Epizootiologic investigations of selected abortive agents in free-ranging alpine ibex (capra ibex ibex) in Switzerland

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Epizootiologic investigations of selected abortive agents in free-ranging alpine ibex (Capra ibex ibex) in Switzerland

Abstract

In the early 2000s, several colonies of Alpine ibex (Capra ibex ibex) in Switzerland ceased growing or began to decrease. Reproductive problems due to infections with abortive agents might have negatively affected recruitment. We assessed the presence of selected agents of abortion in Alpine ibex by serologic, molecular, and culture techniques and evaluated whether infection with these agents might have affected population densities. Blood and fecal samples were collected from 651 ibex in 14 colonies throughout the Swiss Alps between 2006 and 2008. All samples were negative for Salmonella spp., Neospora caninum, and Bovine Herpesvirus-1. Antibodies to Coxiella burnetii, Leptospira spp., Chlamydophila abortus, Toxoplasma gondii, and Bovine Viral Diarrhea virus were detected in at least one ibex. Positive serologic results for Brucella spp. likely were false. Overall, 73 samples (11.2%) were antibody-positive for at least one abortive agent. Prevalence was highest for Leptospira spp. (7.9%, 95% CI 55.0-11.7). The low prevalences and the absence of significant differences between colonies with opposite population trends suggest these pathogens do not play a significant role in the population dynamics of Swiss ibex. Alpine ibex do not seem to be a reservoir for these abortive agents or an important source of infection for domestic livestock in Switzerland. Finally, although interactions on summer pastures occur frequently, spillover from infected livestock to free-ranging ibex apparently is uncommon. Key words: Abortive agents, Alpine ibex, Capra ibex ibex, population dynamics, serology, survey, Switzerland.
EPIZOOTIOLOGIC INVESTIGATIONS OF SELECTED ABORTIVE AGENTS IN FREE-RANGING ALPINE IBEX (CAPRA IBEX IBEX) IN SWITZERLAND

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ABSTRACT: In the early 2000s, several colonies of Alpine ibex (Capra ibex ibex) in Switzerland ceased growing or began to decrease. Reproductive problems due to infections with abortive agents might have negatively affected recruitment. We assessed the presence of selected agents of abortion in Alpine ibex by serologic, molecular, and culture techniques and evaluated whether infection with these agents might have affected population densities. Blood and fecal samples were collected from 651 ibex in 14 colonies throughout the Swiss Alps between 2006 and 2008. All samples were negative for Salmonella spp., Neospora caninum, and Bovine Herpesvirus-1. Antibodies to Coxiella burnetii, Leptospira spp., Chlamydia abortus, Toxoplasma gondii, and Bovine Viral Diarrhea virus were detected in at least one ibex. Positive serologic results for Brucella spp. likely were false. Overall, 73 samples (11.2%) were antibody-positive for at least one abortive agent. Prevalence was highest for Leptospira spp. (7.9%, 95% CI=5.0–11.7). The low prevalences and the absence of significant differences between colonies with opposite population trends suggest these pathogens do not play a significant role in the population dynamics of Swiss ibex. Alpine ibex do not seem to be a reservoir for these abortive agents or an important source of infection for domestic livestock in Switzerland. Finally, although interactions on summer pastures occur frequently, spillover from infected livestock to free-ranging ibex apparently is uncommon.

Key words: Abortive agents, Alpine ibex, Capra ibex ibex, population dynamics, serology, survey, Switzerland.

INTRODUCTION

The Alpine ibex (Capra ibex ibex) disappeared from the Alpine massif between the 16th and 19th century, except in the Gran Paradiso National Park, Italy. In Switzerland, the last ibex were extirpated between 1800 and 1850, and the first reintroductions took place in 1911. Eighty years later, the Swiss ibex population was estimated at 14,000. Reintroduction of ibex to the Swiss Alps was successful, and since 1977, ibex have been subject to controlled hunting (Hindenland and Nievergelt, 1995). Recently, hunting authorities reported a cessation of growth or decrease in numbers of Alpine ibex in some colonies, despite adaptive hunting management strategies (Baumgartner, 2005). Because no abnormal mortality had been observed in these colonies, the question was raised whether reproductive
problems due to infections with abortive agents might be influencing recruitment negatively.

