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ORIGINAL PAPER

Alpine ibex (*Capra i. ibex*) is not a reservoir for chlamydial infections of domestic ruminants and humans

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Abstract *Chlamydophila* (*C.*) *abortus* is the most common infectious abortigenic agent in small domestic ruminants in Switzerland. In contrast, the knowledge about chlamydiae in wild ruminants is scarce. As interactions between livestock and Alpine ibex (*Capra i. ibex*) occur on alpine pastures, the question raises if wild ruminants could play a role as carriers of chlamydiae. Thus, we investigated the prevalence of chlamydiae in Alpine ibex in Switzerland. In total, 624 sera, 676 eye swabs, 84 organ samples and 51 faecal samples from 664 ibex were investigated. Serum samples were tested by two commercial ELISA kits specific for *C. abortus*. Eye swabs, organs and faecal samples were examined by a *Chlamydiaceae*-specific real-time polymerase chain reaction (PCR). Positive cases were further investigated by the ArrayTube (AT) microarray method for chlamydial species determination. Of 624 serum samples investigated, 612 animals were negative, whereas nine sera (1.5%) reacted positively in one of the two tests and three sera showed an inconclusive result. Eye swabs of seven out of 412 ibex (1.7%) were tested positive for *Chlamydiaceae* by real-time PCR. By AT microarray, *Chlamydophila (C.) pecorum* was identified in two animals, *Chlamydophila (C.) pneumoniae* was detected in one animal and a mixed infection with *C. abortus* and *C. pecorum* was found in four animals. Organs and faecal samples were all negative by real-time PCR analysis. In summary, we conclude that *C. abortus* is not a common infectious agent in the Swiss ibex population. To our knowledge, this is the first description of *C. pneumoniae* in ibex. Further studies are necessary to elucidate the situation in other species of wild ruminants as chamois (*Rupicapra r. rupicapra*), red deer (*Cervus elaphus*) and roe deer (*Capreolus c. capreolus*) in Switzerland.

Keywords Alpine ibex · Switzerland · *Chlamydophila abortus* · Abortigenic agent · Interactions between wildlife and domestic ruminants

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Introduction

Chlamydophila (*C.*) *abortus* is the most common infectious abortigenic agent in small domestic ruminants in Switzerland. As reported in a previous study, 39% of examined abortions in sheep and 23% in goats were caused by *C. abortus* (Chanton-Greutmann et al. 2002). In the canton of Grisons (eastern Swiss Alps), economic losses due to chlamydial abortion in small domestic ruminants are significantly higher than in the other cantons

(Grisons, 43% seroprevalence in sheep for *C. abortus*; Borel et al. 2004). Large flocks, extensive animal husbandry and mixing of different sheep flocks during summertime in the mountains enhance the spread of infectious agents. However, seroprevalence for *C. abortus* is much lower in the canton of Valais (western Swiss Alps) although management systems are similar to those in Grisons, suggesting other unknown factors favouring chlamydial abortion in small ruminants in Grisons (Borel et al. 2004). Wild ruminants (especially chamois *Rupicapra r. rupicapra* and Alpine ibex *Capra i. ibex*) graze on the same Alpine summer pastures as domestic sheep and goats. It has been shown that encounters (0–50 m) between domestic and wild ruminants are common events in the Swiss Alps (Ryser-Degiorgis et al. 2002, 2009). In this respect, it must be noted that hybridisation of domestic goats with free-ranging male Alpine ibex was documented (Giacometti et al. 2004). Direct and indirect contacts can facilitate interspecific transmission of infectious agents. For example, using molecular epidemiological markers, interspecific transmission of *Mycoplasma conjunctivae* has been shown to occur between domestic sheep, Alpine ibex and chamois on summer pastures (Belloy et al. 2003). Therefore, the question arises if wild ruminants could play a role in the spread of chlamydioses.

The population of wild ruminants in Switzerland is composed of about 261,000 animals. The most frequent species is the roe deer (*Capreolus c. capreolus*), followed by chamois, red deer (*Cervus elaphus*) and Alpine ibex. The population of wild ruminants in Grisons counts about 60,000 animals (Eidgenössische Jagdstatistik 2008). Around 65,000 wild ruminants (mainly red deer, roe deer and chamois) are hunted per year in Switzerland (11,500 in Grisons). The successful reintroduction of ibex in Switzerland began in 1911 when zoo-born ibexes were released in the canton of St. Gallen (eastern Swiss Alps; Schneider 2006). In 2008, the Swiss Alpine ibex population counted about 16,000 animals, 6,000 of them living in Grisons. As measures to stabilise the stocks have become necessary, about 1,100 ibexes are shot annually in Switzerland, approximately 500 of them in the canton of Grisons.

