

147
55

The microbiology of the normal camelid conjunctival sac

ISU
1991
G437
c. 3

by

Juliet Rathbone Gionfriddo

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
Master of Sciences

Department: Veterinary Clinical Sciences

Major: Veterinary Clinical Sciences

Signatures have been redacted for privacy

Iowa State University
Ames, Iowa
1991

TABLE OF CONTENTS

EXPLANATION OF THESIS FORMAT	1
INTRODUCTION	2
LITERATURE REVIEW	6
PART I. THE BACTERIAL AND MYCOPLASMAL FLORA OF THE NORMAL CAMELID CONJUNCTIVAL SAC	16
SUMMARY	17
INTRODUCTION	18
MATERIALS AND METHODS	20
RESULTS	24
DISCUSSION	30
REFERENCES	34
PART II. THE FUNGAL FLORA OF THE NORMAL CAMELID CONJUNCTIVAL SAC	37
INTRODUCTION	38
MATERIALS AND METHODS	39
RESULTS	40
DISCUSSION	43
REFERENCES	46
PART III. THE BACTERIAL FLORA OF THE NORMAL EYE OF CAPTIVE CAMELIDS IN WINTER	48
RESULTS	49
DISCUSSION	53
PART IV. SEASONAL COMPARISON OF BACTERIOLOGY DATA	56
RESULTS	57

DISCUSSION	61
SUMMARY AND CONCLUSION	63
REFERENCES	64
ACKNOWLEDGEMENTS	71
APPENDIX. MATERIALS AND METHODS	72

EXPLANATION OF THESIS FORMAT

This thesis is presented in the alternate thesis format as defined in the Graduate College Thesis Manual of Iowa State University. The papers included in Parts I and II of this thesis, "The bacterial and mycoplasmal flora of the normal eye of captive camelids" and "The fungal flora of the normal camelid conjunctival sac" have been accepted for publication in the American Journal of Veterinary Research and are scheduled for publication in 1991. Each paper contains its own introduction, materials and methods, results, discussion, and reference sections and its own tables and figures. I am the primary author of each report.

The other sections in the thesis are included to expand upon the information contained in the papers in Parts I and II and to present additional data not included in them. The literature review section covers the pertinent literature on the microbiology of the external eye of domestic animal species. It lays the groundwork for comparisons between captive camelids and other species. Part III "The bacterial flora of the normal camelid eye during winter" provides a supplement to the material presented in Part I. The bacteriology data for the summer appears in Part I. Part IV compares and discusses the winter and summer bacteriological flora of the normal camelid eye. Seasonal comparisons of the fungal flora appear in Part II. The appendix "Materials and Methods" provides a more detailed description of the methods used for isolation and identification of microbes than is presented in the papers.

INTRODUCTION

The growing popularity of South American camelids (llamas, alpacas and guanacos) in the United States has made them important to veterinary medicine. Despite the great economic value of llamas (individual breeding animals have sold for as much as \$60,000) and their significant place as companion and pack animals, llama's eyes have received little attention in the veterinary medical literature. Their ocular anatomy, normal physiological parameters and ocular pathologic processes have not been described in detail. However, a few descriptions of ocular diseases in camelids and surgical procedures on llama and guanaco eyes have appeared in the literature.

Congenital and developmental problems are the most common ophthalmic diseases reported in camelids (Gionfriddo and Friedman 1991). Abnormalities of the lens are frequently observed. These include immature, mature and hypermature cataracts (Barrie 1978, Gionfriddo and Friedman 1991) and lens colobomas (Barrie 1978). Iris hypoplasia and colobomas and optic disc and peripapillary colobomas are congenital uveal defects seen in llama eyes (Gionfriddo and Friedman 1991).

Ocular problems secondary to systemic diseases are becoming more frequently recognized in camelids. Equine herpesvirus I (EHV I) causes severe ocular disease in camelids. Several outbreaks of this disease have been reported (Rebhun et al. 1988, Gionfriddo and Friedman 1991). Camelids acquire EHV I through contact with members of the Equidae family and transmission from both horses and zebras has been reported

(Rebhun et al. 1988, Gionfriddo and Friedman 1991). Blindness due to severe central nervous system derangement and ocular signs of uveitis, vitritis, chorioretinitis and optic neuritis were reported in 21 alpacas and one llama infected with EHV I (Rebhun et al. 1988). In a herd of llamas in Illinois exposed to four zebras with rhinitis, 28 of the 125 animals developed severe neurologic signs (head tilt, nyctagmus, circling and blindness) and inflammatory ocular signs of uveitis and chorioretinitis (Gionfriddo and Friedman 1991). In 1989, Paulsen et al. reported signs of chorioretinitis and optic neuritis in a blind llama. Although a causative organism was not identified, this was most likely a case of EHV I infection.

Numerous cases of uveitis in camelids have been seen which may be secondary to infectious systemic disease (Fowler 1990, Severin 1990). However, attempts at isolation of the causative agent have been inconclusive. These animals have usually had histories of either respiratory or gastrointestinal symptoms and severe panuveitis. Although Klebsiella or Pseudomonas bacteria were isolated from the respiratory system of some of these animals neither was thought to be the primary cause of the uveitis (Fowler 1990). Immunosuppression by a virus (such as a lentivirus) may play a role in these cases (Fowler 1990), but its role is unclear.

Despite the large size and prominent position of camelid eyes, external ocular disease does not seem to be prevalent. According to the Veterinary Medical Database (VMDB) at Purdue University, of 3009 llama admissions to 22 veterinary teaching institutions in the United States

and Canada for the period from January 1980 through December 1990, 60 animals (1.9%) were diagnosed as having keratitis and 23 (0.76%) had conjunctivitis. Only 11 (0.4% of all llamas presented) of the keratitis cases were due to known infectious causes and 10 (0.3% of all llamas presented) of the conjunctivitis cases were infectious. Trauma accounted for 11 (0.4% of all llamas presented) of the animals seen for corneal problems and only 1 case of conjunctival trauma was seen.

Few published reports describe extraocular diseases in camelids. There is one report of keratoconjunctivitis in a llama (Brightman et al. 1982). Staphylococcus aureus was isolated from the conjunctiva and Moraxella liquafaciens was isolated from the cornea of this animal. Direct corneal invasion by bacteria (such as by Moraxella bovis in cattle) has not been reported in camelids, nor has any conjunctival or corneal disease caused by mycoplasma or fungi.

There are several hypotheses which could explain the apparent infrequency of infectious external ocular disease in camelids: 1) Pathogens and/or opportunistic pathogens are not harbored in the camelid external eye because of environmental factors within the eye which are unfavorable to their survival and growth. 2) Pathogens and/or opportunistic pathogens are present in the external eye but are not often allowed to invade the intact or excoriated cornea or conjunctiva due to anti-microbial factors in the tears or physical barriers in the tissues themselves. 3) Camelid eyes are not highly susceptible to direct invasion by pathogens and camelids are not prone to predisposing

corneal trauma which would facilitate invasion by opportunistic pathogens.

This study was conducted to determine the frequency with which captive camelids harbor microorganisms in their external eye and to identify any organisms present. This would help determine if they harbor potential pathogens and identify the microbial flora they have in common with other species of domestic animals.

LITERATURE REVIEW

Bacteria of the conjunctival sac of domestic animals

The bacterial flora of the normal eyes of horses (Banis 1959, Lundvall 1967, Cattalani et al. 1976, Riis 1981, Whitley et al. 1983, Whitley and Moore 1984), cattle (Wilcox 1970), sheep (Spadbrow 1968), dogs (Bistner 1969, Hacker et al. 1973, Mc Donald and Watson 1976, Urban et al. 1972) cats (Campbell 1973) and psitticine birds (Zenoble et al. 1983) have been investigated. These domesticated species harbor a variety of bacteria, some of which are potentially pathogenic. The types of bacteria isolated differ among the domesticated species and even among breeds within a single species (Urban et al. 1972).

In the equine studies, gram positive organisms predominate the conjunctival bacterial flora, although, numerous gram negative bacteria were also isolated. The prevalence of various bacterial species varied among the studies. This variation was attributed to seasonal and geographic differences, differences in methodologies (culture collection and bacterial isolation), and bias in animal testing (Whitley and Moore 1984). Both Lundvall (1967) in the midwestern United States and Cattabiani et al. (1976) in Italy isolated Bacillus species with the greatest frequency while in Yugoslavia, Staphylococcus aureus was the most commonly cultured bacterium (Banis 1959).

Bacterial isolates from normal equine eyes included known pathogens and opportunistic pathogens (McLaughlin et al. 1983, Moore et al. 1983). Streptococcus species and Staphylococcus species were often cultured

from both healthy and diseased eyes of horses (Banis 1959, Lundvall 1967, Cattabiani et al. 1976, McLaughlin et al. 1983, Moore et al. 1983, Whitley et al. 1983). Pseudomonas the bacterial genus most commonly associated with severe ulcerative keratitis in the horse (Moore et al. 1983) has been cultured from normal equine eyes (Lundvall 1967). Moraxella has been isolated from the eyes of several horses with conjunctivitis (Hughes and Pugh 1970, Huntington et al. 1987). It was infrequently found in the conjunctival sac of healthy horses in the United States (Whitley et al. 1983) but commonly isolated from horses in Italy (Cattabiani et al. 1976).

In dogs, gram positive bacteria were consistently the most common bacterial isolates from the conjunctival sac. In the majority of studies, Staphylococcus was the most commonly cultured genus (eg. Bistner 1969, Urban et al. 1972, McDonald et al. 1976). Staphylococcus aureus, thought to be the most common bacterial species causing external ocular disease in the dog (Murphy et al. 1978), was frequently cultured from normal eyes (Bistner 1976, Urban et al. 1972, Murphy et al 1978, Hacker 1979) and is therefore considered an opportunistic pathogen (Murphy et al. 1978). Other potential bacterial pathogens isolated from apparently normal canine eyes included Bacillus species, Pseudomonas species, Escherichia coli, Proteus species and Enterobacter species (Urban et al. 1972, Murphy et al. 1978, Gerding et al. 1988). Urban et al. (1972) noticed considerable differences in the frequencies of isolation of staphylococci, streptococci, pneumococci and Neisseria among the seasons. These researchers also noted a significant variation

in ocular flora among purebred dog breeds. However, very similar bacteria were present in both eyes of any individual dog. Among canine studies, prevalence of bacteria in normal eyes ranged from 78% positive cultures (McDonald and Watson 1976) to 91% positive cultures (Urban et al. 1972).

In contrast to the relatively high incidence of positive bacterial cultures from canine eyes, Campbell et al. (1973) reported that only 42.1% of 240 conjunctival samples from feline eyes were positive. They attributed this low incidence to natural host resistance, tears, phagocytosis and mechanical barriers, but presented no data to substantiate this. The most common bacteria cultured from cats' conjunctivae were Staphylococcus albus and S. aureus. Gerding and Kakoma (1990) also found a lower percentage of positive bacterial cultures from cats' eyes (54%) than dogs' eyes (94%).

The bacteriologic flora of the conjunctiva and cornea of healthy psittacine birds is similar to that of mammals (Zenoble et al. 1983). Gram positive bacteria predominate with, Staphylococcus epidermidis and alpha-hemolytic streptococci comprising the majority of isolates. Gram negative organisms were not commonly found. Frequencies of isolation of bacterial species ranged widely and were correlated with husbandry practices. Those birds from private owners and local aviaries had considerably fewer bacterial isolates than birds cultured at an import station.

In sheep, only 40% of ocular bacterial cultures were positive (Spadbrow 1968). Neisseria ovis, the most commonly isolated organism, was found in 24.1% of the sheep cultured.

Wilcox (1970) identified the bacterial flora of normal cattle eyes and compared it with that of diseased eyes. Normal eyes contained numerous bacteria, including known pathogens such as Moraxella bovis, the causative agent of infectious bovine keratoconjunctivitis (IBK). A definite seasonal pattern of occurrence of Moraxella bovis was evident: it was isolated from up to 50% of the cattle in summer and autumn but was never cultured during winter and spring. This difference was attributed to the presence of flies (which spread the disease) in the summer and autumn but not in winter and spring. In diseased eyes there were higher incidences of M. bovis and Neisseria cararrhialis than in normal eyes. In normal eyes, the number of species recovered from an individual eye was much greater than in diseased eyes, but the total number of bacterial colonies from each culture was less.

Mycoplasma of the conjunctival sac of domestic animals

Mycoplasmas are ubiquitous in the environment and are commonly found in sewage, compost and leaves (Smith 1971). They are also commensals in the gastrointestinal, genital and respiratory tracts of numerous animal species (Carter 1986b). Mycoplasmas are often implicated in many diseases in domestic animals and have been cultured in cases of conjunctivitis and/or keratoconjunctivitis in sheep and goats (Surman 1968, Langford 1971, Barile et al. 1972, Al-Abaidi et al. 1973, Taoudi et al. 1987, Egwu et al. 1989), cattle (Langford and

Dorward 1969, Gourlay 1973, Gourlay and Thomas 1973, Langford and Leach 1973, Rosenbusch and Knudson 1980, Salih and Rosenbusch 1986), dogs (Campbell and Okuda 1975) and cats (Cello 1957, Tan and Markham 1971, Campbell et al. 1973a, Campbell et al. 1973b).

The role of mycoplasmas in ocular disease is not well understood. In sheep, mycoplasmas are known to have a role in infectious ovine keratoconjunctivitis (IOK). However, symptoms of IOK have not been reproduced with pure cultures of mycoplasmas, only with infectious ocular secretions (Pugh 1976). Mycoplasmas may therefore be secondary invaders in bacterially or virally infected eyes or may work in combination with bacteria or viruses to cause IOK.

In cattle, many researchers have found mycoplasmas in cases of infectious bovine keratoconjunctivitis (IBK) and conjunctivitis (Gourlay and Thomas 1969, Rosenbusch and Knudson 1980). Rosenbusch and Knudson (1980) characterized naturally occurring cases of conjunctivitis caused by mycoplasmas. These cases were distinct from IBK. They also experimentally inoculated the conjunctival sacs of calves with Mycoplasma bovoculi. The calves responded with a mild conjunctivitis. When a Ureaplasma sp. was inoculated, a more severe conjunctivitis was induced. They concluded that Mycoplasma bovoculi and Ureaplasma sp. alone were capable of causing a persistent conjunctivitis with high morbidity. They also confirmed that mycoplasmas are commonly present in cases of IBK and suggested that mycoplasmas may play a role in predisposing cattle to invasion by Moraxella bovis.

In cats mycoplasmas have been isolated from cases of conjunctivitis. Cello (1957) first described the isolation of mycoplasmas from 3 cats with conjunctivitis and suggested that this organism was the cause. In 1967, the first detailed description of a mycoplasma of feline origin was published (Cole et al. 1967). Mycoplasmas were found in the saliva of normal cats and in the conjunctival sac of a cat with severe conjunctivitis. Heyward et al. (1969) isolated 148 organisms from 90 apparently healthy, domestic cats. These were classified into 3 different groups of mycoplasmas. The majority of these isolates came from the conjunctiva and/or upper respiratory tract. Blackmore et al. (1971) attempted to isolate mycoplasmas from cats in 3 unrelated cat colonies and 17 households. Over 80% of adult cats had mycoplasmas. Most of the isolates were found in throat swabs. Only 6% of the conjunctival swabs were infected. Seven of the 12 cats from which ocular mycoplasmas were found were apparently normal while 5 had epiphora due to conjunctivitis or keratitis. There was no significant relationship between clinical signs of respiratory disease and conjunctivitis in these cats.

