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Abstract: Mycoplasma conjunctivae was isolated four times from the eyes of nine Alpine ibex (Capra ibex ibex) suffering from keratoconjunctivitis. The animals examined were affected in two different outbreaks in the Swiss Alps. Parasitological and bacteriological studies, including investigations for Chlamydia and mycoplasmas, were performed. The results indicate that M. conjunctivae is the primary pathogenic agent causing infectious keratoconjunctivitis in this species.
Isolation of Mycoplasma conjunctivae from Conjunctival Swabs of Alpine Ibex (Capra ibex ibex) Affected with Infectious Keratoconjunctivitis

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Summary
Mycoplasma conjunctivae was isolated four times from the eyes of nine Alpine ibex (Capra ibex ibex) suffering from keratoconjunctivitis. The animals examined were infected in two different outbreaks in the Swiss Alps. Bacteriological and virological studies, including investigations for Chlamydia and mycoplasmas, were performed. The results indicate that M. conjunctivae is the primary pathogenic agent causing infectious keratoconjunctivitis in this species.

Introduction
Infectious keratoconjunctivitis (IKC) is a frequent disease in cattle (SCHOTTKE-Wagner et al., 1990), sheep (NICOLET et al., 1971; JONES, 1980) and goats (Bass et al., 1977). Of the wild bovidae, IKC is known in Alpine ibexes (Capra ibex ibex) (Klingler et al., 1955), Przewalski's ass (Equus przewalskii) (Teuber et al., 1985), Alpine ibex (Capra ibex ibex) (Reuter et al., 1955; Conti, 1964), muskox (Ovibos moschatus muskatus; Salden et al., 1990) and takin (Budorcas taxicolor taxicolor; Christy, 1963). The first cases in Alpine ibex were reported in 1956 in the colony of the Augustinmühle, Switzerland (Dikaz, 1976). Since then, IKC has regularly occurred in this species (Couturier, 1989; Gaethner, 1991).

The etiology of IKC remains controversial. Several bacteria have been described as being associated with IKC in both domestic and wild ruminants, including Mycoplasma conjunctivae, chlamydia and Brachyspiraferox (Klingler et al., 1964; NICOLET and FREUNDT, 1975; JONES, 1980; Sánchez-Belis and MARTINEZ-fernando, 1984-1990). Additionally, environmental factors that may predispose or aggravate the disease have been discussed (Viglietti and ROY, 1985; Esco et al., 1989). A severe outbreak of IKC in the Alpine colony of the Switzerland Rottolkha-Hochweing (region of Ardez, Graubünden, Switzerland) in the spring of 1999 renewed interest in
disease research in this species. This investigation considers possible infectious pathogens in Alpine hares.

**Materials and Methods**

**Origin of material**

The geographical provenance of the samples is shown in Figure 1. Nine hares infected in two independent outbreaks were examined. The three control animals originated from a colony that has been free of EKC since its establishment in 1970. An overall picture of the material is given in Table 1.

The animals were selected as an initial phase by judging their behaviour in the open country with binoculars magnifying 8-10 times. After approaching the animal, the head, especially the eyes, was inspected with a telescope magnifying up to 10 times. Priority for selection was given to lightly affected animals. Fœtuses and kids could be approached up to a distance of approximately 10 m. Males were approached to within 20 m, thus allowing immobilisation, which was not possible in females and kits. The immobilisation and showing were performed by state gamekeepers. Heilbëuren mixture (1-2 ml per animal) was used to immobilise the animals. [Weischer und von Heijden, 1985]. The syringe was filled with a Teligen-G.U.T. rifle (Teligen-Capito, Röedeleben, Germany) and a Parko-B-MK 24 rifle (Parker Ltd., Thames, New Zealand).

Age was determined using the method described by Ratté and Hargreaves [1977] and refers to fully completed years. A clinical examination with special attention to head and eye was performed on the immobilised mares. Full necropsy was carried out in the field (Cowan, 1971). A gross pathology staging of the eye lesions was established (Table 2), adapted and modified in line with Landmann et al. [1985].

**Specimens and media**

Bacteriological samples were taken from behind the third eyelid of one eye with sterile
Table 2. Gross pathological findings of the left severely affected eye compared with microphthalmological results. 1: no deformal findings; 2: hypoplastic accessory cornea; 3: light opacity in the centre of the cornea; IV: perilimbal neovascularization; V: neovascularization to the edge of widespread corneal opacity and corneal erosion; VI: perforated cornea. OTPC, SRAH as are in Table 1

<table>
<thead>
<tr>
<th>Animal</th>
<th>Stage</th>
<th>M. conjunctiveae</th>
<th>Colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM 6</td>
<td>1</td>
<td>+++</td>
<td>OTPC</td>
</tr>
<tr>
<td>DM 7</td>
<td>1</td>
<td>+++</td>
<td>OTPC</td>
</tr>
<tr>
<td>DM 10</td>
<td>0</td>
<td>Negative</td>
<td>OTPC</td>
</tr>
<tr>
<td>DM 11</td>
<td>0</td>
<td>Negative</td>
<td>OTPC</td>
</tr>
<tr>
<td>DM 12</td>
<td>IV</td>
<td>Negative</td>
<td>SRAH</td>
</tr>
<tr>
<td>DM 13</td>
<td>H</td>
<td>+</td>
<td>SRAH</td>
</tr>
<tr>
<td>DM 14</td>
<td>H</td>
<td>Negative</td>
<td>SRAH</td>
</tr>
<tr>
<td>DM 15</td>
<td>H</td>
<td>Negative</td>
<td>SRAH</td>
</tr>
<tr>
<td>DM 16</td>
<td>H</td>
<td>Negative</td>
<td>Unilateral</td>
</tr>
<tr>
<td>DM 17</td>
<td>0</td>
<td>Negative</td>
<td>Unilateral</td>
</tr>
<tr>
<td>DM 20</td>
<td>0</td>
<td>Negative</td>
<td>Unilateral</td>
</tr>
</tbody>
</table>