So far, the knowledge about chlamydiae in wild ruminants is scarce and often based solely on a serological method with low sensitivity and specificity. A recent study indicated a very low prevalence of chlamydial infection in Alpine ibex in Switzerland (Marreros et al., submitted). However, this data based only on a serological study performed with a single ELISA test. Direct evidence of the infectious agent as assessed by DNA amplification methods from eye swabs, faeces and organs has not been performed in wild ungulates so far. The investigation on prevalence and the range of chlamydial species in wild ruminants will elucidate their potential role as a source of infection for domestic ruminants and the potential zoonotic risk for humans having contact to them (i.e. hunters, game keepers). Transmissions of *C. abortus* to humans have been repeatedly reported. This serious and life-threatening zoonosis affects pregnant women after contact with lambing ewes or goats and leads to severe febrile illness in pregnancy and abortion (Longbottom and Coulter 2003). The first Swiss case of human abortion by a zoonotic infection of a pregnant woman with *C. abortus* from a caprine abortion was reported in the canton of Grisons in autumn 2001 (Pospischil et al. 2002).

The aim of the present study was to determine the prevalence of *Chlamydia* in Alpine ibex in Switzerland and particularly in Grisons, using sensitive and specific methods.

Materials

In total, 624 sera, 676 eye swabs (of 412 ibexes), 84 organ samples (of 24 ibexes) and 51 faecal samples originating from a total of 664 Alpine ibexes were investigated (Table 1). Group 1 refers to samples from 517 ibexes collected during the hunting season in autumn 2007 and 2008 from various cantons of Switzerland. From most animals (n=240), swabs from both eyes were available, whereas from 84 ibexes, only one eye was sampled (total number of swabs=564). Group 2 includes samples from 120 ibexes which were live-trapped in different cantons for ecological studies between 2006 and 2008. Of these animals, 101 sera, 65 eye swabs and 30 faecal samples were investigated. None of the animals of groups 1 and 2 did show any eye symptoms. Group 3 stays for samples of 27 ibexes collected in the Surselva region (canton of Grisons) during the hunting season of autumn 2008. All these animals were clinically healthy except one animal showing signs of infectious keratoconjunctivitis in the left eye. From 16 ibexes, the complete sample spectrum was obtained including serum, eye swabs, organs and faecal samples. The most frequently collected organs were liver, lung, kidney and genital tract. In 11 animals, only limited samples were available (details are given in Table 1). Animals from Groups 1 and 2 were already used in a previous study on abortive agents (ELISA; Marreros et al., submitted), they originate from various colonies throughout the Swiss Alps. Animals from Group 3 are from a geographical region considered as a risk area for chlamydial infections according to studies performed in domestic sheep (Borel et al. 2004).

Table 1 Details of examined samples, originating of 664 ibexes

Origin of the samples	Animals per canton	Serum samples	Eye swabs	Faecal samples	Organ samples
Hunting season	GR *n*=291	1–499	1–306, 500–517	n.a.	n.a.
2007–2008	VS *n*=118	*n*=499	*n*=324		
FIWI Bern	VD *n*=41				
(Group 1)	SG *n*=35				
	BE *n*=31				
	NW *n*=1				
	(*n*=517)				
Live-trapped	VD *n*=66	518–618	541–586, 619-637	569–598	n.a.
Animals	GR *n*=52	*n*=101	*n*=65	*n*=30	
2006–2008	UR *n*=2				
FIWI Bern	(*n*=120)				
(Group 2)					
Surselva	GR *n*=27	638–661	638–658, 663–664	638–653, 658–661, 664	638–656, 659–663
Hunting Season	*n*=27	*n*=24	*n*=23	*n*=21	*n*=24
2008					
(Group 3)					
Total	664	624	412	51	24

GR Grisons, *VS* Valais, *VD* Vaud, *SG* St. Gallen, *BE* Bern, *NW* Nidwalden, *UR* Uri, *n.a.* not available

Methods

DNA extraction

Organ samples and eye swabs were pretreated using a lysis buffer (0.0125 M EDTA, 0.625% Tween 20, 0.0625 M Tris/HCl pH8.0, 0.2 mg/ml Proteinase K (recombinant, PCR Grade, Roche Diagnostics GmbH, Mannheim, Germany)). Approximately 10 mg of fresh organ material or an eye swab was suspended in 400 µl lysis buffer and incubated at 55°C with rotation (550 rpm) overnight, using a thermomixer.

