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LESIONS ASSOCIATED WITH INFECTIOUS KERATOCONJUNCTIVITIS IN ALPINE IBEX

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ABSTRACT: Following a severe infectious keratoconjunctivitis (IKC) epizootic in free-ranging alpine ibex (*Capra ibex ibex*) in Switzerland in 1993, 19 animals were examined from six different populations. Mucopurulent exudates, reddened conjunctiva and mononuclear inflammatory cell infiltration in the conjunctiva and the limbic area were observed in mild cases. In more severe cases, lesions ranged from perilimbal neovascularization to corneal edema, erosion, ulceration and neovascularization accompanied by infiltration of neutrophils. Sometimes an iridocyclitis was observed. In the most advanced stages, the cornea was perforated and an anterior synechia was present. Lesions found in ibex affected with IKC indicated a non-generalized, specific ocular disease. The other organs investigated did not show alterations suggestive of changes induced by agents which might cause IKC, including *Chlamydia* spp. and *Mycoplasma* spp. The microbiological findings indicate that *Mycoplasma conjunctivae* is the primary pathogenic agent causing IKC in this species in Switzerland.

Key words: Alpine ibex, *Capra ibex ibex*, histology, infectious keratoconjunctivitis, *Mycoplasma conjunctivae*, pathology.

INTRODUCTION

Infectious keratoconjunctivitis (IKC) is the most common ocular disease in domestic ruminants (Jones, 1976; Baas et al., 1977; Barber, 1986). Infectious keratoconjunctivitis describes a clinical condition which is contagious, but apparently not always ascribable to the same infectious agent. In domestic sheep, pathogenicity has been demonstrated for only two of the microorganisms which have been isolated from the eyes, *Chlamydia psittaci* and *Mycoplasma conjunctivae* (Jones, 1991), and *Branhamella ovis* may play a role in IKC in domestic sheep (Dagnall, 1994).

In the European Alps, IKC occurs frequently in both free-ranging ibex (*Capra ibex ibex*) and chamois (*Rupicapra rupicapra*) with highest prevalences in summer (Giacometti et al., 1995). When IKC epizootics occur in chamois or ibex, morbidity may attain 85%, but mortality does not exceed 15% (Gauthier, 1991). *Mycoplasma conjunctivae*, previously found in the eyes of alpine chamois (Nicolet and Freundt, 1975), was isolated recently in pure cultures from conjunctival swabs taken from affected alpine ibex

(Mayer et al., 1996). However, the etiology of IKC in ruminants remains controversial. Other etiological agents besides *C. psittaci* and *M. conjunctivae* are involved in ocular disorders in wildlife, including *Moraxella* spp., *Brucella* spp. and the herpesviruses causing malignant catarrhal fever (Bouvier et al., 1954; Heuschele et al., 1988; Garin-Bastuji et al., 1990; Gauthier, 1994; Rossi et al., 1995). Neither *Chlamydia psittaci* nor *Mycoplasma conjunctivae* appear capable in themselves of producing the most severe form of IKC, namely corneal ulceration. This suggests bacterial involvement either as a primary agent or secondary invader (Jones, 1991).

A severe epizootic of IKC in alpine ibex in the region of Arosa, Switzerland, from August through October 1993, renewed interest in disease research in these wild ruminants. Importantly, the course of the disease seems to differ between alpine ibex and alpine chamois (Couturier, 1962). The objective of this study is to describe the lesions associated with naturally acquired IKC in alpine ibex. A detailed gross and histological description is lacking for IKC in alpine ibex. This could contribute

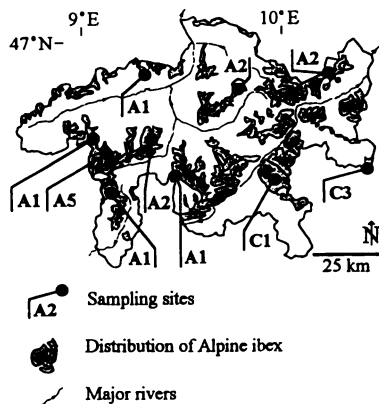


FIGURE 1. Geographical provenance of alpine ibex samples collected for studies on keratoconjunctivitis in Grisons (Switzerland) indicating (A) affected animals, (C) control animals, and (1-5) number of animals examined at each site.

to the understanding of the etiology and pathogenesis of the disease in this species.

