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Serosurvey of Roe Deer, Chamois and Domestic Sheep in the Central Italian Alps

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ABSTRACT: Roe deer (*Capreolus capreolus*), chamois (*Rupicapra rupricapra rupicapra*), and domestic sheep in the Orobie Alps, Italy, were serologically tested for antibodies to selected pathogens that may be transmitted across species. Antibodies against *Brucella* spp. and bovine herpesvirus 1 (roe deer and chamois only) were not detected in any species. In roe deer, antibodies were detected against *Toxoplasma gondii* (13%) and *Neospora caninum* (3%). Chamois tested positive for antibodies to *T. gondii* (5%), *N. caninum* (21%), bovine respiratory syncytial virus (BRSV) (41%), bovine parainfluenza type-3 virus (17%), pestiviruses (18%), and *Mycoplasma conjunctivae* (17%). In the sheep, particularly high antibody prevalence rates were found for *T. gondii* (78%), *Chlamydophila* spp. (20%), pestiviruses (90%), BRSV (82%), and *M. conjunctivae* (81%).

Key words: Alpine chamois, domestic sheep, Italian Alps, roe deer, serosurvey.

Interactions between domestic and wild ungulates represent a potential problem in the Alps, due to livestock summer pasturing. Infectious diseases such as brucellosis (Ferroglio et al., 2000) and infectious keratoconjunctivitis (IKC; Belloy et al., 2003) have been found in wild ruminants due to transmission from livestock. In the Alps, sheep are of particular concern due to large herd sizes and the broad movements that often are associated with sheep management. To better understand the potential for pathogen transmission between sheep and wild ungulates in the Alps, a cross-sectional serologic survey of roe deer (*Capreolus capreolus*), chamois (*Rupicapra rupricapra rupicapra*), and sheep was done during 1998–2001. The study area (45°40′ to 46°10′N, 9°25′ to 10°20′E), situated in the Orobie Alps (northern Italy), has three main valleys (Val Brembana, Val Seriana, and Val di Scalve). There are approximately 4,000 roe deer and 5,000 chamois in this area with the highest densities in Val Brembana; an outbreak of pneumonia and IKC recently caused high mortality in the chamois population in Val Brembana. Large wandering sheep flocks as well as smaller groups (approximately 25,000 sheep in total) share habitats with wild ungulates from June to September.

Blood samples were collected by hunters from 207 roe deer and 236 chamois harvested during the 1998–2001 hunting seasons. Refrigerated blood samples were delivered to the laboratory within 2 days; they were centrifuged and sera were stored at −20 C. Sera were collected from 13,565 sheep in 2000 and were stored at −20 C. From these samples, 352 sheep sera from each valley were randomly selected using a computerized random list produced with the "Random" procedure of the PEPI 3.01 software (Abramson and Gahlinger, 1993–2000). For sample size determination (sheep), we used an expected prevalence of 50% for all the tested pathogens; and based on Graat et al. (1997), this sample size provides a precision of 5% at a 95% confidence level.

Wildlife species were tested for antibodies to *Brucella* spp. by using complement fixation test (CFT) (Ciuchini and Farina, 1991) and the Rose Bengal agglutination test (D.M. 2 Luglio 1992 n.453) depending on the quality and amount of the sera. Ovine sera were tested by CFT; antibody titers ≥20 were considered positive.

For *Toxoplasma gondii* antibody testing, a commercial latex agglutination test (Toxotest-MT®, Eiken Chemical Co., Tokyo, Japan) was used with a cut-off titer of 32 (Walls and Remington, 1983). Antibodies to *Neospora caninumin* in wildlife species were detected with the indirect fluorescent antibody test performed on antigen slides (Conrad et al., 1993) with species-specific conjugates. Antigen consisted of tachyzoites of the NC-PV1 (*N. caninum*) Italian isolate (Magnino et al., 1999). Antibody titers were determined as the last dilution showing whole parasite fluorescence. For sheep, a commercial enzyme-linked immunoadsorbent assay (ELISA; Chekit Neospora®, Bommeli Diagnostics, Liebefeld-Bern, Switzerland) was used.

Roe deer and chamois were tested for *Chlamydophila* spp. antibodies by using a CFT (O.I.E. Manual, 1992); titers $\geq$16 were considered positive. Sheep sera were tested using a commercial ELISA (Chekit Chlamydia®, Bommeli Diagnostics).

A commercial blocking ELISA (Herd-Check Anti-IBR gE®, IDEXX laboratories, Westbrook, Maine, USA) was used to test for antibodies to bovine herpesvirus 1 (BHV-1). Hemagglutination inhibition, by using SF-4 (ATCC VR281) as a reference virus, was used for bovine parainfluenza 3 virus (BPIV-3) antibody testing; 0.5% guinea pig red blood cells were used in this assay (Contini and Cossu, 1975). For pestivirus antibody testing, a blocking ELISA was performed using anti-p80 monoclonal antibodies as described previously (Brocchi et al., 1993); sera were tested at a 1:4 dilution. A blocking ELISA also was used to test for antibodies to bovine respiratory syncytial virus (BRSV; De Simone et al., 1986).