DNA was extracted from 200 µl lysed samples using the MagNA Pure® LC System (Roche Diagnostics, Mannheim, Germany), an automated extraction method, according to the manufacturer's instructions.

Real-time PCR assay for *Chlamydiaceae*

All samples were examined on an ABI 7500 instrument (Applied Biosystems, Foster City, CA, USA) using the 23S rRNA gene-based *Chlamydiaceae* family-specific real-time PCR as described previously (Ehricht et al. 2006). A cycle threshold (Ct value) of <38.00 was considered as positive, and all samples were tested at least 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.

ArrayTube microarray identification of chlamydial species

The samples with at least one positive Ct value were examined using the species-specific 23S ArrayTube (AT) microarray assay as described by Borel et al. (2008).

Antibody assays

The serum samples were tested with two commercial antibody-detecting ELISA assays specific for *C. abortus*:

1. *C. abortus* ELISA (Institut Pourquier, Montpellier, France), validated for sheep, goats and cattle: the test was performed according to the manufacturer's instructions. The final values were determined as 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*.
2. ID Screen® *Chlamydia abortus* indirect ELISA (ID Vet Innovative Diagnostics, Montpellier, France), validated for ruminants, horses and swine. Determination and interpretation of final values were performed as described in (1).

For ibex sera, reference values for goats were applied with both ELISA assays.

Results

Details of positive results are given in Table 2.

Serum samples

Of 624 serum samples investigated, 612 animals were tested negative in both ELISA tests, whereas nine sera (1.5%) reacted positive in one of the two ELISA tests performed. Of these, six animals (1.0%) showed a positive reaction in the Pourquier ELISA only, while three other sera (0.5%) revealed positive results using the ID Screen® ELISA. By Pourquier ELISA, three additional sera showed an inconclusive result.

Eye swabs

Out of 412 ibexes, seven (1.7%) showed positive results in real-time PCR for *Chlamydiaceae* in at least one eye. Using the ArrayTube microarray, *Chlamydophila (C.) pecorum* was identified in two animals, *Chlamydophila (C.) pneumoniae* was detected in one animal and a mixed infection with *C. abortus* and *C. pecorum* was found in four animals. All the positive results were limited to one eye, except of one case with a mixed infection (*C. pecorum* and *C. abortus*) which affected both eyes of ibex No. 3 (Fig. 1).

Organs and faecal samples

Organs (n=84, originating of 24 ibexes) and faecal samples (n=51) were all negative by real-time PCR analysis.

Discussion

The aim of this study was to investigate the prevalence of *C. abortus* in Alpine ibex in Switzerland, in particular in a risk area in Grisons. Seroprevalence for *C. abortus* was very low (1.5%) adding the results of the two different specific ELISA tests. These results confirm those previously obtained (Marreros et al., submitted) and are in contrast with the findings of previous studies in wild ruminants in Switzerland and other countries. High seroprevalence (over 50%) of *Chlamydia* was determined by complement fixation test (CFT) in white-tailed deer (*Odocoileus virginianus*) in USA and Canada (Debbie 1967). A study from Tuscany (Italy) reported 79% of *Chlamydia* (*C.*) *psittaci* seropositive fallow deer (*Dama dama*; Giovannini et al. 1988). In Poland, cross transmission between free-ranging European bisons (*Bison bonasus*) and cattle was suggested as 28 out of 60 bisons were tested serologically positive for *Chlamydia* (Kita and Anusz 1991). In Switzerland (colony Albris, canton of Grisons), 31% of sera from clinically healthy Alpine ibex were found positive by CFT for *C. psittaci* (Giacometti et al. 1995). A sero-epidemiological study of chlamydial infections on 1,244 wild ruminant sera in Spain (CFT) showed 37% seroprevalence in mouflon (*Ovis aries orientalis*), 30%

Table 2 Details of ibexes (n=16) positive by either serology for *C. abortus* or real-time PCR for *Chlamydiaceae*