The pathogenic potential of abortive agents is generally poorly documented in nondomestic ruminants. However, studies in captive (Berri et al., 2004; Kreeger et al., 2004; Passler et al., 2009; Lloyd et al., 2010) or free-ranging ruminants (Gondim, 2006; Pioz et al., 2007, 2008) have suggested reduced reproductive success related to abortive agents. In contrast, infections with abortive pathogens are well-documented for domestic animals in Switzerland. Coxiella burnetii, Leptospira spp., Chlamyphila abortus, Salmonella enterica subspecies enterica serovar Abortusovis (S. Abortusovis), Salmonella spp., Toxoplasma gondii, Neospora caninum, and the Bovine Viral Diarrhea virus (BVDV, a pestivirus) are abortive agents known to circulate in domestic livestock (Luginbühl, 2009; Wirz-Dittus et al., 2010b). Antibody prevalences are high for most agents in cattle (60% for C. burnetii: Ochs, 2007; 19% for Leptospira spp.: Hässig and Lubsen, 1998; 45.6% for T. gondii: Berger-Schoch, et al., 2011; 11.5% for N. caninum: Gottstein et al., 1998; 57.6% for BVDV: Rüfenacht et al., 2000) and in sheep (18% [up to 40% in some areas] for C. abortus: Borel et al., 2004; 61.6% for T. gondii: Berger-Schoch, et al., 2011). According to veterinary monitoring and surveillance, Swiss domestic livestock is officially free of Bovine Herpesvirus 1 (BHV-1), Brucella melitensis, and Brucella abortus (Luginbühl, 2009). An eradication program for BVDV was initiated in 2008 in Swiss cattle (Zimmerli et al., 2009).

Infections caused by most of these abortive agents are notifiable in Switzerland, and wildlife species might be infected by, or even function as reservoirs for, some of these pathogens (Frölich et al., 2002; Simpson, 2002; Madariaga, 2005; Gortázar, et al., 2007). Alpine ibex were found antibody-positive for C. burnetii and Leptospira spp. (Rozo, 1995), S. Abortusovis (Bourgogne, 1990), C. abortus (formerly Chlamydia psittaci serotype 1; Genero et al., 1993; Giacometti et al., 1995), T. gondii (Burnet, 2007), N. caninum (Ferroglio et al., 2001) and pestivirus (Genero et al., 1993) in regions of the Alps. Ferroglio et al. (1998) reported a clinical case of brucellosis in an Alpine ibex in Italy. However, none of these authors investigated whether these pathogens could have an influence on the dynamics of ibex populations. Our goal was to assess infection status of Alpine ibex for selected abortive agents and evaluate whether infectious abortions might have affected ibex population trends.

MATERIALS AND METHODS

Study area

We studied ibex from 14 colonies (biological units: A to N, Fig. 1) in five cantons (political units: Valais, Bern, Vaud, Grisons, St. Gallen, Fig. 1) throughout the Swiss Alps (45°49' to 47°49'N, 5°58' to 10°29'E). We defined a colony as a large group of ibex having regular social contacts, which can be considered as an epizootiologic unit (Ryser-Degiorgis et al., 2009). According to the population censuses from 1998 to 2007, colonies were classified into four categories (Fig. 1): 1) Declining colonies = those experiencing a population decrease in the years 2001–2002 and no recovery since then, despite adapted management strategies; 2) Recovered colonies = those that experienced a decrease in 2001–2002 with subsequent recovery; 3) Stable colonies = those that experienced no population decrease; and 4) Unclear colonies = no clear trends observed in population dynamics due to strong variations in the population censuses.

Animals and samples

From 2006 to 2008, 651 ibex were sampled (344 males, 305 females, and two of unknown sex). Blood samples were obtained from the heart or thoracic cavity of dead ibex or by jugular venipuncture in living ibex and placed into tubes with and without anticoagulant (tripotassic ethylene diamine tetraacetic acid; EDTA). Fecal swabs were taken rectally or from feces collected from the rectum or the ground. Samples were obtained from 526 ibex.
shot during the hunting season, 16 found dead or shot because of disease and submitted to necropsy, 98 living ibex caught with or without subsequent anesthesia (Abderhalden et al., 1998), and 11 nonimmobilized ibex remotely sexed and aged with a telescope (Table 1). Ibex were aged and grouped into five age classes (Ryser-Degiorgis et al., 2009). There were seven kids (<1 yr old), 47 yearlings (1 yr old), 158 young adults (2–3 yr old), 288 middle-aged adults (4–10 yr old), and 140 old adults (>11 yr old). Exact age information was missing for nine adults, and no age data were available for two ibex.

