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Abstract

Because interactions between livestock and chamois occur on Alpine pastures, transmission of infectious diseases is considered possible. Thus, the occurrence of Chlamydiaceae, Mycoplasma conjunctivae, and pestiviruses in Alpine chamois (Rupicapra r. rupicapra) of the Surselva region (eastern Swiss Alps) was investigated. In total, 71 sera, 158 eye swabs, 135 tissue samples, and 23 fecal samples from 85 chamois were analyzed. The sera were tested by 2 enzyme-linked immunosorbent assay (ELISA) kits specific for Chlamyphila abortus. Eye swabs, tissue, and fecal samples were examined by a Chlamydiaceae-specific real-time polymerase chain reaction (PCR). Positive cases were further investigated by microarray method. One serum sample (1.4%) was positive in 1 of the ELISAs. Eye swabs of 3 chamois (3.8%) were positive for Chlamydiaceae. The microarray method revealed the presence of Chlamyphila abortus, C. pecorum, and C. pneumoniae. All tissue and fecal samples were negative. With real-time PCR, 3.9% of the chamois tested positive for Mycoplasma conjunctivae. One chamois had a simultaneous infection with M. conjunctivae and 2 chlamydial species (C. abortus, C. pecorum). Skin and tongue tissue samples of 35 chamois were negative for pestivirus antigen by immunohistochemistry. It was concluded that in contrast to the findings in Pyrenean chamois (Capra p. pyrenaica) of Spain, the occurrence of Chlamydiaceae in Alpine chamois of the Surselva region is low, and the transmission between domestic and wild Caprinae seems not to be frequent. Comparably, persistent pestiviral infections do not seem to be common in chamois of the Surselva region.
Occurrence of *Chlamydiaceae, Mycoplasma conjunctivae, and pestiviruses in Alpine chamois (Rupicapra r. rupicapra) of Grisons, Switzerland*

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Abstract. As interactions between livestock and chamois occur on Alpine pastures, transmission of infectious diseases is considered possible. Thus, the occurrence of Chlamydiaceae, Mycoplasma conjunctivae and pestiviruses in Alpine chamois (Rupicarpa rupicapra) of the Surselva region (eastern Swiss Alps) was investigated. In total, 71 sera, 158 eye swabs, 135 tissue samples and 23 fecal samples from 85 chamois were analyzed. The sera were tested by two ELISA kits specific for Chlamyphila abortus. Eye swabs, tissue and fecal samples were examined by a Chlamydiaceae-specific real-time PCR. Positive cases were further investigated by ArrayTube microarray method. One serum sample (1.4%) was positive in 1 of the ELISAs. Eye swabs of 3 chamois (3.8%) were positive for Chlamydiaceae. The ArrayTube microarray method revealed the presence of Chlamyphila abortus, Chlamyphila pecorum and Chlamyphila pneumoniae. All tissue and fecal samples were negative. 3.9% of the chamois were positive for Mycoplasma conjunctivae by real-time PCR. One chamois had a simultaneous infection with Mycoplasma conjunctivae and 2 chlamydial species (Chlamyphila abortus, Chlamyphila pecorum). Skin and tongue tissue samples of 35 chamois were negative for pestivirus antigen by immunohistochemistry. It was concluded that in contrast to the findings in Pyrenean chamois (Capra p. pyrenaica) of Spain, the occurrence of Chlamydiaceae in Alpine chamois of the Surselva region is low and transmission between domestic and wild Caprinae seems not to be frequent. Comparably, persistent pestiviral infections do not seem to be common in chamois of that region.

Key words: Alpine chamois, Chlamydiaceae, Mycoplasma conjunctivae, pestivirus, Switzerland
*Chlamydia abortus* is the most common infectious abortigenic agent in small domestic ruminants in Switzerland. A previous study revealed that 39% of examined abortions in sheep and 23% in goats were caused by *C. abortus*. Economic losses due to chlamydial abortion in small domestic ruminants are significantly higher in the canton of Grisons (eastern Swiss Alps) than in other Swiss regions (Grisons: 43% seroprevalence in sheep for *C. abortus*). The question was raised whether wild ruminants may play a role in the transmission of chlamydiosis.