Tan and Miles (1972) reported a 30% incidence of Mycoplasma felis in healthy cats' eyes, but isolated it in almost 100% of cats with conjunctivitis (Tan and Miles 1974). The high incidence of mycoplasmas in diseased eyes suggested a pathogenic role for the organism. However, the role of Mycoplasma felis as a primary or secondary invader was not established and its significance as the sole cause of conjunctivitis in these cases remains uncertain.

A role for mycoplasmas in the pathogenesis of chronic keratitis in the dog was proposed by Campbell and Okuda (1975) but it was not well supported. These researchers isolated mycoplasmas from 9 of 101 adult dogs but did not record whether or not the eyes were normal. The mycoplasmas isolated from the dogs were used to sensitize guinea pigs to the mycoplasmal antigen and then the guinea pigs were challenged with intracorneal inoculations of the antigen preparations. Although this method induced an immune mediated keratitis, the condition did not resemble reported clinical cases of keratitis in dogs. Few conclusions could be drawn from this study.

Fungi of the conjunctival sac of domesticated animals

Fungi are very common in the environment, and large numbers of potentially pathogenic and saprophytic fungi can be isolated from feed, soil, and bedding of animals (Bonner and Fergus 1959, Christensen 1965). It is thought by many researchers that fungal organisms found in the conjunctival sacs of most animal species represent transient, random seeding from the environment (Riis 1981, Samuelson et al. 1984, Gionfriddo and Gabal 1991).

Fungal organisms in the eye rarely cause disease unless there is a history of trauma (Eastwood 1969, Forster and Rebell 1975) and/or previous treatment with antibiotics or corticosteroids (Moore et al. 1983, Samuelson et al. 1984, Whitley and Moore 1984, Eichenbaum et al. 1987). Horses seem especially prone to keratomycoses (Gwin 1981, Kern

et al. 1983, Moore et al. 1983, Gerding and Kakoma 1990). This may be due to the propensity of these animals to get mechanical ocular injuries which later may be invaded by fungi. Fungal spores may already be present in the conjunctival sac or may be on the material causing the injury.

The genera of fungi responsible for ocular disease in horses are diverse. Moore et al. (1983) cultured fungi from 15 equine eyes with ulcerative keratitis. Seven genera were isolated and Aspergillus was the most prevalent. Penicillium sp., Alternaria sp. and Mucor sp. were other filamentous fungi isolated and Candida was the only yeast found. McLaughlin et al. (1983) commonly found Aspergillus in horses with external ocular disease. Fusarium has also been reported as a cause of keratomycosis in horses (Mitchell and Attleberger 1973, McLaughlin 1983).

The same genera of fungal organisms found in diseased eyes, along with other common fungi, are often cultured from normal equine eyes. Lundvall (1967) isolated Aspergillus, Penicillium, Fusarium and Absidia most frequently in horses from Iowa. Riis (1981) found Aspergillus and Cladosporium in over one-third of normal equine eyes. Samuelson et al. (1984) isolated fungi from 95% of horses sampled in Florida. Aspergillus (56% of eyes) and Penicillium (51% of eyes) were the bacteria he most commonly found and Alternaria and Cladosporium were frequently present. Of the isolates found by Samuelson et al. (1984), 13% were yeasts.

In an extensive study, Eastwood (1969) cultured 216 horse eyes and recovered fungal organisms from 212 of them. Three hundred and seventy nine fungal isolates were found which represented 26 different genera and 110 different species. Seasonal frequencies in genera of fungi isolated tended to be different. Absidia, Aspergillus, Mucor and Rhizopus were found primarily in the winter sampling. This was attributed to the exposure of horse to these organisms in storage hay and grain. Cladosporium, Curvularia and Stemphylium occurred chiefly in summer samples. These are all saprophytes on plant residues and may be pasture plant pathogens. Some genera were found in large numbers in both seasons, although species of those genera generally differed among seasons.

Primary and secondary fungal infections of the external eye rarely have been reported in small animals (Schmidt 1974). This probably reflects the fact that dogs and cats are often kept in indoor environments and receive considerably less exposure to environmental fungi than do food animals or horses. Additionally, because of the relatively small size of the eyes of dogs and cats there may be less surface area exposed to the environment and potential trauma (Samuelson et al. 1984). Aspergillus and Candida species are the most common isolates in cases of fungal keratitis in small animals (Gerding 1990). These fungi are usually secondary invaders in wounds or ulcers that have been treated with antibiotics or corticosteroids (Eichenbaum 1987).

The few published studies show that normal eyes of dogs and cats harbor fungal spores, but to a lesser extent than normal eyes of horses

and cattle. In one study, fungi were cultured from 22% of 50 normal dogs and 40% of 25 normal cats (Samuelson et al. 1984). The most common isolate from both cats and dogs was Cladosporium oxysporum. Aspergillus species were found in 8% of the cats and none of the dogs, in contrast to 56% of the horses and 100% of the cows in the same study.

All of the 25 cattle in the study by Samuelson et al. (1984) had at least 2 species of fungi isolated from their conjunctival sacs with a mean of 4 per animal. The most common isolates were Cladosporium and Penicillium. Yeasts were also found in 12% of the animals. The types of fungal organisms found in cattle were similar to those in horses but the prevalence of each type differed between the two. This was attributed to possible differences in husbandry, type of bedding and feed, and individual habitat.

Despite the high numbers of fungal spores found in cattle eyes, there are few reports of keratomycosis in cattle (Samuelson et al. 1984). Samuelson et al. (1984) attributed this to a paucity of predisposing corneal trauma in cattle or the fact that cattle are often not closely observed, allowing infected wounds to go unnoticed. Additionally, cattle eyes are less frequently treated with antibiotics and corticosteroids, drugs which may favor fungal growth in the eyes of other domesticated species.

PART I. BACTERIAL AND MYCOPLASMAL FLORA OF THE
NORMAL CAMELID CONJUNCTIVAL SAC

J.R. Gionfriddo, DVM; R. Rosenbusch, DVM, PhD; J.M. Kinyon, BS, MS;
D.M. Betts, DVM, MS; T.M. Smith, BS

From the Department of Veterinary Clinical Sciences (Gionfriddo, Betts),
and the Department of Veterinary Microbiology and Preventive Medicine
(Rosenbusch, Kinyon), College of Veterinary Medicine, Iowa State
University, Ames, Iowa 50011.

T.M. Smith was a first year veterinary student at the time of this
writing.

SUMMARY

The normal conjunctival sacs of 88 individuals of 3 species of captive camelids (Lama glama, Lama guanicoe, Lama pacos) and llama-guanaco hybrids were sampled for bacterial and mycoplasmal flora. No mycoplasma were isolated from any animals sampled. Eleven genera of bacteria were isolated. The most frequent isolates were Staphylococcus epidermidis and Pseudomonas spp. Nine different varieties of Pseudomonas were found which represented at least 3 Pseudomonas species. Many of the bacterial isolates (especially the pseudomonads) are potential pathogens in the eyes of these camelids.

INTRODUCTION

There are 4 species of South American camelids, the llama (Lama glama), the guanaco (Lama guanicoe), the alpaca (Lama pacos) and the vicuna (Vicuna vicuna or Lama vicuna). Of these only the llama, guanaco and alpaca are present in significant numbers in the United States. The vicuna is an endangered species and its export from South America has been banned. South American camelids are becoming very popular in the U.S. as pack animals, show animals, and pets. Their value continues to rise. There are currently about 30,000 head in North America, mostly in private herds. As they become more popular and more valuable, veterinarians will be called upon more frequently to treat them. Many reports of normal blood values and normal anatomy are appearing in the literature. Additionally, diseases of llamas, guanacos, and alpacas are being reported. There are several accounts of ocular diseases. Many of these are congenital malformations such as lens colobomas (Barrie et al. 1978) and cataracts (Ingram et al. 1983), but reports of ocular lesions due to infectious agents such as equine herpesvirus-1 (Rebhun et al. 1988) also exist.

The incidence of external eye disease in South American camelids is unknown. Keratoconjunctivitis in a llama has been reported and 2 bacterial species (Staphylococcus aureus and Moraxella liquefaciens) were isolated from the conjunctiva and cornea (Brightman et al. 1981).

The bacterial flora of the normal eye of horses (Lundvall 1967, Whitley et al. 1983), cattle (Wilcox 1970), and sheep (Spadbrow 1968) has been investigated. These studies recovered both gram-positive and gram-negative organisms, and these included both opportunistic and known

pathogens. It is important to know which bacteria domestic camelids harbor in their healthy conjunctival sacs in order to document potential pathogens they carry and to know which organisms they have in common with other species of domestic livestock.

There are no reports of isolation of mycoplasmas from eyes of South American camelids. Mycoplasma organisms are ubiquitous in the environment, commonly found in sewage, compost, and leaves (Smith 1971). Several species have been documented as the cause of conjunctivitis and keratoconjunctivitis in sheep, goats, and cattle (Barile et al. 1972, Gourlay 1973, Al-Aubaidi et al. 1973, Rosenbusch and Knudtson 1980, Taoudi et al. 1987). It is not known if camelids are susceptible to the eye diseases caused by mycoplasmas or if they normally harbor them in their conjunctival sacs. The purpose of this study was to survey the bacterial and mycoplasmal flora of the healthy conjunctival sacs of captive camelids in the midwestern United States.

MATERIALS AND METHODS

Six herds of captive camelids in Iowa and northern Missouri were sampled (Figure 1-1). The sampling was done in June and July 1989. The eyes of each animal were briefly examined for the presence of external ocular disease and only apparently normal eyes were sampled. One eye was cultured for aerobic and anaerobic bacteria. The other eye was sampled for mycoplasmas. In each eye, a sterile, moistened cotton swab was rotated in the inferior conjunctival fornix, anterior to the nictitating membrane. Care was taken to avoid touching the eyelid margin with the swab. The swab for bacterial isolation was placed in a tube containing a small amount of sterile thioglycollate transport medium. The tubes were transported to the laboratory in a GasPak anaerobic jar¹ packed in ice. The swabs were streaked on anaerobic and aerobic 5% bovine blood agar plates and incubated at 37⁰ C. Bacterial colonies which grew were subcultured, gram stained and identified by conventional tube biochemical methods. Additionally, gram negative organisms were identified with the API 20E² and Minitex systems.³ Antibiotic susceptibility testing of the Pseudomonas and Pasteurella isolates was done with the Kirby-Bauer disk diffusion system.⁴

1 Marion Scientific, Kansas City, MO

2 Analytab Products, Ayerst Laboratories, Plainview, NY

3 Becton Dickinson Co., Cockeysville, MD

4 Becton Dickinson Co., Cockeysville, MD

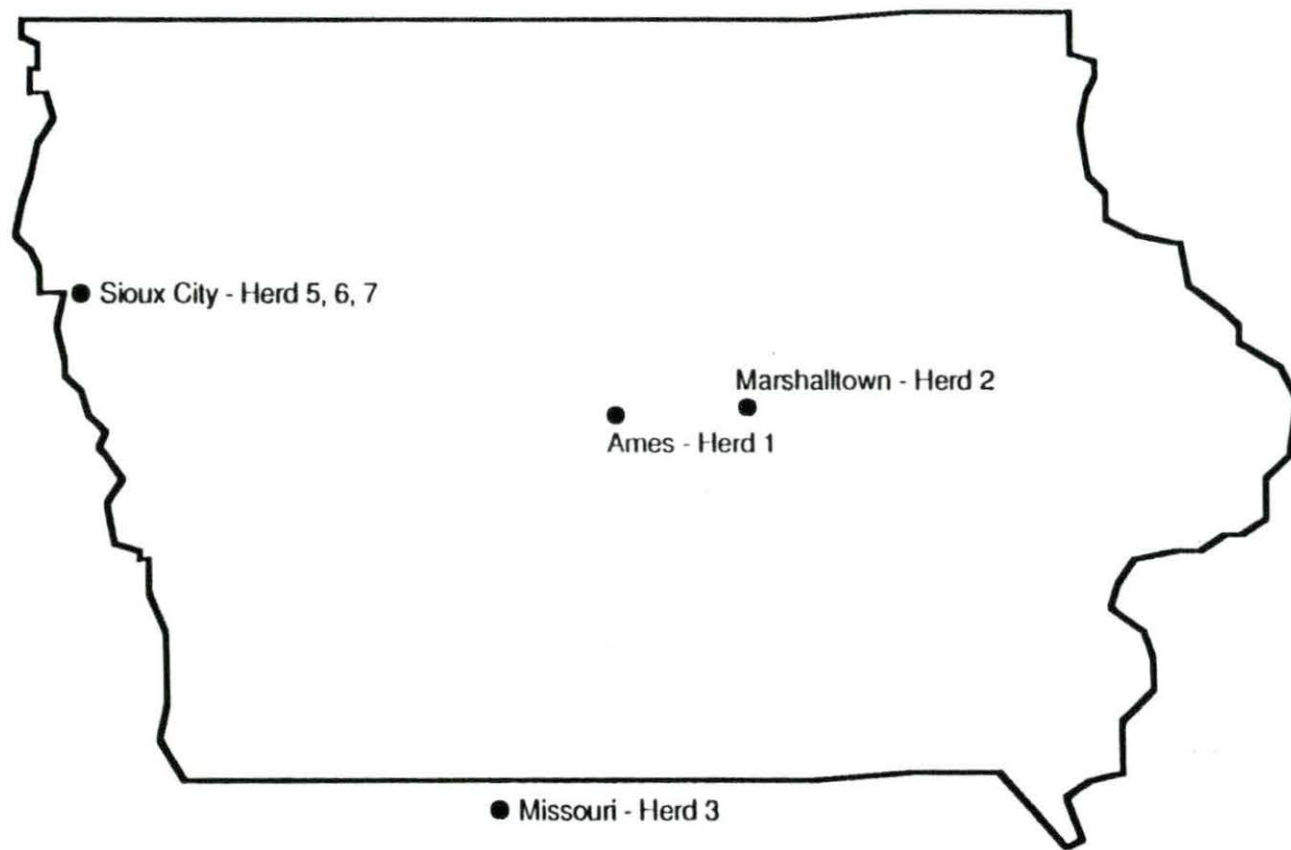


Figure 1-1. Map of Iowa and northern Missouri showing locations of herds sampled.