Case no. (Group no.)	Serology Canton	ELISA Pourquier (% Value)	ELISA ID screen (% Value)	Eye swabs Real-time PCR (ø ct value)	AT species identification assay
1 (1)	Grisons	neg (0.81)	neg (12.12)	pos (35.5)	*C. pecorum*
2 (1)	Grisons	neg (0.51)	neg (12.88)	pos (34.0)	*C. pecorum*
3 (1)	Grisons	neg (0.81)	neg (15.18)	pos (23.8/24.9)[a]	*C. abortus*, *C. pecorum*
4 (1)	Grisons	neg (1.19)	neg (12.23)	pos (22.3)	*C. abortus*, *C. pecorum*
5 (1)	Valais	neg (1.75)	neg (13.01)	pos (34.4)	*C. abortus*, *C. pecorum*
256 (1)	St. Gallen	neg (1.16)	neg (12.46)	pos (36.9)	*C. abortus*, *C. pecorum*
648 (3)	Grisons	neg (1.13)	neg (13.58)	pos (36.6)	*C. pneumoniae*
6 (1)	Grisons	neg (0)	pos (63.34)	neg	
265 (1)	Vaud	pos (90.64)	neg (10.81)	neg	
587 (2)	Vaud	pos (73.18)	neg (12.69)	neg	
642 (3)	Grisons	pos (60.8)	neg (21.23)	neg	
214 (1)	Valais	neg (1.66)	pos (136.16)	n.a	
475 (1)	Vaud	pos (62.15)	neg (11.52)	n.a	
483 (1)	Bern	pos (81.32)	neg (11.9)	n.a	
484 (1)	Bern	pos (111.46)	neg (14.78)	n.a	
518 (2)	Grisons	neg (10.96)	pos (96.46)	n.a	

