INJURY THRESHOLD AND ECONOMIC IMPACT OF FACE FLIES ON BEEF CATTLE

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INJURY THRESHOLD AND ECONOMIC IMPACT OF FACE FLIES ON BEEF CATTLE

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CHAPTER I

INTRODUCTION

The face fly, Musca autumnalis (DeGeer), has been a pest of livestock in parts of North America since its introduction to this continent sometime in the late 1940's or early 1950's. By 1964 this species had been recorded from all but 14 states of the continental United States (Sabrosky, 1961; USDA, 1965). Between 1964 and 1969 the face fly had been recorded from nine of the remaining 14 states including Washington, Oregon, California, Idaho, Nevada, Utah, Oklahoma, Arkansas, and Mississippi (USDA, 1966, 1967, 1968, 1969; Poorbaugh and Smith, 1968). The face fly was first found in a few northeastern counties of Louisiana in 1975 (Meek, 1976), and is currently well established across northern and eastern Arkansas (Boyer et al., 1975), and in 23 counties in the northeastern corner of Oklahoma (Wright and Arends, 1979).

Reviews of the field behavior and biology of the face fly in North America are by Pickens and Miller (1980), Teskey (1960, 1969), Ode and Matthysse (1967), and Killough et al. (1965). The life history of the face fly in the laboratory was reported by Wang (1964).

The face fly does not bite, but annoys livestock by congregating around the eyes and muzzle to feed on muscous secretions or on blood from any wound or insect bite on the animal. Much of the damage to cattle has been attributed to the general irritation caused by crawling and feeding face flies, use of energy in fighting flies, and loss in feeding

and grazing time caused by such irritation (Benson and Wingo, 1963; Ode and Matthysse, 1967; Teskey, 1969). Recent research (Schmidtmann, et al., 1981) indicated there was little difference in total grazing time between cattle heavily infested with face flies and those not, and we may have to revise our basis for estimating losses. Despite the high estimates of losses caused by face flies (USDA, 1968; Steelman, 1976) and the continued annual effort to control this pest, there is no evidence as to how much direct damage the face fly causes or of the injury threshold of this species (Steelman, 1976).

A review by Steelman (1976) on the effects of external and internal arthropod parasites on livestock production indicates there are no good economic thresholds for any of these parasites. In fact there is little data relating directly to the economic importance of any of the biting or nuisance flies attacking cattle. An early paper by Freeborn et al. (1928) indicated that milk losses to house flies, horn flies, and stable flies were overestimated and that milk losses were increased when the test animals were treated. Bruce and Decker (1947) found that dairy cattle protected from house flies and stable flies produced significantly more milk and in a three-year test from 1955-1957 and concluded that 10% to 20% increases in milk production should result from proper fly protection (Bruce and Decker, 1958). Granett and Hansens (1956, 1957) showed that a group of biting flies including horn flies, stable flies, horse flies, and mosquitoes caused reductions in milk production. Miller et al. (1973) indicated little effect on milk production as a result of stable flies feeding but their tests were done under controlled environmental conditions and a high energy ration which does not occur under normal dairy conditions.

Several studies have shown the advantage of controlling various species of biting flies on beef cattle. Laake (1946) found a 30-60 lb weight gain per animal in animals protected from horn flies. Cheng (1958) showed an average weight gain of 0.47 lb/animal/day in animals protected from horn flies, stable flies, and horse flies, whereas Cutkomp and Harvey (1958) found weight gains of 0.25 lb/head/day, 0.67 lb/head/day in cattle protected from horn flies and stable flies in 1954, 1955, and no gain in protected cattle in 1956. Roberts and Pund (1974) found that protecting cattle from horn flies and horse flies in Mississippi with weekly spray application resulted in increased gains of 0.20 and 0.23 lb/animal/day in 1969 and 1970, respectively. Haufe (1974) reported a 40% increase in weight gains (0.64 lb/animal/day) in heifers protected from horn flies in Alberta, and Campbell (1976) found a 12.9 lb per calf difference in weaning weights of calves protected from horn flies in Nebraska.

All the data from these studies was obtained with cattle on pasture where it was difficult to determine the impact of a single pest species from the combined effect of all biting flies. Thus it is very difficult to determine an actual economic threshold for a species under pasture conditions, but it is important to try to determine such thresholds under conditions that are as natural as possible. In order to more accurately determine economic or injury thresholds of some biting species, researchers have conducted studies with cattle in screened cages under natural conditions using feed rations designed to duplicate weight gains of pasture situations. Steelman et al. (1972 and 1973) have shown that mosquito populations caused significant reduction in weight gains in unprotected cattle as compared to cattle protected in screened cages.

Campbell et al. (1977) showed that stable flies caused significantly reduced weight gains and reduced feed efficiency in cattle on growing and finishing rations during 100 day-feeding trials. In a similar study, Campbell et al. (1981) found that house flies did not effect the weight gain on feed efficiency of cattle under simulated feedlot conditions. These studies have shown that this technique is more accurate in determining the actual impact of a pest species than are field trials where there are many variables that can confound the results.

Reviews of literature concerning infectious bovine keratoconjunctivitis (IBK) by Wilcox (1968) and Baptista (1979) indicate that IBK is worldwide in distribution and occurs primarily in the warmer months of the year coinciding with the fly season. It is possible that the greatest impact of the face fly may be its ability to transmit organisms causing eye disorders in cattle. This species has been directly incriminated with the transmission and spread of pinkeye in cattle (Steve and Lilly, 1965; Cheng, 1967; Brown and Adkins, 1972; Gerhardt et al., 1976), but its true role as a vector under pasture conditions is not fully understood. It appears that only a few face flies per animal can cause eye damage that make animals more susceptible to one of the causative organism Moraxella bovis. Shugart et al. (1979) found that one face fly per animal for 33 days can cause mechanical damage to the eyes of cattle which may predispose the eye for entrace of pathogens.

IBK is an important ocular disease of cattle and of great economic importance wherever it occurs (Baptista, 1979). Thrift and Overfield (1974) found that IBK reduced weight gains in heifers and bulls, 36 and 40 lbs, respectively, in 205 day weaning weights. Under Oklahoma pasture conditions, Cobb et al. (1976) found that Hereford and Angus

calves gained 33 lbs less at 205 days than animals that did not have clinical IBK. Bilateral IBK was found to decrease calf performance more severely than unilateral IBK by Hughes et al. (1976) and Killinger et al. (1977) in a 4-year study found an 11 lb suppression for calves with unilateral IBK, and 35 lbs for bilateral IBK at an adjust 205 day weaning weights.

The objectives of this study were to evaluate the performance of Hereford heifers while under the stress of face flies under disease free conditions, to evaluate the ability of the face fly to transmit Moraxella bovis in the laboratory, to investigate the transmission of M. bovis by face flies to heifers in screened cages, and to determine the prepatent period for the establishment of M. bovis in the eye and the development of clinical symptoms of infectious bovine keratoconjuctivitis (IBK).

CHAPTER II

ECONOMIC IMPACT OF FACE FLIES ON BEEF CATTLE

Introduction

The face fly, Musca autumnalis (DeGeer), is a pest of livestock in southern Canada and all of the continental United States except Arizona, Texas, New Mexico, Alaska and Florida (Pickens and Miller, 1980). The face fly is not a biting fly but rather annoys livestock by congregating around the nose and eyes to feed on mucous secretions. Due to these feeding habits, much of the effects on cattle by face flies has been attributed to the use of energy in fighting flies and loss of grazing and feeding time (Benson and Wingo, 1967; Ode and Matthysse, 1967; Teskey, 1969). In fact, there is very little data relating directly to the economic impact of non-biting flies on cattle. Campbell et al. (1981) found that house flies did not effect the weight gain or feed efficiency of cattle under simulated feedlot conditions. Shugart et al. (1979) found face fly feeding for an equivalent of 33 days caused lesions on the eye conjunctiva of cattle and proposed an injury threshold level of 1 fly/face for 33 days. Research on the effects of the face fly on total grazing time between cattle infested with face flies and cattle that are fly free showed no significant difference between the two (Schmidtmann et al., 1981).

