through the 30th
Animal #535 Animal over 1 year old with history of a mild case of Pinkeye as a small calf	7-15-57 RE -- LE -- 7-16-57 RE -- LE -- 7-17-57 RE -- LE --	7-17-57 RE	7-18-57 RE ++++ LE -- 7-19-57 RE ++ LE --	7-19-57 RE - 25,000 units penicillin LE - None	7-20-57	-- / - Both eyes remained negative through the 30th

TABLE VII (Continued)

History of animal used	Results of initial swabs	Inoc. with 1 ml susp of A.T.C.C.	Results of swabs after inoculation	Treatment	Results of swabs after treatment	
					Date	Rt. eye / Lt. eye
Animal # 534 Animal over 1 year old with history of a mild case of Pinkeye as a small calf	7-15-57 RE -- LE -- 7-16-57 RE -- LE -- 7-17-57 RE -- LE --	7-17-57 Rt. eye	7-18-57 RE ++ LE -- 7-19-57 RE ++ LE --	7-19-57 RE - 10 mgs Terramycin LE - None	7-20-57	-- Both eyes remained negative through the 30th --
Animal #554 Animal over 1 year old with history of a mild case of Pinkeye as a small calf	7-15-57 RE -- LE -- 7-16-57 RE -- LE -- 7-17-57 RE -- LE --	7-17-57 Rt. eye	7-18-57 RE +++ LE -- 7-19-57 RE ++ LE --	7-19-57 RE - 10 mgs Erythromycin LE - None	7-20-57	-- Both eyes remained negative through the 30th --
Animal #559 Animal over 1 year old with history of a mild case of Pinkeye as a small calf	7-15-57 RE -- LE -- 7-16-57 RE -- LE -- 7-17-57 RE -- LE --	7-17-57 Rt. eye	7-18-57 RE ++ LE -- 7-19-57 RE ++ LE -- 7-20-57 RE +++ LE -- 7-22-57 RE ++ LE -- 7-24-57 RE ++ LE -- 7-26-57 RE +++ LE -- 7-30-57 RE + LE --	7-19-57 No treatment on either eye	7-20-57	

TABLE VII (Continued)

History of animal used	Results of initial swabs	Inoc. with 1 ml susp. of A.T.C.C.	Results of swabs after inoculation	Treatment
Animal # 543 Animal over 1 year old with history of a mild case of Pinkeye as a small calf	7-15-57 RE -- LE -- 7-16-57 RE -- LE -- 7-17-57 RE -- LE --	7-17-57 Rt. eye	7-18-57 RE +++ LE -- 7-19-57 RE ++ LE -- 7-20-57 RE ++ LE -- 7-22-57 RE + LE -- 7-24-57 RE -- LE -- 7-26-57 RE -- LE -- 7-30-57 RE -- LE --	No treatment
Animal # 532 Animal over 1 year old with history of a mild case of Pinkeye as a small calf	7-15-57 RE -- LE -- 7-16-57 RE -- LE -- 7-17-57 RE -- LE --	7-17-57 Rt. eye	7-18-57 RE ++ LE -- 7-19-57 RE +++ LE -- 7-20-57 RE +++ LE -- 7-22-57 RE ++ LE -- 7-24-57 RE +++ LE -- 7-26-57 RE ++ LE -- 7-30-57 RE ++ LE --	No treatment

TABLE VII (Continued)

History of animal used	Results of initial swabs	Inoc. with 1 ml susp. of A.T.C.C.	Results of swabs after inoculation	Treatment
Animal #527 Animal over 1 year old with history of a mild case of Pinkeye as a small calf	7-15-57 RE -- LE -- 7-16-57 RE -- LE -- 7-17-57 RE -- LE --	7-17-57 RT. eye	7-18-57 RE -- LE -- 7-19-57 RE -- LE -- 7-20-57 RE -- LE -- 7-22-57 RE -- LE -- 7-24-57 RE -- LE -- 7-26-57 RE -- LE -- 7-30-57 RE -- LE --	No treatment

Code for Table VII

RE Right eye
LE Left eye
-- Negative for M. bovis
+ 1 to 6 colonies of M. bovis on plate
++ 7 to 20 colonies of M, bovis on plate
+++ 21 to 50 colonies of M. bovis on plate
++++ Over 50 colonies of M. bovis on plate

* The eye was inoculated with 1 ml of a suspension of Moraxella bovis obtained from the American Type Culture Collection.

