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Epidemic Caprine Keratoconjunctivitis: Recovery of *Mycoplasma conjunctivae* and Its Possible Role in Pathogenesis

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Clinical, microbiological, serological, histological, and therapeutic aspects of two separate outbreaks of caprine keratoconjunctivitis are described. The disease was characterized by a high rate of contagion, rapid onset, intense lacrimation, conjunctival hyperemia, and corneal opacity with neovascularization. In addition, many of the animals developed respiratory illness during the second epidemic. The only organism consistently isolated was *Mycoplasma conjunctivae*. A total of 23 strains were isolated from 18 inflamed conjunctivae, one normal conjunctiva, and the nasal secretions of four goats with concomitant respiratory illness. The convalescent sera of goats in the first outbreak had neutralizing antibody titers to *M. conjunctivae* that ranged from 1:32 to 1:256. In the milder second outbreak the antibody titers ranged from 1:4 to 1:32 in animals with only ocular disease and from 1:4 to 1:64 in animals with only respiratory disease. Whereas little change was noted in antibody titers of goats with only localized eye disease, 43% of the goats with respiratory disease showed significant fourfold rises. The histological picture was consistent with acute corneal infection. Animals requiring antibiotic treatment appeared to respond favorably to a combination of oxytetracycline and polymyxin B, but not to penicillin. These findings suggest that *M. conjunctivae* is one cause of epidemic caprine keratoconjunctivitis.

A number of mycoplasmas have been implicated in the pathogenesis of various inflammatory ocular diseases of man and animals (1, 2, 4, 6-12, 14-17). The implied role of mycoplasma is based on the frequent isolation of this organism from inflamed eyes and on limited attempts to induce ocular disease experimentally. A recent epidemic of caprine keratoconjunctivitis prompted a more detailed investigation of the natural disease. Two previous outbreaks of caprine and ovine pink-eye led to the isolation and characterization of a new mycoplasma species, *Mycoplasma conjunctivae* (2). This report describes the microbiological and clinical features of naturally occurring caprine keratoconjunctivitis observed during two outbreaks involving separate goat populations. The results of this study support the view that *M. conjunctivae* is one agent responsible for epidemic keratoconjunctivitis.

MATERIALS AND METHODS

Media. The broth and agar media used have been described earlier (2). The broth medium consisted of 70% Mycoplasma Broth Base (Baltimore Biological Laboratory, Baltimore, Md.), 10% fresh yeast extract (Microbiological Associates, Bethesda, Md.), 20% heat-inactivated and pretested horse serum (Flow Laboratories, Rockville, Md.), 0.5% dextrose, 0.2% arginine, and antibacterial agents (0.025% thallium acetate and 100 U of penicillin G per ml). Purified Ionagar no. 2 (Oxoid, distributed by K. C. Biologicals, Lenexa, Kan.) was added for preparation of semisolid broth (0.05%) and solid (0.75%) media. Penicillin and thallium acetate were omitted from the media when they were used for bacterial and fungal isolation. Bacteria and fungi were identified on the bases of morphology and biochemical reactions, using a variety of differential media. Primary African green monkey kidney cell and rabbit kidney cell cultures were also used for isolation of mycoplasmas from several selected specimens. Viral and chlamydial studies were not performed.

Isolation of mycoplasma. Conjunctival specimens were obtained for culture by firmly rubbing a sterile cotton-tipped applicator in a rotating motion across the upper bulbar conjunctiva. Nasal specimens obtained with sterile cotton-tipped applicators were also examined. Swabs were streaked in duplicate onto agar media, and the cotton tip was then placed in broth medium. Broth cultures were incubated aerobically at 36 ± 1°C and subcultured to agar media, either when the culture became turbid or at weekly intervals for at least 3 weeks. One of the agar cultures was incubated aerobically, and the other was incubated in an atmosphere of 5% carbon dioxide and 95% nitrogen.

Identification of mycoplasma. The agar plate epiimmunofluorescence procedure was used to identify the mycoplasmas isolated (5).

Serology. Sera obtained from clinically normal and from diseased animals were tested for the presence of specific neutralizing antibodies to *M. conjunctivae* strain DBS 694, originally isolated from a goat with keratoconjunctivitis (2) by the metabolic inhibition test procedure (19). The sera were obtained from goats during convalescence in the first epidemic and from goats during the early (but not necessarily acute) and the convalescent (late) stages of disease in the second epidemic.