The swabs for mycoplasma isolation were placed in tubes containing 1 ml of sterile saline and the tubes were packed in ice for transport to the laboratory. Within 4 hours of sampling, the tubes were centrifuged at 1000 rpms for 3 minutes to segregate the heavier detritus and bacteria to the bottom of the tube. Two-tenths mililiter of saline was pipetted from the center of each tube of saline and placed into either modified Friis medium (Knudtson et al. 1986) or Hayflicks broth (Knudtson et al. 1986). Ten-fold serial dilutions were made in each medium until the final dilution was 10^{-5} . The tubes were incubated at 37°C in 5% CO_2 and observed for mycoplasma growth as indicated by pH changes (acid or base production by the organism) seen as color changes of the medium. Growth was also indicated by increased turbidity of the medium. If these changes occurred, a few drops of the final dilution were inoculated onto Friis soft agar plates to promote colony growth for identification. In addition, conjunctival scrapings (using a Cytology Brush)⁵ from 6 animals were deposited on slides, fixed with methanol and stained with 4'-6'-diamidino'-2-phenylindole (DAPI)⁶, a DNA specific fluorochrome. The slides were examined by fluorescence microscopy (Russel et al. 1975). This was done to detect any prokaryotic DNA associated with conjunctival epithelial cells which could be mycoplasma organisms.

5 Medical Packaging Co., Camarillo, CA

6 Boehringer Mannheim, Indianapolis, IN

Chi square analysis was used to determine if there were any significant variations from the expected even distributions of each of the bacterial isolates among the camelid species, among the herds, among age groups or between the sexes. Significance was defined as $P < 0.05$.

RESULTS

Eighty-eight animals were sampled for bacteria. These represented 3 species (llama, alpacas and guanacos) and llama-guanaco hybrids. Of these 54 were females and 34 were males (Figure 1-2). Mycoplasma isolation was attempted on 69 animals. Of these, 52 samples were inoculated into Friis medium and 17 into Hayflicks broth.

No mycoplasmas were isolated from any of the animals sampled. No extranuclear DNA (other than that identified as bacterial DNA) was seen in conjunction with the conjunctival epithelial cells.

Many bacteria were isolated from the camelids sampled (Table 1-1). Eleven genera of bacteria were represented. All the isolates were aerobes or facultative anaerobes. No strict anaerobes were found. The most frequent isolates from the camelids were Staphylococcus epidermidis, Pseudomonas spp. and Bacillus spp.

Nine different varieties of Pseudomonas organisms were isolated representing at least 3 different species. The most frequent Pseudomonas isolate was identified as CDC group E-4. It was present in 33% of the animals sampled and represented 76% of all Pseudomonas isolates. Three morphologically different colonies (2 yellow pigmented and 1 tangerine colored) were identified as CDC group E-4 and may represent different subspecies or varieties. Pseudomonas maltophilia was also commonly isolated (in 8% of the animals sampled representing 19% of Pseudomonas isolates).

Herd differences in the frequencies of total bacteria isolated were significant (Figure 1-3). Herds 1,3, and 5 were the largest herds,

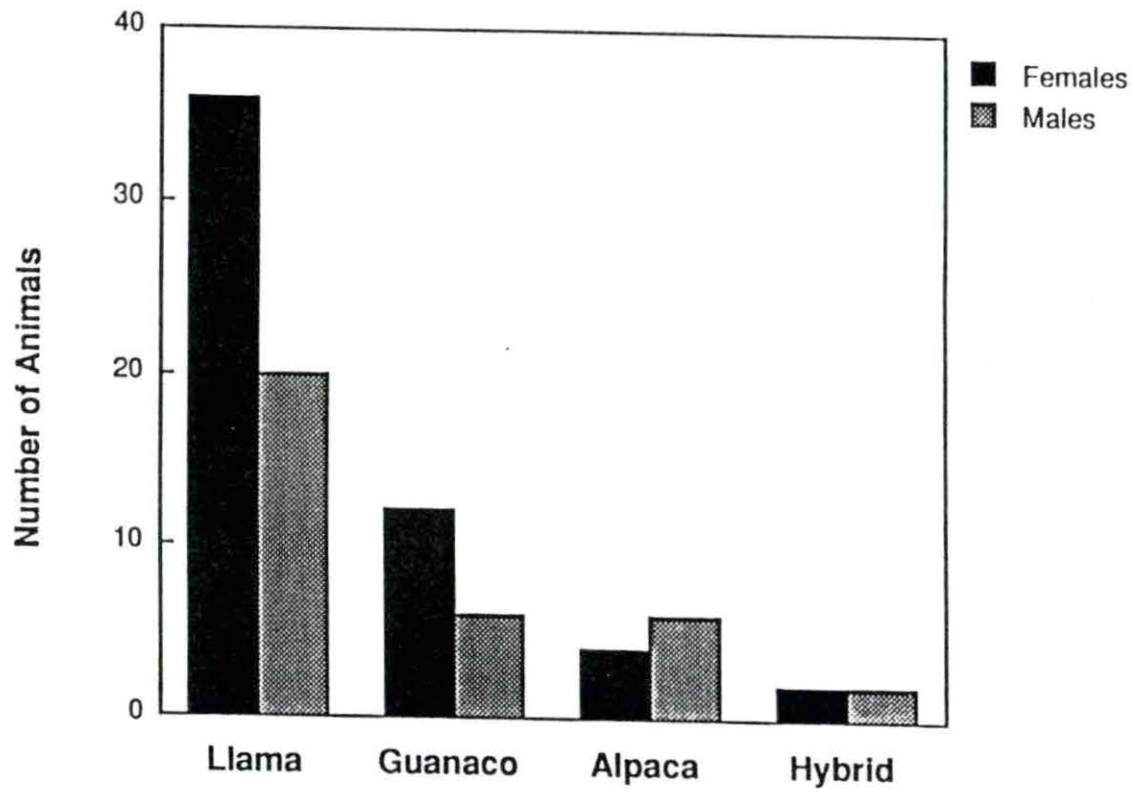


Figure 1-2. Species and sex distribution of the camelids sampled in summer.

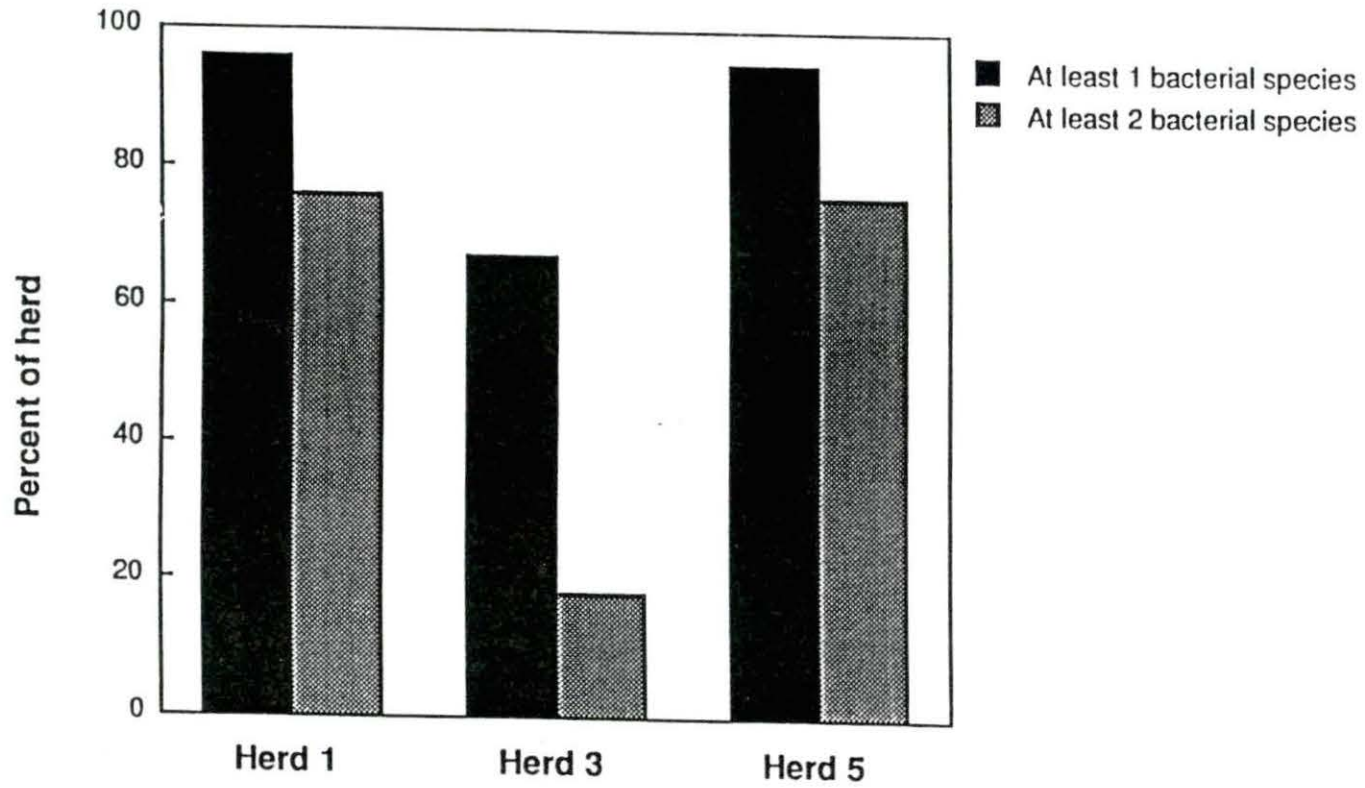


Figure 1-3. Frequencies of bacteria isolated from the 3 largest camelid herds.

each having about 25 head. Significantly fewer ($P = 0.013$) animals in Herd 3 had bacterial isolates than either Herd 1 or Herd 5.

A highly significant deviation ($P = 0.008$) from the expected even distribution of Bacillus spp. between the sexes was found. Bacillus spp. were found more often in females of all the camelids sampled.

Other statistically significant deviations from expected even distributions were found in the Pseudomonas and Pasteurella species isolated. There was not an even distribution of Pseudomonas across age groups. Fewer very young camelids (less than 6 months) had Pseudomonas isolates than any other age group ($P = 0.03$). A highly significant variation from an expected even distribution among the camelid species was found for Pseudomonas spp. ($P = 0.008$) and Pasteurella ureae ($P = 0.001$). A smaller percentage of llamas had these 2 genera (Pseudomonas = 26.8%, Pasteurella = 1.8%) than the other camelids and a large percentage of guanacos harbored these microorganisms (Pseudomonas = 66.7%, Pasteurella = 33.3%). There was also a highly significant deviation from an even distribution of these bacteria across the herds (Pseudomonas spp. $P = 0.001$), Pasteurella spp. $P = 0.008$). A high percentage of herds 1 and 5 had Pseudomonas isolates (68% and 61.9% respectively) while only 7.1% of herd 3 had Pseudomonas isolates. Pasteurella ureae isolates were primarily confined to herd 1 in which 28% of the animals harbored the organism.

Antibacterial sensitivities of the most frequent Pseudomonas isolates, CDC groups and Pasteurella ureae are shown in Table 1-2.

Table 1-1. Types and frequencies of bacterial isolated from captive camelids (n = 88).

Bacterial Isolates	Percent of animals cultured
<u>Staphylococcus epidermidis</u>	52
<u>Pseudomonas</u> spp.	41
<u>Bacillus</u> spp.	28
<u>Streptomyces</u> spp.	18
alpha hemolytic <u>Streptococcus</u> spp.	13
<u>Pasteurella ureae</u>	9
<u>Corynebacterium</u> spp.	8
<u>Staphylococcus aureus</u>	6
<u>Planococcus</u> spp.	5
<u>Micrococcus</u> spp.	2
<u>E. coli</u>	2
<u>Klebsiella</u> spp.	2

Table 1-2. Antibacterial sensitivity of the commonly isolated Pseudomonas sp., CDC groups and Pasteurella ureae.

Bacteria	Antibiotic											
	CE	CH	GE	TE	TR	AM	N	S	CL	E	L	P
<u>Pseudomonas maltophilia</u>	R	S	S	S	R	I	S	R	R	S	R	R
CDC Group E-4 bright yellow colonies	S	S	I	S	R	S	S	R	R	S	R	S
CDC Group E-4 small yellow colonies	S	R	S	R	S	S	S	S	R	S	R	S
CDC Group E-4 tangerine Colonies	S	S	S	S	R	S	S	R	R	S	S	S
<u>Pasteurella ureae</u>	S	S	S	S	S	S	S	S	S	S	S	S

CE = Cephalothin
 CH = Chloramphenicol
 GE = Gentamicin
 TE = Tetracycline
 TR = Tribriksen
 AM = Ampicillin

N = Neomycin
 S = Sulfa
 CL = Cloxacillin
 E = Erythromycin
 L = Lincomycin
 P = Penicillin

S = Sensitive
 R = Resistant
 I = Intermediate
 Sensitivity

DISCUSSION

The bacterial organisms isolated from the conjunctival sacs of these captive camelids are similar to those isolated from other domestic animals and man (Lundvall 1967, Urban et al. 1972, Perkins et al. 1975, McDonald et al. 1976, Whitley and Moore 1984, Wilcox 1970). Gram positive organisms predominate the microbial flora of most normal animals tested. Staphylococcus spp., Streptococcus spp., and Bacillus spp. are often the most frequent isolates. However, in normal cattle and sheep eyes, investigators have also found large numbers of gram-negative organisms. Most of these are Moraxella and Neisseria species (Wilcox 1970, Spadbrow 1968, Whitley and Moore 1984). Reports of Pseudomonas isolates from normal eyes of dogs (Urban et al. 1972), and cattle (Wilcox 1970) were found but these organisms are not common isolates from normal eyes. It is unusual, therefore, to find Pseudomonas organisms with high frequency in normal camelids conjunctival sacs.

Most of the Pseudomonas organisms isolated from these camelids are considered non-pathogens (Gardner et al. 1970) and are often associated with plants. They produce yellow or peach-colored pigments and are considered less virulent than the Pseudomonas organisms producing blue-green pigment (Bistner et al. 1969). However, a Pseudomonas organism may have the potential to become pathogenic secondary to trauma or other ocular disease (Bistner et al. 1969). Pseudomonas maltophilia is a known pathogen. It has been isolated from river water, rabbit and human feces, raw milk, blood, pericardial and ascitic fluid and human oropharyngeal swabs (Hugh and Gilardi 1980). It has been implicated as

a cause of pneumonia and infections associated with tracheostomy and endotracheal tubes (Gardner et al. 1970, Hugh and Gilardi 1980) and could be a potential ocular pathogen.

Pasteurella ureae is most commonly isolated from the upper respiratory tract of humans and isolates from sites other than the respiratory tract are rare (Weaver and Hollis 1980). The studies by Moore et al. (1983) and McLaughlin et al. (1983) on bacterial isolates from horses with external eye disease isolated Pasteurella organisms. These were not found in normal equine eyes and may represent pathogens in horses. The Pasteurella ureae isolates may be potential pathogens in the camelid eye.

Staphylococcus aureus, Klebsiella spp., Escherichia coli, Corynebacterium spp. and Bacillus spp. have all been isolated from both healthy and diseased eyes in other species (Urban et al. 1972, Perkins et al. 1975, McDonald et al. 1976, Moore et al. 1983, McLaughlin et al. 1983) and may represent opportunistic pathogens in camelids.