pos positive, *neg* negative, *n.a.* not available

[a] Both eyes positive

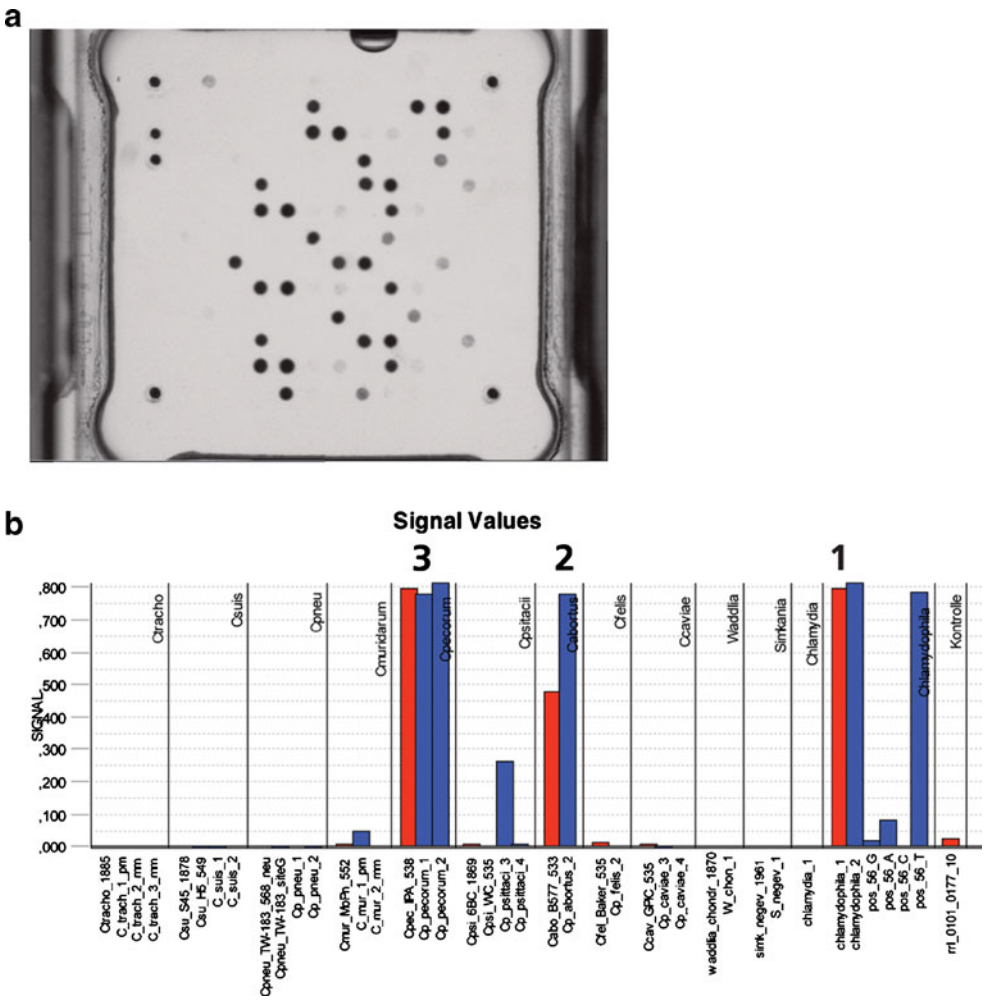

Fig. 1 DNA-based examination of the eye swab (right eye) from ibex No. 3 using ArrayTube species identification assay. **a** Microarray image. **b** Barplot showing specific signals for genus *Chlamydophila* (*1*) and species *C. abortus* (*2*) and *C. pecorum* (*3*)

in fallow deer and 24% in both red deer and Iberian ibex (*Capra pyrenaica hispanica*) (Cubero-Pablo et al. 2000). The prevalence rates were significantly higher in wild ruminants inhabiting the peripheral region of the study area, which simultaneously served as pasture for sheep and goats, while in the central areas, where no domestic ruminants were present, the prevalence was lower. According to these findings, an inter-transmission of *Chlamydia* sp. between wild and domestic ruminants was concluded. A French study investigated the influence of bacterial abortive infections on reproduction success in Alpine chamois. The prevalence of antibodies against *Salmonella enterica* serovar Abortusovis, *Chlamydophila abortus* and *Coxiella burnetii* were found to explain 36% of the annual variation in reproductive success of the population (Pioz et al. 2008a). In a follow-up study, the relationship between the serological status concerning the three bacteria and the reproductive success was investigated. It was shown that females with high antibody-titers against *Salmonella* underwent a decrease in their reproductive success, while antibody-titers against *Chlamydophila* and *Coxiella* were not related to the reproductive success of female chamois (Pioz et al. 2008b). The CFT used in the above mentioned studies is of inferior sensitivity and specificity and cross-reactivity to other species of the family *Chlamydiaceae* is well known (Markey et al. 1993; Donn et al. 1997; Jones et al. 1997; Rodolakis et al. 1998). Thus, false positive results due to cross-reaction with *C. pecorum*, a widely spread bacterium in domestic ruminants, could have led to such high seroprevalence results. In contrast, in the present study, false positive results were avoided applying ELISA assays specific for *C. abortus*. Therefore, seroprevalence estimates are more precise in our study compared to previous reports.

We applied two different ELISA tests: the Pourquier and the ID Screen ELISA, with variable results. Although both tests revealed a similarly low seroprevalence for *C. abortus* (1.0% by Pourquier ELISA and 0.5% by ID Screen ELISA), none of the animals showed a positive result in both assays. Non-congruent results could possibly be explained by the fact that different specific antigens were used in the two tests. The Pourquier ELISA is based on a recombinant fragment of polymorphic outer membrane protein (POMP), while in the ID Screen ELISA, a fragment of major outer membrane protein (MOMP) is applied. The

Pourquier ELISA is a highly specific test for the detection of *C. abortus* infections, but it is known to be of inferior sensitivity when used on field sera, compared to sera of experimentally infected animals (Wilson et al. 2009). The authors state that the choice of recombinant POMP fragment has a great impact on specificity and sensitivity of the test, as in a previous study wide variation was demonstrated when assessing overlapping recombinant fragments of the POMP90 protein (Longbottom et al. 2002). No current published data is available on the test performance of the ID Screen ELISA, making comparisons difficult. However, its antigen MOMP is used in a competitive ELISA as well, which proved to be a sensitive and specific test if applied on sheep and goat sera (Salti-Montesanto et al. 1997).

In contrast to the studies cited before, a recent study (Salinas et. al. 2009) applied an in-house ELISA specific for *C. abortus* directed against POMP. Thus, results of this study can be compared with our findings. Sera from 434 Spanish wild ungulates of eight species including red deer, roe deer, Pyrenean chamois (*Rupicapra p. pyrenaica*) and Iberian ibex revealed a seroprevalence of 18.9%. Of these, the Iberian ibex was the only wild ungulate species which showed no positive serum for *C. abortus* (30 sera). This finding is comparable to the low seroprevalence (1.5%) found in Alpine ibex in our study.