Goat herds. The goats were held at the National Institutes of Health (NIH) Animal Center, Poolesville, Md., and consisted of Nubian, Alpine, Saanen, Toggenberg, La Mancha, and crossbreed females and castrated males, 4 months to 6 years of age. The animals were housed in either semi-enclosed outdoor concrete pens or barns, which opened into pasture. The goat areas bordered a pasture and pens housing sheep, but were physically separated from all other animals on the farm. The herd varied in size from 30 to 90 goats during the 7-year study period. The goats present during the first outbreak were no longer part of the herd during the second outbreak. Periodically, a goat or sheep purchased from local suppliers arrived at the farm with a mild case of keratoconjunctivitis, or developed the disease shortly thereafter. Also, an occasional healthy animal developed a spontaneous mild conjunctivitis with no apparent evidence of exposure to other infected animals.

Drug therapy. Attempts to abort disease during the first epidemic included one or several of the following drugs: (i) subconjunctival injections of 1 ml of dihydrostreptomycin sulfate (150 mg/ml), penicillin G (200,000 U/ml), chloromycetin (10 mg/ml), dexamethasone (2 mg/ml), or methylprednisolone acetate (40 mg/ml); (ii) topical administration of ophthalmic ointments, including chloromycetin (10 mg/g), sodium sulfacetamide (100 mg/g), neomycin sulfate (2.5 mg/g) with prednisolone acetate (5 mg/g), or oxytetracycline (5 mg/g) with polymyxin B (10,000 U/g). Atropine sulfate (1%) was given topically to relieve pain in cases of severe eye disease.

Drug treatment was generally withheld in the sec-

TABLE 1. *Microbiological and serological aspects of caprine keratoconjunctivitis: first epidemic*[a]

Goat identification				Eye involved	Mycoplasma eye isolate	Neutralizing convalescent MI antibody titer
No.	Strain	Age (yrs)	Sex			
139	N	6	F	L, R	—	1:32
140	N	5	F	L	—	1:128
150	T	5	F	R	—	1:64
164	T	3	F	L, R	—	1:256
192	T	3	M		*M. conjunctivae*	ND
207	N	6	M	L	—	>1:256
210	T	4	F	L, R	—	1:32
228	T	3	F	L	*M. conjunctivae*	1:128
231	T	3	F	R	—	1:64
235	T	3	F	R	—	1:64
236	T	3	F	R	*M. conjunctivae*	1:64
237	T	2	F	L	—	1:64
240	N	4	M	R	*M. conjunctivae*	1:128
242	N	4	F	R	*M. conjunctivae*	1:64
250	N	4	F	L	—	1:128
258	T	4	F	L	—	1:64
263	T	3	F	L, R	—	1:64
264	T	4	F	L	—	1:64
265	T	5	F	L	*M. conjunctivae*	1:128
266	T	4	F	L	*M. conjunctivae*	ND
296	T	4	F	L	*M. conjunctivae*	1:64
297	T	3	F	L, R	—	1:128
300	T	3	M	R	—	ND
303	T	3	F	R	—	1:64
307	T	5	F	R	—	1:128
310	N	3	F	R	*M. conjunctivae*	1:64
311	N	3	F	L, R	—	1:128
313	N	4	F	R	—	1:128
319	N	4	M		—	>1:256
320	N	3	F	R	*M. conjunctivae*	ND
324	T	4	F	L, R	—	1:64

[a] Abbreviations: N, Nubian; T, Toggenberg; F, female; M, male; L, left eye; R, right eye; MI, metabolic inhibition; ND, not done. —, Negative.

ond epidemic. However, a few animals did receive oxytetracycline with polymyxin B topically.

Histological examination. The infected right eye of a yearling male goat with a moderately severe case of keratoconjunctivitis was enucleated 7 days after onset and was prepared for examination as follows. The eye was placed in 10% neutral buffered Formalin for 4 days and then cut into several central and paracentral vertical sections. The Formalin-fixed sections were embedded in paraffin, sectioned, and stained by the hematoxylin and eosin, periodic acid-Schiff, or Brown and Brenn (3) procedure.

RESULTS

Epidemics. The two separate outbreaks investigated occurred within a 6-year interval in the goat herd at NIH. The first epidemic occurred between October 1968 and April 1969, spreading rapidly throughout the entire herd. Fifty goats, 94% of the herd, were affected. The epidemic form of disease appeared to be much more severe than the mild conjunctivitis observed sporadically. Various medications were administered during the epidemic (see below). The epidemic abated after about 6 months, leaving a few animals with scarred corneas. A summary of the microbiological and serological findings in this outbreak is presented in Table 1.

The second epidemic in the NIH herd occurred between August and September 1974. The epidemic began shortly after the arrival of two lambs with severe cases of keratoconjunctivitis. The inflamed eyes of both lambs were positive for *M. conjunctivae* by direct culture procedures. Fourteen of 32 goats examined (43% of the herd) developed keratoconjunctivitis (Tables 2, 3, and 4). Five goats had both eye and respiratory disease. Two of the goats with eye disease developed a moderately severe inflammatory arthritic condition.