A commercial ELISA (Chekit *M. conjunctivae*®, Bommeli Diagnostics) was used to test sheep for antibodies to *Mycoplasma conjunctivae* (Belloy et al., 2001). For chamois, a monoclonal antibody to sheep and goat IgG that was conjugated to horseradish peroxidase was used (Giacometti et al., 2002).

Because of inadequate serum quantity and poor quality due to hemolysis, individual sera were often not included in all assays. In particular, tests for antibodies against BHV-1 and BPIV-3 virus were not performed for the sheep.

For all antibody prevalence estimates, a 95% confidence interval (CI) was estimated according to a binomial distribution (Henken et al., 1997). Chi-square tests were used to test for differences in seroprevalence between valleys. The Cramer Φ coefficient was calculated according to Jekel et al. (1996) to value the effect of the sample size on the estimate of χ^2.

Results are shown in Table 1. Negative results for *Brucella* spp. antibodies are consistent with observed absence of brucellosis in sheep in this area since 1997. In sheep, antibody prevalence was >70% for *T. gondii*, pestiviruses, respiratory syncytial virus, and *M. conjunctivae*. Significant differences in antibody prevalence between sheep sampled in different valleys were detected for *T. gondii* ($\chi^2=18.9_{(df=2)}$, $P<0.0001$), *Chlamydophila* spp. ($\chi^2=10.2_{(df=2)}$, $P=0.006$), and *M. conjunctivae* ($\chi^2=21.0_{(df=2)}$, $P<0.0001$; Table 2). In all cases, however, contingence coefficients were low (Cramer $\Phi_{Toxoplasmosis}=0.13$, Cramer $\Phi_{Chlamydiosis}=0.10$, and Cramer $\Phi_{Keratoconjunctivitis}=0.14$).

In roe deer, antibodies were only detected for *T. gondii* (13%) and *N. caninum* (3%); chamois also tested seropositive for *T. gondii* (5%) and *N. caninum* (21%). These protozoa infect a wide range of intermediate hosts, and definitive hosts, which include domestic cats, dogs, and other carnivores (Dubey, 1993; Gondim et al., 2004); these species are present in large parts of the study area. Our data agree with previously reported seropositive results from wild ruminants from the Alps (Gennero et al., 1993; Ferroglio and Rossi, 2001).

The prevalence of *M. conjunctivae*-seropositive chamois observed in this study was higher than prevalence estimates from the eastern Swiss Alps (Gia-

TABLE 1. Number of animals tested, positive animals, and overall seroprevalences for selected pathogens in three ruminants species in 1998–2001 in the Province of Bergamo (Italy).

	Roe deer			Chamois			Sheep		
	No. of animals		Prevalence (%)	No. of animals		Prevalence (%)	No. of animals		Prevalence (%)
Pathogen	Positive	Tested	(95% CI)[a]	Positive	Tested	(95% CI)[a]	Positive	Tested	(95% CI)[a]
Brucella spp.	0	50	0 (0.0–7.1)	0	49	0 (0.0–7.2)	0	1,056	0 (0.0–0.3)
Toxoplasma gondii	27	207	13 (8.7–18.4)	5	108	5 (1.5–10.4)	821	1,056	78 (75.1–80.2)
Neospora caninum	4	117	3 (0.9–8.5)	14	67	21 (11.9–32.5)	22	1,010	2 (1.3–3.2)
Chlamydophila spp.	0	90	0 (0.0–4.0)	0	106	0 (0.0–3.4)	199	1,013	19.6 (17.2–22.2)
BHV-1	0	128	0 (0.0–2.8)	0	100	0 (0.0–3.6)	nd[b]	nd	nd
BPIV-3	0	128	0 (0.0–2.8)	15	90	17 (9.6–26.0)	nd	nd	nd
Pestiviruses	0	138	0 (0.0–2.6)	18	98	18 (11.2–27.4)	954	1,056	90 (88.4–92.0)
BRSV	0	131	0 (0.0–2.7)	36	88	41 (30.5–51.9)	879	1,056	82 (80.8–85.4)
M. conjunctivae	nd	nd	nd	40	236	17 (12.5–22.4)	857	1,056	81 (78.6–83.4)

[a] Binomial exact one-sided, 97.5% CI.

[b] nd=not done.

TABLE 2. Number of animals tested, positive animals, and seroprevalences for selected pathogens in sheep in 2000 in three valleys of the Province of Bergamo (Italy).