This study was conducted to determine the impact of face flies on

yearling Hereford heifers enclosed in screened cages which allowed the evaluation of the effect of a single pest species on cattle. The impact of the face fly on the heifers was determined by measuring total weight gain, feed consumption, and feed conversion.

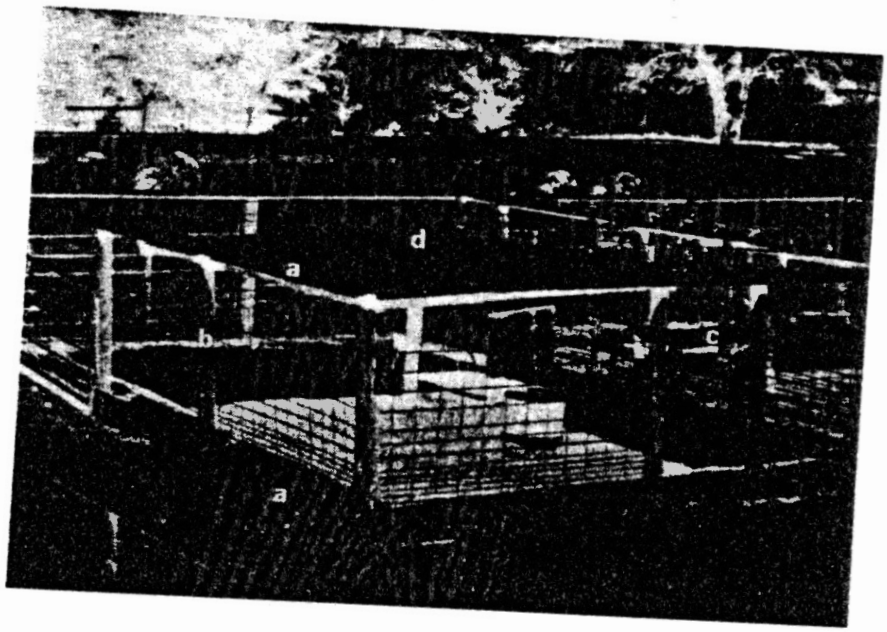
Materials and Methods

Face fly rearing techniques were modified during 1979 to enable production of 35,000 to 45,000 face fly pupae daily (Arends and Wright, 1981). These numbers of pupae were necessary to supply 4-5 day old adult face flies for release daily on Hereford heifers enclosed in screened pens, as well as adult flies needed to maintain the stock colony. The release flies were reared under the same conditions using the same techniques as the stock colony (Arends and Wright, 1981) except they were held in smaller cages (40.6 x 35.5 x 30.4 cm) which facilitated handling and release. Four to five day old flies were released because at this age the adults have mated and the females are strongly attracted to the eyes and nose of bovines in search of a protein food source needed for egg maturation (Lodha et al., 1970).

In 1979, 8 pens (6.1 x 8.5 x 1.8 m) were constructed at the Animal Science Range Cow Research Center, Stillwater, Oklahoma. The top of each pen was framed with 5 x 10.1 cm (2" x 4") to form the support for the screened cages and the bottom of each pen was framed with 5 x 30.4 cm (2" x 12") to which the bottom of the screened cages were fastened with 2.5 x 10.1 cm (1" x 4") (Figure 1a). Steel wire cattle panels 1.5 x 4.9 m were fastened to the inside of 2.4 m posts forming the perimeter of each pen (Figure 1b). A 1 m wide gate provided access to each pen (Figure 1c) and opened into the alleyway between the 2 rows of 4 pens.

Figure 1. Cattle pen, 6.1 x 8.5 x 1.8 m, with screened cage supports used to enclose heifers in economic impact study. a-SARAN® cage supports, b-posts, c-door, d-fiberglass roofing panels.

Figure 2. Cattle pen, 6.1 x 8.5 x 1.8 m, with SARAN® screened cage placed over it providing a fly proof environment.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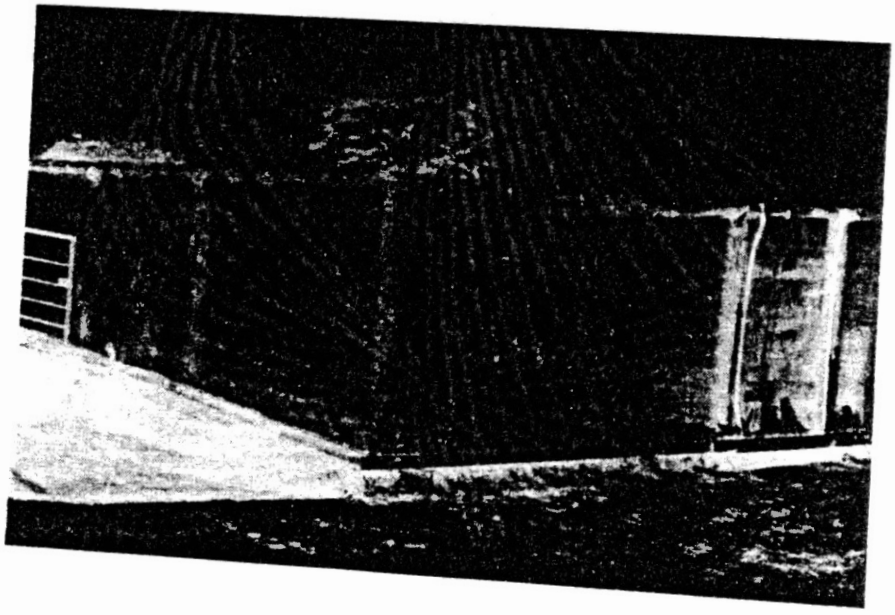


Shade was provided in each pen by covering the southwest one-quarter of each pen with fiberglass roofing panels built on a 5 x 10.1 cm (2" x 4") frame (Figure 1d). Each pen was constructed so that when the presewn SARAN® (Chicopee Manufacturing, Cornelia, Georgia) screened cages were placed over the 5 x 10.1 cm (2" x 4") frame each pen was a fly proof environment (Figure 2). The bottoms of the cages were fastened to the 5 x 30.4 cm (2" x 12") with 2.5 x 10.1 cm (1" x 4") to seal the bottoms. The bottom of the screen doors were sealed with a hinged 5 x 30.4 cm (1" x 4") backed with 5 cm foam. To supply extra support for the cage roofs, nylon line was strung across the width and length of the cages. Water was supplied to each pen with automatic waterers and feed was supplied free choice in a 422.88 l (12 bu) creep feeder in each pen. In 1980, 4 additional pens were constructed in the same pattern as 1979 except that 15.2 cm to 20.3 cm of fine chat-screening rock was placed in all pens as a base. This provided a surface that did not become wet and sloppy following rain and made the pens much easier to clean. Feed and water were supplied in the same manner as in 1979.

The rations used in 1979 and 1980 were designed to give 0.68 kg to 0.81 kg per day gain. In both years the rations were placed in each feeder every 2-3 days, with all feed carefully weighed so feed consumption could be monitored throughout the study. The ration used in 1979 consisted of: 30.0% cottonseed hulls, 30.0% alfalfa meal pellets, 24.7% cracked corn, 7.0% cottonseed meal, 7.0% molasses, 0.3% plain salt, 0.5% dicalciumphosphate-calcium carbonate and Vitamin A added to give 35,000 IU/S per animal/day. Due to higher than anticipated feed consumption in 1979, the ration used in 1980 was changed to decrease feed consumption to: 40.0% ground corn, 35.0% ground alfalfa hay, 21.75% cottonseed hulls,

and 3.0% cane molasses and 0.25% salt. Manure was removed from each pen 3 days a week to control fly breeding and reduce attraction of house flies to the pens. Care was taken to exclude flies as much as possible when entering or leaving the cages. The average number of face flies per heifer per day was determined by averaging 3 face counts of face flies on the faces of the animals. The counts were taken prior to release, immediately following release and at 1 hour post-release.