Clinical aspects. The clinical course of keratoconjunctivitis in both epidemics was similar and was characterized by rapid onset, with initial signs showing increased lacrimation, photophobia, and congestion of conjunctival vessels (Fig. 1). Over the next several days, the reaction progressed to marked erythema, edema, and papillary hypertrophy of conjunctival tissue. By day 5, the lacrimal secretions acquired a mucoid appearance, and the cornea developed peripheral vascularization and clouding. Vascularization began as a "brush border" of vessels from the conjunctiva and spread centrally into the cornea. Cloudiness also began peripherally in advance of the vessels and spread to involve most of the cornea. The mild form of the disease appeared to be self-limiting, with the usual course of disease taking about 10 days. In severe cases, the disease persisted for as long as 12 weeks.

Three of the goats in the first epidemic devel-

TABLE 2. *Clinical, microbiological, and serological aspects of goats in the herd with overt eye disease: second epidemic*[a]

Goat identification				Eye involved	Severity of eye disease	Concurrent respiratory and arthritic (A) disease	Isolation of mycoplasmas[b]		*M. conjunctivae* MI antibody titer	
No.	Strain	Age (yrs)	Sex				Eye	Nasopharynx	Early	Convalescent
652	S	1	F	L, R[c]	+++, +	-	-	ND	ND	32
654	CB	3	M	R	++	+	-	ND	4	16
658	S	1	M	R	+++	-	*M. conjunctivae*	ND	4	8
668	N	4	M	R	+++	-	*M. conjunctivae*	ND	8	4
678	N	4	M	L, R L[c]	+++, +++ +++	-	ND	ND	ND	16
697	A	3	M	R	+++	+	-	ND	4	4
707	N	0.5	M	R, L[c]	+++, +	+	*M. conjunctivae*	*M. conjunctivae*	8	8
715	S	0.5	M	R	+++	-	*M. conjunctivae*	ND	4	16
721	CB	2	F	L	+++	-, (A)	ND	ND	8	8
723	N	0.5	F	R	+++	-	ND	ND	8	16
726	CB	2	M	R	+	+	*M. conjunctivae*	-	8	4
728	S	3	F	R, L[c]	++, ++	-	*M. conjunctivae, A. laidlawii*	ND	16	32
730	N	0.5	M	L, R[c]	+, +	+	*M. conjunctivae, A. laidlawii*	-	8	32
735	N	0.5	M	R	+++	-, (A)	ND	ND	4	4

[a] Abbreviations: N, Nubian; T, Toggenberg; S, Saanen; CB, crossbreed; A, Alpine; F, female; M, male; L, left eye; R, right eye; MI, metabolic inhibition; ND, not done. -, Negative; +, mild disease; ++, moderate disease; +++, severe disease.

[b] In addition, *M. conjunctivae* was isolated from the inflamed eyes of two lambs housed in adjoining areas.

[c] Recurrence of eye disease after complete recovery of the initial eye involvement.

TABLE 3. *Clinical, microbiological, and serological aspects of goats in the herd with respiratory disease but without overt eye disease: second epidemic*[a]

Goat identification				Respiratory disease	Isolation of mycoplasmas		*M. conjunctivae* MI antibody titer	
No.	Strain	Age (yrs)	Sex		Eye	Nasopharynx	Early	Convalescent
653	S	3	M	+	ND	-	ND	8
663	S	3	M	+	ND	-	4	16
667	T	3	M	+	ND	-	8	16
669	A	4	F	+	ND	-	8	8
670	A	4	F	+	ND	-	4	8
672	T	2	F	+	ND	-	4	8
696	N	2	F	+	ND	-	4	8
710	N	1	M	+	*M. conjunctivae*	ND	4	32
716	N	1	F	+	ND	-	8	32
719	N	1	F	+	ND	-	16	64
720	N	2	F	+	-	*M. conjunctivae*	4	8
724	T	2	F	+	ND	*M. conjunctivae*	2	8
725	LM	2	F	+	ND	-	8	16
733	N	0.5	M	+	-	*M. conjunctivae*	8	32

[a] Abbreviations: N, Nubian; T, Toggenberg; S, Saanen; CB, crossbreed; A, Alpine; LM, La Mancha; F, female; M, male; MI, metabolic inhibition; ND, not done. +, Positive; -, negative.