	Val Brembana			Val Seriana			Val di Scalve		
	No. of animals		Prevalence (%)	No. of animals		Prevalence (%)	No. of animals		Prevalence (%)
Pathogen	Positive	Tested	(95% CI)[a]	Positive	Tested	(95% CI)[a]	Positive	Tested	(95% CI)[a]
Brucella spp.	0	352	0 (0.0–1.0)	0	352	0 (0.0–1.0)	0	352	0 (0.0–1.0)
Toxoplasma gondii	301	352	86 (81.4–89.0)	264	352	75 (70.1–79.4)	256	352	73 (67.7–77.3)
Neospora caninum	12	352	3 (1.7–5.8)	6	348	2 (0.6–3.7)	4	310	1 (0.3–3.2)
Chlamydophila spp.	63	352	18 (14.0–22.3)	87	348	25 (20.5–30.0)	49	313	16 (11.8–20.1)
Pestiviruses	322	352	91 (88.0–94.1)	315	352	90 (80.7–88.5)	317	352	90 (86.4–92.9)
BRSV	286	325	81 (76.7–85.1)	299	352	85 (80.7–88.5)	294	352	84 (79.2–87.2)
M. conjunctivae	290	352	82 (78.0–86.2)	260	352	74 (69.0–78.4)	307	352	87 (83.2–90.5)

[a] Binomial exact one-sided, 97.5% CI.

TABLE 3. Number of animals tested, positive animals, and seroprevalences for selected pathogens in chamois in three valleys of the Province of Bergamo (Italy).

	Val Brembana			Val Seriana			Val di Scalve		
	No. of animals		Prevalence (%)	No. of animals		Prevalence (%)	No. of animals		Prevalence (%)
Pathogen	Positive	Tested	(95% CI)[a]	Positive	Tested	(95% CI)[a]	Positive	Tested	(95% CI)[a]
Brucella spp.	0	13	0 (0.0–24.7)	0	17	0 (0.0–19.5)	0	19	0 (0.0–17.6)
Toxoplasma gondii	1	24	4 (0.1–21.1)	3	32	9 (19.8–25.0)	1	50	2 (0.0–10.6)
Neospora caninum	4	30	13 (3.7–30.7)	6	16	38 (15.2–64.5)	4	22	18 (5.1–40.2)
Chlamydophila spp.	0	31	0 (0.0–11.2)	0	32	0 (0.0–10.8)	0	43	0 (0.0–8.2)
BHV-1	0	23	0 (0.0–14.8)	0	27	0 (0.0–12.7)	0	50	0 (0.0–7.1)
BPIV-3	0	13	0 (0.0–24.7)	1	27	4 (0.0–19.0)	14	50	28 (16.2–42.5)
Pestiviruses	0	25	0 (0.0–13.7)	0	27	0 (0.0–12.7)	18	46	39 (25.0–54.6)
BRSV	5	20	25 (8.6–49.1)	10	26	38 (20.2–59.4)	21	50	42 (28.1–56.8)
M. conjunctivae	34	202	16 (12.0–22.8)	2	18	11 (1.9–36.0)	4	16	25 (8.3–52.5)

[a] Binomial exact one-sided, 97.5% CI.

cometti et al., 2002). This is probably due to an IKC outbreak that occurred in the Val Brembana immediately before the collection of our samples.

For chamois, antibody prevalence estimates for pestivirus ($\chi^2=24.9_{(df=2)}$, $P<0.0001$) and BPIV-3 ($\chi^2=10.5_{(df=2)}$; $P=0.005$) differed significantly between the valleys (Table 3). However, we found only moderate values for the contingence coefficients (Cramer $\Phi_{Pestivirus}=0.54$ and Cramer $\Phi_{PI3}=0.34$). Antibodies to pestiviruses have been detected in chamois in the western Alps (Olde Riekerink, 2005), and pestivirus infections and related disease have been reported from Pyrenean chamois (Hurtado et al., 2004). The presence of antibodies to BRSV in chamois also may be significant, because this virus may have been associated with epidemic pneumonia in chamois in an area bordering west Val Brembana (Citterio et al., 2003).

Antibodies to *Chlamydophila* spp. were not found in either chamois or roe deer in this study, but seropositive results have been reported for these species (Cubero-Pablo et al., 2000). In addition, *Chlamydophila abortus* was recently detected by polymerase chain reaction from a mummified fetus from a healthy roe deer from our study area (Gaffuri et al., 2003). Antibodies to BHV-1 also were not detected, and although serologic cross-reactions can occur among alphaherpesviruses (Keuser et al., 2004), we cannot discount the possible presence of other herpesviruses in these populations.

Based on the higher prevalence of antibodies in sheep compared with both roe deer and chamois, it is probable that sheep represent a potentially important source of infection for wild ruminants. However, these high prevalence estimates from sheep may be partially influenced by herd reorganization and the routine mixing of small groups of animals. Our results confirm the absence of brucellosis in this area but demonstrate the potential circulation and transmission of several impor-

tant pathogens between species. To better understand the nature and significance of these interactions, additional clinical data are needed as well as the isolation and molecular characterization of these etiological agents.

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