TABLE VIII

THE RESULTS OF ANTIBIOTIC THERAPY ON ANIMALS WITH NO PREVIOUS "PINKEYE" INFECTION

History	Results of initial swabs	Inoc. with* 1 ml susp. of A.T.C.C.	Results of swabs after inoculation	Treatment	Results of swabs after treatment		
					Date	Rt. eye	Lt. eye
Calf #625 Small Holstein calf with no previous history of "pinkeye"	7-15-57 RE -- LE --	7-16-57 Both eyes inoculated	7-17-57 RE ++ LE -- 7-18-57 RE ++ LE --	7-18-57 RE - 10 mgs Terramycin LE - None	7-19-57 7-20-57 7-21-57 7-22-57 7-23-57 7-24-57 7-25-57 7-26-57 7-27-57 7-29-57	-- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- --
Calf #624 Small Holstein calf with no previous history of "pinkeye"	7-15-57 RE -- LE --	7-16-57 Both eyes inoculated	7-17-57 RE ++ LE ++ 7-18-57 RE +++ LE +++	7-18-57 RE - 10 mgs Aureomycin LE - 25,000 units pen-icillin 7-25-57 RE - 25,000 Units pen-icillin LE - None 8-1-57 RE - 10 mgs Erythromycin LE - 10 mgs Erythromycin	7-19-57 7-20-57 7-21-57 7-22-57 7-23-57 7-24-57 7-25-57 7-26-57 7-27-57 7-29-57 7-30-57 7-31-57 8-1-57 8-2-57 8-3-57	-- -- -- + ++ ++ +++ -- -- -- + + ++ + ++	-- -- -- + +++ ++ +++ +++ ++++ ++++ ++++ +++ ++++ + ++

*

TABLE VIII

History	Results of initial swabs	Inoc. with 1 ml susp of A.T.C.C.	Results of swabs after inoculation	Treatment	Results of swabs after treatment Date	Rt. eye	Lt. eye
Calf #628	7-15-57	7-16-57	7-17-57	7-18-57	7-19-57	--	--
Small	RE --	Both eyes	RE ++++	RE - 10 mgs	7-20-57	--	--
Holstein	LE --	inoculated	LE +++	Chloromycetin	7-21-57	--	+
calf with				LE - 10 mgs	7-22-57	+	++
no previous			7-18-57	Erythromycin	7-24-57	+++	++
history of			RE +++		7-25-57	+++	++
"pinkeye"			LE +++	7-25-57			
				RE - 10 mgs	7-26-57	--	++
				Aureomycin	7-27-57	--	+
				LE - None	7-29-57	--	+
					7-30-57	+++	+
				8-1-57	8-1-57	++	++++
				RE - 25,000			
				units pen-	8-2-57	--	--
				icillin	8-3-57	+	+++
				LE - 25,000			
				units pen-			
				icillin	8-5-57	+++	++
					8-6-57	+++	--
				8-5-57	8-7-57	+++	--
				RE - None	8-8-57	+	+
				LE - 20 mgs	8-9-57	--	+
				Erythromycin	8-10-57	+	+
					8-12-57	++++	--
				8-22-57	8-13-57	++++	+
				RE - 20 mgs	8-14-57	--	+
				Chloromycetin	8-15-57	++++	++++
				LE - 20 mgs			
				Chloromycetin	8-22-57	++++	++++
					8-23-57	--	--
					8-24-57	--	--
					8-26-57	--	--
					8-27-57	--	+
					8-30-57	--	+

TABLE VIII (Continued)