MATERIALS AND METHODS

From August 1994 through August 1996, 19 wild alpine ibex were shot in six colonies in Grisons (46°10' to 47°03'N, 8°46' to 10°20'E), Switzerland (Fig. 1). Fifteen animals were clinically affected with IKC, but the duration of the disease process could not be determined. The control group consisted of four uninfected animals. Three control animals were from a colony where IKC has not been reported and one animal was from a colony free of IKC in alpine ibex and alpine chamois for a 2-yr-period. Animals were selected and approached as described in Mayer et al. (1996), with the objective to obtain a range of ocular lesions. Shooting was performed by state gamekeepers. The age of individual animals was determined by counting horn annuli (Ratti and Habermehl, 1977), and refers to fully completed years. Conjunctival swabs from the eyes of 15 animals were taken and examined for bacteria, including *Chlamydia* spp. and *Mycoplasma* spp. Methods used concerning cultivation, isolation and identification are reported in Bannerman and Nicolet (1971) and Mayer et al. (1996). All animals were then necropsied in the field within 1 hr after death (Cowan, 1971). Both eyes were enucleated and fixed in NaPO₄-buffered Bouin's solution (Denk et al., 1989). In order to enhance fixation of the inner eye, Bouin's solution was instilled with a syringe into the vitreous body. Slices ≤ 44 mm wide from the following organs were fixed in 4% NaPO₄-buf-

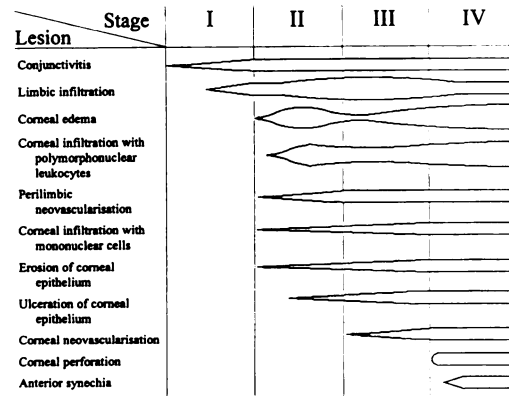


FIGURE 2. Schematic staging of ocular lesions in alpine ibex with keratoconjunctivitis. Stages I-IV refer to classification of lesions as described in the text. The thickness of the bars represents the intensity of the lesions (semiquantitative).

fered formalin: liver, lung, kidney, spleen, heart through papillary muscle, diaphragm, reticulum, rumen, omasum, abomasum, jejunum, caecum, colon, mandibular lymph node, mesenteric lymph node, and testicle with epididymis or uterus. Samples were collected from the same location in each animal. Also, samples from apparent lesions were obtained. The brain of one animal which showed severe corneal opacity was studied. For ocular sections, one-half of an eye was embedded in methylmethacrylate (Schenk et al., 1984), or smaller sections were embedded in paraffin. Other organ samples were embedded in paraffin. Histologic sections of 4 to 6 μm were cut and stained with hematoxylin and eosin. Additional stains (Burck, 1973) included Azan (eyes), van Gieson elastica (kidney, lung, heart), periodic acid-Schiff's reaction (kidney), and Ziehl-Neelson, Köster and Giemsa (various organs).

RESULTS

We investigated 12 affected male animals and 3 females aged 0 to 14 yr. Eyes in different stages of lesion progression were examined, but early lesions were less frequent. Ocular lesions were bilateral. Eyes from the four control animals were macroscopically and histologically normal. Often corneal vascularization was observed, and in three animals the cornea was perforated. Based on macroscopic and histological findings, eye lesions were classified into stages I to IV (Fig. 2).

In stage I, reddened conjunctiva and

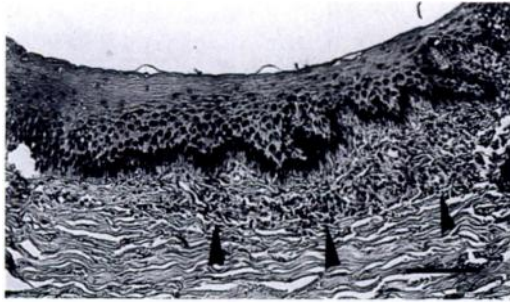


FIGURE 3. Limbic area of a stage I lesion of keratoconjunctivitis in an alpine ibex. Mild limbic infiltration (◆) and pigmented epithelium is still present. H&E stain. Bar = 120 μ m.

mild mucopurulent effusion were present in the least affected eyes. Histologically, hyperemic conjunctiva and mild infiltration with lymphocytes, plasma cells, macrophages and monocytes (mononuclear cell infiltration) in conjunctiva and limbic area (Fig. 3) were observed.