In 1979, 24 yearling Hereford heifers from 5 sires were grouped by weight and sire into 4 replicates, 6 animals per replicate, 3 animals per pen. Each replication consisted of 2 pens, one randomly designated fly free and one as fly stressed. The heifers were paired according to sire and initial weight, with one heifer from each pair randomly placed into a fly free or fly stressed pen and the other into the remaining pen. In 1979, we used 3 heifers per pen. The animals were pre-weighed after a 12-hour shrink the day before the test started on June 5, 1979. The heifers were weighed at 3-28 day intervals following 12-hour shrinks at each weigh date.

In 1980, the number of Hereford heifers in each pen was reduced to 2 per pen, but with the addition of 4 additional pens the total number of heifers used in the study remained at 24, chosen from 3 related sires. The heifers were grouped by sire and shrink weight into 6 replicates, 4 heifers per replicate, 2 heifers per pen. The heifers were pre-weighed after a 12-hour shrink on May 2, 1979 and randomly placed in fly free or fly stressed pens as in 1979. Animals were weighed at 4-28 day intervals, but only the final 112-day weight was a weight following a 12-hour shrink. The experiment was set up in a randomized block design and an Analysis of variance performed.

Results and Discussion

In 1979, the initial releases of face flies into the fly stressed pens caused obvious irritation to the heifers. Many animals had lacrimation from both eyes to the jawline within the first 24 hours after the intial release. Other indications of irritation were the swinging of heads to dislodge flies, excessive mucous from the nose and the huddling of the heifers together to dislodge flies. After the first 48 hours, the number of flies in each pen decreased rapidly and signs of irritation to the heifers also decreased. Subsequent releases later in the study produced similar irritation and behavior, however, as the length of time the heifers were under face fly stress increased, the irritation from the pests seemed to decrease. In 1979 during the first 28 days, face flies were released 5 times with an average of 7,000 flies per cage in each release which resulted in an average number of face flies per face of 7. In the second 28-day release period, flies were released 8 times, 4,087 flies per release with an average of 15 flies per face. During the last 28-day release period, flies were released 13 times with an average of 4,753 flies per release which produced an average of 17 flies per face. Throughout the release period the number of flies per face was lower than anticipated with the number of flies released. Even though eye irritation was easily observed, no cases of clinical infectious bovine keratoconjunctivitis (IBK) were observed in the fly stressed heifers.

The impact of face flies on the performance of the caged heifers was evaluated using 3 criteria: feed consumption, feed conversion, and weight gain, both total and average daily gain (ADG). The average daily feed consumption of heifers in fly free and fly stressed pens was 10.42 kg and 10.46 kg per day, respectively. The difference of 0.04 kg per day

not statistically significant. The feed conversion ratio, kg feed consumed per kg gain for the fly free and fly stressed heifers was 9.26 kg and 9.42 kg, respectively (Table I) and this difference was not statistically significant with an AOV analysis. The fly free animals gained 94.57 kg or 1.12 kg per day during the study period while the fly stressed animals gained 93.42 kg or 1.11 kg per day (Table I) and this difference was not statistically different (AOV).

In 1980 face fly releases were made on a daily basis for the 112-day study period. In each fly stressed pen an average of 3,500 adult face flies were released 7 days a week. As in 1979, the initial pest level on the heifers cause obvious irritation and as in 1979 after the initial 2 weeks of releases the heifers became acclimated to the pest load and irritation decreased even though pest load did not. Face counts on individual animals ranged from 0 to 50 flies per face with an average of 12 flies per face for the 112-day average. As in 1979 no cases of clinical IBK were observed in any of the fly stressed animals.

In 1980 the fly free and fly stressed heifers consumed an average of 11.0 kg and 10.3 kg of feed per day with feed conversions of 9.32 kg and 9.19 kg, respectively, and those differences were not statistically significant. The mean total weight gain for the fly stressed animals was 128.6 kg (ADG of 1.15 kg) while the fly free animals gained 133.1 kg (ADG of 1.18 kg) (Table II). The differences of 4.5 kg total gain for the fly free animals was not statistically significant. In 1979 in 2 replications, the fly free heifers gained an average of 10.4 kg more than the fly stressed. In the other 2 replications just the reverse was true with the fly stressed heifers gaining an average of 6.9 kg more than the fly free. In 1980 a similar trend was observed as in 4 replications the

TABLE I

COMPARISONS OF WEIGHT GAINS AND FEED EFFICIENCY
OF FACE FLY FREE AND FACE FLY STRESSED
HEREFORD HEIFERS IN 1979

Replication	Treatment	*Initial **Weight (kg)	Final Weight (kg)	Total Gain (kg)	Average Daily Gain (kg)	Feed Efficiency (kg feed/kg gain)
1	Fly Free	213.2	311.6	98.4	1.17	8.95
	Fly Stressed	210.9	297.1	86.2	1.02	9.42
2	Fly Free	244.9	334.7	89.8	1.05	10.00
	Fly Stressed	248.5	343.8	95.3	1.13	9.68
3	Fly Free	237.2	335.6	98.4	1.17	9.03
	Fly Stressed	236.7	343.8	107.0	1.27	8.68
4	Fly Free	235.8	327.5	91.7	1.09	9.29
	Fly Stressed	233.6	318.8	85.2	1.01	10.36
Average	Fly Free			94.57	1.12	9.26
	Fly Stressed			93.42	1.11	9.42

*All weights after 12-hour shrink
**Average 3 animals/pen

TABLE II

COMPARISONS OF WEIGHT GAINS AND FEED EFFICIENCY
OF FACE FLY FREE AND FACE FLY STRESSED
HEREFORD HEIFERS IN 1980

Replication	Treatment	*Initial **Weight (kg)	Final Weight (kg)	Total Gain (kg)	Average Daily Gain (kg)	Feed Efficiency (kg feed/kg gain)
1	Fly Free	176.0	314.3	138.3	1.23	8.72
	Fly Stressed	173.0	298.9	125.9	1.12	9.09
2	Fly Free	183.4	323.3	139.9	1.24	8.92
	Fly Stressed	183.0	331.5	148.5	1.32	8.72
3	Fly Free	195.3	316.1	120.8	1.07	8.99
	Fly Stressed	195.5	321.1	125.6	1.12	8.66
4	Fly Free	210.7	348.6	137.9	1.23	9.65
	Fly Stressed	210.0	335.2	125.2	1.11	9.40
5	Fly Free	191.8	325.4	133.6	1.19	10.12
	Fly Stressed	191.4	315.5	124.1	1.10	8.75
6	Fly Free	198.7	326.8	128.1	1.14	9.53
	Fly Stressed	198.4	321.1	122.7	1.07	9.76
Average	Fly Free			133.1	1.18	9.32
	Fly Stressed			128.6	1.15	9.19

*All weights after 12-hour shrink
**Average 2 animals/pen

fly free heifers outgained the fly stressed animals by an average of 10.04 kg and in 2 replicates fly stressed heifers gained an average of 7.7 kg more than the fly free. While these differences were not statistically significant, they do indicate that there is some stress applied by pest loads of 12-15 flies per face in 2 of 4 replicates in 1979 and 4 of 6 replicates in 1980.

During 1980, siblings of the heifers used in the study (on native Oklahoma pasture) were monitored for weight gains. They were weighed on the same dates as the study animals and averaged 0.5 kg per day gain for the 112-day period as compared to the 1.13 kg to 1.17 kg ADG of the test animals. This gain is the sort one would expect to see from animals on native pasture in Oklahoma and is the same average gains we had hoped to reproduce in the study.

Low activity level and boredom of the heifers may have partially accounted for their high feed consumption and consequent high weight gains. Utilizing the 1.11 kg per day gain of the heifers on native pasture as a guide, our study heifers would have needed to consume 4.65 kg per day instead of the 11.0 kg of feed per day to have a similar gain as those heifers on native pasture.