TABLE 4. *Microbiological and serological aspects of asymptomatic goats in the herd: second epidemic*[a]

Goat identification				Isolation of mycoplasmas		*M. conjunctivae* MI antibody titer	
No.	Strain	Age (yrs)	Sex	Eye	Nasopharynx	Early epidemic	Post-epidemic
700	S	4	F	-	ND	4	8
709	LM	0.5	M	*M. conjunctivae*	ND	16	16
711	N	2	M	-	ND	16	16
729	N	0.5	F	-	ND	2	8

[a] Abbreviations: S, Saanen; LM, La Mancha; N, Nubian; M, male; F, female; MI, metabolic inhibition; ND, not done. -, Negative.

oped severe keratoconjunctivitis with ulceration of the cornea, which did not perforate but resulted in corneal opacity. Most animals developed unilateral ocular disease, involving either eye with equal frequency, but bilateral involvement was seen in 12 of 43 (28%) goats examined in the second epidemic. Several cases of recurrent infection (or relapse) were also observed. There were five cases of recurrent eye disease in the second epidemic, and one of these appeared in the same eye (Table 2). The severity of the recurrent disease was comparable in three goats and of less severity in two goats. In the second outbreak, 5 of 14 diseased goats developed a concurrent generalized respiratory and/or arthritic disease. The tissues examined from some of these animals showed the development of glomerulonephritis. Radiologically confirmed ossification of the involved joint developed in two of the three arthritic goats 4 months after the onset of keratoconjunctivitis.

Microbiological aspects. *M. conjunctivae* was the only agent that was consistently and readily isolated from inflamed conjunctival tissues during the two outbreaks. A total of 17 strains of *M. conjunctivae* were isolated. Ten strains were isolated from the inflamed conjunctivae of goats in the first epidemic (Table 1), and seven strains were isolated from the inflamed eyes of 10 goats (70%) in the second epidemic (Table 2). *Acholeplasma laidlawii* was also isolated from two of these inflamed eyes (Table 2). *M. conjunctivae* was isolated from both the inflamed eye and nasal secretions of one of three goats examined that had both ocular and pulmonary disease. Further isolations were made from the inflamed eyes of three of five goats with recurrent eye disease.

Five additional strains of *M. conjunctivae* were isolated from 18 additional goats examined during the second epidemic. Fourteen of these animals had severe respiratory disease with no overt eye disease (Table 3). One strain was isolated from the eyes of three goats, and three strains were isolated from the nasal secretions of 13 goats with respiratory disease only (Table