In a previous study, a low prevalence of *Chlamydiaceae* was demonstrated in Alpine ibex (*Capra i. ibex*) of Switzerland, but the situation in other wild ruminants still remains to be elucidated. Alpine chamois (*Rupicapra r. rupicapra*) are known to get in contact with domestic sheep and goats on Alpine pastures and could therefore possibly play a role in distribution and transmission of infectious diseases. Infectious keratoconjunctivitis (IKC) is an ocular disease affecting domestic as well as wild *Caprinae* such as chamois and Alpine ibex. To date, *Mycoplasma conjunctivae* is regarded as the major cause of IKC, but chlamydiae have also been reported to be involved in the etiology of IKC. For example, *Chlamydia sp.* was isolated from the conjunctiva of 2 out of 7 free-ranging mule deer (*Odocoileus hemionus*) with IKC in a National Park in Utah, USA, and was detected in the eyes of bighorn sheep (*Ovis canadensis*) of the Yellowstone National Park, Wyoming, USA, affected by IKC. Eradication of bovine viral diarrhea (BVD) is currently performed in Switzerland. Before eradication, the prevalence of persistent infections in cattle of Switzerland was around 1%. However, wild ruminants are not tested during this program as they are not believed to have a significant epidemiological impact. Bovine viral diarrhea virus (BVDV) and border disease virus (BDV) are not strictly host-specific and transmission between species has been described. In wildlife, infection with pestiviruses has been demonstrated worldwide in serologic surveys. A very recent study revealed a
seroprevalence of 4.3% for BVDV in Alpine ibex of Switzerland (Marreros et al., submitted), but data on the prevalence of pestiviruses in chamois are lacking so far. The aim of this study was to determine the occurrence of Chlamydiaceae in Alpine chamois of Grisons (eastern Swiss Alps), using sensitive and specific methods. Furthermore, eye swabs were investigated for M. conjunctivae by real-time PCR and cryosections of skin and tongue tissue were tested by immunohistochemistry for the presence of pestiviral antigen.

All samples (n=387) available for the study originated from a total of 85 Alpine chamois. The material consisted of 71 sera, 158 eye swabs (originating from 79 chamois), 135 organ samples (out of 42 chamois) and 23 fecal samples. The samples were collected in autumn 2008 in the Surselva region (canton of Grisons, eastern Swiss Alps, 46°36' to 46°48' N, 8°42' to 9°20' E). This area is known as a risk area for chlamydial abortion in sheep and interactions between wild and domestic ruminants occur regularly (Brosi and Thoma, personal communication, 2008). The animals were either shot during the hunting season (September 2008, mainly healthy animals) or were killed by game wardens due to disease symptoms from September to November 2008. Two chamois were found dead. For each chamois, a form was completed by the sampling person providing information about the animal and recording disease symptoms. Of 19 chamois, the complete sample spectrum was obtained including serum, eye swabs, organs and fecal samples. The most frequently collected organs included liver, lung, kidney and genital tract as well as skin and tongue for the examination on pestiviruses. Of 66 animals, only limited samples were available. DNA extraction was performed using the MagNA Pure LC System as previously described followed by real-time PCR for Chlamydiaceae on an ABI 7500 instrument using the previously described 23S rRNA gene-based Chlamydiaceae family-specific real-time PCR. A cycle threshold (Ct value) of < 38.00 was considered as positive, and
all samples were tested in duplicate. The results were interpreted as questionably positive if one Ct value was less than 38 and the other sample showed no Ct value. If one Ct value was above 38 and the other sample showed no Ct value, the result was interpreted as questionably negative. The samples with at least one positive Ct value were examined using 23S ArrayTube microarray assay\(^c\) for chlamydial species identification as described previously.\(^5\) The serum samples were tested with two commercial antibody-detecting ELISAs specific for *C. abortus*: (i) Pourquier\(^{®}\) ELISA *Chlamyphila abortus* serum verification\(^d\), validated for sheep, goats and cattle; and (ii) ID Screen\(^{®}\) *Chlamydia abortus* indirect ELISA\(^e\) validated for ruminants, horses and swine. The two ELISA tests were performed according to the manufacturer’s instructions. In both cases, the final values were determined as a ratio between the corrected optical density (OD) of the sample (S) and the mean corrected optical density of the positive control (P), expressed as S/P\%. Sera with S/P%-values equal to or lower than 50\% were interpreted as negative, sera with an S/P% between 50\% and 60\% were classified as doubtful and sera with an S/P% higher or equal to 60\% were considered positive for antibodies against *C. abortus*. For chamois sera, reference values for goats were applied with both ELISAs. Presence of *M. conjunctivae* in the eye swabs was examined by TaqMan real-time PCR using an *lppS*-directed hydrolysis probe, as previously described.\(^{21,29}\) The assay included the presence of an exogenous internal positive control in each reaction to check for the presence of eventual PCR inhibitors and to prevent false-negative responses. Immunohistochemistry for pestiviruses was performed using frozen samples of skin and tongue tissue by two antibodies specific for BVDV and pestiviruses, respectively, as described previously.\(^{15}\)