Under our simulated range conditions, with unlimited feed and water and with the face fly the only arthropod pest stressing the heifers there were no significant differences in any of the criteria we used to evaluate the impact of face flies on the heifers. To gain a more precise estimate of the impact of face flies on pastured heifers the intake of feed will have to be adjusted to meet the 4.5-5.5 kg per day to give an ADG of 0.5 kg - 0.6 kg. To accomplish this the animals will have to be on a limited intake of feed rather than the free choice system used

in this study. Under our conditions, with no disease complications, pest loads of 12 flies per face did not significantly affect animal performance or behavior. If there was a stress placed on the heifers by the level of pest intensity in this study, the heifers were able to compensate for any stress due to the face flies.

CHAPTER III

TRANSMISSION OF *MORAXELLA BOVIS* IN THE LABORATORY BY THE FACE FLY *MUSCA AUTUMNALIS*

Introduction

Infectious Bovine Keratoconjunctivitis (IBK) is an eye disorder of cattle frequently associated with temporary or permanent blindness (Pugh and Hughes, 1975) and weight loss (Hughes et al., 1976). Many authorities suggest that the bacteria *Moraxella bovis* (Hauduroy) is the causative agent most commonly associated with IBK (Hughes et al., 1965; Baptista, 1979; Wilcox, 1968), however, many authorities believe that the bovine eye needs to be predisposed by irritation prior to infection. Hughes et al. (1965), Pugh and Hughes (1975), Wilcox (1968), and Baptista (1979) suggest ultraviolet radiation, dust and fly irritation as possible predisposing factors.

Since its introduction into North America in 1952 in Nova Scotia (Vockeroth, 1953) the face fly, *Musca autumnalis* (DeGeer), has spread to 45 states and all of Canada. The spread of the face fly has been accompanied with reported increases of eye disorders in cattle (IBK) from Missouri (Benson and Wingo, 1963), Pennsylvania (Cheng, 1967), and Tennessee (Gerhardt et al., 1976). Steve and Lilly (1965) isolated *M. bovis* from face flies that had been allowed to walk on the exudate from severely infected eyes and found the bacteria could be isolated

from the flies for up to 3 days, but did not elucidate as to the total number of bacteria the face fly could transmit nor to the percentage of flies from which the bacteria was isolated. Brown and Adkins (1972) released non-infected face flies on caged cattle and the mechanical irritation to the eyes from the feeding of face flies caused irritation that could be classified as beginning IBK symptoms. However, when face flies contaminated with M. bovis were released onto caged cattle, M. bovis was isolated from the eyes but no symptoms of IBK developed. Shugart et al. (1979) speculated that an average of 1 face fly per animal for an equivalent of 33 days caused lacrimation and lesions on the eyes that could predispose the animal to pathogens that cause IBK. Therefore, there is some controversy as to the role of the face fly in the transmission of pathogens causing IBK.

The purpose of this study was to quantitatively measure the ability of the face fly to transmit M. bovis over time, to determine an exposure technique that would allow the maximum number of bacteria to be transmitted by the face fly, and to determine how long the face fly can efficiently transmit M. bovis in the laboratory.

Materials and Methods

A stock culture of M. bovis, strain FLA-64, was obtained from Dr. G. W. Pugh, Jr. (NADL, Ames, Iowa) and maintained in trypticase soy broth (TSB) frozen at -60°C. To supply the M. bovis cultures needed for face fly exposure one tube of FLA-64 was thawed, streaked on blood agar plates (BAP) and incubated at 37°C for 24 hours. Following incubation, 5-10 typical smooth colonies were picked from the BAP with an inoculating loop, suspended in TSB and agitated on a mechanical mixer

to assure even distribution in the TSB. This TSB suspension was used to prepare subsequent BAP inoculations for use in face fly exposures.

M. bovis cultures were prepared and face flies exposed by four techniques to determine which exposure technique facilitated transmission of the greatest number of organisms for the longest period of time. For exposure techniques A and B, BAP were inoculated from the M. bovis-TSB suspension and incubated at 37°C for 24 hours. In exposure technique A, 2 of the BAP were placed into a cage of face flies for 3 hours immediately after incubation. In exposure technique B, 2 of the BAP were overlaid with 2 ml of TSB immediately after incubation and then placed into a cage of face flies for 3 hours. The TSB overlay produced serous-like surfaces on the plates which were similar to bovine eye secretions.

M. bovis culture materials for exposure techniques C and D were prepared by inoculating a series of blood agar plates with the M. bovis-TSB suspension and incubating them at 37°C for 24 hours. Following incubation, the growth from 10 plates was scraped with an inoculating loop into a test tube with 10 ml of TSB (Pugh and Hughes, 1969). The growth from 10 other plates was scraped into a test tube containing 10 ml of TSB + 0.1% agar which prevented agglutination. Both of these tubes were stored at -60°C until used for exposure to face flies. In exposure technique C, a tube of the M. bovis-TSB + 0.1% agar was thawed, agitated, and 5 ml poured onto each of 2 blood agar plates which were then placed in a cage of face flies for 3 hours. In exposure technique D, a tube of the M. bovis-TSB suspension was thawed, agitated, and 5 ml poured onto each of 2 blood agar plates which were exposed in a cage of face flies for 3 hours.

Face flies used in this study were reared according to Arends and

Wright (1981) and were not allowed to oviposit, as exposure to ovipositional material increased the possibility for bacterial contamination. In each of the two replications of the study, 4,000 to 5,000 adult face flies, 5-6 days old (post-eclosion) were placed in each cage and exposed to M. bovis culture for 3 hours with 1 of 4 exposure techniques assigned to the cages in a randomized manner.

Prior to exposure, 2 randomly chosen flies from each cage were placed individually on sterile BAP for 10 minutes. Following incubation these plates were examined to determine if flies in a cage were contaminated with any organism that would make it difficult to determine if M. bovis was transmitted to the BAP. Immediately after the 3 hour exposure to the M. bovis cultures (0 hours post-exposure), 5 randomly selected flies from each exposure cage were placed individually on BAP for 10 minutes, with the same number of flies tested in the same manner at 1, 3, 6, and 12 hours post-exposure. Following the 10 minute exposure on the BAP, flies were removed and the BAP incubated at 37°C for 24 hours. At this time the total number of B-hemolytic colonies per plate were counted and this data was analyzed by an analysis of variance procedure. Typical colonies from each plate were tested further to confirm them as M. bovis. Confirmation was based on B-hemolysis, growth appearance, negative gram stain, oxidase test, catalase test, 3% KOH Gram reaction test, and the ability to digest casien in 2% milk agar plates.

Results and Discussion

Plates exposed to face flies prior to the M. bovis exposure showed no bacterial contamination in any of the cages. The maximum number of colonies of M. bovis transmitted by face flies occurred at 0 hours

post-exposure with all 4 exposure techniques (Table III). The highest number of colonies transmitted by face flies was 123.7 colonies per fly with technique B. The TSB overlay on the BAP provided a viscous surface that remained wet for 2 hours. Evidently the flies were able to pick up the bacteria while the plates were still covered by the TSB which attributed to the large number of colonies transmitted. One hour after exposure there were decreases in the number of colonies transmitted of 75%, 60%, and 79% in exposure techniques B, C, and D, whereas there was only a 33% decrease in exposure technique A. At 0, 1, and 3 hours post-exposure, 100% of all sampled flies from each cage transmitted M. bovis to BAP. By 3 hours post-exposure, less than 5 colonies were transmitted per fly in exposure techniques C and D, the frozen preparations (Table III). However, the flies exposed to the plate preparations (techniques A and B) transmitted greater than 12 colonies per fly at 3 hours post-exposure. After 6 hours post-exposure only the flies exposed to M. bovis by technique A still transmitted 4.9 colonies per fly or more. By 12 hours post-exposure, the average number of colonies transmitted from exposure techniques B, C, and D, were less than 1, whereas those transmitted in technique A was 1.4 (Table III).