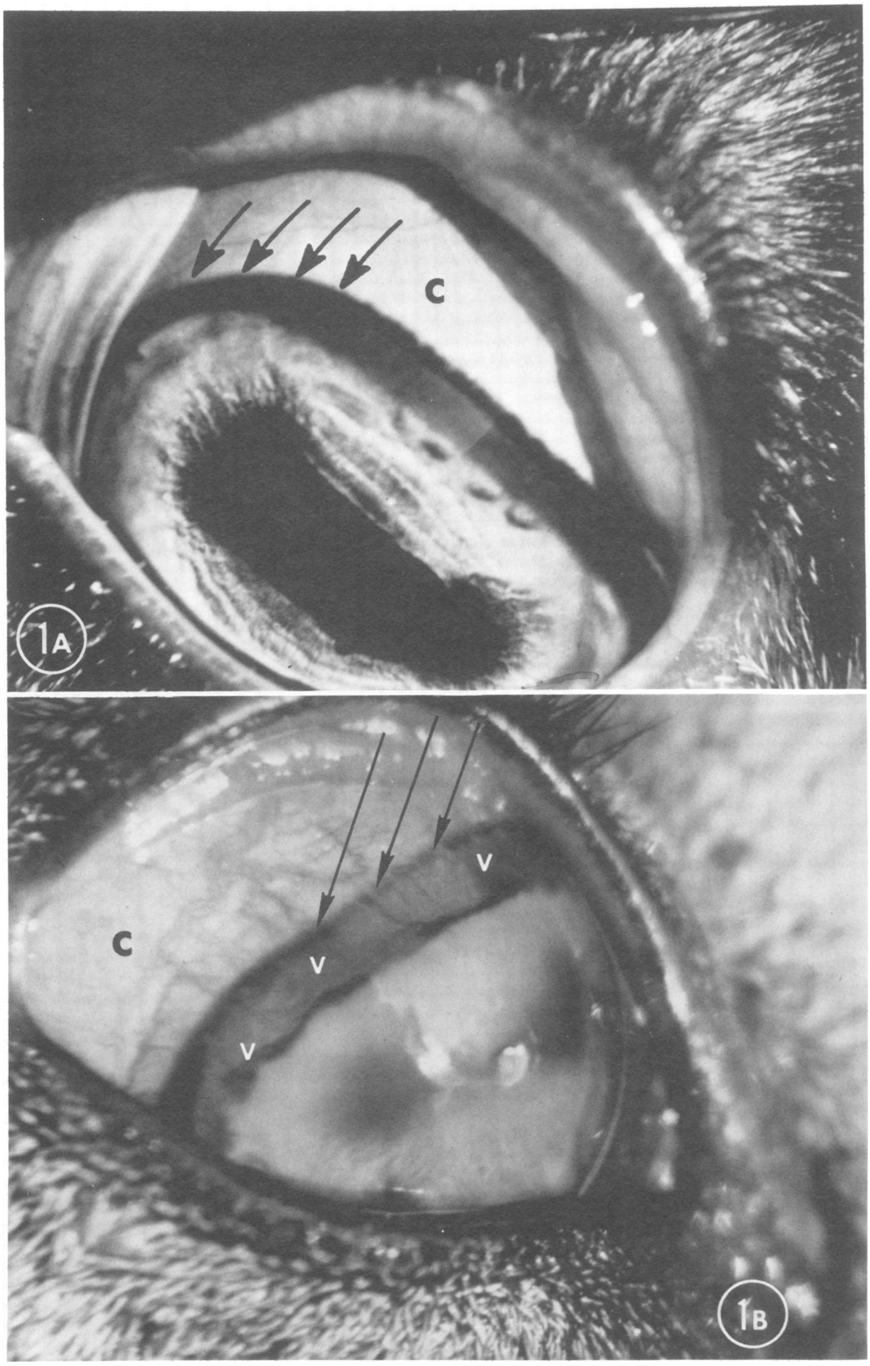

FIG. 1. *Normal left eye (A) and infected right eye (B) of a goat with keratoconjunctivitis ("pink-eye"). The normal conjunctiva (c) is characterized by a very fine vascular network and a dark band of pigmentation circumscribing the corneoscleral junction (arrows). The iris, pupil, and anterior chamber are readily visible through the clear cornea. The infected eye (B) has an intense hyperemia of the conjunctival vascular network (c). Note the neovascularization (v) adjacent to the pigment band (arrows) and the clouding of the cornea. Excessive lacrimation (wetting) can be observed at the medial canthus.*

3). One more strain was also recovered from the eyes of four asymptomatic goats in the herd (Table 4). Occasionally a bacterial or fungal organism (such as a saprophytic fungus of the *Aspergillus* species, *Staphylococcus albus*, *Escherichia coli*, and *Pseudomonas* species) was isolated from the conjunctivae of either diseased or asymptomatic goats.

Serology. Preinfection or acute-phase sera were not available from all animals. However, convalescent sera taken from 27 goats during the first outbreak had neutralizing titers to *M. conjunctivae* that ranged from 1:32 to 1:256 (the 2-sigma lower and upper limits were 1:24 to 1:363), with a geometric mean titer of 1:94. Early and convalescent sera were also obtained from 14 goats with overt eye disease in the second epidemic, and the antibody titers are shown in Tables 2, 3, and 5. Three of 14 goats (21%) seen with eye disease either alone or with respiratory disease and 8 of 19 goats (42%) seen with respiratory disease either alone or with keratoconjunctivitis developed significant fourfold rises. One of seven goats (14%) with only ocular disease showed a fourfold rise in antibody titer (Table 3). Two of five goats with both ocular and respiratory disease showed a fourfold response (Table 5). The geometric mean titers of sera obtained from goats during early and late phases of infection with only ocular disease were 7.4 and 12.6 ($P = 0.1$), whereas the mean titers of goats with respiratory disease were 6.3 and 17.8 ($P = 0.001$). One of the four asymptomatic goats also had a fourfold rise in serum titer (Table 5). In addition, specific neutralizing antibody to *M. conjunctivae*, with mean titers of 1:16, was detected in the synovial fluids of the two arthritic goats examined.

Histology. Examination of the inflamed eye (Fig. 2) showed a subepithelial mononuclear-cell infiltrate in the conjunctiva, consisting primarily of macrophages with occasional plasma cells, lymphocytes, and some polymorphonuclear leukocytes (Fig. 3). The cornea showed a slight intracellular edema, with an intense mixed-cell infiltrate in the anterior half of the stroma. The inflammatory cells were primarily polymorphonuclear leukocytes, with macrophages constituting the majority of the remaining invading mononuclear cells (Fig. 4). A few plasma cells and lymphocytes were present. Numerous small and large vessels were observed throughout the anterior stroma, some of which were surrounded with a cuff of mononuclear cells. The central and posterior corneal stroma showed scattered polymorphonuclear leukocytes. Descemet's membrane and the corneal endothelium appeared normal. The anterior chamber appeared clear, but the trabecular meshwork at the corneoscleral junction showed a mild-to-moderate mononuclear-cell infiltration (Fig. 5). A mild mononuclear-cell infiltration was also observed in the iris and ciliary body. The remainder of the eye appeared normal. Careful examination of the tissues with special stains revealed no bacterial or fungal organisms.