History	Results of initial swabs	Inoc. with* 1 ml susp. of A.T.C.C.	Results of swabs after inoculation	Treatment	Results of swabs after treatment		
					Date	Rt. eye	Lt. eye
Calf #627 Small Holstein calf with no previous history of "pinkeye"	7-22-57 RE -- LE --	7-23-57 Both eyes inoculated	7-24-57 RE +++ LE ++ 7-25-57 RE +++ LE ++	7-25-57 RE-10 mgs Erythromycin LE - None 8-1-57 LE - 10 mgs Chloromycetin RE - None	7-26-57 7-27-57 7-29-57 7-30-57 7-31-57 8-1-57 8-2-57 8-3-57 8-5-57 8-7-57 8-9-57 8-10-57 8-12-57 8-14-57 8-16-57	-- -- -- ++ + + + -- ++ -- + ++ ++ +++ ++++	++ + -- +++ +++ ++ -- -- -- -- -- -- ++ + +++
Calf #636 Small Holstein calf with no previous history of "pinkeye"	7-22-57 RE -- LE --	7-23-57 Both eyes inoculated	7-24-57 RE ++ LE + 7-25-57 RE +++ LE --	7-25-57 RE - 10 mgs Chloromycetin LE - None 8-22-57 RE - 50,000 units penicillin LE - 50,000 units penicillin	7-26-57 7-27-57 7-29-57 7-30-57 8-22-57 8-23-57 8-24-57 8-26-57 8-27-57 8-30-57	-- -- -- + + -- + -- -- --	-- -- -- -- + -- + -- -- --

TABLE VIII (Continued)

History	Results of initial swabs	Inoc. with 1 ml susp. of A.T.C.C.	Results of swabs after inoculation	Treatment	Results of swabs after treatment		
					Date	Rt. eye	Lt. eye
Calf #634	7-22-57	7-23-57	7-24-57	7-25-57	7-26-57	+	--
Small	RE --	Both eyes	RE +	RE - None	7-27-57	+++	--
Holstein	LE --	inoculated	LE ++++	LE - 10 mgs	7-29-57	++	+
calf with				Terramycin	7-30-57	++	+
no previous			7-25-57		8-1-57	++	+
history of			RE +	8-1-57			
"pinkeye"			LE +++	RE - 10 mgs	8-2-57	--	+
				Terramycin	8-3-57	+	++++
				LE - None	8-5-57	+	+
					8-7-57	--	--
					8-9-57	--	--
					8-10-57	--	--
					8-12-57	+	++
					8-13-57	++	--
					8-14-57	++	+
					8-15-57	+	--
					8-22-57	--	++
				8-22-57			
				RE - 50,000	8-23-57	--	--
				units pen-	8-24-57	+	+
				icillin	8-26-57	--	+
				LE - 50,000	8-27-57	--	+
				units pen-	8-30-57	--	--
				icillin			

TABLE VIII (Continued)

History	Results of initial swabs	Inoc. with 1 ml susp. of A.T.C.C.	Results of swabs after inoculation	Treatment	Results of swabs after treatment Date	Rt. eye	Lt. eye
Calf #629 Small Holstein calf that was purchased 7-16-57 and put in same pen with the rest of the small calves being used.	7-30-57 RE +++ LE --	not inoculated		7-31-57 RE - 10 mgs Terramycin LE - None	8-1-57	++	++++
					8-2-57	+++	+++
					8-3-57	+	++++
	7-31-57 RE ++++ Le ++				8-5-57	--	--
				8-3-57 RE - 20 mgs Terramycin LE - 20 mgs Terramycin	8-6-57	--	--
					8-7-57	--	--
					8-8-57	--	--
					8-9-57	--	--
					8-10-57	+	--
					8-12-57	+	--
					8-13-57	++	+
					8-14-57	++	+
					8-15-57	+++	++
				8-22-57 RE - 20 mgs Terramycin LE - 20 mgs Terramycin	8-22-57	+	+++
					8-23-57	--	--
					8-24-57	--	--
					8-26-57	--	--
					8-27-57	--	+++
					8-30-57	--	++++
Calf #635 Small Holstein calf that was purchased on 8-6-57 and put in same pen with the rest of the small calves being used	8-22-57 RE ++++ LE ++++	not inoculated		8-22-57 RE - 20 mgs Erythromycin LE - 20 mgs Erythromycin	8-23-57	--	--
					8-24-57	--	+
					8-26-57	--	--
					8-27-57	--	--
					8-30-57	--	--