Our inability to isolate mycoplasma may be due to the need of the organisms for different culture techniques or possibly or healthy camelid eyes in the midwestern U.S. do not harbor mycoplasma organisms. Great care was taken in handling the mycoplasma swabs, however, a very fragile organism may have not survived the trip to the laboratory. Modified Friis medium was chosen because it has been shown to support and allow growth of conjunctival mycoplasma isolates from cattle (Rosenbusch and Knudtson 1980, Knudtson et al. 1986) and modified Hayflicks medium was chosen for its ability to support ureaplasma from sheep and goats with keratoconjunctivitis and from calves with pneumonia

(Barile et al. 1972, Knudtson et al. 1986). A DNA specific cytochrome stain (DAPI) was used to identify the presence of mycoplasmal DNA in association with the conjunctival cell membrane. The fact that none was found suggests that these organisms may not be present in the normal conjunctival sacs of camelids.

The differences in the frequencies of bacteria isolated from herds 1,3 and 5 were most likely a result of management differences among the herds. Herd 3 had less bacterial isolates and was the only herd kept on grass pasture. Herds 1 and 5 were kept on dirt lots and were subject to much blowing dust which may have exposed them to more bacteria. Feeding, vaccination and other management practices were similar among the herds.

Since herds 1,3 and 5 were composed of different species, the variables of species and herd were not independent. Thus it was impossible to determine statistically which factor (herd or species) was most responsible for the deviations from the expected even distributions of Pseudomonas and Pasteurella organisms across the herds. However, herd management differences were also suspected to have contributed to the different types of bacteria isolated. Herd 3 had the most animals less than 6 months old and also the fewest number of bacterial isolates and Pseudomonas isolates. Thus, age variation was also mostly likely related to herd variation. No reasons for the sex difference in the frequency of Bacillus isolates were evident. Each of the herds had similar sex distributions.

This study shows that captive camelids harbor potentially pathogenic bacteria in their conjunctival sacs. This may be an

indication for the use of topical antibiotics prior to ocular surgery in these species. The reason for the high incidence of Pseudomonas isolates is unknown but may be due to the prevalence of the organism in the environment in which the animals are kept. Conjunctival cultures of other domestic livestock species in close proximity to the herds is necessary to investigate this.

The failure to isolate any mycoplasmas suggests that these animals do not normally harbor them in their eyes. However, the ability of mycoplasmas to cause ocular disease in captive camelids remains to be investigated.

REFERENCES

- Al-Aubaidi, J. M., Dardiri, A. H., Muscoplatt, C. C., and E. H. McCauley. 1973. Identification and characterization of Acholeplasma oculusi spec. nov. from the eyes of goats with keratoconjunctivitis. Cornell Vet. 63:117-129.
- Barile, M. F., Del Giudice, R. A., and J. G. Tully. 1972. Isolation and characterization of Mycoplasma conjunctivae sp. n. from sheep and goats with keratoconjunctivitis. Infect. Immun. 5:70-76.
- Barrie, K. P., Jacobson, E., and R. L. Peiffer Jr. 1978. Unilateral cataract with lens coloboma and bilateral corneal edema in a guanaco. J. Am. Vet. Med. Assoc. 173:1251-1252.
- Bistner, S. I., Roberts, S. R., and R. P. Anderson. 1969. Conjunctival bacteria: clinical appearances can be deceiving. Mod. Vet. Pract. 45-47.
- Brightman, A. H., McLaughlin, S. A., and V. Brumley. 1981. Keratoconjunctivitis in a llama. Vet. Med. Sm. An. Clin. 1776-1777.
- Gardner, P., Griffin, W. B., Swartz, M. N., and L. J. Kunz. 1970. Nonfermentative gram-negative bacilli of nasocomial interest. Am. J. Med. 48:735-749.
- Gourlay, R. N. 1973. Significance of mycoplasma infections in cattle. J. Am. Vet. Med. Assoc. 163:905-909.
- Holmes, B., Lapage, S. P., and B. G. Easterling. 1979. Distribution in clinical material and identification of Pseudomonas maltophilia. J. Clin. Path. 32:66-72.
- Hugh, R., and G. L. Gilardi. 1980. Pseudomonas. Pages 288-317 in E.H. Lennette, ed. Manual of Clinical Microbiology, 3rd. ed. American Society of Microbiology, Washington DC.
- Ingram, K. A., and R. L. Sigler. 1983. Cataract removal in a young llama. Proc. Ann. Meet. Am. Assoc. Zoo Vets. 95-97.
- Knudtson, W. U., Reed, D. E., and G. Daniels. 1986. Identification of Mycoplasmatales in pneumonic calf lungs. Vet. Microbiol. 11:79-91.
- Lundvall, R. L. 1967. The bacterial and mycotic flora of the normal conjunctival sac in the horse. Proc. Am. Assoc. Equine Prac. 101-107.
- McDonald, P. J., and A. J. D. Watson. 1976. Microbial flora of normal canine conjunctivae. J. Small An. Pract. 17:809-812.

- McLaughlin, S. A., Brightman, A. H., Helper, L. C., Manning, J. P. and J. E. Tomes. 1983. Pathogenic bacteria and fungi associated with extraocular disease in the horse. J. Am. Vet. Med. Assoc. 182:241-242.
- Moore, C. P., Fales, W. H., Whittington, P., and L. Bauer. 1983. Bacterial and fungal isolates from Equidae with ulcerative keratitis. J. Am. Vet. Med. Assoc. 182:600-603.
- Perkins, R. E., Knudsin, R. B., Pratt, M. V., Abrahamsen, I. and H. M. Leibowitz. 1975. Bacteriology of normal and infected conjunctiva. J. Clin. Microb. 1:147-149.
- Rebhun, W. C., Jenkins, D. H., Riis, R. C., Dill, S. G., Dubovi, E. G., and A. Torres. 1988. An epizootic of blindness and encephalitis associated with a herpesvirus indistinguishable from equine herpesvirus 1 in a herd of alpacas and llamas. J. Am. Vet. Med. Assoc. 192:953-956.
- Rosenbusch, R. F., and W. U. Knudtson. 1980. Bovine mycoplasmal conjunctivitis: experimental reproduction and characterization of the disease. Cornell Vet. 70:307-320.
- Russel, W. C., Newman, C., and D. H. Williamson. 1975. A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasma and viruses. Nature. 253:461-462.
- Smith, P. F. 1971. The Biology of Mycoplasmas. Academic Press, New York. 257 pp.
- Spadbrow, P. 1968. The bacterial flora of the ovine conjunctival sac. Aust. Vet. J. 44:117-120.
- Taoudi, A., Johnson, D. W., Kheyyali, D., and H. Kirchhoff. 1987. Pathogenicity of Mycoplasma capricolum in sheep after experimental infection. Vet. Microbiol. 14:137-144.
- Urban, M., Wyman, M., Rheins, M., and R. V. Marrarol. 1972. Conjunctival flora of clinically normal dogs. J. Am. Vet. Med. Assoc. 161:201-206.
- Weaver, R. E., and D. G. Hollis. 1980. Gram-negative fermentative bacteria and Francisella tularensis. Pages 242-262 in E.H. Lennette. Manual of Clinical Microbiology 3rd ed. Washington DC: American Society of Microbiology, 1980.
- Whitley, R. D., and C. P. Moore. 1984. Microbiology of the equine eye in health and disease. Vet. Clinics No. Am. Large An. Pract. 6:451-466.

- Whitley, R. D., Burgess, E. C., and C. P. Moore. 1983. Microbial isolates of the normal equine eye. Equine Vet. J. Suppl. 2. 138-140.
- Wilcox, G. E. 1970^a. An examination of Moraxella and related genera commonly isolated from the bovine eye. J. Comp. Path. 80:65-74.
- Wilcox, G. E. 1970^b. Bacterial flora of the bovine eye with special reference to the Moraxella and Neisseria. Aust. Vet. J. 46:253-257.

PART II. THE FUNGAL FLORA OF THE NORMAL CAMELID CONJUNCTIVAL SAC

Juliet R. Gionfriddo, DVM;
Moustafa A. Gabal, BVM; & S, Dr. Med Vet

From the Department of Veterinary Clinical Sciences (Gionfriddo) and
Department of Veterinary Microbiology and Preventive Medicine (Gabal),
Iowa State University, Ames, Iowa 50011

INTRODUCTION

Mycotic keratitis is uncommon in domestic animals except the horse (Moore et al. 1983, Gerding and Kakoma 1990, Slatter 1990). Infection may occur in all species, however, following eye injury by plant material such as branches and hay. It also may follow long term topical or systemic corticosteroid or antibiotic therapy (Moore et al. 1983, Samuelson et al. 1984, Eichenbaum et al. 1987). Infrequency of keratomycosis in captive camelids may be attributed to fewer traumatic injuries or external eye conditions which require long term drug therapy. Alternatively, camelids may harbor few opportunistic fungi in the conjunctival sac or be resistant to fungal invasion of the cornea.

There are 3 species of captive South American camelids in the United States, the llama (Llama glama) the alpaca (L. alpaca) and the guanaco (L. guanacoe). Llamas are commonly owned as pack animals and pets. Some breeding animals are very valuable. Alpacas are also becoming popular and are kept as pets and for their fine wool. Guanacos are present in many zoos and a few private herds across the United States. Since camelids are valuable and increasingly common, veterinary practitioners should recognize normal anatomy and physiology as well as disease to treat the animals properly. Knowledge of organisms found in the normal eye of camelids is important for detection of potential pathogens and interpretation of ocular culture results. The objectives of this study were to characterize the fungal flora of the normal camelid eye in the midwestern United States and identify potential opportunistic pathogens.

MATERIALS AND METHODS

Six herds of captive camelids (llamas, alpacas, and guanacos) were sampled for conjunctival fungal flora in conjunction with a study of the bacterial flora of these animals (Gionfriddo et al. 1991). Three herds were sampled in summer (June and July 1989) and 6 herds were sampled in winter (February and March 1990). The herds were located in Iowa and northern Missouri.

One eye of each animal was sampled by rotating a moistened, sterile cotton swab in the inferior fornix of the conjunctiva just anterior to the nictitating membrane without touching the eyelid margin. Only those eyes free of apparent external ocular disease were cultured. Swabs were placed in test tubes containing 0.5 ml of Stuart's transport medium and transported to the laboratory in a Gas-Pak anaerobic jar¹ packed in ice. Swabs were streaked on Sabouraud's dextrose agar plates and incubated at 25⁰ C for 3 to 4 weeks. Fungal colonies were identified by colony growth rate, gross morphology and microscopic appearance as described in Barnett and Hunter (1972). Culture plates were further incubated for 6 additional weeks to isolate slow growing organisms. Those fungi which failed to form conidia were subcultured onto fresh Sabouraud's agar and reincubated to promote conidia formation. Morphologic species of the genus Aspergillus were identified as described by Thom and Raper (1945). Because of the small sample size of the summer cultures, statistical comparisons of the seasonal fungal organisms were not meaningful.

¹

Scott Laboratories Inc. Fiskeville, RI

RESULTS

Ninety-three (30 males and 63 females) animals were cultured in winter and 34 (16 males, 18 females) in summer. Eighteen animals were sampled in both seasons. Ages ranged from 1 day to 12 years. Eighteen females and 16 males were cultured in the summer, and 63 females and 30 males in the winter. In summer at least 1 fungal organism was recovered from the conjunctival sac of 56% of animals tested; 6% had more than 1 organism. In winter 53% of the cultures were positive for 1 organism and 9% had more than 1 organism.

Fungal species from 10 genera were isolated (Table 2-1). In both winter and summer Aspergillus spp. were the most common isolates. Nine species of Aspergillus were identified (Table 2-2). Aspergillus organisms not be identified to the species level were designated "Aspergillus spp". Rhinocladiella and Fusarium also were commonly isolated in both seasons (Table 2-1). Penicillium was isolated in the winter but not in the summer. Darkly pigmented fungi which failed to produce characteristic conidia on repeated subculturing were placed in the category "dematiaceous fungi".

Eighteen camelids from 3 herds (5, 6, and 7) were cultured in both seasons. The conjunctival sac of only one had the same fungal species in both winter and summer. Two other camelids yielded different species of Aspergillus seasonally. The conjunctival sacs of four animals were cultured negative for fungi in both seasons. One animal had no fungi in the winter but 3 species in the summer. The remaining 10 camelids harbored different fungal species in summer and winter.

Table 2-1. Proportion of animals sampled with fungus present.

Fungus	Winter				Summer	
	Llama n=65	Alpaca n=7	Guanaco n=20	Hybrid n=3	Llama n=20	Alpaca n=10
<u>Aspergillus</u> spp.	0.35	0.43	0.35	0.33	0.4	0.20
<u>Fusarium</u> spp.	0.02	-	0.05	-	0.3	0.20
<u>Rhinochadiella</u> spp.	0.08	0.14	-	-	0.25	
<u>Curvularia</u> spp.	-	-	-	-	0.15	-
<u>Penicillium</u> spp.	0.03	0.14	0.15	0.33	-	-
Mycelia Sterilica	-	-	-	-	0.05	-
<u>Mucor</u> sp.	0.05	0.14	-	-	-	-
<u>Trichoderma</u> spp.	-	-	0.10	-	-	-
<u>Hormodendrum</u> <u>compactum</u>	-	-	-	-	0.05	
<u>Alternaria</u> <u>alternaria</u>	-	-	-	-	0.05	-
Dematiaceous fungi	0.12	-	-	0.33	-	-

Table 2-2. Proportion of animals with Aspergillus spp. present.

Fungus	Winter				Summer	
	Llama n=65	Alpaca n=7	Guanaco n=20	Hybrid n=3	Llama n=20	Alpaca n=10
<u>Aspergillus</u> spp.	0.08	0.30	0.05	--	0.15	0.10
<u>Aspergillus</u> <u>versicolor</u>	0.05	--	0.25	--	0.15	0.1
<u>Aspergillus</u> <u>niveus</u>	0.02	--	--	--	--	--
<u>Aspergillus</u> <u>candidus</u>	0.02	--	--	0.33	0.05	--
<u>Aspergillus</u> <u>fumigatus</u>	0.06	0.14	--	--	--	--
<u>Aspergillus</u> <u>oryzae</u>	0.03	--	--	--	--	--
<u>Aspergillus</u> <u>niger</u>	0.02	0.14	--	--	--	--
<u>Aspergillus</u> <u>ustus</u>	--	--	--	--	0.05	--
<u>Aspergillus</u> <u>amstelladomi</u>	0.03	--	--	--	--	--
<u>Aspergillus</u> <u>lutescens</u>	0.02	--	--	--	--	--

DISCUSSION

The fungal organisms isolated in this study are similar to those found in normal eyes of other domestic animals (Eastwood 1969, Urban et al. 1972, Whitley et al. 1983, Samuelson et al. 1984, Moore et al. 1988). Aspergillus and Fusarium are usually the most common inhabitants of the eyes of most mammalian species. Alternaria, Penicillium and Trichoderma have also been isolated from equine eyes (Riis 1981, Kern et al. 1983, Samuelson et al. 1984) and Curvularia has been isolated from the bovine and canine eye (Samuelson et al. 1984). Rhinochadiella, Mucor and Hormodendrum have not been reported in normal eyes, although they are common on plants. Their presence in the camelid eyes suggests an environmental seeding.