Laboratory analysis

Sera were centrifuged at 2,000 \( \times G \) for 20 min, immediately after arrival at the laboratory (hunted ibex) or within 12 hr after collection (captures, necropsies). Blood and serum samples in EDTA were stored at \( -20^\circ C \) until laboratory analysis. One aliquot of each serum showing no or only moderate hemolysis

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**Table 1.** Number of sampled Alpine ibex (Capra ibex ibex) in Switzerland originating from colonies A to N collected in 2006–2008 and of samples analyzed for abortive infectious agents. Ibex were shot during hunting season (hunted), sampled alive (alive), or shot because of disease symptoms and sent for pathologic examination (diseased).

<table>
<thead>
<tr>
<th>Group</th>
<th>Origin</th>
<th>No. ibex</th>
<th>Blood samples</th>
<th>Fecal swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Hunted</td>
<td>All colonies</td>
<td>526</td>
<td>260</td>
<td>258</td>
</tr>
<tr>
<td>Alive</td>
<td>A, C, D, F, K</td>
<td>109</td>
<td>61</td>
<td>37</td>
</tr>
<tr>
<td>Diseased</td>
<td>F, I, K, L</td>
<td>16</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>651</td>
<td>331</td>
<td>295</td>
</tr>
</tbody>
</table>

\(^a\) Two blood samples were taken from ibex of unknown sex.

\(^b\) Fecal swabs from hunted ibex originated from colonies A, B, C, F, and N.

\(^c\) Fecal swabs from living ibex originated from colonies F and K.

\(^d\) Blood sample from diseased ibex originated from colonies F, K, and L.
was kept unfrozen for serologic testing for *Leptospira* spp.

Table 2 shows the list of agents and the tests used to demonstrate active or former infection. Commercially available tests were performed according to the manufacturer's instructions. Fecal swabs were used for culture of *Salmonella* spp. In order to increase sensitivity of the culture, a selective enrichment for *Salmonella* spp. was performed prior to culture (World Organisation for Animal Health, 2005; Table 2).

Sera with noninterpretable results for *Brucella* spp. by the rose bengal test (RBT) were filtered using a sterile 0.2-μm pore membrane (Acrodisc® Syringe Filter, Pall Corporation, Ann Arbor, Michigan, USA) to remove interfering debris and tested again. Sera with positive or unclear reactions in the RBT also were tested using a commercial indirect enzyme-linked immunosorbent assay (iELISA). We extracted DNA from 199 of 200 randomly selected blood samples and tested them for *Brucella* spp. by polymerase chain reaction (PCR). Of these, 95 showed noninterpretable results in the RBT, and two were positive by ELISA. Whenever a confirmation test was applied, the calculated seroprevalence was based on these results.

**Statistical analysis**

We determined the sample size necessary for detection of positive individuals in the ibex population according to Cannon and Roe (1982) using WinEpiscope 2.0 software. Doubtful and noninterpretable test results were not included in statistical analyses. Due to low sample size (*n*=2), ibex originating from colony N also were excluded from calculations. The two-tailed Fisher's exact test was used to test differences in prevalence between colonies. The Fisher's exact test and calculation of confidence intervals (CI) of estimated prevalences were performed using NCSS 2007 software (NCSS, LLC, Kaysville, Utah, USA). Statistical significance was set at *P*<0.05.

**RESULTS**

All samples were negative for *C. burnetii* (PCR), *Brucella* spp. (PCR), S. Abortusovis, *Salmonella* spp., *N. caninum*, and BHV-1 (Table 3). All interpretable results for *Brucella* spp. (RBT, 339 of 572 samples) were negative. There was at least one antibody-positive result for *C. burnetii* (ELISA), *Leptospira* spp., *C. abortus*, *B. melitensis/B. abortus* (ELISA), *T. gondii* (ELISA and indirect fluorescent antibody test), and BVDV (ELISA and serum neutralization test). All confirmatory and PCR tests revealed lower prevalences than screening tests. Antibody-positive reactions against *Leptospira* spp. included all tested serovars. Thirteen ibex were positive for only one serovar (Tarassovi, Pomona, Bataviae, Australis, Bratislava, or Autumnalis). Three ibex were simultaneously positive for two serovars, and six were positive for six or more serovars. Titers ranged from 1:100 to 1:300. Overall, 73 of 651 tested ibex (11.2%) had antibody to at least one of the selected abortive agents. One ibex simultaneously was antibody-positive for BVDV and *L. Bataviae*, and another for BVDV and *L. Australis*. No ibex had antibodies to more than two agents.