When the total number of colonies transmitted by flies exposed to M. bovis by techniques A and B were compared with the number transmitted by flies exposed by techniques C and D (Figure 3), a significant difference ($P < 0.02$) in the total number of colonies transmitted was observed. The number of colonies transmitted by the flies from the BAP preparations was 2,924 vs 1,819 colonies from the frozen preparations. The log count vs log time plot (Figure 3) of these data show a linear relationships between the number of colonies transmitted over time.

TABLE III

AVERAGE NUMBER OF COLONIES OF MORAXELLA BOVIS TRANSMITTED TO BLOOD AGAR PLATES BY THE FACE FLY AFTER EXPOSURE TO FOUR CULTURE PREPARATIONS

Hours Post-Exposure	Exposure Technique A	B	C	D
0	60.6*	123.7	43.8	88.5
1	40.7	30.9	17.1	18.7
3	13.3	12.1	2.7	3.8
6	4.9	2.7	3.9	1.6
12	1.4	0.1	0.6	0.8

*Average number colonies per plate, 2 rep.-5 plates/exposure period/exposure technique/rep.

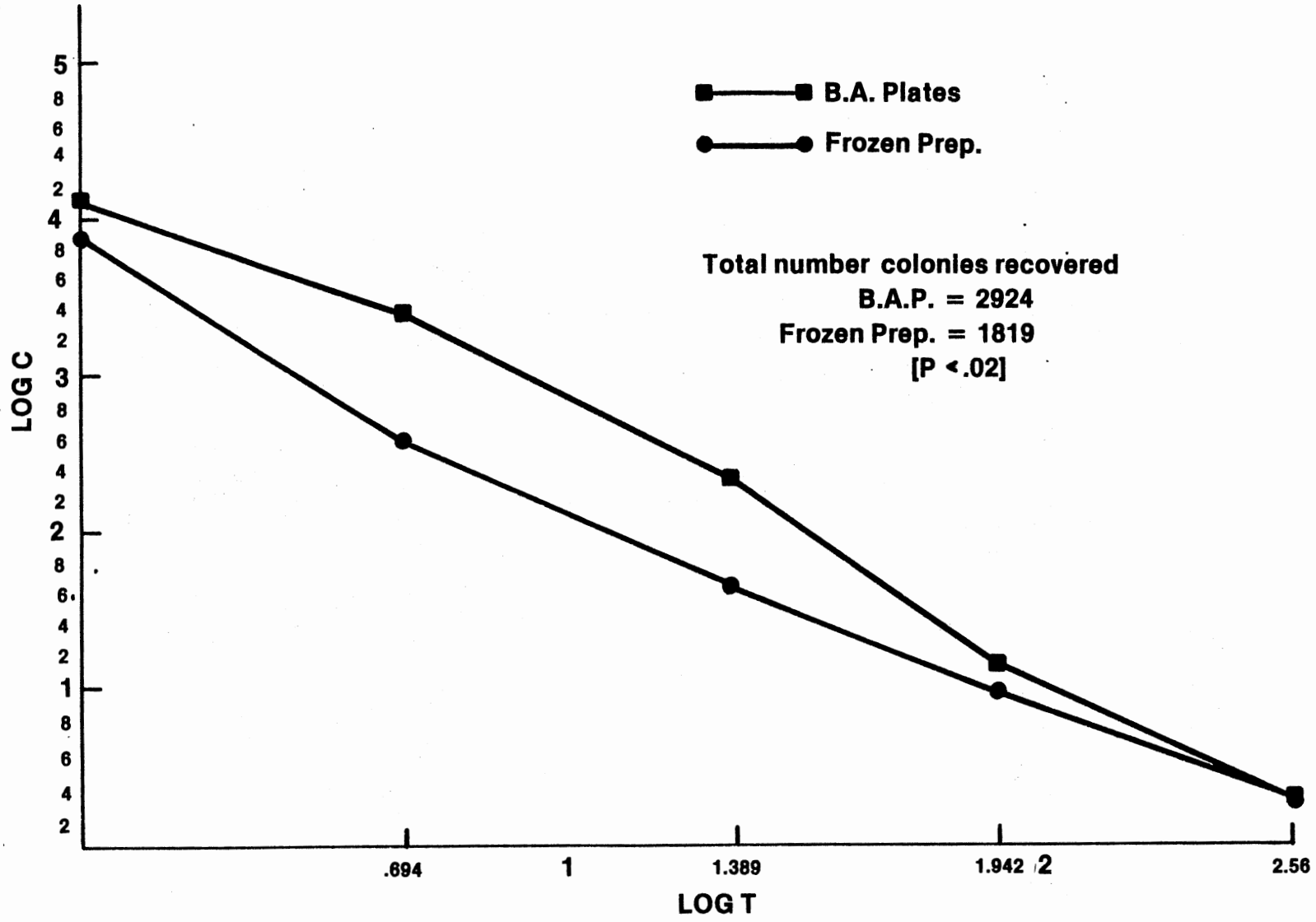
A-2 M. bovis cultures on BAP

B-2 M. bovis cultures on BAP + 2 ml trypticase soy broth overlay

C-Frozen M. bovis culture suspended in trypticase soy broth + 0.1% agar

D-Frozen M. bovis culture suspended in trypticase soy broth

Figure 3. Comparison of the log of the total number of Moraxella bovis colonies recovered from BAP over time following exposure of face flies to Moraxella bovis-BAP or Moraxella bovis-frozen preparation.


B.A. Plates
Frozen Prep.
Total number colonies recovered
B.A.P. = 2924
Frozen Prep. = 1819
[P < .02]
LOG C
LOG T
.694
1
1.389
1.942
2
2.56

In theory, the frozen preparation contained 5 times the number of bacteria than the plate methods, as each tube of the preparation contained the growth from 10 M. bovis-BAP plates. However, the face flies were not able to transmit the bacteria from the frozen preparations with the same efficiency as from the plate preparations. The frozen preparation is used as a standard inoculum for infecting bovines with M. bovis by injecting directly on the eye (Hughes et al., 1976; Pugh and Hughes, 1975). However, our data show it would not be the best method available to expose face flies to M. bovis for transmittance to bovines. A threshold of 5 bacteria per predisposed bovine eye has been proposed as the number needed for M. bovis to become established in the bovine eye. Face flies exposed to M. bovis by techniques A and B were able to transmit more than 5 colonies/fly until 6 hours post-exposure; whereas the flies exposed to techniques C and D fell below this level at 3 hours post-exposure. All face flies sampled from the cages in all 4 techniques were able to transmit M. bovis to BAP.

If similar percentages of face flies in nature are contaminated with M. bovis following feeding on an infected bovine eye, one face fly could transmit sufficient numbers of M. bovis for 6 hours after feeding for establishment in the eye. The high percentage of flies transmitting M. bovis in the laboratory coupled with the feeding behavior of the face fly further illustrates the apparently important role the face fly plays in the epidemology of IBK in face fly infested areas.

CHAPTER IV

ABILITY OF THE FACE FLY TO TRANSMIT *MORAXELLA BOVIS* UNDER FIELD CONDITIONS

Introduction

The face fly, *Musca autumnalis* (DeGeer), was introducted in Nova Scotia (Vockeroth, 1953) and has since spread to 44 states (Pickens and Miller, 1980) and all of southern Canada (Depner, 1969). This advancement of the face flies distribution has been accompanied with reported increases of eye disorders of cattle (Benson and Wingo, 1963; Cheng, 1967; Gerhardt et al., 1976).

Infectious bovine keratoconjunctivitis (IBK) is the most common eye disorder of cattle and has been associated with temporary and permanent blindness (Pugh and Hughes, 1976) and weight loss (Hughes et al., 1976; Killinger et al., 1977; Cobb et al., 1976 Thrift and Overfield, 1974).