Data from this study support Riis' (1981) hypothesis that the fungal organisms found in the external eye may represent transient, random seeding from the environment. While the numbers of animals positive for fungal organisms were similar seasonally, the genera and species of the organisms they harbored varied. The finding that only one of 18 of the animals cultured in both seasons had the same fungal species isolated twice also supports Riis' (1981) contention. The fungal isolates in this study apparently followed no pattern of herd or species distribution. In contrast with Whitely et al's. (1983) results¹¹, numerous organisms were cultured in the winter and the numbers of positive animals were similar between seasons. In the present study, various Aspergillus species were present in all camelid species and all herds in both seasons. This probably reflects the high number of Aspergillus organisms in the environment and the changes in

the distributions of Aspergillus species across seasons and geographic locales. The presence of Penicillium only in summer may reflect a seasonal fluctuation in the population levels of these organisms.

Aspergillus and Fusarium are commonly responsible for keratomycosis in most animal species and man (Francois 1968, Mitchell and Attleberger 1973, Riis 1981, Samuelson et al. 1984). Mucor and Penicillium have also been implicated in ulcerative keratitis in the horse (Riis 1981, Samuelson et al. 1984). No published reports were found of mycotic ocular disease in captive camelids. However, several fungal organisms (Rhodotorula, Acremonium, Scopulairopsis and Aspergillus) were found in two llamas with conjunctivitis and superficial keratitis.² The significance of the fungal isolates is unknown because bacteria were recovered from the same eyes and it was impossible to determine the role of the fungal organisms in the pathogenesis of the disease. Moore et al. (1983) found that the most severe ulcerative keratitis in horses occurred with mixed infections of Aspergillus and a gram negative bacteria. The fungal organisms may similarly have contributed to the pathogenesis of the disease in the llamas.

The low incidence of mycotic external ocular disease in camelids despite the presence of potentially pathogenic fungi in their conjunctival sac, may reflect their low frequency of predisposing causes

² Gionfriddo J.R. Unpublished data, 1990.

to mycotic ocular invasion in these animals. As more camelids are bred and maintained however, the incidence of mycotic infections may increase. Intraocular surgeries for cataract removal may often be necessary in the future (llamas have a high incidence of cataracts)³, and prophylaxis against secondary mycotic infection may be an important consideration prior to surgery.

³ Friedman D. University of Wisconsin, Madison, WI: Personal communication 1990.

REFERENCES

- Barnett, H. L., and B. B. Hunter. 1972. Illustrated genera of imperfect fungi. Burgess Pub. Co., Minneapolis MN. 241 pp.
- Eastwood, B. J. 1969. Mycotic flora of the apparently normal equine eye. Masters thesis. Iowa State University, Ames, Iowa.
- Eichenbaum, J. D., Lavach, J. D., Severin, G. A. and M. E. Paulsen. 1987. Immunology of the ocular surface. Compend. Cont. Educ. Pract. Vet. 9:1101-1104.
- Francois, J. 1968. Les Mycoses Ocularies. Masson and Cie, Paris. 134-155.
- Gerding, P. A., and I. Kakoma. 1990. Pages 616-625 in Microbiology of the canine and feline eye. Vet. Clin. of N. Am.: Small Anim. Pract. W. B. Saunders, Philadelphia.
- Gionfriddo, J. R., Rosenbusch, R., Kinyon, J. M., Betts, D. M. and T. M. Smith. 1991. The bacterial and mycoplasmal flora of the normal camelid conjunctival sac. Am. J. Vet. Res. in press.
- Kern, T. J., Brooks, D. E., and M. M. White. 1983. Equine keratomycosis: current concepts of diagnosis and therapy. Equine Vet. J. Suppl. 2. 33-38.
- Mitchell, J. S., and M. H. Attleberger. 1973. Fusarium keratomycosis in the horse. Vet. Med. Sm. An. Clin. 1257-1260.
- Moore, C. P., Fales, W. H., Whittington, P., and L. Bauer. 1983. Bacterial and fungal isolates from Equidae with ulcerative keratitis. J. Am. Vet. Med. Assoc. 182:600-603.
- Moore, C. P., Heller, N., Majors, C. J., et al. 1988. Prevalence of ocular microorganisms in hospitalized and stabled horses. Am. J. Vet. Res. 49:773-777.
- Riis, R. C. 1981. Equine ophthalmology. Pages 569-605 in K.N. Gelatt ed. Veterinary Ophthalmology, Lea and Febiger, Philadelphia.
- Samuelson, D. A., Andresen, T. L., and R. M. Gwin. 1984. Conjunctival fungal flora in horses, cattle, dogs and cats. J. Am. Vet. Med. Assoc. 184:1240-1242.
- Slatter, D. H., 1990. Fundamentals of Veterinary Ophthalmology. 2nd ed. W.B. Saunders, Philadelphia. 628 pp.
- Thom, C., and K. B. Raper. 1945. A manual of the Apergilli. Williams and Wilkins Co, Baltimore. 373 pp.

- Urban, M., Wyman, M., Rheins, M., and R. V. Marraro. 1972. Conjunctival flora of clinically normal dogs. J. Am. Vet. Med. Assoc. 161:201-206.
- Whitley, R. D., Burgess, E. C., and C. P. Moore. 1983. Microbial isolates of the normal equine eye. Equine Vet. J. Suppl. 2. 138-140.

PART III. THE BACTERIAL FLORA OF THE NORMAL EYE
OF CAPTIVE CAMELIDS IN THE WINTER

RESULTS

Cultures were taken from ninety-four animals from 6 herds during winter (February-March 1990). Sixty-two females and 32 males representing 3 different species (llamas, alpacas, guanacos) and guanaco-llama hybrids were sampled (Figure 3-1).

Many bacteria were isolated from the camelid eyes. Ninety-two percent of all animals cultured in winter had at least one species of bacteria in the conjunctival sac. The numbers of bacterial species isolated per animal are presented in Figure 3-2. Fourteen genera, 3 different Center for Disease Control (CDC) groups of gram negative rods, and 3 gram negative rods (which could not be identified) were isolated (See Table 4-2). All the isolates were aerobes or facultative anaerobes; no strict anaerobes were found. The most common bacterial isolate, coagulase negative Staphylococcus, was found in 63% of the camelids sampled. Other common isolates included Micrococcus spp. (30%), hemolytic Bacillus spp. (27%) and Streptomyces spp. (16%). Pseudomonas spp. were found in 5 animals (5%); and 3 of these pseudomonads were identified as Pseudomonas maltophilia. Branhamella spp. were found in 6% of the 94 camelids sampled.

Several differences in the frequency of occurrence of the various bacterial species among the herds were significant. Statistically significant deviations from expected even distributions across the herds were found in Micrococcus, Streptomyces, Branhamella and coagulase negative Staphylococcus. Micrococci were present in a higher percentage of animals in herds 3 (50%) and 7 (46%) than the other herds ($P=0.01$). Staphylococci were more significantly more common in herds 1 (88%), 3 (78%) and 6 (60%).

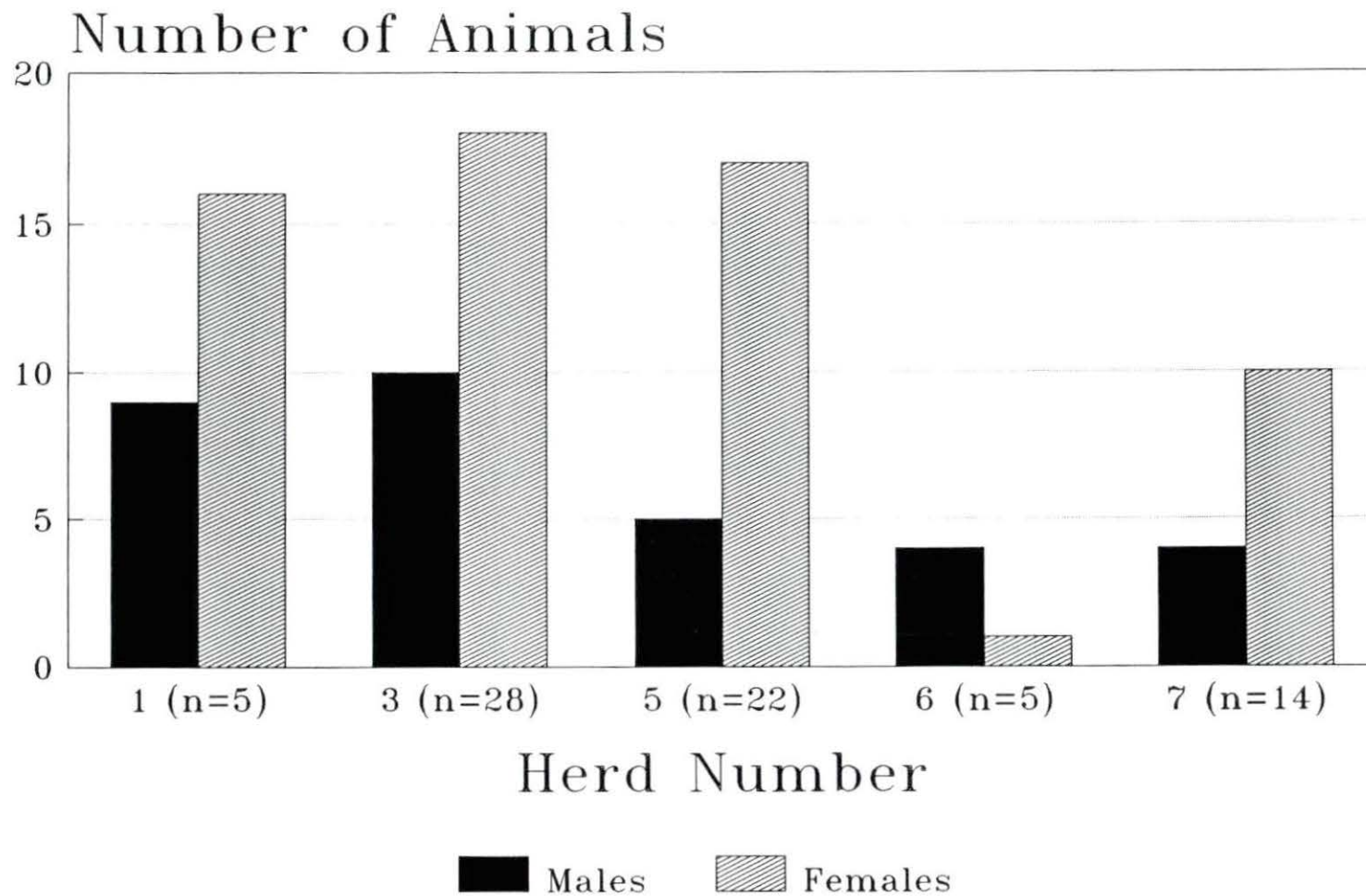


Figure 3-1. Number of camelids sampled per herd in winter

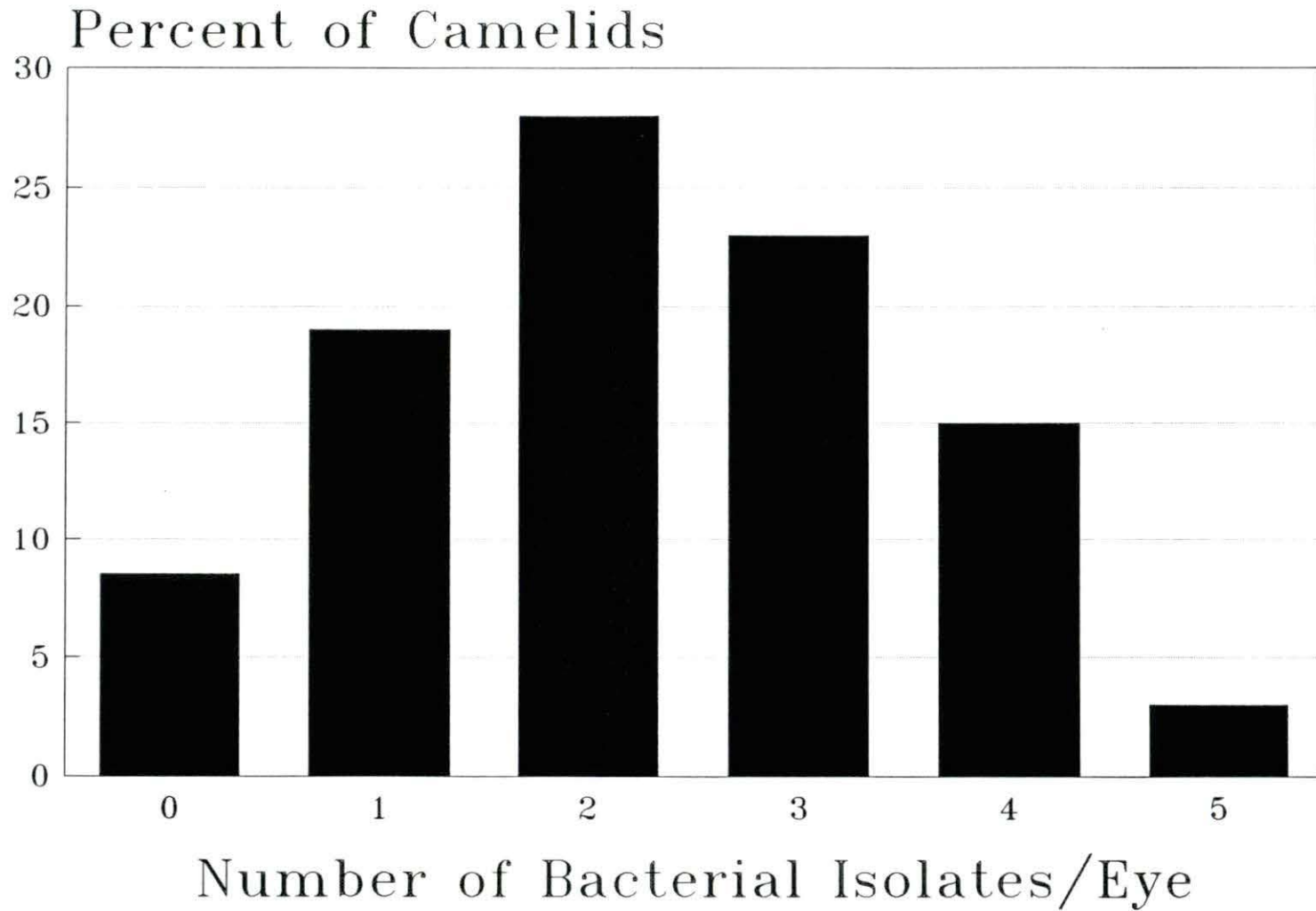


Figure 3-2. Numbers of bacterial species isolated per camelids' eye in winter

Camelids in herd 5 had a significantly higher incidence of Streptomyces isolation (43%) than the other herds.

