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Short communication

Detection of *Mycoplasma conjunctivae* in the eyes of healthy, free-ranging Alpine ibex: Possible involvement of Alpine ibex as carriers for the main causing agent of infectious keratoconjunctivitis in wild Caprinae

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ABSTRACT

Mycoplasma conjunctivae is considered the major cause of infectious keratoconjunctivitis (IKC) in Alpine ibex (*Capra i. ibex*) and chamois (*Rupicapra r. rupicapra*). While it is known that domestic sheep can act as healthy carriers for *M. conjunctivae*, this question has not been addressed in wild ungulates so far. In this study, bacteriological investigations and field observations were performed to assess whether free-ranging Alpine ibex can be healthy carriers of *M. conjunctivae*. Among 136 ibex without clinical signs of IKC, *M. conjunctivae* was identified 26 times (19.1%) by TaqMan PCR. To assess the potential pathogenicity of *M. conjunctivae* strains isolated from asymptomatic eyes, strains from three healthy ibex and from 15 IKC-ibex and IKC-chamois were analysed genetically by DNA sequence analysis of the variable part of the *lppS* gene. No significant differences were observed between strains from asymptomatic and clinically affected animals, reflecting the assumption that healthy ibex may act as carriers for *M. conjunctivae* strains that may be pathogenic for other individuals. Our results further indicate that development of IKC is associated with *M. conjunctivae* load in the eyes. In addition, a questionnaire survey revealed that IKC is generally less common in ibex than chamois and that infection in wild ungulates is not necessarily linked to the presence of sheep. These data support the hypothesis that apparently healthy ibex may be important in the epizootiology of IKC and indicate that host predilection may play a role in IKC development.

1. Introduction

Mycoplasma conjunctivae has been implicated as etiological agent of infectious keratoconjunctivitis (IKC) in Alpine ibex (*Capra i. ibex*; Mayer et al., 1996; Giacometti et al., 1998; Grattarola et al., 1999), Alpine chamois (*Rupicapra r. rupicapra*; Nicolet and Freundt, 1975; Grattarola et al., 1999; Degiorgis et al., 2000b; Tschopp

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et al., 2005), and moufflon (*Ovis orientalis musimon*; Terrier, 1998) in the Alps. Spontaneous recovery appears to be the most common disease outcome in these species (Gauthier, 1991; Terrier, 1998), however, high mortality resulting from blindness is also observed (Hars and Gauthier, 1984; Lanfranchi et al., 1985; Grattarola et al., 1999; Degiorgis et al., 2000b). Domestic sheep, in which infection with *M. conjunctivae* is widespread, are regarded as maintenance hosts and probable source of infection for wildlife (Giacometti et al., 2002a). Interspecific transmission between domestic sheep, ibex and chamois has indeed been shown to occur on summer pastures (Belloy et al., 2003a).

Based on a serological study in chamois (Giacometti et al., 2002b), it has been proposed that the infection is not self-maintained in wild Caprinae. However, other authors (Terrier, 1998; Tschopp et al., 2005) suggested that ibex may be carriers of the disease agent. The existence of healthy carriers for *M. conjunctivae* has been previously reported in domestic sheep (e.g. Naglić et al., 2000; Baker et al., 2001; Janovsky et al., 2001; Åkerstedt and Hofsagen, 2004; Vilei et al., 2007) but not in wild Caprinae so far. Recently, an ibex, a chamois and a roe deer (*Capreolus c. capreolus*) kept in neighbouring enclosures at a wildlife rehabilitation centre were all tested positive for *M. conjunctivae* by PCR in absence of clinical signs; in the ibex, *M. conjunctivae* persisted in the eyes for several months (M.-P. Ryser-Degiorgis, L. Zimmermann and E.M. Vilei, unpublished obs.). Moreover, game-wardens report close spatial interactions between healthy ibex and chamois severely affected with IKC, further indicating that the potential role of ibex in the epizootiology of IKC needs to be elucidated.

The aim of this study was to investigate whether apparently healthy ibex are infected with *M. conjunctivae* in the wild, and to discuss the potential role of ibex in the epizootiology of IKC in the Alps, based on (1) the obtained prevalence of healthy carriers, (2) the comparison of DNA sequences of strains from healthy ibex and from clinically affected animals, (3) the determination of a correlation between development of IKC and *M. conjunctivae* loads in conjunctival swabs, and (4) field observations regarding interspecific interactions of ungulates on Alpine pastures and occurrence of IKC epidemics in the study areas.

2. Materials and methods

2.1. Animals and sample collection

Eye swabs were collected from 136 ibex without IKC symptoms, i.e., excessive lachrymation (pathognomonic) with or without associated corneal opacity and/or perforation (Couturier, 1962; Mayer et al., 1997). One animal (asymptomatic ibex #1) presented a unilateral, mild corneal opacity in absence of lachrymation and was also considered as asymptomatic. Seventy-four animals were shot, and 62 ibex were caught alive, with or without subsequent anaesthesia (Abderhalden et al., 1998).

The ibex originated from 12 different colonies (A–L, Table 1) in the Swiss Alps. An ibex colony is defined as a group of ibex having regular social contacts, whereas animals from different colonies have no or only exceptional contacts with each other (usually due to geographical barriers). A colony can therefore be considered as an epizootiological unit. A single colony can be further subdivided in several subcolonies: intraspecific interactions are much more frequent between animals belonging to the same subcolony than between animals of different subcolonies.

The living animals were from the subcolonies A1, A2 and F1 (Table 1). Except for seven ibex sampled in the subcolony F1 in May–June 2006, all samples were taken from January to December 2007. Overall, there were 79 males, 56 females, and no data on sex was available for one ibex. Age was determined based on horn growth (Habermehl, 1985). Five age classes were determined according to social segregation, sexual maturity and aging process (Bon et al., 2001; Meile et al., 2003): yearlings (1-year-old,

Table 1
Prevalence of infection with *M. conjunctivae* in free-ranging ibex from the Swiss Alps without clinical signs of infectious keratoconjunctivitis, as determined by TaqMan analysis

Ibex colony		Canton[a]	Positive/tested	% Positive
Albris-SNP	A	GR	13/43	30.2
"Swiss National Park" subcolony	A1	GR	0/10	0
"Piz Albris" subcolony	A2	GR	13/33	39.4
Julier	B	GR	2/10	20.0
Flüela-Rätikon	C	GR	0/10	0
Oberalp Tödi-Calanda/Foostock/Graue Hörner	D	GR-SG	0/6	0
Churfirsten	E	SG	0/2	0
Cape au Moine-Chaussy/Pierreuse-G./Wittenberg	F	VD-BE	7/28	25.0
"Cape au Moine" subcolony	F1	VD	7/25	28.0
"Wittenberg" subcolony	F2	BE	0/3	0
Brienzer Rothorn/Augstmatthorn	G	BE	0/5	0
Schwarzmönch	H	BE	0/5	0
Chablais	I	VS	1/5	20.0
Valais central, left riverside	J	VS	1/10	10.0
Oberwallis, left riverside	K	VS	1/7	14.3
Oberwallis, right riverside	L	VS	1/4	25.0
(Unknown)		VS	0/1	0
Total			26/136	19.1

[a] BE: Bern; GR: Grisons; SG: St. Gallen; VD: Vaud; VS: Valais.