TABLE VIII (Continued)

History	Results of initial swabs	Inoc. with 1 ml susp. of A.T.C.C.	Results of swabs after inoculation	Treatment	Results of swabs after treatment		
					Date	Rt. eye	Lt. eye
Calf #631 Small Holstein calf that was purchased 7-16-57 and put in same pen with the rest of the small calves being used	7-30-57 RE ++ LE +	not inoculated		7-31-57 RE - 10 mgs Aureomycin LE - None	8-1-57	--	++++
					8-2-57	++	++++
					8-3-57	+	++++
	7-31-57 RE +++ LE ++				8-5-57	++++	--
				8-3-57 RE - None LE - 20 mgs Chloromycetin	8-6-57	++	++
					8-7-57	+	+
					8-8-57	--	--
					8-9-57	--	+
					8-10-57	++	+
					8-12-57	++	--
					8-13-57	+++	++
					8-14-57	++++	++++
				8-22-57 RE - 50,000 units penicillin LE - 50,000 units penicillin	8-16-57	+++	++
					8-22-57	++	++
					8-23-57	--	+
					8-24-57	--	+
					8-26-57	--	++
					8-27-57	--	++
					8-30-57	--	++++
Calf #649 Small Holstein calf that was purchased on 8-6-57 and put in same pen with the rest of the small calves being used	8-22-57 RE + LE ++++	not inoculated		8-22-57 RE - 50,000 units penicillin LE - 50,000 units penicillin	8-23-57	--	--
					8-24-57	+	--
					8-26-57	--	--
					8-27-57	--	--
					8-30-57	--	+

TABLE VIII (Continued)

History	Results of initial swabs	Inoc. with 1 ml susp. of A.T.C.C.	Results of swabs after inoculation	Treatment	Results of swabs after treatment Date	Rt. eye	Lt. eye
Calf #632							
Small Holstein calf that was purchased on 7-16-57 and put in same pen with the small calves being used	7-30-57			7-31-57	8-1-57	+++	++
	RE +	Not		RE - None	8-2-57	++++	++
	LE ++	inoculated		LE - 10 mgs Chloromycetin	8-3-57	+++	+++
	7-31-57						
	RE +++			8-3-57	8-5-57	--	+
	LE ++++			RE - 20 mgs	8-6-57	--	--
				Erythromycin	8-7-57	--	--
				LE - None	8-8-57	--	--
					8-9-57	--	--
					8-10-57	--	--
					8-12-57	--	+
					8-13-57	+	+
					8-14-57	+	++
					8-15-57	+	--
					8-22-57	++	++
				8-22-57			
				RE - 50,000	8-23-57	--	--
				units penicillin	8-24-57	+	+
					8-26-57	++++	--
				LE - 50,000	8-27-57	++	+
				units penicillin	8-30-57	+	+

TABLE VIII (Continued)

History	Results of initial swabs	Inoc. with 1 ml susp. of A.T.C.C.	Results of swabs after inoculation	Treatment	Results of swabs after treatment		
					Date	Rt. eye	Lt. eye
Calf #633 Small Holstein calf that was purchased on 7-16-57 and put in same pen with the rest of the small calves being used	7-30-57 RE + LE +++ 7-31-57 RE + LE +++	Not inoculated		7-31-57 RE - None LE - 10 mgs Aureomycin 8-3-57 RE - 20 mgs Chloromycetin LE - None 8-22-57 RE - 20 mgs Aureomycin LE - 20 mgs Aureomycin	8-1-57 8-2-57 8-3-57 8-5-57 8-6-57 8-7-57 8-8-57 8-9-57 8-12-57 8-13-57 8-14-57 8-15-57 8-22-57 8-23-57 8-24-57 8-26-57 8-27-57 8-30-57	++++ +++ +++ + + -- + -- ++ ++++ ++++ +++ + -- -- -- -- --	-- -- -- -- -- -- -- + ++ + ++ +++ + -- -- -- + +

Code for abbreviations

RE	Right eye
LE	Left eye
--	Negative for M. bovis on plate
+	1 to 6 colonies of M. bovis on plate
++	7 to 20 colonies of M. bovis on plate
+++	21 to 50 colonies of M. bovis on plate
++++	Over 50 colonies of M. bovis on plate

* The eyes were inoculated with 1 ml of a suspension of Moraxella bovis obtained from the American Type culture collection.