**Effect of age**

Except for three ibex (two yearlings, one of which had antibodies to *C. burnetii* and the other to BVDV, and one ibex of unknown age, which had antibodies to BVDV), all ibex antibody-positive for any of the selected agents were adult, with 60 of 72 (83.3%) antibody-positive animals in the middle-age or old-age category. Overall, antibody prevalence was significantly higher in these two categories compared to younger animals (*P*=0.001). This difference also was significant when considering BVDV only (*P*=0.042).

**Distribution among colonies**

Ibex had antibodies to *Leptospira* spp. in eight colonies, BVDV in seven, *C. abortus* in four, *C. burnetii* in three, and *T. gondii* in two colonies (Table 4). Antibody-positive ibex originated from three declining colonies, four recovered, one stable, and three unclear colonies. All ibex originating from colony E (stable) and colonies G and M (unclear) were negative for all tested agents. Prevalences differed
Table 2. Summary of methods used to detect evidence of contact with abortive agents in Alpine ibex between 2006–2008, showing sample type, threshold for serology (if quantitative or different from reference), secondary antibodies (if different from reference), and media used for culture.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Sample</th>
<th>Assay</th>
<th>Detects</th>
<th>Name of commercial kit, antigen, secondary antibody, culture media, cut-off values (serology)</th>
<th>Manufacturer or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB</td>
<td>Serum</td>
<td>iELISA</td>
<td>Ab</td>
<td>CHEKIT* Q-Fever</td>
<td>IDEXX Laboratories B.V., Schiphol-Rijk, the Netherlands</td>
</tr>
<tr>
<td>CB</td>
<td>EDTA-blood</td>
<td>PCR</td>
<td>Bacterial genome</td>
<td></td>
<td>Willems et al., 1994</td>
</tr>
<tr>
<td>LS</td>
<td>Serum</td>
<td>MAT</td>
<td>Ab</td>
<td>C. abortus ELISA</td>
<td>World Organisation for Animal Health (OIE), 2005</td>
</tr>
<tr>
<td>CA</td>
<td>Serum</td>
<td>iELISA</td>
<td>Ab</td>
<td>C. abortus ELISA</td>
<td>Institut Pourquier, Montpellier, France</td>
</tr>
<tr>
<td>BM/BA</td>
<td>Serum</td>
<td>RBT</td>
<td>Ab</td>
<td>75 µl of serum; brucellosis antigen rose bengal; positive control for brucellosis; negative control for brucellosis</td>
<td>Institut Pourquier, Montpellier, France; OIE, 2005</td>
</tr>
<tr>
<td>BM/BA</td>
<td>Serum</td>
<td>iELISA</td>
<td>Ab</td>
<td>CHEKIT* Brucellose Serum</td>
<td>IDEXX Laboratories B.V., Schiphol-Rijk, the Netherlands</td>
</tr>
<tr>
<td>BS</td>
<td>EDTA-blood</td>
<td>PCR</td>
<td>Bacterial genome</td>
<td></td>
<td>Hinic et al., 2009</td>
</tr>
<tr>
<td>SA</td>
<td>Serum</td>
<td>iELISA</td>
<td>Ab</td>
<td>Tetrathonate broth incubated at 37 C for 24 hr; brilliant green agar and brilliance salmonella agar, incubated at 37 C for 24 hr, biochemical tests, determination of serotype by agglutination</td>
<td>Institut Pourquier, Montpellier, France; OIE, 2005</td>
</tr>
<tr>
<td>SS</td>
<td>Fecal swabs</td>
<td>Culture</td>
<td>Whole organism</td>
<td></td>
<td>Oxoid, Wesel, Germany; Kauffmann-White scheme, Institut Pasteur, Paris Cedex, France; Murray et al., 2003</td>
</tr>
<tr>
<td>TG</td>
<td>Serum</td>
<td>iELISA</td>
<td>Ab</td>
<td>Native, affinity-purified T. gondii P30 antigen; alkaline phosphatase labeled rabbit-anti-goat IgG (Cat. A4187)</td>
<td>SR2B, Avrilhé, France; Sager et al., 2003; Gottstein et al., 1998; Sigma, Buchs, Switzerland</td>
</tr>
<tr>
<td>TG</td>
<td>Serum</td>
<td>IFAT</td>
<td>Ab</td>
<td>Tachyzoites obtained from cell cultures; Fluorescein-Isothiocyanate-conjugated rabbit-anti-goat IgG antibody (Cat. F2016)</td>
<td>Gottstein et al., 1998; Sigma, Buchs, Switzerland</td>
</tr>
<tr>
<td>NC</td>
<td>Serum</td>
<td>iELISA</td>
<td>Ab</td>
<td>Soluble somatic Nc1-tachyzoite lysate; alkaline phosphatase-labeled rabbit-anti-goat IgG (Cat. A4187)</td>
<td>Gottstein et al., 1998; Sigma, Buchs, Switzerland</td>
</tr>
<tr>
<td>NC</td>
<td>Serum</td>
<td>IFAT</td>
<td>Ab</td>
<td>Tachyzoites obtained from cell cultures; Fluorescein-Isothiocyanate-conjugated rabbit-anti-goat IgG antibody (Cat. F2016)</td>
<td>Gottstein et al., 1998; Sigma, Buchs, Switzerland</td>
</tr>
<tr>
<td>BVDV</td>
<td>Serum</td>
<td>biELISA</td>
<td>Ab</td>
<td>Optical density, 450 nm = 0.5</td>
<td>Canal et al., 1998</td>
</tr>
<tr>
<td>BVDV</td>
<td>Serum</td>
<td>SNT</td>
<td>Ab</td>
<td>Titer values ≥1:8</td>
<td>Steck et al., 1980; Spearman, 1908; Kaerber, 1931; Köppel, 2006; Danuser et al., 2009</td>
</tr>
<tr>
<td>BHV-1</td>
<td>Serum</td>
<td>biELISA</td>
<td>Ab</td>
<td>HerdCheck* IBRgB</td>
<td>IDEXX Switzerland AG</td>
</tr>
</tbody>
</table>
at both cantonal and at colony levels (Table 5). We found no relationship between prevalence of infection and trends in population dynamics.