In field observation performed by hunters and game wardens, 64 out of 85 investigated chamois were declared healthy, whereas pathological changes were reported in 21 animals. Eye
lesions such as corneal ulceration, corneal opacity, mucopurulent discharge or blindness were
found in 11 animals (12.9%), whereas poor body condition of unknown cause was reported in
seven chamois. Figure 1 shows an example of a chamois with severe eye lesions. Furthermore,
pulmonary lesions consistent with pneumonia were seen in 2 chamois. One of these chamois
was found dead and the other was shot due to poor body condition. In addition, 1 chamois found
dead showed abscesses in lung and liver. Details of results from 6 chamois (no. 1, 2, 3, 4, 35 and
82) that were positive for at least 1 test are given in Table 1. All 71 serum samples investigated
were negative for antibodies against C. abortus by Pourquier® ELISA, while in the ID Screen®
ELISA, 1 serum (1.4%) reacted positive (animal no. 4, clinically healthy). Eye swabs (left and
right) from 79 chamois were available for the Chlamydiaceae real-time PCR. Of these, 3 animals
(3.8%) showed positive results. By ArrayTube microarray investigation, the following
chlamydial species could be identified: mixed infection with C. abortus and C. pecorum in one
eye and C. pecorum monoinfection in the other eye of chamois no.1; C. pneumoniae unilateral in
animal no. 2; one eye of chamois no. 3 was positive by real-time PCR for Chlamydiaceae but the
chlamydial species could not be determined by ArrayTube microarray (Table 1). Eye swabs from
76 chamois were analyzed by real-time PCR for Mycoplasma conjunctivae: three chamois had to
be completely excluded from the study due to insufficient quality of the samples for this assay.
Furthermore, for 8/76 animals only 1 eye swab per chamois could be included in this study.
Three out of 76 animals (3.9%) were positive for M. conjunctivae. Animals no. 1 and no. 82
were positive in both eyes, while chamois no. 35 was only positive in 1 eye (Table 1).
Remarkably, in chamois no. 1, a mixed infection with C. abortus, C. pecorum and M.
conjunctivae was found (Table 1). This animal was blind, showed corneal lesions in both eyes
and was emaciated. Chamois no. 35 and 82 showed eye symptoms as well, while chamois no. 2
and 3 were healthy. All organs (n = 135, originating from 42 chamois) and fecal samples (n = 23) were negative by real-time PCR analysis for *Chlamydiaceae*. Among the group of 64 healthy animals, 3 (4.7%) were positive for *Chlamydiaceae* by either PCR or ELISA, while in the group of animals showing eye lesions 3 out of 11 chamois (27.3%) were positive for *Chlamydiaceae* and/or *M. conjunctivae*. All 68 skin and tongue tissue samples of 35 chamois were tested negative for pestivirus antigen by immunohistochemistry.