TABLE IX

INHIBITION ZONES PRODUCED BY ANTIBIOTICS USING MORAXELLA BOVIS

(ATCC) AS THE TEST ORGANISM

Antibiotic	0.0625 ug per 0.1 ml	0.125 ug per 0.1 ml	0.25 ug per 0.1 ml	0.50 ug per 0.1 ml	1.0 ug per 0.1 ml	2.0 ug per 0.1 ml
Achromycin	0.88-0.92*	0.95-0.97	1.10-1.10	1.30-1.50	1.76-1.84	2.03-2.07
	0.90**	0.96	1.10	1.40	1.80	2.05
Albamycin	0.80-0.80	0.80-0.80	0.80-0.80	0.94-1.00	1.30-1.30	1.45-1.55
	0.80	0.80	0.80	0.97	1.30	1.50
Aureomycin	1.20-0.98	1.00-1.40	1.30-1.50	1.50-1.90	2.02-2.08	2.30-2.50
	1.09	1.20	1.40	1.70	2.05	2.40
Carbomycin	0.85-0.95	1.00-1.00	1.10-1.30	1.30-1.34	1.64-1.60	1.84-2.01
	0.90	1.00	1.20	1.32	1.62	1.92
Chloromycetin	0.80-0.80	0.80-0.80	0.80-0.84	1.30-1.50	2.10-1.90	2.80-2.60
	0.80	0.80	0.82	1.40	2.00	2.70
Erythromycin	0.81-0.81	1.22-1.24	1.48-1.46	1.73-1.71	1.70-2.10	2.30-2.30
	0.81	1.23	1.47	1.72	1.90	2.30
Magnamycin	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80
	0.80	0.80	0.80	0.80	0.80	0.80
Neomycin	0.80-0.80	0.94-0.90	1.08-1.10	1.25-1.35	1.42-1.58	1.80-1.70
	0.80	0.92	1.09	1.30	1.50	1.75
Penicillin	1.50-1.70	2.10-2.10	2.40-2.44	2.75-2.79	2.86-3.03	3.15-3.25
	1.60	2.10	2.42	2.77	2.94	3.20
Polymyxin B-Sulfate	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80
	0.80	0.80	0.80	0.80	0.80	0.80
Streptomycin	0.80-0.80	0.80-0.80	0.95-1.05	1.50-1.50	1.76-1.80	1.70-1.90
	0.80	0.80	1.00	1.50	1.78	1.80
Bacitracin	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	0.88-0.92
	0.80	0.80	0.80	0.80	0.80	0.90
Terramycin	0.80-0.80	0.86-0.90	1.00-1.00	1.20-1.40	1.70-1.80	1.90-2.10
	0.80	0.88	1.00	1.30	1.75	2.00

TABLE IX (Continued)