**DISCUSSION**

To our knowledge, this is the first survey for a broad spectrum of abortive agents in free-ranging Alpine ibex and the first study of the relationship between infection status and population dynamics in this species. Our sample included a large number of colonies in the Swiss Alps, and the results provide a good overview of the situation in ibex in Switzerland.

As is the case for numerous wildlife species, commercial tests are not validated for Alpine ibex, and we cannot exclude the possibility that sensitivity or specificity of the tests we used were lower than described for domestic ruminants. However, serologic cross-reactions have been demonstrated between closely related wild and domestic ungulate species, including between domestic goat and Spanish ibex (*Capra pyrenaica hispanica*; Lastras et al., 2000). Alpine ibex are closely related to domestic goats (Giacometti et al., 2004) and secondary antibodies specific against sheep and goat IgG were successfully applied on Alpine ibex sera (Degiorgis et al., 2000). Serologic tests used in this study were validated for goats or sheep except for the BHV-1 blocking ELISA. Nevertheless, results from blocking ELISAs, as well as from microagglutination tests, cultures, and PCR, are not expected to be influenced by the host species. Therefore, these tests are expected to provide reliable results when applied to ibex samples.

**Prevalences of infection and interactions with domestic livestock**

Our study indicates that most infectious agents we surveyed are not widespread in the Swiss Alpine ibex population. Given the low prevalences in comparison to Swiss domestic livestock, Alpine ibex cannot be considered as a reservoir or a
relevant infection source for these agents. Although most of these pathogens are widespread in domestic livestock (Gottstein et al., 1998; Rüfenacht et al., 2000; Borel et al., 2004; Luginbühl, 2009; Berger-Schoch et al., 2011) and interactions between livestock and ibex occur on Alpine pastures (Ryser-Degiorgis et al., 2002, 2009), spillover from domestic animals seems to be infrequent.

The reason for higher prevalences of antibody to *C. burnetii* and *Leptospira* spp. in ibex from the canton of Valais is unclear. Higher prevalences of infection with these agents in domestic animals in Valais have not been reported (Luginbühl, 2009). Unlike in domestic sheep (Borel et al., 2004), prevalence of antibody to *C. abortus* in ibex was not higher in Grisons than in other cantons. High prevalences of antibody to BVDV were found in small domestic ruminants from the cantons of Glarus, Grisons, Ticino and St. Gallen, but not Valais (Danuser et al., 2009), where most positive ibex were found. Thus, factors other than the presence of infected livestock might influence the situation in Alpine ibex. Further investigations also should consider ibex from adjoining regions in neighboring countries.