The occurrence of *C. abortus* antibodies assessed in the present work by two different specific ELISA tests was low (0% and 1.4%). These results are comparable to those recently obtained in Swiss Alpine ibex using the same tests (seroprevalence of 1.5%). The 2 ELISA tests gave slightly different results: when using the Pourquier® ELISA kit all 72 sera were tested negative, while a single serum reacted positive in ID Screen ELISA. The non-congruent results in 1 serum could possibly be explained by the fact that different specific antigens (polymorphic outer membrane protein, POMP, versus major outer membrane protein, MOMP) were used. In contrast to our results, a recent study reported a very high seroprevalence (40%) in Pyrenean chamois (*Rupicapra p. pyrenaica*) from Spain. Those authors used a *C. abortus* specific in-house ELISA directed against POMP which is somewhat comparable to the Pourquier® ELISA used in this study. Hence we conclude that the differing seroprevalences are more likely due to the different geographical localization of the two chamois subspecies than to differing performance of laboratory tests. Using a real-time PCR for the detection of *Chlamydiaceae* in eye swabs, 3 chamois showed positive results, two of them in a single eye and one in both eyes. ArrayTube microarray revealed three different chlamydial species: *C. pecorum, C. abortus* and *C. pneumoniae*. In 1 chamois, the chlamydial species could not be determined whereas in another animal, a mixed infection with *C. abortus* and *C. pecorum* was detected, which is consistent with
the previous finding of four ibexes showing mixed infections by the same chlamydial species in the Surselva region.\textsuperscript{16} Interestingly, 1 chamois was positive for \textit{C. pneumoniae}; this chlamydial species has been detected in an ibex of the same region as well.\textsuperscript{16} Neither the ibex nor the chamois infected with \textit{C. pneumoniae} showed any clinical eye lesions, which is consistent with the findings of a previous study where no association between the presence of chlamydial DNA (\textit{C. abortus, C. pecorum, C. suis}) in the eyes of sheep and the onset of clinical disease was found.\textsuperscript{20} To our knowledge, this is the first description of \textit{C. pneumoniae} in chamois. Pathogenic role and zoonotic potential of \textit{C. pneumoniae} in wild ruminants remains to be elucidated. In this study, we found no correlation between positive PCR results for \textit{Chlamydiaceae} and positive \textit{C. abortus} serology. These findings confirm the results of a previous study, where the authors found no agreement between positive PCR results for \textit{Chlamydia} in the male genital tract and semen of small ruminants and positive serology results for \textit{C. abortus}.\textsuperscript{25} However, good correlation between the presence of \textit{C. abortus} DNA in conjunctival swabs of sheep and seropositivity was reported previously.\textsuperscript{20} All organs (n = 135) and fecal samples (n = 23) analyzed were negative by real-time PCR, indicating that there was neither systemic chlamydial infection nor intestinal infection with fecal shedding. In summary, considering the results obtained by ELISA and real-time PCR, we conclude that, similarly to the situation in Alpine ibex, \textit{C. abortus} is a very rare infectious agent in the Swiss Alpine chamois population. Thus, Alpine chamois are not responsible for the high seroprevalence observed in sheep in Grisons. Further studies are planned to elucidate the situation in red deer (\textit{Cervus elaphus}) and roe deer (\textit{Capreolus c. capreolus}) of Switzerland. In the Swiss Alps, infectious keratoconjunctivitis (IKC) is frequent in free-ranging Alpine chamois and ibex.\textsuperscript{12} IKC describes a contagious medical condition and several infectious agents can be involved. Other microorganisms isolated from ruminants with symptoms of
keratoconjunctivitis were for instance *Moraxella (Branhamella) ovis, Listeria monocytogenes* or *Chlamydia psittaci*, although it is now generally approved that *M. conjunctivae* is the main causing agent of IKC. Simultaneous infections with *M. conjunctivae* and *Chlamydiaeae* have not yet been reported in the literature. In this study, one chamois (animal no.1) showed a mixed infection with *M. conjunctivae* and two *Chlamydiaeae*-species (*C. abortus* and *C. pecorum*). This animal was emaciated and had severe eye symptoms. Although it has been reported that *M. conjunctivae* load in the eyes seems to be responsible for development of IKC, one cannot exclude that clinical symptoms may also be enhanced by simultaneous infection with *M. conjunctivae* and *Chlamydiaeae*. For single infections with *M. conjunctivae*, frequencies of occurrence are difficult to estimate as outbreaks are associated to the presence of infected sheep on Alpine pastures during summer months. In our study, 3 out of 11 chamois affected by ocular disease (27.3%) were positive for *M. conjunctivae*, which is comparable to a previous study, where among a sample of 28 chamois affected by IKC, *M. conjunctivae* was identified in 14 animals (50%) by conventional PCR, but which is inconsistent with more recent studies using real-time PCR whereby the frequency of *M. conjunctivae* in the eyes of IKC-affected animals was reported to be much higher. The presence of pestiviruses in wildlife populations should be monitored in order to make eradication programs successful in the long term. In the United States, where persistently infected (PI) white-tailed deer (*Odocoileus virginianus*) were detected, there is major concern that those animals could endanger a complete eradication of BVDV. In addition, persistent infections have been described in other wild ruminant species such as mouse deer (*Tragulus javanicus*) and eland (*Taurotragus oryx*). During investigations on the reason for population decreases in Pyrenean chamois in the French and Spanish Central Pyrenees, 21 animals found dead or dying were necropsied. Organs of all
chamois were positive for pestivirus by immunohistochemistry.\(^{17}\) As PI animals are important regarding BVD eradication programmes, we used an immunohistochemistry method which is able to detect PI animals. In this study, all skin and tongue tissue samples (n=68), originating of 35 chamois, were negative for BVDV and pestivirus antigen when investigated by immunohistochemistry. The low number of positive samples found in this study contrasts with previous reports, most of them based on serology. For example, 16% seropositive chamois were found in the Central Pyrenees, and 25.5% of the investigated chamois were positive in an Italian study.\(^{2,19}\) There, the authors stated that presence of BDV among extensively kept domestic sheep may be the cause for the high pestivirus antibody prevalence in that chamois population. A recent Swiss study in sheep of Grisons showed a seroprevalence of 32.4 and 33.7% for BVDV-1 and BDV-1, respectively.\(^9\) Although no evidence for persistently infected chamois could be found \textit{in the present study}, the existence of PI chamois in Grisons can not be excluded, as the number of sampled animals was small (35 chamois). In summary, despite the limited number of samples investigated, \textit{it is concluded} that the occurrence of \textit{Chlamydiaceae} and pestiviral infections in chamois of the Surselva region (Grisons, Switzerland) is low and transmission between domestic and wild Caprinae seems unlikely.