In addition to conjunctivitis, animals in stage II had more severe mucous or mucopurulent effusion, which sometimes formed crusts on the hair under their eyes. Histologically, mild to moderate mononuclear cell infiltration of the stroma and, sometimes, the epithelium was present in the conjunctiva. A few eyes revealed mononuclear cell infiltration combined with neutrophils (mixed cell infiltration) in the conjunctiva and, occasionally, the infiltration formed lymphatic follicles. Perilimbic, but not vertic, neovascularization was frequently observed macroscopically and was consistently detected histologically, mainly in the outer stromal layers. The limbic infiltration was more intense and additionally neutrophils were present. The mononuclear part of the infiltrate did not extend substantially beyond the neovascularization. Partial or complete loss of pigmentation of the epithelium in the limbic area and erosions of the limbic epithelium were observed, especially in the area where the eyes normally have thin epithelium over corium-papillae-like processes of the stroma. Although less distinct, these weak points of the limbic epithelia also

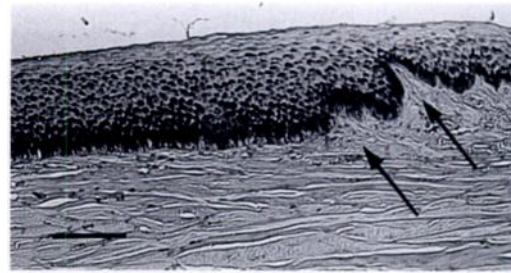


FIGURE 4. Limbic area of an unaffected eye of an alpine ibex. Pigmented epithelium and thin epithelium over corium-papillae-like processes (\rightarrow) of the stroma. H&E stain. Bar = 120 μ m.

were present in the unaffected animals (Fig. 4). Some eyes revealed a discrete opacity in the center of the cornea. Other eyes had more widespread corneal opacity and few corneas showed gross erosions in the vertex. The corneas with eroded or ulcerated epithelium were moderately to severely infiltrated by neutrophils and moderately to severely edematous and necrotic in the outer layers of the stroma (Fig. 5). The inner stromal layers consistently revealed less edema than outer layers. Mild to severe mononuclear cell infiltration was present in some irises and in the corneoscleral trabecular meshwork (iridocyclitis).

For stage III, the alterations of stage I and II were accompanied by neovascularization to the vertex area of the cornea

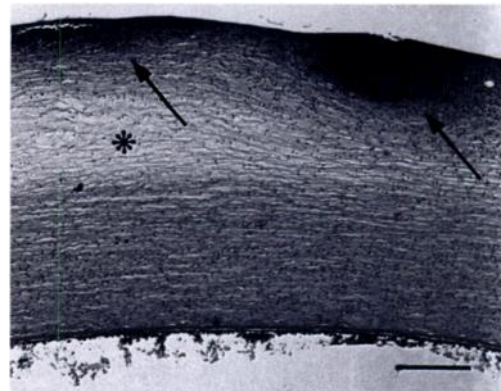


FIGURE 5. Vertic area of a stage II lesion of keratoconjunctivitis in an alpine ibex showing severe edema (*) with infiltration, loss of epithelium and severe multifocal infiltration with necrosis (\rightarrow). H&E stain. Bar = 300 μ m.

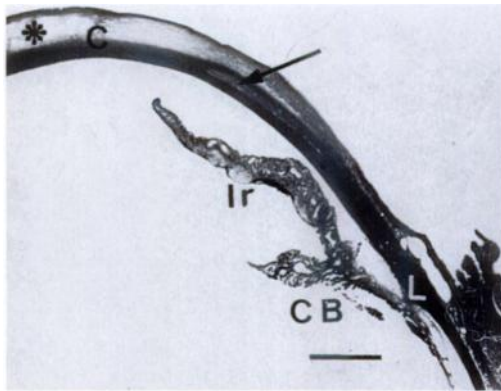


FIGURE 6. General view of a stage III lesion of keratoconjunctivitis in an alpine ibex with severe corneal edema (*) and neovascularization (→) to the vertex area showing the (C) cornea, (Ir) iris, (CB) ciliary body, and (L) limbus. H&E stain. Bar = 2 mm.



FIGURE 7. Vertic area of a stage III lesion of keratoconjunctivitis in an alpine ibex demonstrating vertic neovascularization (→), mixed cellular infiltration and mild edema. H&E stain. Bar = 180 μ m.

(Fig. 6, 7). The corneal epithelium was often eroded or ulcerated and mixed inflammatory cell infiltration and necrosis were present in the stroma and epithelium. Corneas with only minor epithelial erosion or with continuous epithelium showed intense neovascularization and predominantly mononuclear cell infiltration. The infiltration tended to aggregate in foci regardless of the condition of the epithelium and formed an irregular corneal surface. Edema was from mild to severe (Fig. 6, 7). In one eye, the vertex area revealed a mild epithelial pigmentation. In a second eye, a foreign body was present in the ulcerated area.

Eyes in stage IV, the most advanced type, had a perforated and opaque cornea with staphyloma. Histologically, these eyes revealed ruptured Descemet's membrane, an anterior synechia in the vertex area with an incarcerated iris, intense melanin deposition and melanophages, and concomitant necrosis and regeneration processes (Fig. 8). The neovascularization in the area of the synechia was more distinct than in the neighboring corneal stroma (Fig. 8). One eye, although it had a continuous corneal epithelium, showed a ruptured Descemet's membrane and an anterior synechia. In this eye, necrosis was almost ab-

sent and regeneration processes were very distinct.