### Significant differences in antibody prevalence between colonies

*Leptospira* spp. seem to be the most widespread and prevalent of the pathogens we surveyed. However, antibody prevalence was low (<7%) in most colonies. In four colonies, prevalence was higher (12.5–40.0%) but except for colony K, sample size was low (5–14 individuals). A previous study reported an absence of antibody to *Leptospira* spp. in colony A (Giacometti et al., 1995) but

### Table 3. Overall prevalence of infections with selected abortive agents in free-ranging Alpine ibex (*Capra ibex ibex*) from Switzerland, 2006–2008.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Methoda</th>
<th>No.</th>
<th>Positive</th>
<th>Prevalence</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Coxiella burnetii</em></td>
<td>ELISA</td>
<td>551</td>
<td>10</td>
<td>1.8</td>
<td>0.9–3.3</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>10</td>
<td>0</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td><em>Leptospira</em> spp.</td>
<td>MAT</td>
<td>280</td>
<td>22</td>
<td>7.9</td>
<td>5.0–11.7</td>
</tr>
<tr>
<td><em>Chlamydothila abortus</em></td>
<td>ELISA</td>
<td>598</td>
<td>6</td>
<td>1.0</td>
<td>0.4–2.2</td>
</tr>
<tr>
<td><em>Brucella abortus/B. melitensis</em></td>
<td>RBT</td>
<td>339</td>
<td>0</td>
<td>0.0</td>
<td>0–1.1</td>
</tr>
<tr>
<td></td>
<td>ELISA</td>
<td>111</td>
<td>8d</td>
<td>NCd</td>
<td>NCd</td>
</tr>
<tr>
<td><em>Brucella</em> spp.</td>
<td>PCR</td>
<td>196</td>
<td>0</td>
<td>0.0</td>
<td>0–1.8</td>
</tr>
<tr>
<td><em>Salmonella</em> Abortusovis</td>
<td>ELISA</td>
<td>555</td>
<td>0</td>
<td>0.0</td>
<td>0–0.7</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>Culture</td>
<td>157</td>
<td>0</td>
<td>0.0</td>
<td>0–2.3</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>ELISAb</td>
<td>562</td>
<td>10</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>IFATc</td>
<td>14</td>
<td>5</td>
<td>0.9f</td>
<td>0.3–2.1</td>
</tr>
<tr>
<td><em>Neospora caninum</em></td>
<td>ELISAb</td>
<td>562</td>
<td>2</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>IFATc</td>
<td>9</td>
<td>0</td>
<td>0.0g</td>
<td>0–0.7</td>
</tr>
<tr>
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<td>ELISAb</td>
<td>553</td>
<td>77</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>SNTc</td>
<td>77</td>
<td>24</td>
<td>4.3h</td>
<td>2.8–6.4</td>
<td></td>
</tr>
<tr>
<td>Bovine Herpesvirus-1</td>
<td>ELISA</td>
<td>553</td>
<td>0</td>
<td>0</td>
<td>0–0.7</td>
</tr>
</tbody>
</table>

a ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; MAT = microscopic agglutination test; RBT = rose bengal test; IFAT = indirect fluorescent antibody test; SNT = serum neutralization test; NC = not calculated.
b Screening test.
c Confirmation test.
d Given RBT and PCR results, ELISA positive results considered false.
e Not calculated because of small sample size.
f Based on 5 confirmed positive of 562 screened.
g based on 0 confirmed positive of 562 screened.
h Based on 24 confirmed positive of 553 screened.
these authors used an endpoint titer of 1:500 as the cutoff. Because seven of 89 ibex (7.9%) had endpoint titers of 1:100, which usually is considered as evidence of past exposure (Levett, 2001), the antibody prevalence obtained in that study was similar to ours, suggesting that occurrence of infection with *Leptospira* spp. in colony A has not varied over time. Our results are in accordance with the situation in the domestic animal population, in which *Leptospira* spp. infection has remained at a low level for several years (Luginbühl, 2009). The low antibody prevalence in ibex also could be due to the high altitude of the ibex natural habitat (Hindenland and Nievergelt, 1995), because temperature is a limiting factor for survival of leptospires in the environment (Levett, 2001). Positive reactions to more than one serovar most likely are due to strong cross-reactivity between serovars (Levett, 2001).

According to the overall antibody prevalence, BVDV was the second-most widespread abortive agent in this survey. Because antibody to BVDV was found in all four colony categories, with highest prevalences in colony I (stable) and B (declining), any effect of a BVD outbreak on ibex population dynamics seems to be unlikely. Most of the BVDV–antibody-positive ibex were older than 4 yr (data not shown), corresponding to the age category of ibex that survived the population decline in 2001. However, the number of antibody-positive ibex was very low (even in the old-age category), and old ibex are more likely to have been in contact with pathogens during their life span than young ones. The absence of detectable antibody in younger ibex and the generally low antibody prevalences suggest that BVDV does not persist in ibex populations. Nevertheless, further investigations, in particular regarding the occur-