The face fly has been incriminated as a vector of *Moraxella bovis* (Hauduroy), a causative agent of IBK, by Steve and Lilly (1965) and Brown and Adkins (1972). Damage to the eye by face fly feeding, which may predispose the eye for entrance of pathogens that cause IBK was reported by Shugart et al. (1979) and Steve and Lilly (1965) were able to isolate *M. bovis* from flies allowed to walk on the exudate of severely infected eyes. Brown and Adkins (1972) released face flies on cattle

in screened enclosure and found that face fly feeding produced symptoms comparable to mild IBK, and when face flies contaminated with M. bovis were released on the cattle, M. bovis was isolated from their eyes but no clinical symptoms of IBK were found.

The objectives of this study were to determine the ability of the face fly to transmit M. bovis from laboratory cultures to animals and determine the incidence of subsequent clinical IBK resulting from such transmission.

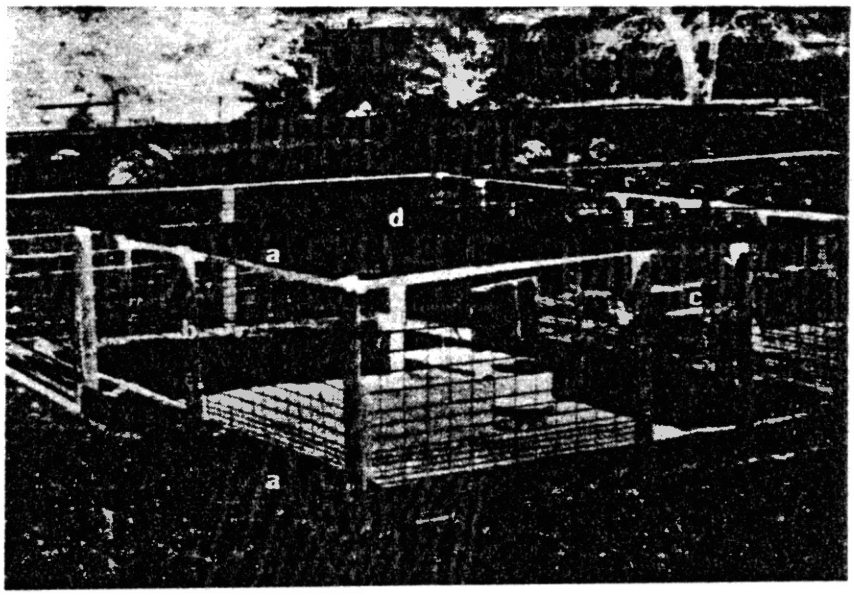
Materials and Methods

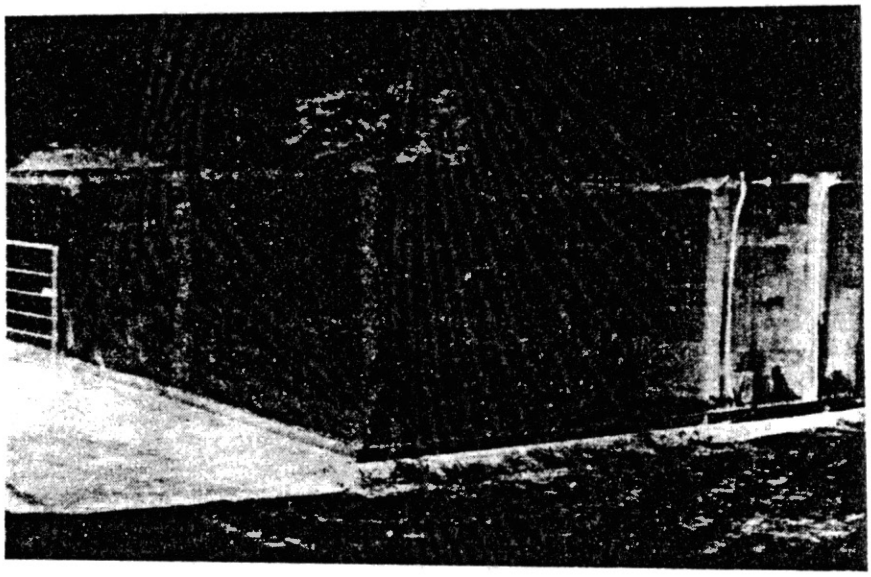
Two pens, 6.1 x 8.5 x 1.8 m, were constructed at the Animal Science Range Cattle Research Center, Stillwater, Oklahoma. The top of the pens were framed with 5 x 10.1 cm (2" x 4") and the bottoms with 5 x 30.4 cm (2" x 12") (Figure 4a) and 1.5 x 4.8 m steel wire cattle panels (Figure 4b) were stapled to the inside of 2.4 m posts forming the perimeter of each pen. Access to each pen was through a 1 m gate (Figure 4c) and one-quarter of each pen was shaded by fiberglass roofing panels built on a 5 x 10 cm (2" x 4") frame (Figure 4d). Each pen was covered with a pre-sewn SARAN® screened cage (Chicopee Manufacturing, Cornelia, Georgia), designed to fit over the framework of the pens (Figure 5) providing a fly proof environment. Water and feed were supplied to each cage free choice.

Four yearling Hereford heifers in which had no signs of clinical IBK were placed into 2 screened cages, 2 heifers per cage. To determine that the animals used were not harboring M. bovis, their eyes were swabbed 3 times at 3-day intervals, with sterile cotton-tipped applicators. The swabs were immediately placed into a screw-cap vial containing 1 ml

Figure 4. Cattle pen, 6.1 x 8.5 x 1.8 m, with screened cage support used in field transmission of Moraxella bovis to Hereford heifers. a-SARAN® cage supports, b-posts, c-door, d-fiberglass roofing panels.

Figure 5. Cattle pen, 6.1 x 8.5 x 1.8 m, with SARAN® screened cage placed over it providing a fly proof environment.


d
a
c
b
a



trypticase soy broth (TSB) after collection to prevent drying out. Following return to the laboratory, the swabs were used to streak blood agar plates (BAP) which were incubated at 37°C for 24 hours and examined for M. bovis.

Cultures of M. bovis denoted FLA-64 and EPP-300 frozen in TSB at -60°C were obtained from Dr. G. W. Pugh, Jr. (NADL, Ames, Iowa.). To supply the M. bovis needed for fly exposure, the cultures were thawed, streaked on BAP, and incubated at 37°C for 24 hours. Five to ten typical smooth colonies were picked from the BAP with an inoculating loop and suspended in 2 ml of TSB and agitated on a mechanical mixer to assure an even distribution in the TSB to supply inoculum for the future M. bovis cultures needed for fly exposure. Cultures were made by inserting a sterile swab into the inoculum and swabbing a BAP and incubating it at 37°C for 24 hours.

Five thousand 4-5 day old (post-eclosion) face flies were released in the pens with heifers for 7 consecutive days. On each day flies were counted to determine the level of pest intensity immediately prior to release, immediately following release and at 1 hour post release. The 3 fly counts were averaged to give the daily fly level for each heifer. Face flies were exposed to M. bovis by placing 2 - 24 hour cultures of M. bovis on BAP in a cage of 5,000, 4-5 day old face flies for 3 hours and then immediately releasing the contaminated flies into the screened cage. Moraxella bovis contaminated face flies were released on the heifers for 10 consecutive days following the initial 7 day fly stress period. Fly counts were taken daily to determine the level of pest intensity and the heifers were examined daily for signs of IBK. On days 3, 5, 7, and 9, post-exposure to contaminated flies, the eyes of the

heifers were swabbed and cultured to determine if M. bovis had become established in the eyes of the exposed heifers. Control animals that were in adjacent screened pens were swabbed at selected intervals to determine if the M. bovis was being transmitted by other means than the contaminated flies.

The severity of the lesions on the cornea of the eyes of the heifers were scored according to the system developed by Killinger et al. (1976). A score of 10=normal eye, 11=an active lesion involving less than one-third of the cornea, 12=an active lesion involving one-third to two-thirds of the cornea, 13=an active lesion involving more than two-thirds of the cornea and 14=perforation of cornea.