Significant differences were found in the distributions of some bacteria between sexes and age groups. Males tended to have more Micrococcus (44%) than females (23%) ($P = 0.03$). More alpha hemolytic streptococci were present in young animals (≤ 3 years) than in older animals (> 3 years).

Among species, there were several significant deviations from even distributions of the bacteria. Alpacas had fewer coagulase negative staphylococci ($P = 0.004$) but more Streptomyces ($P = 0.011$) and Branhamella ($P = 0.0001$) than the other camelid species. The corynebacteria also were not evenly distributed across camelid species being found in larger percentages of llamas and hybrids than alpacas or guanacos ($P = 0.01$).

DISCUSSION

As in the other animal species which have been investigated, gram positive bacteria dominated the bacterial flora of the conjunctival sacs of captive camelids. Gram negative organisms also were found but in fewer camelids.

Although most bacteria are opportunistic pathogens, several of the organisms found in this study have commonly been reported to cause disease in animals or man. In man, Pseudomonas maltophilia has been associated with primary pneumonia and opportunistic infections of many tissues including the conjunctiva (Gilardi 1985). Pseudomonas paucimobilis and Pseudomonas pseudoalkaligenes have been found in cases of meningitis and septicemia in man (Gilardi 1985). Branhamella ovis has been cultured from cases of conjunctivitis in sheep (Riou et al. 1982) and goats (Bulgin and Dubose 1982). However, its pathogenicity has not been established. Pitman and Reuter (1988) found Branhamella ovis in 47 of 60 (78%) normal eyes of Angora goats. They suggested that further studies were warranted before B. ovis could be considered the primary cause of conjunctivitis in domestic livestock. Data from the present study support the contention that B. ovis can be present in normal eyes.

Since the species composition of each herd was not independent of herd number (e.g., all alpacas were in herd 5 and all guanacos and hybrids were in herd 1), statistically significant Chi square values for species and herds were difficult to interpret. The significant differences in the distributions of individual bacterial species among the herds is probably due to variation in bacterial population sizes and distributions in the

environment. Micrococci and staphylococci are common inhabitants of skin and mucus membranes. They are routinely isolated from conjunctivae of animals of all species. Although several coryneform bacteria can be important pathogens, most species are saprophytes or plant pathogens (Coyle et al. 1985). The relative abundance of these saprophytes among herds is probably not clinically significant.

Chi square analysis revealed that those bacterial species whose frequencies of occurrence were not evenly distributed among camelid species also were not evenly distributed among herds. The fact that alpacas had significantly fewer coagulase negative staphylococci and more streptomycetes than the other camelid species may be a herd difference rather than a species difference. All the alpacas were in herd 5, and statistically significant Chi square values indicated that these bacteria were also more frequently found in herd 5. The possibility that real differences in conjunctival flora exist among camelid species cannot be dismissed, however. Other animal species have differential susceptibility to invasion by various ocular bacteria. Moraxella bovis frequently causes severe keratoconjunctivitis in cattle, but only rarely causes conjunctivitis in other species (Huntington et al. 1987). Species differences in susceptibility to bacterial invasion probably reflect differences in ocular immunity (Eichenbaum et al. 1978). Ocular immunity undoubtedly also plays an important role in the types and numbers of bacteria found in the normal eye.

The fact that Branhamella spp. were found almost exclusively in alpacas in herd 5 could be due to either herd or species differences. Alpacas may be more likely than other camelids to have Branhamella in their

eyes, or perhaps their environmental exposure was greater. Since Branhamella are cultured from eyes of sheep and goats, exposure of the alpacas to these other animals was suspected but not confirmed. No sheep or goats were kept with the camelids in herd 5, but several recently acquired members of the herd could may have been exposed to Branhamella via exposure to other livestock at the sale barn from which they were purchased.

Winter feeding methods in herd 5 were different from those of the other herds and may have contributed to the significantly greater frequency of Streptomyces in herd 5 alpacas. In herd 5 hay was fed in wire bunkers raised above the ground to about the height of the heads of the animals. Dirt and dust from the hay bales could easily have fallen into the animals' eyes. Since Streptomyces is a common inhabitant of dirt and plants (Gordon 1985), its abundance in hay was probably very high. This could have led to much seeding of the bacteria into the eye. Most streptomycetes are not known to cause human or animal disease (Gordon 1985), and their presence in large numbers of animals in herd 5 probably has no clinical significance.

The differences in occurrence of bacterial species among the camelid sex and age classes cannot be explained. There are no apparent reasons why more micrococci would be present in males than in females or why young animals might be more likely to harbor streptococci in their eyes. These patterns may be statistical artifact but more culturing needs to be done to investigate this.

Part IV. SEASONAL COMPARISON OF BACTERIOLOGY DATA

RESULTS

Ninety-four animals from 6 herds were sampled in the winter and 88 animals from 5 herds were sampled in the summer. Forty-five animals from herds 1,3,5,6,and 7 were cultured in both seasons (Table 4-1).

The most commonly isolated bacteria were found with very similar frequencies in both seasons (Table 4-2) except for Micrococcus spp., which were present more frequently during winter ($P=0.0001$), and the Pseudomonas spp. which were found more frequently in summer ($P=0.0001$). Gram positive bacteria were the most common bacterial type cultured in both seasons, however, gram negative bacteria were also commonly found, especially in summer. Many of the Enterobacteriaceae were found only in one season or the other, but they were found in so few animals that this pattern was not considered important.

More animals had bacterial isolates in winter (92%) than in summer (82%). Also, the frequency of isolation of 2 or more bacterial species per camelid was greater in winter (73%) than in summer (62%). Neither of these relationships was statistically significant, however ($P=0.1$).

Initial bacterial colony frequencies on blood agar isolation plates generally reflect abundances of the various bacterial species present in the eye. In the present study, except for the staphylococci, the majority of bacteria were present in small numbers on the initial plates (Table 4-3).

Table 4-1. Number of animals cultured in each season.

Herd	Summer	Winter	Both
1	25	25	13
2	5	0	0
3	28	28	19
5	21	22	11
6	4	5	2
7	5	14	2
	—	—	—
Total	88	94	45

Table 4-2. Comparison of the frequencies of bacteria isolated seasonally.

Bacteria	Winter n = 94	Summer n = 88
<u>Micrococcus</u> spp.	0.30	0.034
Coagulase neg. <u>Staphylococcus</u> spp.	0.63	0.51
<u>Staphylococcus aureus</u>	0.09	0.06
Hemolytic <u>Bacillus</u> spp.	0.27	0.31
<u>Streptomyces</u> sp.	0.11	0.18
<u>Corynebacterium</u> spp.	0.16	0.08
alpha hemolytic <u>Streptococcus</u> spp.	0.20	0.14
<u>Acinotabacter coalceticus</u>	0.01	0
CDC group E2	0.02	0.02
CDC group 2J	0.01	0
CDC group EF4	0.01	0
<u>Gardenerella vaginalis</u>	0.03	0
<u>Pseudomonas</u> spp.	0.06	0.42
<u>Pasteurella</u> spp.	0.01	0.10
<u>Serratia rubideae</u>	0.02	0
<u>Branhamella</u> sp.	0.06	0
<u>Flavobacterium</u> sp.	0.01	0
<u>Enterobacter agglomerans</u>	0.02	0
Unidentified gram - rods	0.03	0
<u>Escherichia coli</u>	0	0.02
<u>Klebsiella</u> spp.	0	0.06
<u>Planococcus</u> spp.	0	0.06
CDC Group E5	0	0.09

Table 4-3. Counts of bacterial colonies present on initial blood agar isolation plates. The number in the table are the number of animals in both seasons having that bacterial count.

Bacteria	Number of Colonies on blood agar plates				
	≤10	10-15	16-30	31-50	≥50
<u>Micrococcus</u> sp.	24	1	4	0	2
coagulase neg. <u>Staphylococcus</u> spp.	55	23	15	13	6
<u>Staphylococcus aureus</u>	8	2	4	0	0
<u>Bacillus</u> spp.	55	1	3	0	1
<u>Streptomyces</u> spp. alpha hemolytic	22	4	3	0	1
<u>Streptococcus</u> spp.	11	6	6	7	2
<u>Corynebacterium</u> spp.	13	3	2	2	2
<u>Pseudomonas</u> spp.	24	4	7	0	0

DISCUSSION

Seasonal comparisons of the conjunctival bacterial isolates revealed few differences in the frequencies in which most of the major bacteria were isolated. Although more micrococci were found in winter, this was probably an artifact of the methods used to identify them. In the analysis of winter samples, biochemical tests (described in the appendix) were used to separate the gram positive catalase positive cocci. When summer samples were analyzed morphology alone was used to do this. As a result, some micrococci may have been classified as staphylococci in the summer analysis.

The presence of significantly more Pseudomonas spp. in the summer samples is difficult to explain. Methods used to identify these organisms in the 2 seasons were identical. Possibly, the warmer, moister summer conditions led to a greater proliferation of pseudomonads in the vegetation or water in the animals' surroundings. Most common bacteria have higher metabolic rates and reproduce more rapidly in warmer, moister environments. Thus, the pseudomonads found in the summer may have been more metabolically active and thus more readily propagated in the laboratory. Why this would be true of only the Pseudomonas spp. and not the other bacteria is unknown. It is also possible that a several concentrated reservoirs of pseudomonads were present near the herds in the summer. This hypothesis is supported by the observation that the 2 herds with the highest numbers of animals having Pseudomonas organisms (herd 1 in Ames, Iowa and herd 5 in Sioux City, Iowa) had similar environmental conditions (see Part I).

Thus, it may be considered "normal" to find small numbers of both gram negative and gram positive organisms in healthy camelid eyes. On the other hand, coagulase negative staphylococci were found in both small and very large numbers and in numerous healthy camelid eyes. This suggests that the "normal" ocular flora of camelids may also include large numbers of coagulase negative staphylococci.

SUMMARY AND CONCLUSIONS

Captive camelids in the midwestern United States have many species of bacteria and fungi in their conjunctival sacs. These microbes are similar to other organisms found in the conjunctivae of other domestic animals and include both commensals and opportunistic pathogens.

Several herd, species and seasonal differences were present in the species of bacteria and fungi isolated, most notably in the pseudomonads. Although the causes of these differences are not known, they probably reflect differences in the relative abundance of the various bacterial species in the local environments around the herds. Husbandry techniques probably played a large role in determining the environmental concentrations of some of the bacteria and fungi.

Since camelids harbor numerous conjunctival microorganisms, yet are not prone to ocular infection, their ocular defense systems may be very efficient in preventing invasion by these organisms. Also, the fact that camelids are apparently not often exposed to ocular trauma which facilitates invasion by opportunistic organisms undoubtedly plays a major role in their low incidence of external ocular infection. To examine this further, specific components of camelid ocular immunity such as the lysozyme, immunoglobulin and cellular composition of tears need to be investigated.

Because of the small sample sizes in this study, general conclusions as to the occurrence of ocular mycoplasmas in camelids cannot be made. Further culturing of camelid conjunctivae for these organisms is necessary.

REFERENCES

- Al-Aubaidi, J. M., Dardiri, A. H., Muscoplatt, C. C., and E. H. McCauley. 1973. Identification and characterization of *Acholeplasma oculusi* spec Nov. from the eyes of goats with keratoconjunctivitis. Cornell Vet. 63:117-129.
- Banett, H. L. and Hunter, B. B. 1972. Illustrated genera of Imperfect Fungi. Burgess Pub. Co. Minneapolis, MN. 241 pp.
- Banis, J. 1959. Microflora in normal and pathological conjunctivae in the horse and dog. ACTA Vet. Belgrades 9:97-108. (In Russian).
- Barile, M. F., DelGudice R. A., and J. G. Tully. 1972. Isolation and characterization of *Mycoplasma conjunctivae* sp. n. from sheep and goats with keratoconjunctivitis. Inf. and Immun. 5(1):70-76.
- Barrie, K. P., Jacobson, E., and R. L. Peiffer Jr. 1978. Unilateral cataract with lens coloboma and bilateral corneal edema in a guanaco. J. Am. Vet. Med. Assoc. 179(9):1251-1252.
- Bistner, S. I., Roberts, S. R., and R. P. Anderson. 1969. Conjunctival bacteria: clinical appearances can be deceiving. Mod. Vet. Pract. Dec: 45-47.
- Blackmore, D. K., Hill, A., and O. F. Jackson. 1971. The incidence of mycoplasma in pet and colony maintained cats. J. Small An. Pract. 12:207-216.
- Bonner, R. D. and C. L. Fergus. 1959. The fungus flora of cattle feeds. Mycologia. 51:855-869.
- Bovre, K. 1985. Family Nisseriaceae. Pages 288-290 in N. R. Creig and J. G. Holt eds. *Bergey's Manual of Systematic Bacteriology*, Volume 1. Williams and Wilkins, Baltimore, MD.
- Brightman, A. H., McLaughlin, S. A., and V. Brumley. 1986. Keratoconjunctivitis in a llama. Vet. Med. Small An. Clin. 1776-1777.
- Bulgin, M. S. and D. A. Dubose. 1982. Pinkeye associated with *Branhamella ovis* infection in dairy goats. Vet. Med. Small An. Clin. 77:1791-1793.
- Campbell, L. H., Fox, J. G., and S. B. Snyder. 1973^a. Ocular bacteria and mycoplasma of the clinically normal cat. Fel. Pract. Nov-Dec: 10-12.
- Campbell, L. H., Snyder, S. B., and C. Reed. 1973^b. *Mycoplasma felis* - associated conjunctivitis in cats. J. Am. Vet. Med. Assoc. 163:991-995.

- Campbell, L. H. and H. R. Okuda. 1975. Cultivation of mycoplasma from conjunctiva and production of corneal immune response in Guinea pigs. Am. J. Vet. Res. 36:893-897.
- Carter, G. R. 1986. Essentials of Veterinary Bacteriology and Mycology. 3rd ed. Lea and Febiger, Philadelphia. 261 pp.
- Cattabiani, F., Cabassi, E., Allodi, C. and F. Gianelli. 1976. Bacterial flora of the conjunctival sac of the horse Ann. Sclavo. 18:91-191. (In Italian)
- Cello, R. M. 1957. Association of pleuropneumonia-like organisms with conjunctivitis in cats. Am. J. Ophthalmol. 43:296-297.
- Christensen, C. M. 1965. Fungi in cereal grain and their products. In G. N. Wogan ed. Mycotoxins in foodstuffs. Massachusetts Institute of Technology Press, Cambridge, MA.
- Cole, B. C., Golightly, L. and J. R. Ward. 1967. Characterization of mycoplasma strains from cats. J. Bact. 94:1451-1458.
- Collins, M. D. and C. S. Cummins. 1986. Genus *Corynebacterium*. Pages 1266-1283 in J.G. Holt ed. Bergey's Manual of Systematic Bacteriology, Volume 2. Williams and Wilkins, Baltimore, MD.
- Coyle, M. B., Hollis, D. G. and N. B. Groman. 1985. *Corynebacterium* spp. and other coryneform organisms. Pages 193-204 in E.H. Lennette, A. Balows, W. J. Hausler and H. J. Shadomy eds. Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
- Eastwood, B. J. 1969. Mycotic flora of the apparently normal equine eye. Masters thesis, Iowa State University, Ames, IA. 37 pp.
- Eichenbaum, J. D., Lavach, J. D., Severin, G. A. and M. E. Paulsen. 1987. Immunology of the ocular surface. Compend. Cont. Ed. Pract. Vet. 9(11):1101-1104.
- Forster, R. K., and G. Rebell. 1975. The diagnosis and management of keratomycoses I. Cause and diagnosis. Arch. Ophthalmology 93:975-978.
- Fowler, M. E. 1990. Personal communication.
- Gerding, P. A., McLaughlin, S. A. and M. W. Troop. 1988. Pathogenic bacteria and fungi associated with external ocular diseases in dogs: 131 cases (1981-1986). J. Am. Vet. Med. Assoc. 193:242-244.
- Gerding, P. A. and I. Kakoma. 1990. Microbiology of the canine and feline eye. Vet. Clin. No. Am. Small An. Pract. 20(3):615-625.