<table>
<thead>
<tr>
<th>Agent</th>
<th>Test</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
<th>M</th>
<th>N</th>
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</thead>
<tbody>
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<td><em>Coxiella burnetii</em> ELISA</td>
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<td>0/63</td>
<td>0/54</td>
<td>0/34</td>
<td>0/5</td>
<td>0/102</td>
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<td>2/14</td>
<td>0/7</td>
<td>0/2</td>
<td></td>
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<tr>
<td><em>Leptospira</em> spp. MAT</td>
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<td>2/31</td>
<td>1/22</td>
<td>3/14</td>
<td>0/1</td>
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<td>0/8</td>
<td>2/5</td>
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<td>1/8</td>
<td>0/2</td>
<td>0/2</td>
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<td><em>Chlamydophila abortus</em> ELISA</td>
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<td>0/63</td>
<td>0/54</td>
<td>0/35</td>
<td>0/6</td>
<td>3/109</td>
<td>0/11</td>
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<td>0/10</td>
<td>0/20</td>
<td>0/67</td>
<td>0/16</td>
<td>0/7</td>
<td>1/2</td>
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<td>0/29</td>
<td>0/12</td>
<td>0/1</td>
<td>0/73</td>
<td>0/8</td>
<td>0/7</td>
<td>0/7</td>
<td>0/12</td>
<td>0/44</td>
<td>0/11</td>
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<tr>
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<td>0/13</td>
<td>1/5</td>
<td>1/13</td>
<td>0/5</td>
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<td>0/3</td>
<td>0/5</td>
<td>nd</td>
<td></td>
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<td>0/6</td>
<td>0/9</td>
<td>0/20</td>
<td>0/32</td>
<td>0/12</td>
<td>0/5</td>
<td>nd</td>
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<tr>
<td><em>Salmonella Abortusovis</em> ELISA</td>
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<td>0/64</td>
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<td>0/2</td>
<td>nd</td>
<td>0/1</td>
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<tr>
<td><em>Toxoplasma gondii</em> ELISA, IFAT</td>
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<td>0/65</td>
<td>0/52</td>
<td>0/35</td>
<td>0/6</td>
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<td>0/7</td>
<td>0/2</td>
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</tr>
<tr>
<td><em>Neospora caninum</em> IFAT</td>
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<td>0/52</td>
<td>0/35</td>
<td>0/6</td>
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<td>0/16</td>
<td>0/7</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>Bovine Viral Diarrhea virus SNT</td>
<td>3/153</td>
<td>6/61</td>
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<td>1/35</td>
<td>0/6</td>
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<tr>
<td>Bovine Herpesvirus-1 ELISA</td>
<td>0/151</td>
<td>0/64</td>
<td>0/54</td>
<td>0/33</td>
<td>0/6</td>
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<td>0/63</td>
<td>0/16</td>
<td>0/7</td>
<td>0/2</td>
<td></td>
</tr>
</tbody>
</table>

* ELISA = enzyme-linked immunosorbent assay; MAT = microscopic agglutination test; RBT = rose bengal test; PCR = polymerase chain reaction; IFAT = indirect fluorescent antibody test; SNT = serum neutralization test.

b Not done.
rence of persistently infected ibex, are necessary to draw conclusions.

Colonies with higher antibody prevalence for \textit{C. burnetii} all were considered as declining or recovered, and a negative influence of this agent on local population dynamics cannot be excluded. However, these prevalences were not significantly higher than in the stable colony I, and low antibody prevalences were found in other declining/recovered colonies. Therefore, it is unlikely that \textit{C. burnetii} has lead to a general decrease of Alpine ibex abundance in Switzerland. Tissue samples are necessary to detect chronic infection with \textit{C. burnetii} (Maurin and Raoult, 1999), but such samples were not available in our survey. Nevertheless, PCR analysis on blood is useful for detecting acute infections (Schneeberger et al., 2010). The negative PCR results in antibody-positive ibex thus suggest a chronic infection or past exposure in these animals.

\textbf{Pathogens without differences in antibody prevalences between colonies}

The 1.0\% overall antibody prevalence and the local prevalence of 0.5\% for \textit{C. abortus} in colony A are well below the prevalence reported by Giacometti et al.
(1995) in this same colony (31%) and by Genero et al. (1993; 52%) in Piemonte, Italy. However, these authors used the less specific complement fixation test (Sachse et al., 2009), and antibody prevalences might have been overestimated. In our study, antibody-positive ibex were dispersed among colonies showing different trends, indicating a sporadic occurrence of infection with *C. abortus*.