Drug therapy. The regimen of drugs used in the first outbreak was mostly ineffective in altering the course of disease. It was our clinical impression that the topical administration of oxytetracycline in combination with polymyxin B (neither was tried alone) reduced the severity of disease. Only those eyes treated with these antibiotics showed a consistent decrease in the intensity of disease, with a more rapid recovery. Nonetheless, a critical double-blind study was not feasible, and no definite conclusions can be made regarding therapy. Most of the animals involved in the second goat epidemic had a spontaneous resolution of disease, but several animals did receive topical oxytetracycline with polymyxin B. Again, it was our impression that this treatment promoted recovery.

DISCUSSION

M. conjunctivae has been repeatedly isolated from the inflamed conjunctivae of goats, sheep, and chamois during separate and geographically distant outbreaks of keratoconjunctivitis in Maryland (2), Canada (10), Switzerland (15), and Australia (16, 17). In addition, experimentally induced keratoconjunctivitis was produced in chamois with *M. conjunctivae* by Klingler et al. (9). These findings suggest a major role for this agent in the etiology of this disease. In the present study, two naturally occurring outbreaks provided an opportunity to conduct a detailed study on the role of *M. conjunctivae* in the etiology of caprine keratoconjunctivitis.

TABLE 5. *Relationship between systemic disease and significant serum antibody response[a]: second epidemic*

Clinical disease	No. of animals tested	No. with ≥4-fold rise in antibody titer (%)
No apparent disease	4	1 (25)
Keratoconjunctivitis only	7	1 (14)
Keratoconjunctivitis with or without respiratory disease	14	3 (21)
Respiratory disease only	14	6 (43)
Respiratory disease with or without keratoconjunctivitis	19	8 (42)
Respiratory disease and keratoconjunctivitis	5	2 (40)

[a] Metabolic inhibition test with *M. conjunctivae*.

The ocular lesion began as a conjunctivitis, which progressed to a deep keratitis within a few days. The lesion slowly regressed within a 6-week period, with residual changes in the inflamed tissue reflecting the intensity of the initial reaction. *M. conjunctivae* was consistently and almost exclusively isolated from the acute eye lesions of the animals examined. In many instances complicating respiratory and, occasionally, arthritic signs were also seen. Cultures from the nasopharynx of animals showing respiratory illness were frequently positive for *M. conjunctivae*. Although cultures of synovial tissues were negative for *M. conjunctivae*, two of three animals examined showed significant levels of specific antibody in the synovial fluids. The association of respiratory, rheumatic, and mucosal disease has been documented in other mycoplasma infections (7, 18, 21).

Animals involved in the second caprine epidemic showed a milder form of disease than those in the first outbreak. Fewer animals showed clinical signs of eye involvement, and convalescent sera had lower antibody titers. However, animals with respiratory signs did produce significant antibody titers. Factors responsible for the differences in severity between the two goat epidemics were not clear from these studies. The studies do suggest that systemic involvement, such as the associated respiratory infection, may be necessary for stimulation of high serum antibody titers.

The recurrence of the eye disease in five animals tends to indicate that little protective immunity is obtained after keratoconjunctivitis, or that the immunity is of short duration. Because increased antibody levels were found during respiratory disease, the development of systemic disease may be required for protection, but this aspect was not examined during this study. Some degree of protection may have occurred in some animals, because two cases of recurrent disease appeared to be less severe.