- Gilardi, G. L. 1985. *Pseudomonas*. Pages 350-372 in Manual of Clinical Microbiology. E. H. Lennette, A. Balows, W. J. Hausler, Jr. and H. J. Shadomy eds. 4th ed. American Society for Microbiology, Washington D.C.
- Gionfriddo, J. R. and M. A. Gabal. 1991. The fungal flora of normal camelid conjunctival sac. Am. J. Vet. Res. In press.
- Gionfriddo, J. R. and D. S. Friedman. 1991. Ophthalmology of non-domestic ruminoids: llamas, alpacas, guanacos, and vicunas. In Current Vet Therapy II, Large Animal Pract. In press.
- Gordon, M. A. 1985. Aerobic pathogenic Actinomycetaceae. Pages 249-232, in Manual of Clinical Microbiology, 4th ed. E. H. Lennette, A. Balows, W. J. Hausler, Jr. and H. J. Shadomy eds. American Society for Microbiology, Washington D.C.
- Gourlay, R. N. and L. H. Thomas. 1969. The isolation of large colony and T-strain mycoplasmas from cases of bovine keratoconjunctivitis. Vet. Res. 84:416-417.
- Gourley, R. N. 1973. Significance of mycoplasma infections in cattle. J. Am. Vet. Med. Assoc. 163:905-909.
- Greenwood, J. R. and M. D. Pickett. 1986. Genus Gardenerella. Pages 1283-1286 in J. G. Holt ed. Bergey's Manual of Systematic Bacteriology, Volume 2. Williams and Wilkins, Baltimore MD.
- Griffith, R. 1989. Flow chart for the identification of gram positive cocci. Page 29 in K. H. Schleifer, R. D. Packer, and R. Griffith eds. Veterinary Bacteriology Laboratory Manual for the Identification of Pathogenic Bacteria of Veterinary Significance. Iowa State University, Laboratory Notes.
- Gwin, R. M. 1981. Equine fungal keratitis: diagnosis and treatment. Equine Vet. Sci. 1:66-69.
- Hacker, D. V., Jensen, H. E., and L. A. Selby. 1973. A comparison of conjunctival culture techniques in the dog. J. Am. An. Hosp. Assoc. 5:223-225.
- Hardie, J. M. 1986. Genus Streptococcus. Pages 1043-1071 in J. G. Holt ed. Bergey's Manual of Systematic Bacteriology, Volume 2. Williams and Wilkins, Baltimore, MD.
- Heyward, J. T., Sabry, M. Z. and W. R. Dowdle. 1969. Characterization of mycoplasma species of feline origin. Am. J. Vet. Res. 30(4):615-622.

- Hughes, D. E. and G. W. Pugh Jr. 1970. Isolation and description of a Moraxella from horses with conjunctivitis. Am. J. Vet. Res. 31(3):457-462.
- Huntington, P. J., Coloe, P. J., Bryden, J. D., and F. MacDonald. 1987. Isolation of a Moraxella sp. from horses with conjunctivitis. Aust. Vet. J. 64(4):118-119.
- Kern, T. J., Brooks, D. E. and M. M. White. 1983. Equine keratomycosis: Current concepts of diagnosis and therapy. Equine Vet. J. Suppl 2. 33-38.
- Langford, E. V. and W. J. Dorward. 1969. A mycoplasma isolated from cattle with infectious bovine keratoconjunctivitis. Can. J. Comp. Med. 33:275-279.
- Langford, E. V. 1971. Mycoplasma and associated bacteria isolated from ovine pink-eye. Can. J. Comp. Med. 37:18-21.
- Langford, E. V. and R. H. Leach. 1973. Characterization of a mycoplasma isolated from infectious bovine keratoconjunctivitis: M. bovoculi Spec. Nov. Can. J. Microbiol. 11:1435-1444.
- Lundvall, R. L. 1967. The bacterial and mycotic flora of the normal conjunctival sac in the horse. Proc. Am. Assoc. Equine Pract. 101-107.
- MacFadden, L. F. 1980. Biochemical Tests for Identification of Medical Bacteria. 2nd ed. Williams and Wilkins, Baltimore, MD. 527 pp.
- McCauley, I. H., Surman, P. G. and D. R. Anderson. 1971. Isolation of mycoplasma from goats during an epizootic of keratoconjunctivitis. Am. J. Vet. Res. 32:861-870.
- McDonald, P. J. and A. D. J. Watson. 1976. Microbial flora of normal canine conjunctivae. J. Small An. Pract. 17:809-812.
- McLaughlin, S. A., Brightman, A. H., Helper, L. C., Manning, J. P. and J. E. Tomes. 1983. Pathologic bacteria and fungi associated with extraocular disease in the horse. J. Am. Vet. Med. Assoc. 182(3):241-242.
- Mitchell, J. S. and M. H. Attleberger. 1973. Fusarium keratomycosis in the horse. Vet. Med. Sm. An. Clin. Nov:1257-1260.
- Moore, C. P., Fales, W. H., Whittington, P., and L. Bauer. 1983. Bacterial and fungal isolates from equidae with ulcerative keratitis. J. Am. Vet. Med. Assoc. 182:600-603.

- Murphy, J. M., Lavach, J. D., and G. A. Severin. 1978. Survey of conjunctival flora in dogs with clinical signs of external eye disease. 1978. J. Am. Vet. Med. Assoc. 172(1):66-68.
- Parry, J. M., Turnbull, P. C. D. and J. Gibson. 1983. A Colour Atlas of Bacillus Species. Wolfe Medical Publications Ltd., London. 272 pp.
- Paulsen, M. E., Young, S., Smith, J. A., and G. A. Severin. 1989. Bilateral chorioretinitis, centripital optic neuritis and encephalitis in a llama. J. Am. Vet. Med. Assoc. 194(9):1305-1308.
- Pitman, D. R. and R. Reuter. 1988. Isolation of Branhamella ovis from conjunctivae of Angora goats. Aust. Vet. J. 65:91.
- Pugh, G. W. 1976. Mycoplasmas and conjunctivitis. Pages 52-66 in O. H. V. Stalheim ed. Laboratory Diagnosis of Mycoplasmosis in Food Animals. The American Association of Veterinary Laboratory Diagnosticians, Madison WI.
- Rebhun, W. C., Jenkins, D. H., Riis, R. C., Dill, S. G., Dubori, R. J., and A. Torres. 1988. An epizootic of blindness and encephalitis associated with a herpes virus indistinguishable from equine herpesvirus I in a herd of alpacas and llamas. J. Am. Vet. Med. Assoc. 192(7):953-956.
- Riis, R. C. 1981. Equine ophthalmology. Pages 309-605 in K. N. Gelatt, ed., Textbook of Veterinary Ophthalmology. W. B. Saunders Co, Philadelphia.
- Riou, J. Y., Bind, J. L., Guibourdenche, M., and P. Bovet. 1982. Diagnostic bacteriologique de Neisseria ovis. A propos d'une souche isolee de keratoconjunctivie de l' agneau. Pathol. Biol. 30(10):859-860.
- Rosenbusch, R. F. and W. U. Knudtson. 1980. Bovine mycoplasmal conjunctivitis: experimental reproduction and characterization of the disease. Cornell Vet. 70(4):307-320.
- Russell, W. C., Newman, C., and D. H. Williamson. 1973. A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses. Nature. 253:461-462.
- Salih, B. A. and R. F. Rosenbusch. 1986. Antibody response in calves experimentally or naturally exposed to Mycoplasma bovoculi. Vet. Microbiol. 11:93-102.
- Samuelson, D. A., Andersen, T. L. and R. M. Gwin. 1984. Conjunctival fungal flora in horses, cattle, dogs, and cats. J. Am. Vet. Med. Assoc. 184:1240-1242.

- Schleifer, K. H. 1986. Family 1: Micrococcaceae. Pages 999-1034 in J. G. Holt ed. Bergey's Manual of Systematic Bacteriology, Volume 2. Williams and Wilkins, Baltimore, MD.
- Schmidt, G. M. 1974. Mycotic keratoconjunctivis. Vet. Med. Small An. Clin. 69:1177-1179.
- Severin, G. A. 1990. Personal communication.
- Smith, P. F. 1981. The Biology of Mycoplasmas. Academic Press, New York. 257 pp.
- Sneath, P. H. A. 1986. Endospore forming gram-positive rods and cocci. Pages 1104-1105 in J. G. Holt ed. Bergey's Manual of Systematic Bacteriology, Williams and Wilkins Baltimore, MD.
- Spadbrow, P. 1968. The bacterial flora of the ovine conjunctival sac. Aust. Vet. J. 44:117-119.
- Surman, P. G. 1968. Cytology of "pinkeye" of sheep including a reference to trachoma of man, by employing acridine orange and iodine stains, and isolation of mycoplasma agents from infected sheep eyes. Aust. J. Biol. Sci. 21:447-467.
- Tan, R. J. S. and J. Markham. 1971. Isolation of mycoplasma from cats with conjunctivitis. New Zealand Vet. J. 19:28-32.
- Tan, R. J. S. and J. A. R. Miles. 1972. Mycoplasma isolations from clinically normal cats. Br. Vet. J. 128:87-90.
- Tan, R. J. S. and J. A. R. Miles. 1974. Incidence and significance of mycoplasmas in sick cats. Res. Vet. Sci. 16:27-34.
- Taoudi, A., Johnson, D. W., Kheyyali, D and H. Kirchoff. 1987. Pathogenicity of Mycoplasma capricolum in sheep after experimental infection. Vet. Microbiol. 14:137-144.
- Thom, C. and K. B. Raper. 1945. A Manual of Aspergilli. Williams and Wilkins, Baltimore MD. 373 pp.
- Urban, M., Wyman M., Rheins, M. and R. V. Marvaro. 1972. Conjunctival flora of clinically normal dogs. J. Am. Vet. Med. Assoc. 161(2):201-206.
- Whitley, R. D., Burgess, E. C. and C. P. Moore. 1983. Microbioal isolates of the normal equine eye. Equine Vet. J. Suppl. 2. 138-140.
- Whitley, R. D. and C. P. Moore. 1984. Microbiology of the equine eye in health and disease. Vet. Clin. No. Am. Large An. Pract. 6(3):451-466.

- Wilcox, G. E. 1970. Bacterial flora of the bovine eye with special reference to Moraxella and Neisseria. Aust. Vet. J. 46:253-257.
- Yedloutschnig, R. G. 1976. Mycoplasmas and diseases of sheep and goats. Pages 67-71 in O. H. V. Stalheim ed. Laboratory Diagnosis of Mycoplasmosis in Food Animals. The American Association of Veterinary Laboratory Diagnosticians, Madison, WI.
- Zenoble, R. D., Griffith T. W., and S. C. Clubb. 1983. Survey of bacteriologic flora of conjunctiva and cornea in healthy psittacine birds. Am. J. Vet. Res. 44(1):1966-1967.

ACKNOWLEDGEMENTS

Many people helped to make this project possible. I especially appreciate the efforts of Joanne Kinyon who started me on the project and assisted me in identifying the bacteria. She was always willing to help and always listened with interest and good humor. I also thank Dr. T. Kramer, then chairman of the Department of Microbiology and Preventive Medicine at Iowa State University, for making all the resources of the his department at my disposal even though I wasn't a microbiology major.

Thank you to all my committee members, Dr. Ricardo Rosenbusch and Dr. M.A. Gabal for assisting me with the identification of the mycoplasma and fungi, Dr. R.W. Carithers for helping me with neuroophthalmology, Dr. Yosiya Niyo for helping me learn pathology and for being my friend, and Dr. Daniel Betts who deserves special thanks for his patience and understanding through my years as an ophthalmology resident, especially in teaching me ophthalmic surgery.

I am grateful to Sandy Popelka who put in many hours of her own time helping me prepare the manuscript and to Mary Ann Delva, Tom Loughin and Barbara Worth for statistical assistance. Last and probably most I thank my husband Jim Gionfriddo for hours of reading, revising and consoling. He helps me learn to strive for excellence in everything I do.

APPENDIX

Materials and Methods

Sample collection

Six herds of captive camelids in Iowa and northern Missouri were sampled in the summer and 5 herds were sampled in the winter. The summer sampling was done in June and July 1989 and the winter sampling was done in February and early March 1990. Each animal's eyes were examined briefly for the presence of external ocular disease. Only eyes that appeared normal were sampled. In the summer, one eye of each animal in every herd was cultured for aerobic and anaerobic bacteria. In addition the same eye of animals 3 herds (5, 6, and 7) was sampled for fungal organisms. The other eye of 69 animals from all herds was sampled for mycoplasma. For the winter sampling, one eye of each animal in all 6 herds was cultured for aerobic and anaerobic bacteria and fungi. In each eye, a sterile, moistened cotton swab was rotated in the inferior conjunctival fornix, anterior to the nictitating membrane. Care was taken to avoid touching the eyelid margin with the swab. The swab for bacterial and fungal isolation was placed in a tube containing a small amount of Stuart's transport medium. The tubes were transported to the laboratory in a Gas-Pak anaerobic jar packed in ice. The swabs were streaked on aerobic and anaerobic 5% bovine blood agar plates and Sabourad's dextrose agar and incubated at 37°C.

Bacterial identification

Bacterial colonies which grew were subcultured, gram stained and identified by colony and bacterial morphology and routine tube biochemical methods, following the flowcharts illustrated in Figure A-1,

A-2 and A-3. These charts were compiled from flow charts, tables and texts in MacFadden (1980) Carter (1986), Collins and Cummins (1986), Greenwood and Pickett (1986), Hardie (1986), Schleifer (1986), and Sneath (1986).