Antibody results confirmed a possible role of Alpine ibex as a host for *T. gondii*. However, in our study, the prevalence was lower than previous reports in ibex from Italy and France (9.5% and 23.8%, respectively; Genero et al., 1993; Burnet, 2007) and in domestic livestock in Switzerland (Berger-Schoch et al., 2011). This could be due to differences in density of final hosts in the natural habitat of the ibex, or in prevalence of infection in final hosts. Additionally, because the climate plays an important role in survival of oocysts (Dubey, 1998), infection with *T. gondii* might be influenced by differences in meteorologic conditions among regions.

**Investigations of Brucella**

Diagnosis of *Brucella* spp. is difficult; use of several tests and consideration of combined results are recommended (Godfroid, 2002). Our noninterpretable results by RBT likely were due to severe hemolysis in samples from hunted ibex. Few ibex were positive by ELISA, but these results should be interpreted with caution because cross-reactive bacteria such as *Yersinia enterocolitica* 09, *Escherichia coli* 0157, and *Stenotrophomonas maltophilia* occur in Swiss domestic and wild animals (Corbel, 1997; Zweifel et al., 2006; Fredriksson-Ahomaa et al., 2007, 2009; Albini et al., 2009) and also might infect ibex. In contrast, PCR has good sensitivity and specificity on blood samples (Hinic et al., 2009). Considering the results of all tests applied, our data suggest that infection with *Brucella* spp. does not occur in Swiss Alpine ibex, as is the case for domestic livestock.

**Pathogens with negative results**

To our knowledge, this is the first survey on *S. Abortusovis* in free-ranging Alpine ibex in Switzerland. In France, Bourgogne (1990) reported finding few antibody-positive ibex by seroagglutination but suspected possible cross-reactions. Our negative culture results in fecal samples suggest that infections with *Salmonella* spp. are uncommon in Swiss ibex. Investigation of fecal samples should be reliable because fecal shedding of *Salmonella* spp. occurs in cattle after abortions due to *Salmonella* spp. and in cattle of the same herd without clinical signs (D. Hüsey, unpubl. data). Fecal shedding of *S. Abortusovis* also has been documented in cases of abortion (Pardon et al., 1988) and after experimental mucosal infection (Sanchis et al., 1995) of domestic sheep.

In contrast to the situation in Gran Paradiso National Park (Ferroglio et al., 2001), we found no antibodies to *N. caninum* in ibex. As with *T. gondii*, absence of infection with *N. caninum* in Swiss ibex could be due to differences in population density of final hosts in the natural habitat of the ibex, or in prevalence of infection in final hosts.

Our negative findings for BHV-1 are in accordance with the status of domestic livestock and previous studies in ibex (Hasler and Engels, 1986; Giacometti et al., 1995; Lugniubühl, 2009). One ibex with antibody to BHV-1 was reported in Italy (Genero et al., 1993), but the authors argued that the result could have been related to cross-reactivity with cervid herpesvirus 1 (CvHV-1, formerly herpesvirus of Cervidae typ 1, HVC-1) or caprine herpesvirus 1 (CpHV-1, formerly bovid herpesvirus 6, BHV-6). Thiry et al. (1988) found an Alpine ibex with antibodies to BHV-6 in France, but no ibex were antibody-positive for BHV-
1. Apparently, Alpine ibex do not become infected with BHV-1, which is consistent with the reported host-specificity of the virus (Muylkens et al., 2007).

CONCLUSION

Prevalences of antibody to notifiable abortive agents are low in Swiss ibex, suggesting that this species does not play a relevant role in the epizootiology of these pathogens. The infection of ibex apparently is not related to the presence of infected domestic livestock. Most importantly, the abortive agents we studied do not appear to play a significant role in the population dynamics of Swiss ibex colonies. Future investigations should consider other biotic and abiotic factors that might have influenced mortality and recruitment in the reintroduced ibex population.

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We thank the hunting authorities, game wardens, and hunters of the cantons of Grisons, Valais, Vaud, St. Gallen, and Bern for excellent collaboration and for providing ibex samples. We acknowledge all students and temporary assistants for their support in the field and laboratory work, David Ortega for serologic analysis on BVDV, and Helmut Segner and two anonymous reviewers for constructive comments on the manuscript. Data on population dynamics in the selected colonies were kindly provided by Iris Biebach and Lukas Keller (Zoological Museum, University of Zurich, Switzerland). Positive and negative *T. gondii* and *N. caninum* control sera were a gift from Kirsten Simon (University of Veterinary Medicine, Hannover, Germany). This study was supported by the Swiss Federal Office for Environment.

LITERATURE CITED


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