Release 1 (using M. bovis strain FLA-64) began on June 3, 1980 and ended on June 24, 1980. Release 2 was started on July 10, 1980 and ended July 31, 1980, and release 3 began on May 31, 1981 and ended June 20, 1980. The M. bovis strain used in releases 2 and 3 was EPP-300.

Results and Discussion

During release 1, an average of 3,460 face flies were released daily into each of the 2 pens for 21 days. During the AM hours of the study there was an average of 8.5 flies per face. The released FLA-64 strain was isolated from each eye of each animal from the 5th day after release of infected flies and remained established in the eyes throughout the study (Table IV). Heifers #3 and #4 did not develop clinical IBK but lesions did develop in the left eyes of animals #1 and #2 (Table IV) which shared the same pen. An initial eye score of 11 (Killinger et al., 1970) was assigned to both lesions (Figure 6) on day 5 after exposure. The lesion in the left eye of heifer #1 did not progress

TABLE IV

INCIDENCE OF MORAXELLA BOVIS RECOVERED FROM THE EYES OF CATTLE FOLLOWING EXPOSURE TO FACE FLIES CONTAMINATED WITH MORAXELLA BOVIS

Release	Heifer Number	Days After Exposure 3 Eye Right	3 Eye Left	5 Eye Right	5 Eye Left	7 Eye Right	7 Eye Left	9 Eye Right	9 Eye Left
1	1	-	-	+	+(11)*	+	+	+	+
M. bovis	2	-	-	+	+(11)*	+	+(12)**	+	+(13)***
strain	3	-	-	+	+	+	+	+	+
FLA-64	4	-	-	+	+	+	+	+	+
2	5	-	-	+(12)**	+	+	+	+	+
M. bovis	6	-	-	+	+	+	+	+	+
strain	7	-	-	+	+	+	+	+	+
EPP-300	8	-	-	-	+	-	+	-	+
3	9	-	-	-	-	-	+	-	+
M. bovis	10	-	-	-	-	-	-	+	-
strain	11	-	-	-	-	+	-	+	-
EPP-300	12	-	-	+(11)*	-	+	-	+	-

*(11) - lesion that involves less than 1/3 of the cornea
**(12) - lesion that involves 1/3 to 2/3 of the cornea
***(13) - lesion that involves more than 2/3 of the cornea

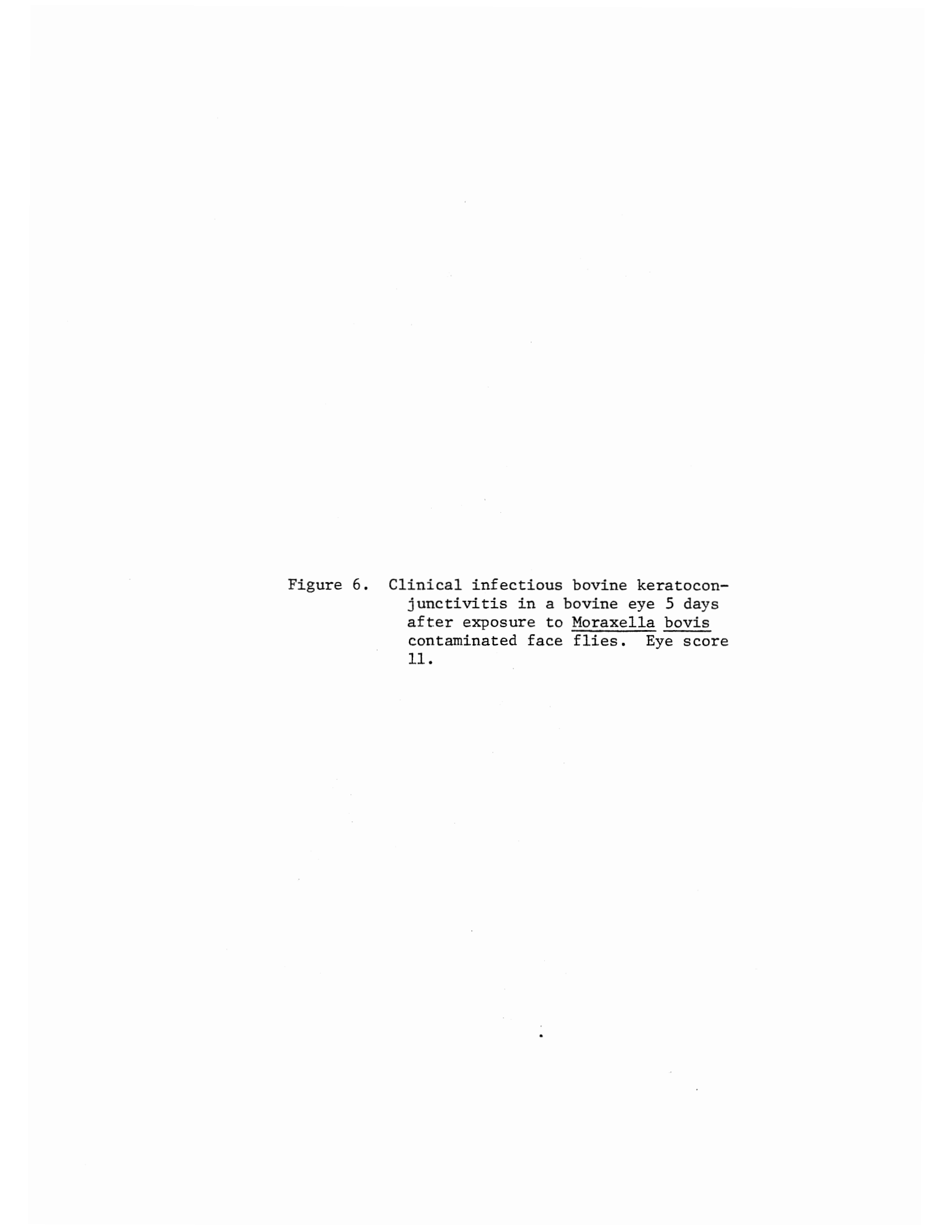
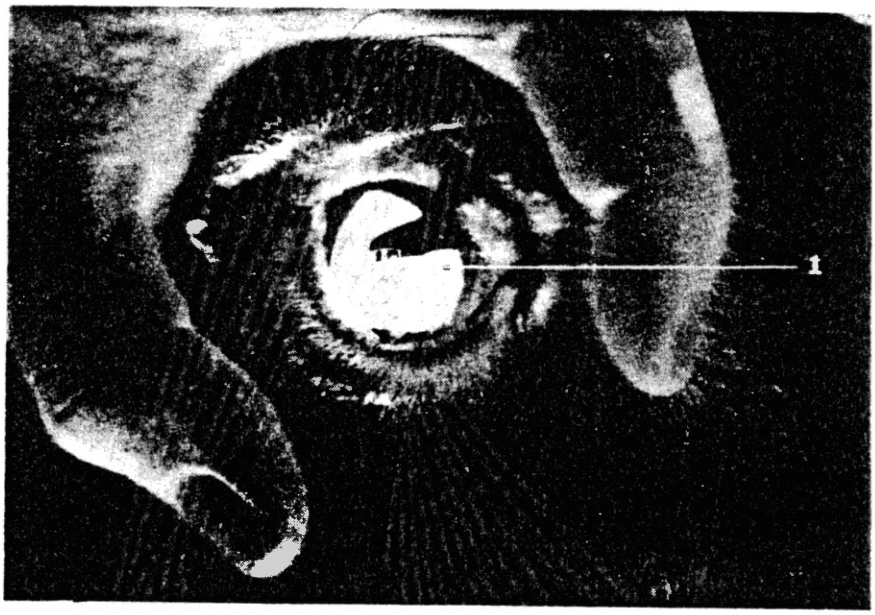


Figure 6. Clinical infectious bovine keratoconjunctivitis in a bovine eye 5 days after exposure to Moraxella bovis contaminated face flies. Eye score 11.