To differentiate between the gram positive catalase positive genera Staphylococcus and Micrococcus, several different methods were used. Positive grow on furazolidone agar and failure to grow on either 6.5% or 10% NaCl containing agar indicated that the microorganism was of the genus Micrococcus (MacFadden 1980, Schleifer 1986, Griffith 1989). Positive growth on 6.5% and/or 10% NaCl agar and no growth on furazolidone agar identified the organism as Staphylococcus (MacFadden 1980, Griffith 1989, Schleifer 1986). In addition, these organisms were differentiated by their ability (Staphylococcus) or inability (Micrococcus) to ferment glucose anaerobically (Schleifer 1986).

In order to facilitate identification of the gram negative rods the API 20 E system¹ was used. This system was used primarily to identify gram negative organisms which were not members of the Enterobacteriaceae family and could not be differentiated on Kligler, SIMs and urea agars. The Minitex system² was used to identify organisms which could not be identified on the API 20 E system or to confirm the identification of the API system.

The gram negative cocci were difficult to identify and were placed in the genus Branhamella primarily on the basis of morphology. The

¹Analytab Products Ayerst Labs., Plainview, N.J.

²Becton Dickinson Co., Cockeysville, M.D.

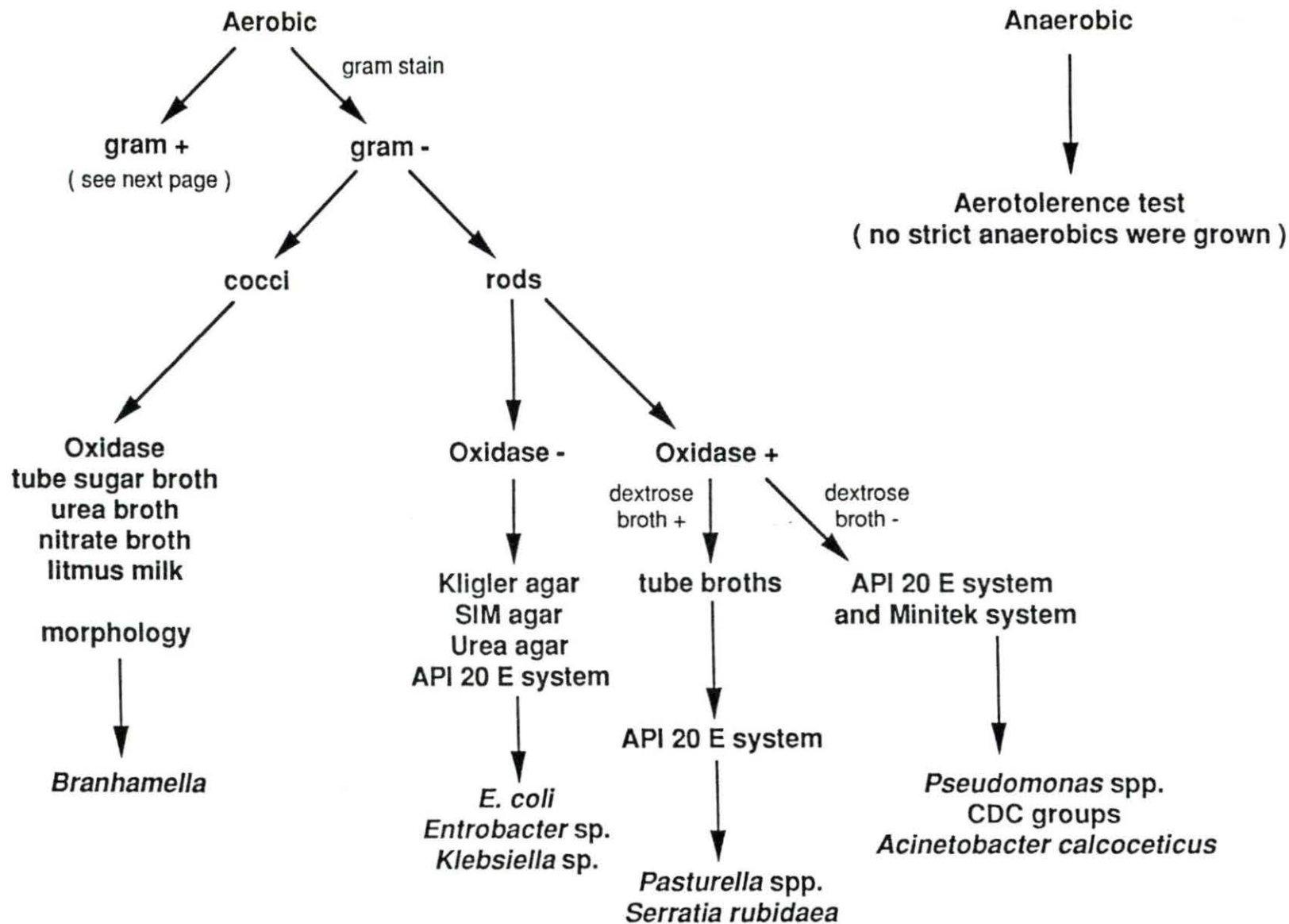


Figure A-1. Flow chart for the identification of bacteria.

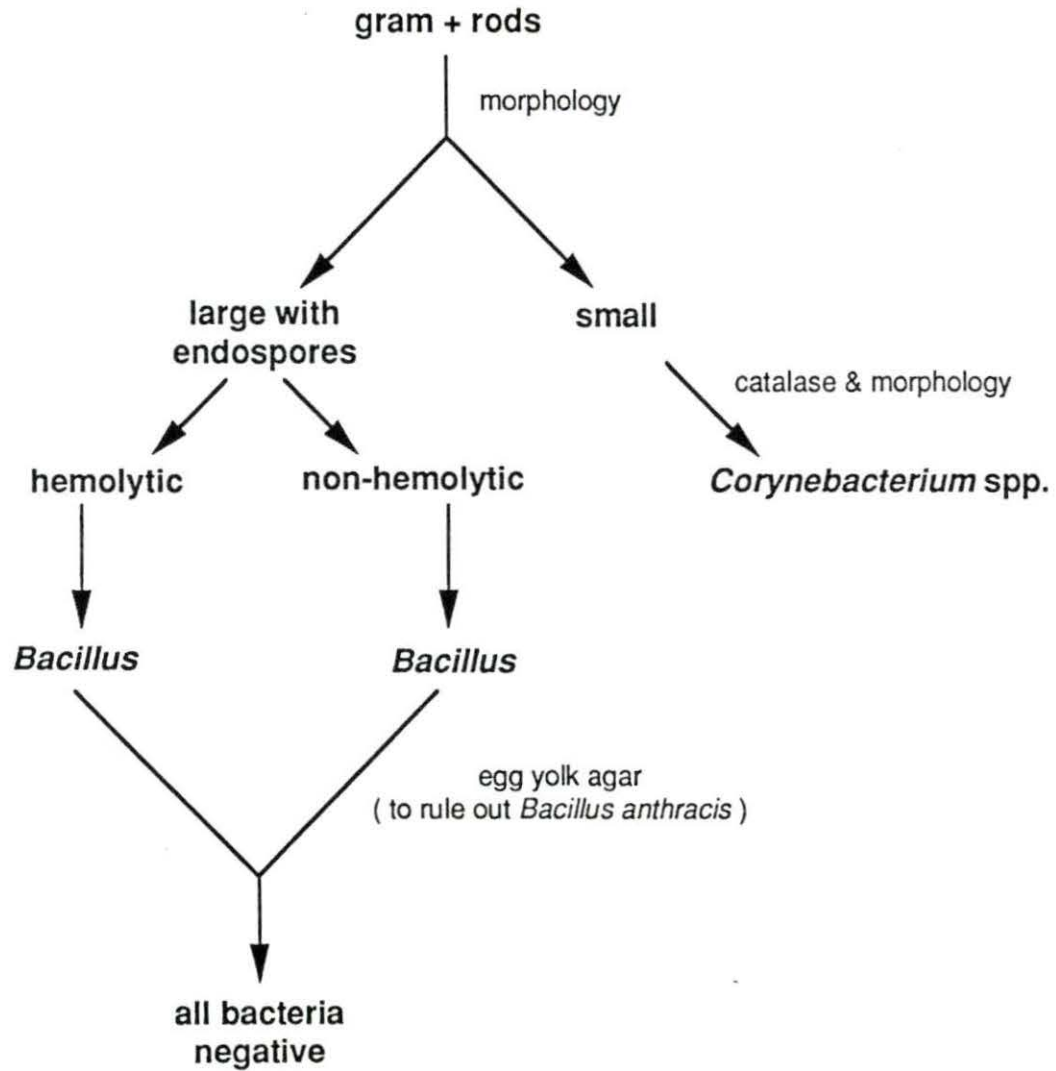


Figure A-2. Flow chart used for the identification of gram + rods.

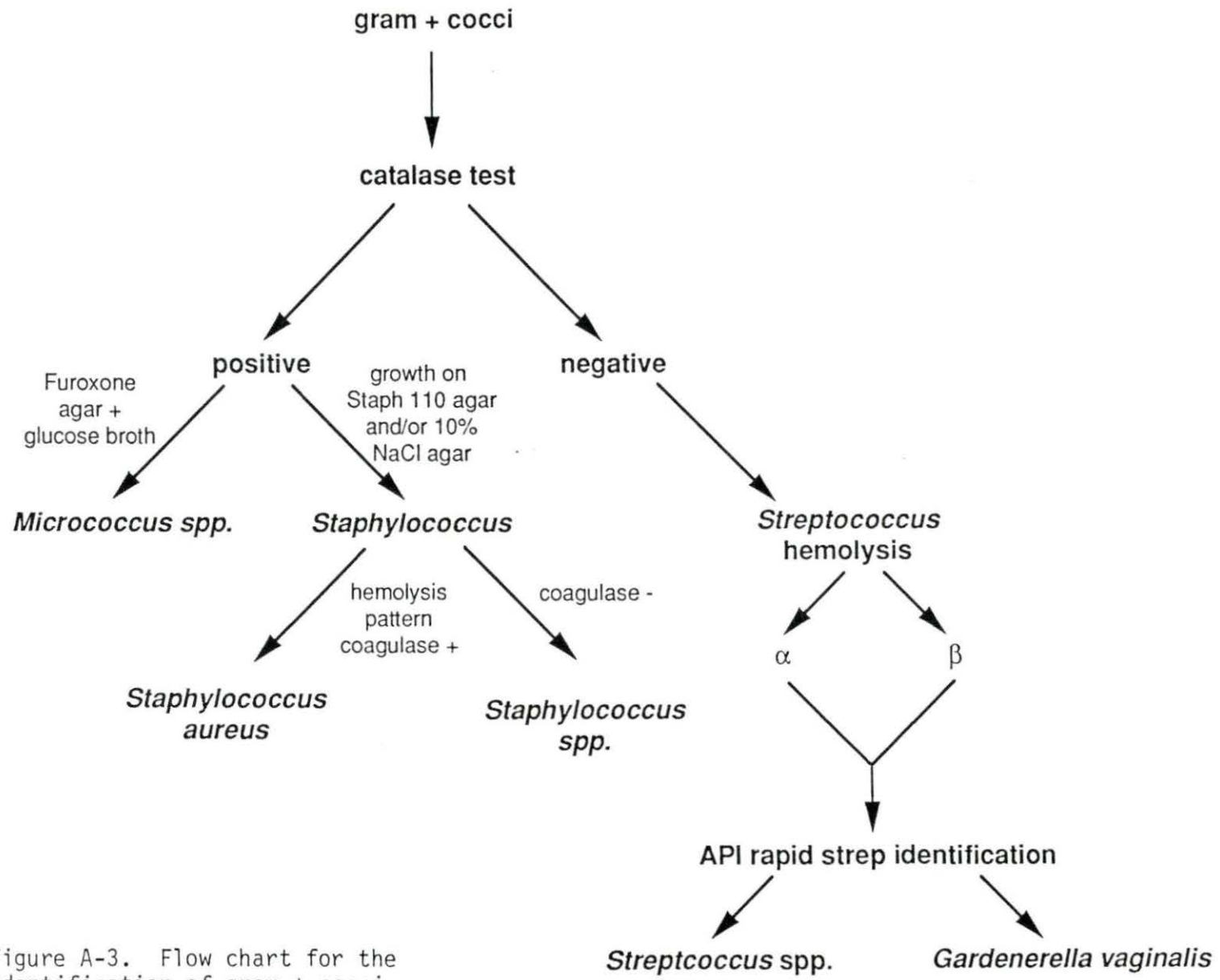


Figure A-3. Flow chart for the identification of gram + cocci.

organisms isolated in this study were round and tended to mould together in diads. Other gram negative cocci with which these organisms could be confused include members of the genera Neisseria and Moraxella.

Moraxella organisms are classified as either rod shaped or coccobacillary. Neisseria organisms may be either round or coccobacillary but are less likely to be found in the eye. Biochemical tests for differentiating among these 3 species were attempted but were not definitive. The 3 organisms react similarly on most commonly available tests (MacFadden 1980, Boure 1984). To identify the species of Streptococcus organisms the API 30 Strep³ identification test was used.

Non-pathogenic and pathogenic Bacillus species were differentiated on the basis of colony morphology, hemolysis on 5% bovine blood agar and lecithinase production on egg yolk agar (Parry et al. 1983).

Mycoplasmal identification

Attempted culture and propagation of mycoplasmas was done according to methods reported by Pugh (1976) and Yedloutschnig (1976). The swabs for mycoplasma isolation were placed in tubes containing 1 ml of sterile saline and were packed in ice for transport to the laboratory. Within 4 hours of sampling, the tubes were centrifuged at 1000 rpms for 3 minutes to segregate the heavier detritus and bacteria to the bottom of the tube. Two-tenths mililiter of saline was pipetted from the center of each column of saline and placed into either modified

³Analytab Products, Ayerst Labs. Plainview, N.J.

Friis medium or Hayflicks broth. Ten-fold dilutions were made in each medium until the final dilution was 10^{-5} . The tubes were incubated at 37°C in 5% CO_2 and observed for mycoplasmal growth as indicated by pH changes (acid or base production by the organism) seen as color changes of the medium. Growth was also indicated by increased turbidity of the medium. If these changes occurred, a few drops of the final dilution were filtered through a milipore filter to reduce the possibility of bacterial contamination and then inoculated onto Friis soft agar plates to promote colony growth for identification. In addition, conjunctival scrapings (using a Cytology Brush) from 6 animals were stained with 4'-6'-diamidino'-2-phenylindole (DAPI) a DNA specific fluorochrome and examined by fluorescence microscopy (Russell et al. 1975). This was done to detect any prokaryotic DNA associated with conjunctival epithelial cells which could be mycoplasmas.

Fungal identification

The swabs for fungal culture were streaked onto Sabourand's dextrose agar and incubated at 37°C . The plates were examined weekly for fungal growth for a minimum of 6 weeks. Fungal organisms were identified by colony and organismal morphology according to Thom and Raper (1945) and Banett and Hunter (1972).

Statistical analysis

Chi square analysis was used to determine if each bacterium (in winter and in summer) and fungus (in winter) was evenly distributed between the sexes, and age classes (less than 3 years or greater than 3 years) and among the 6 herds and 4 species (llamas, guanacos, alpacas and hybrids). Significance was defined as $P < 0.05$.