Various approaches to therapy were at-

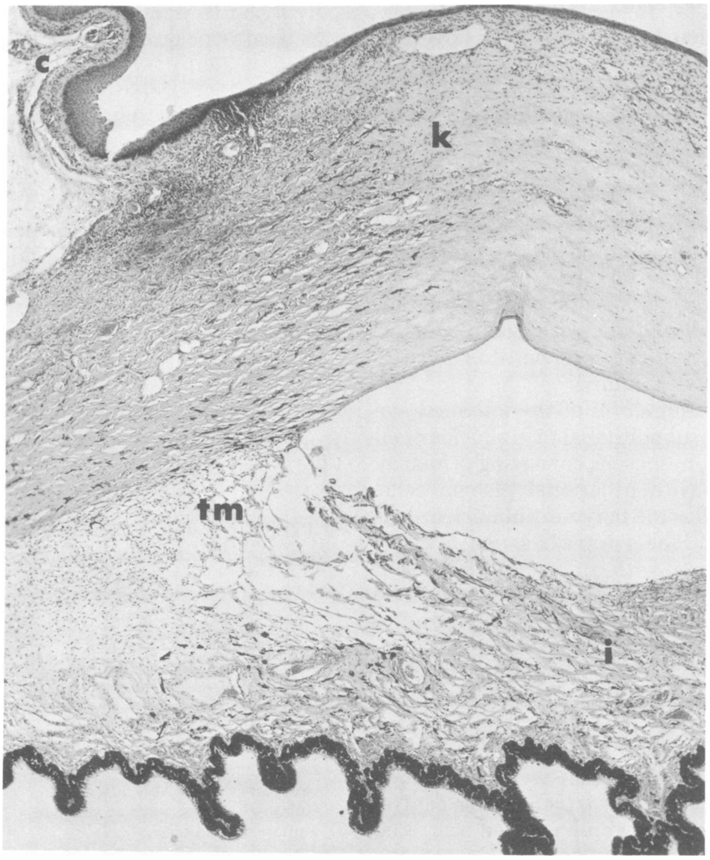

FIG. 2. *Portion of anterior segment of eye from yearling goat at peak of clinical disease. Inflammatory cells are present in the conjunctiva (c), cornea (k), trabecular meshwork (tm), and iris (i). (Hematoxylin and eosin, ×40.)*

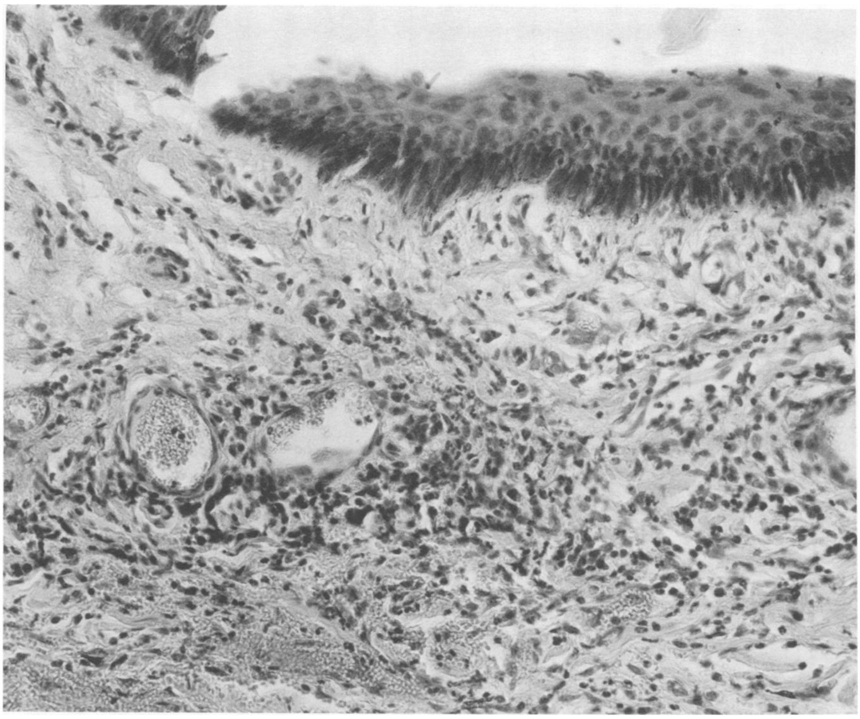

FIG. 3. *Mixed inflammatory cell infiltrate within the conjunctiva is composed primarily of mononuclear cells, with an occasional polymorphonuclear leukocyte. (Hematoxylin and eosin, ×240.)*