current trials. If there was a difference in stages of the disease between the left and right eye, the animal's less severely affected eye was tested. The swab was dipped into Tris-wash transport medium (Medical Wire & Equipment Co. Ltd., Corsham, England). A second swab was put into a sodium-potassium-glutamate (SPG) transport medium for chlamydia (BOWDEN et al., 1982). Using a third swab, smears were carried out on three microscopes slides. The eye was then immersed in a 0.5% NaCl solution. The smearing solution was collected on a slide and a drop of concentrated formalin was added in order to fix any parasites that might be present. These samples were taken within 15 min of stunning the animals. A piece of the lung was also collected and put aside for bacteriological investigation. The samples were sent to the laboratory overnight and examined within 24 h of collection.

**Bacteriology**

Smears were stained with Gram, Giemsa and Wiegmann stains, and examined for the presence of bacteria, mycoplasma and chlamydia. Swabs were inoculated onto 5% sheep's blood agar and 9% CBA agar (NOCERI et al., 1974) and incubated at 3% CO₂ atmosphere at 37°C. Bacterial and mycoplasma growths were identified according to established diagnostic subcultures. Material from the SPG transport medium was inoculated onto vero-cell monolayers on coverslips and examined for chlamydia after 5 days (SNIPA, 1993).

**Parasitology**

The fluid obtained from the retina was centrifuged in a Beckman Model 21 centrifuge with a JA-14 rotor at 200 x g (Beckman Inc., Palo Alto, CA, USA). The sediment was macroscopically examined at a magnification of 100 times.

**Results**

**Behaviour and clinical findings**

The affected animals had an unstable gait; they often stumbled and got stuck in difficult terrain. They became exhausted after a short run. They blushed frequently. Their conjunctivae were sometimes swollen and reddish, and the fur under their eyes, toward the corners of the mouth, was wet or encrusted. Occasionally, their eyes were opaque.

**Gross pathological findings**

The stages of HCC found in the ibex are documented in Table 2. The lungs of two
Iberian lambs (LM 5 and LM 14) showed parasitic alterations in the apical and middle lobes. No other organs showed any leukocytic infiltrates or KKC.

**Bacteriology**

No common pathogenic bacteria, such as E. coli or other vegetative bacteria, such as *Staphylococcus aureus*, were isolated from conjunctival swabs. *Mycoplasma conjunctivae* was isolated from four of the 12 swabs (Table 2). No mycoplasma effect (MCE) was observed on the cell cultures and no leukocytes were isolated. All lung samples were negative for the presence of pathogenic bacteria and mycoplasmas.

**Parasitology**

No parasites were found in the nasal fluid from the eyes.

**Discussion**

*Mycoplasma conjunctivae* was suspected to be the causative agent of IKC in Alpine ibex (Costa, 1986). *Mycoplasma conjunctivae* was the only pathogen to be isolated from the affected eyes of Alpine ibex in this study. A *virulent* strain, as described by Rigonna et al. (1983), was not found. No Chlamydia parasites, such as was found in Pyrenean chamois in the studies by Salazar-Belda and Martinez-Fernando (1992) and Tournier et al. (1992), was isolated.

Although no transmission for the presence of a virus was performed in this study, there was no indication for a primary viral infection since no CPE on cell cultures was observed. In contrast, Costa (1986) described a CPE on cell cultures, but no virus was isolated from chamois or deer affected with IKC. Early stages of the disease seem to facilitate the isolation of *M. conjunctivae*. Proper sampling and a short time interval between sampling and bacteriological examination diminish the possibility of contamination and thus increase the chance of detecting this organism.

In chamois, *M. conjunctivae* was isolated from the lungs (Krzyczańska et al., 1989). This was not the case in Alpine ibex, although some lungs showed parasitic alterations. An ecology for these alterations was not detectable. The clinical findings of IKC appear to be more severe in chamois and ibex than in domestic ruminants. This could mean that the causative agent is less well adapted in chamois and ibex than in domestic ruminants, or that the wild hosts are more exposed to environmental predisposing factors.

Those findings indicate that *M. conjunctivae* is the causative agent of IKC in Alpine ibex, since it was the only mycoplasma organism isolated from the eyes of these animals. This hypothesis is supported by the fact that, in all cases, isolates originated from animals in an early stage of the disease. The same mycoplasmas were recovered from many small ruminants affected with IKC.

To elucidate the role of *M. conjunctivae* as the primary pathogenic agent of IKC, more research is necessary, such as the experimental infection that was carried out in domestic ruminants (Tipton et al., 1977; Th.LAAS, 1988; Whitemore et al., 1990).

Further epidemiological studies should be carried out in order to investigate the possible transmission of IKC between ibex, chamois and sheep.

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