The other organs investigated in infected and control animals did not show lesions suggestive of changes induced by agents which might cause IKC, including *Chlamydia* spp. or *Mycoplasma* spp. In two affected ibexes described here (ocular lesions corresponding to stage I and III,



FIGURE 8. Vertic area of a stage IV lesion of keratoconjunctivitis in an alpine ibex showing ruptured and twisted Descemet's membrane (♦), melanin deposit (→) and pigment cell migration (▼). The neovascularization is more distinct in the synechia area than in the neighbored corneal stroma. H&E stain. Bar = 360 μ m.

respectively), *Mycoplasma conjunctivae* was isolated from conjunctival swabs.

DISCUSSION

Each animal was examined at only one point of time. Since lesion severity is variable and of a progressive nature (Gauthier, 1991), determination of the precise manner of lesion development or the course of disease is limited by this type of sampling. In addition, four times as many male animals as females were examined because it is harder to approach female than male alpine ibex (Couturier, 1962). However, we were able to point out characteristic lesions associated with different stages of the disease.

The first visible signs of IKC in alpine ibex were mucopurulent effusions and mononuclear inflammatory cell conjunctivitis, which also can persist chronically without any sign of keratitis. *Mycoplasma conjunctivae* has been isolated from four alpine ibexes with earlier cases (Mayer et al., 1996), two of them described here in. Mild keratitis was characterized by either edema with accompanying infiltration of neutrophils or by perilimbal neovascularization with mostly mononuclear inflammatory infiltration. Thus, perilimbal neovascularization can occur without preceding corneal edema in the vertex area when keratitis is mild. Vascularization occurs in response to a variety of cytokines released by damaged corneal epithelium, stromal keratocytes, or immigrant leukocytes, rather than as a response to the edema (Friedlander et al., 1995). In contrast to our results, Lanfranchi et al. (1985) consistently found central corneal edema preceding perilimbal neovascularization in alpine chamois affected with IKC. Superficial edema was variable in the more advanced cases, but was always severe either with marked neovascularization or necrosis. When neovascularization extended to the ulcerated area, mixed cellular infiltration was present. According to Wilcock (1992), ulcerated epithelia lead to infiltration with neutrophils. Neutrophils may be present

in response to secondary bacterial infection. In sheep and alpine chamois affected with IKC, a relatively heterogeneous flora was isolated in addition to *M. conjunctivae*, including *Staphylococcus aureus*, *Haemophilus* sp., *Actinobacillus ligneresii*, *Corynebacterium pyogenes*, *Moraxella ovis*, streptococci, and micrococci (Nicolet and Freundt, 1975; Gauthier, 1994). All ruptured corneas showed anterior synechia and melanin deposits forming staphyloma. Couturier (1962) stated that IKC would take a milder course in alpine ibex than in alpine chamois. However, this study showed that IKC in alpine ibex also can progress and lead to perforated corneas.

In clinically healthy alpine ibex, 31% of sera analyzed were found positive for *C. psittaci* (Giacometti et al., 1995) and this organism is reported as a cause of follicular conjunctivitis in lambs showing polyarthritis (Hopkins et al., 1973). Moreover, *Chlamydia* spp. has been isolated in wild ruminants affected with IKC, including pyrenean chamois (Tournut et al., 1985), bighorn sheep (Meagher et al., 1992), and black-tailed deer (Taylor et al., 1996). However, *M. conjunctivae*, but not *C. psittaci*, was isolated from the eyes in some of the ibexes described here in. Thus, the follicular nature of IKC as well as the tendency toward focal infiltration might not be characteristic for a specific agent, since some of the ibexes in this study showed follicular conjunctivitis as well as irregular corneal surfaces and focal corneal infiltration.

Lanfranchi et al. (1985), and Bassano et al. (1994) found brain lesions which were interpreted as associated with IKC in alpine chamois and alpine ibex. However, in this study lesions were not detected either in the caudal aspects of the eyes, in the optical nerves or in the brain. Consequently, the involvement of the central nervous system seems unlikely in IKC in ibex in Switzerland. Our results indicate that IKC in alpine ibex is a specific ocular disease.

The question remains why different in-

dividuals affected with keratoconjunctivitis develop distinct patterns of ocular disease. Whether or not these distinct patterns are due to involvement of different species or strains of the etiologic agent(s) (Dagnall, 1994), to superinfection with pyogenic bacteria (Egwu et al., 1989), to the immune status of individual animals (Baas et al., 1977), or to damage induced by flies or to environmental predisposing factors (Wilcox, 1968) will be the subject of future studies.

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