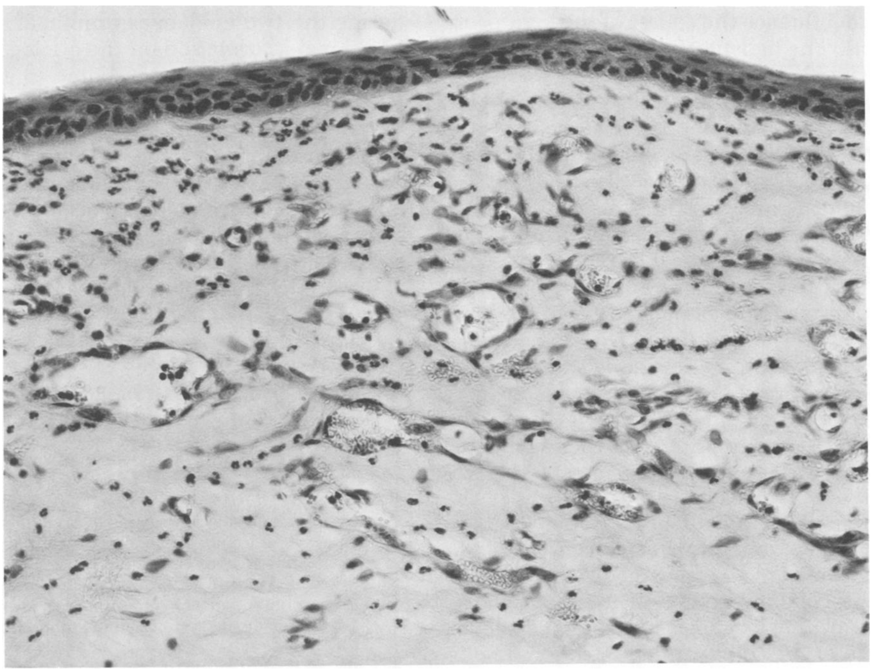

FIG. 4. *Anterior cornea (epithelial side) is infiltrated primarily with polymorphonuclear leukocytes. Neovascularization is prominent. (Hematoxylin and eosin, ×240.)*

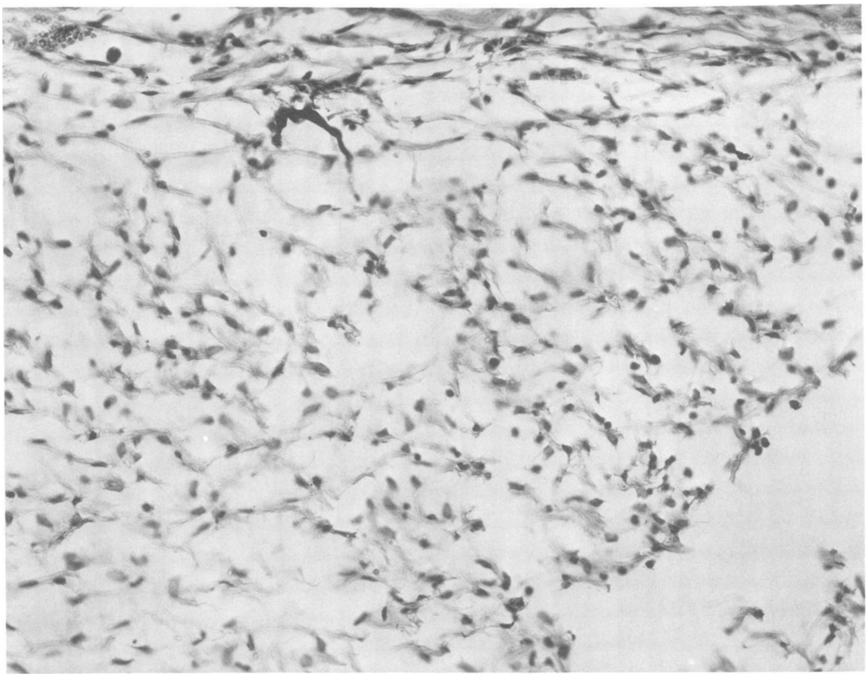

FIG. 5. *Mononuclear cells are scattered throughout the trabecular meshwork. (Hematoxylin and eosin, ×240.)*

tempted, but only the topical application of an oxytetracycline and polymyxin B combination seemed to influence the course of the keratoconjunctivitis. The beneficial effect of tetracyclines and the ineffectiveness of penicillin for mycoplasma diseases has been well established (13). Oxytetracycline was not tried alone, but the failure to respond to penicillin supports a mycoplasma etiology for the disease.

Although the mode of transmission of the *M. conjunctivae* into the herd was not firmly established, the second caprine outbreak was associated with the arrival into the herd area of sheep with *M. conjunctivae*-associated keratoconjunctivitis. However, *M. conjunctivae* was also isolated from asymptomatic animals, and recurrence of disease and the resulting epidemic may reflect reactivation of a latent infection. The presence of a carrier state is further supported by the occurrence of sporadic cases of keratoconjunctivitis, which appeared throughout the 6-year interval between the two goat epidemics. Once a virulent organism establishes itself in the herd, the rapid spread of disease may be due to the common source of food and water. Common grazing land has been implicated as the source of *M. conjunctivae* infection among sheep and chamois herds in Switzerland (15).

The findings reported provide strong presumptive evidence to implicate *M. conjunctivae* as the agent of epidemic keratoconjunctivitis during the two epidemics examined. To obtain additional information on the etiological role of this agent, studies were initiated to experimentally induce keratoconjunctivitis in goats, using a pure cloned culture of *M. conjunctivae*. The results presented in the accompanying paper (20) indicate that the clinical, histological, serological, and microbiological aspects of experimentally induced keratoconjunctivitis are similar or identical to the naturally occurring disease observed in the epidemics examined in this study.

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