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grateful to ID Vet diagnostics for providing of ELISA kits and to the laboratory technical staff of
the Institute of Veterinary Pathology and of the Clinical Laboratory, Vetsuisse Faculty,
University of Zurich for technical help.

Sources and manufacturers

a Roche Diagnostics GmbH, Mannheim, Germany.
b Applied Biosystems, Foster City, CA, USA.
c Clondiag Chip Technologies GmbH, Jena, Germany.
d Institut Pourquier, Montpellier, France.
e ID Vet Innovative Diagnostics, Montpellier, France.

References

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   Microbiol 69:1913-1919.


Table 1. Details of chamois (n = 6) positive by either serology for *Chlamydia abortus* or 
real-time PCR for *Chlamydiaceae* and/or real-time PCR for *Mycoplasma conjunctivae*.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Serology ELISA Pourquier® (% value)</th>
<th>Serology ELISA ID Screen® (% value)</th>
<th>Eye swabs Real-time PCR <em>Chlamydiaceae</em> (ø ct value)</th>
<th>AT species identification assay <em>Chlamydiaceae</em></th>
<th>Eye swabs Real-time PCR <em>M. conjunctivae</em> (ø ct value)</th>
<th>Eye lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n.a.</td>
<td>n.a.</td>
<td>pos (36.2/37.6)*</td>
<td>C. abortus, C. pecorum</td>
<td>pos (24.8/28.6)*</td>
<td>Corneal lesions, blindness*</td>
</tr>
<tr>
<td>2</td>
<td>neg</td>
<td>neg</td>
<td>pos (26.8)</td>
<td>C. pneumoniae</td>
<td>neg</td>
<td>no</td>
</tr>
<tr>
<td>3</td>
<td>neg</td>
<td>neg</td>
<td>pos (28.2)</td>
<td></td>
<td>neg</td>
<td>no</td>
</tr>
<tr>
<td>4</td>
<td>neg</td>
<td>pos (84.6)</td>
<td>neg</td>
<td>n.a.</td>
<td>n.a.</td>
<td>no</td>
</tr>
<tr>
<td>35</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>n.a.</td>
<td>pos (34.5)</td>
<td>no</td>
</tr>
<tr>
<td>82</td>
<td>n.a.</td>
<td>n.a.</td>
<td>neg</td>
<td>n.a.</td>
<td>pos (23.8/24.9)*</td>
<td>Purulent keratoconjunctivitis</td>
</tr>
</tbody>
</table>

3 pos = positive
4 neg = negative
5 n.a. = not available
6 * Both eyes affected
Figure 1. Chamois severely affected by infectious keratoconjunctivitis showing broad corneal ulceration and high amount of mucopurulent exudate.