1

beyond an eye score of 11, but the lesion in the left eye of #2 had increased to a score of 12 (Figure 7) by day 7 and to a score of 13 (Figure 8) by day 21. At this time the study was terminated and all eyes treated and lesions cleared up.

Strain EPP-300 M. bovis was used in the second test in 1980. An average of 3,956 face flies were released daily and an average of 12.5 per face were present in AM counts. Moraxella bovis was reisolated from all eyes of all heifers except the right eye of heifer #8 by the 5th day after release of infected flies. The bacteria was present in the eyes except the right eye of #8 throughout the remainder of the study. Lesions developed in only one eye, the right eye, of heifer #5 (Table IV). It advanced to an eye score of 12 (Figure 7).

Moraxella bovis, strain EPP-300, was used in release 3 in 1981. An average of 4,125 face flies were released daily and an average of 6.5 flies per face were present in the AM counts. Moraxella bovis was isolated from the right eye of heifer #12 on the 5th day after release of infected flies (Table IV). A lesion was observed on the right eye of heifer #12 at the same time and an eye score of 11 was given to the eye. The lesion did not progress any further even though M. bovis was isolated from the right eye for the rest of the study (Table IV).

Moraxella bovis was isolated from the left eye of heifer #9 and the right eye of heifer #11 7 days after the release of infected flies and was isolated from the right eye of heifer #10 at 9 days post release (Table IV).

There were no isolations of M. bovis from the control animals in any of the releases, confirming that the only mode of transmittance in our study was by face flies.

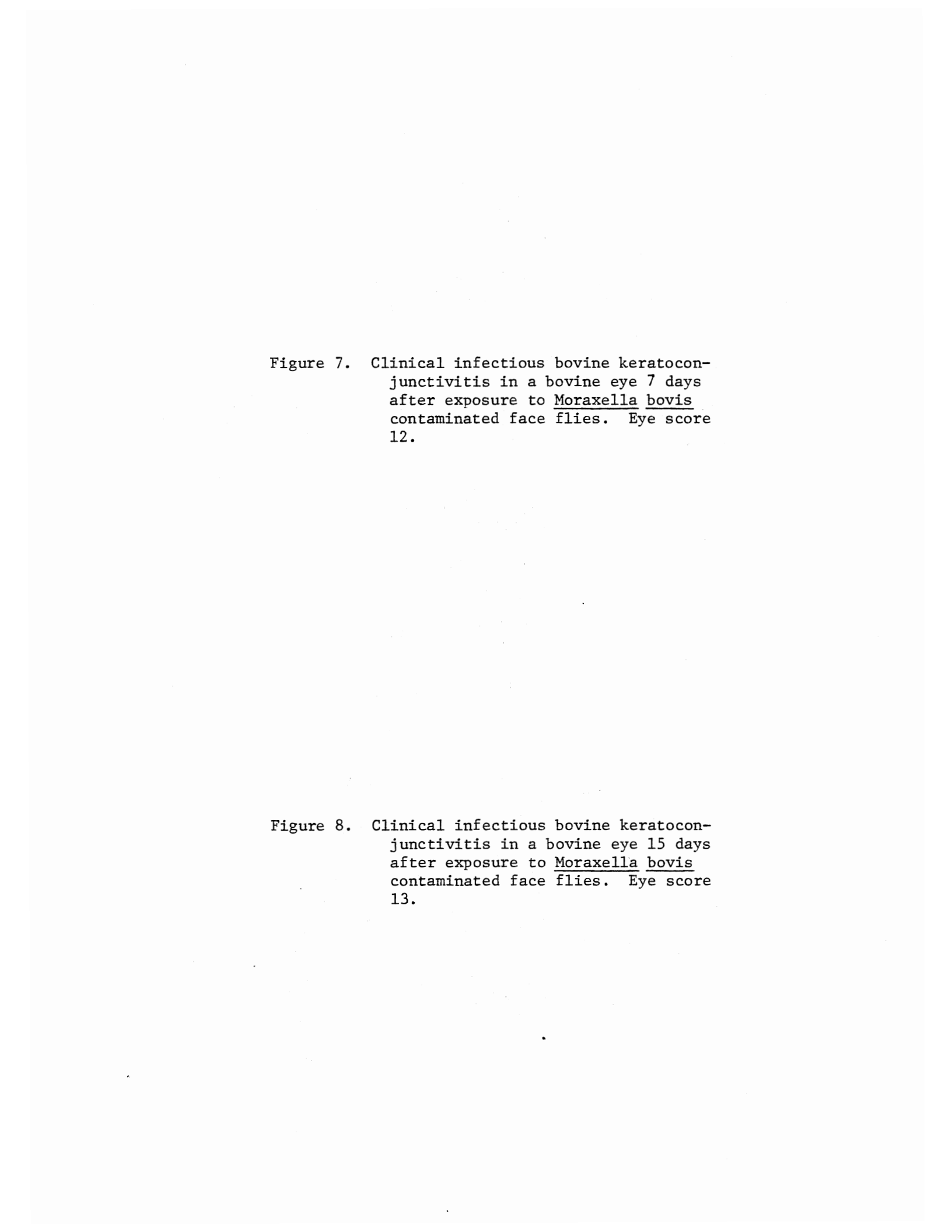
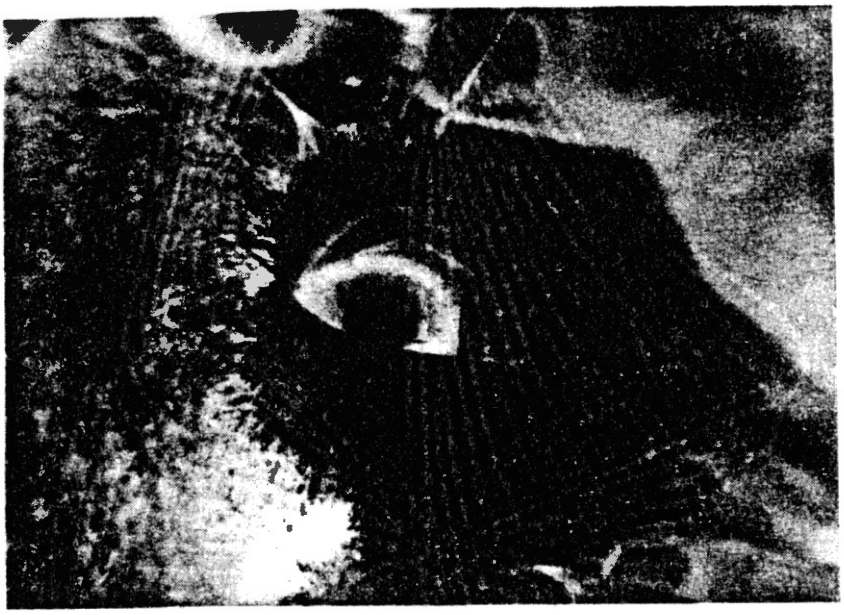


Figure 7. Clinical infectious bovine keratoconjunctivitis in a bovine eye 7 days after exposure to Moraxella bovis contaminated face flies. Eye score 12.

Figure 8. Clinical infectious bovine keratoconjunctivitis in a bovine eye 15 days after exposure to Moraxella bovis contaminated face flies. Eye score 13.



Using the face fly as the vector of M. bovis we were able to establish M. bovis in 19 of 24 bovine eyes exposed to contaminated face flies. Subsequent development of clinical IBK was observed in 4 of the 19 eyes in which M. bovis was established and the incubation period we observed of 5-6 days was near the lower limit of the reported incubation period of 1-20 days (Pugh and Hughes, 1975). We feel that the combination of damage to the eye due to fly feeding and the efficiency of the face fly as a vector of M. bovis attributed to the rapid establishment and development of clinical IBK.

These results unquestionably incriminate the face fly as a vector of M. bovis and further illustrate the importance of the role the face fly plays in the epidemology of IBK in face fly areas.

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