Using a real-time PCR for the detection of *Chlamydiaceae*, seven ibexes showed positive results in eye swabs, six of them in a single eye and one in both. AT microarray revealed three different chlamydial species: *C. pecorum* (n=6), *C. abortus* (n=4) and *C. pneumoniae* (n=1). Surprisingly, a mixed infection with *C. abortus* and *C. pecorum* was found in four ibexes.

Ocular infections by chlamydiae are associated with ocular disease manifestations like conjunctivitis or keratitis in humans and animals. A recent study by Polkinghorne et al. (2009) found no association between the presence of chlamydial DNA in the eyes of sheep and onset of clinical disease. The authors suggest that the biodiversity of chlamydiae in the eyes of sheep is greater than previously thought and state that further investigations will be necessary to determine whether a causal relationship between infection by chlamydiae and ocular disease exists in these animals. *Chlamydia* sp. was isolated from the conjunctiva of two out of seven free-ranging mule deer (*Odocoileus hemionus*) with infectious keratoconjunctivitis (IKC) in a National Park, Utah, USA (Taylor et al. 1996). Furthermore, *Chlamydia* sp. was detected in the eyes of Pyrenean chamois (Tournut et al. 1985) and bighorn sheep (*Ovis canadensis*) of Yellowstone National Park, WY, USA, affected by IKC (Meagher et al. 1992). In contrast, following a severe infectious keratoconjunctivitis (IKC) epizootic in free-ranging Alpine ibex in Grisons in 1993, conjunctival swabs of 15 affected animals examined were negative for *Chlamydia* (Mayer et al. 1997).

Knowledge about mixed infections with different chlamydial species in vivo is scarce. New methods such as the ArrayTube microarray System are able to detect mixed infections as shown in a previous study, where simultaneous infections with *C. abortus* and *C. pecorum* were demonstrated in 16 conjunctival and nasal swabs of calves (Borel et al. 2008).

Interestingly, one ibex originating from the Surselva region (canton of Grisons) was positive for *C. pneumoniae*. To our knowledge, this is the first description of *C. pneumoniae* in ibex. In the past, *C. pneumoniae* was assumed to infect exclusively humans, being transmitted by aerosol and causing acute and chronic respiratory disease (Saikku 1992). Recently, *C. pneumoniae* was shown to have the widest host range of all chlamydial species including mammals as horses and marsupials (particularly koalas), but also amphibians and reptiles (Bodetti et al. 2002). In contrast to *C. abortus*, transmission of *C. pneumoniae* between animals and humans has not yet been documented. In a recent study based on genomic and phylogenetic evidence, the authors hypothesise that *C. pneumoniae* was originally derived from an animal source (Myers et al. 2009).

In our study, positive PCR results did not correlate with positive serology. These findings are consistent with a previous study, where no agreement was found between positive PCR results for *Chlamydia* in the male genital tract and semen of small ruminants, and positive serology results for *C. abortus* (Teankum et al. 2007). In contrast, the aforementioned study by Polkinghorne et al. (2009) found good correlation between the presence of *C. abortus* DNA in conjunctival swabs of sheep and seropositivity.

All organ (n=84) and faecal samples (n=51) analysed were negative by real-time PCR, indicating *C. abortus* having a strong tropism for eye infections.

The positive results obtained by PCR and ELISA were equally distributed among the three groups and there was no significant difference in prevalence observed between the cantons of Grisons and Valais (Marreros et al., submitted; this study) as it was described previously in sheep (Borel et al. 2004). In domestic ruminants, it is well known that antibody titers are highest during lambing period and also direct antigen detection by PCR should be performed around birth time. However, most of the samples in the present study (groups 1 and 3) were collected in autumn during the hunting season. This could possibly have caused an underdetection of chlamydial infections.

In summary, considering the results obtained by ELISA and real-time PCR, we conclude that *C. abortus* is a very rare infectious agent in the Swiss Alpine ibex population.

Hence, there is limited evidence that Swiss Alpine ibex could act as a reservoir for *C. abortus* in domestic small ruminants, and transmission of *C. abortus* between ibexes and domestic ruminants seems hardly to occur. Thus, Alpine ibex are not responsible for the high seroprevalence observed in sheep in Grisons. Further studies are necessary to elucidate the situation in the other species of wild ruminants (chamois, red deer and roe deer) in Switzerland.

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