Antibiotic	40.ug per 0.1 ml	10.0 ug per 0.1 ml	20.0 ug per 0.1 ml	30.0 ug per 0.1 ml	40.0 ug per 0.1 ml	50.0 ug per 0.1 ml	60.0 ug per 0.1 ml
Achromycin	2.40-2.40 2.40	2.70-2.90 2.80	2.96-2.98 2.97	3.40-3.00 3.20	3.30-3.50 3.40	3.63-3.67 3.65	
Albamycin	1.80-1.80 1.80	1.80-1.84 1.82	2.00-2.20 2.10	2.20-2.20 2.20	2.20-2.40 2.30	2.94-2.96 2.95	
Aureomycin	2.70-2.70 2.70	3.04-3.06 3.05	3.20-3.40 3.30	3.47-3.51 3.49	3.64-3.66 3.65	4.00-3.80 3.90	
Carbomycin	2.40-2.50 2.45	2.75-2.75 2.75	2.94-3.00 2.97	2.90-3.01 2.95	3.40-3.00 3.20	3.20-3.30 3.25	
Chloromycetin	3.05-3.05 3.05	3.05-3.15 3.10	3.45-3.35 3.40	3.70-3.66 3.68	3.87-3.83 3.85	3.90-4.10 4.00	
Erythromycin	2.70-2.50 2.60	2.85-2.95 2.90	3.25-3.35 3.30	3.75-3.55 3.65	3.90-3.70 3.80	3.95-4.05 4.00	
Magnamycin	0.80-0.80 0.80	0.80-0.80 0.80	0.80-0.80 0.80	0.80-0.80 0.80	0.80-0.80 0.80	0.80-0.82 0.81	0.85-0.89 0.87
Neomycin	1.90-1.90 1.90	2.05-2.11 2.08	2.30-2.20 2.25	2.40-2.35 2.357	2.40-2.60 2.50	2.85-2.55 2.70	
Penicillin	3.42-3.58 3.50	3.60-3.80 3.70	3.90-4.00 3.95	3.98-4.00 3.99	4.20-4.22 4.21	4.40-4.44 4.42	
Polymyxin B - Sulfate	0.80-0.80 0.80	0.85-0.85 0.85	1.05-0.95 1.00	1.10-1.10 1.10	1.34-1.32 1.33	1.34-1.41 1.375	
Streptomycin	2.00-2.00 2.00	2.25-2.35 2.30	2.40-2.60 2.50	2.72-2.88 2.80	2.90-3.01 2.955	3.04-3.06 3.05	
Bacitracin	1.23-1.27 1.25	1.20-1.40 1.30	1.50-1.54 1.52	1.68-1.70 1.69	1.70-1.70 1.70	1.70-1.71 1.705	
Terramycin	2.47-2.49 2.48	2.60-2.70 2.65	2.90-3.00 2.95	3.04-3.13 3.085	3.20-3.26 3.23	3.34-3.26 3.30	

* Diameter of the inhibition zones (cms) test run in duplicate

** The average diameter of the two zones (cms)

TABLE X

INHIBITION ZONES PRODUCED BY SULFONAMIDES USING MORAXELLA BOVIS (ATCC) AS THE TEST ORGANISM

Sulfonamide	25 ug per 0.1 ml	50 ug per 0.1 ml	100 ug per 0.1 ml	150 ug per 0.1 ml	200 ug per 0.1 ml	250 ug
Sulfadiazine	0.80-0.80*	0.80-0.80	0.80-0.80	0.80-0.80	1.30-1.70	1.50-1.70
	0.80**	0.80	0.80	0.80	1.50	1.60
Sulfamerazine	0.80-0.80	0.80-0.80	0.80-0.80	0.80-0.80	1.00-1.40	1.50-1.50
	0.80	0.80	0.80	0.80	1.20	1.50
Sulfapyridine	0.82-0.84	1.60-1.80	2.00-2.00	2.20-2.40	2.55-2.65	2.71-2.79
	0.83	1.70	2.00	2.30	2.60	2.75
Sulfanilamide	0.80-0.80	0.80-0.80	0.80-0.80	0.88-0.90	1.00-1.00	1.30-1.40
	0.80	0.80	0.80	0.89	1.00	1.35
Sulfathiazole	0.80-0.80	1.10-1.30	1.50-1.50	1.85-1.95	2.00-2.20	2.21-2.29
	0.80	1.20	1.50	1.90	2.10	2.25

* Diameter of the inhibition zones (cms) test run in duplicate

** The average diameter of the two zones (cms)

VITA

Ira O. Kliewer

Candidate for the Degree of

Master of Science

Thesis: THE *IN VIVO* AND *IN VITRO* SUSCEPTIBILITY OF *MORAXELLA BOVIS* TO SELECTED ANTIBIOTICS AND SULFONAMIDES

Major Field: Bacteriology

Biographical and Other Items:

Personal data: Born November 25, 1918 at Great Bend, Kansas.

Education: Undergraduate study, Bethel College 1938-1942. University of Wichita 1945-1946. Received the Bachelor of Arts degree from University of Wichita, 1946.

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