

Serological survey of *Neospora caninum* in free-ranging wild ruminants

Marco Bregoli^{1*}, Capelli Gioia², Nardelli Stefano², Cova Mariapia¹, and Pasolli Claudio¹

¹Istituto Zooprofilattico Sperimentale delle Venezie – AT5 Trento, Italy

²Istituto Zooprofilattico Sperimentale delle Venezie – Legnaro (PD), Italy

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ABSTRACT

Neospora caninum is a protozoan parasite, affecting several domestic species as intermediate-hosts. It is a recognised major cause of abortion in cattle worldwide. Definitive and intermediate hosts have been found in wildlife. A retrospective serological survey was conducted on a total of 694 samples collected during the period 2000-2003 from hunted alpine chamois (*Rupicapra rupicapra*), red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) for antibodies to *N. caninum* by a competitive enzyme-linked immunosorbent assay (cELISA, VMRD®). This first investigation of the presence of *N. caninum* in free-ranging wild ruminants in the Trentino (north-eastern Italy) showed a prevalence of 1.4% in alpine chamois, 3.2% in red deer and 7.6% in roe deer. These results indicate the exposure of wild ruminants living in the study area to *N. caninum* infection.

Key words: *Neospora caninum*, wild ruminants, cELISA, north-eastern Italy

Introduction

Neospora caninum is a cyst-forming protozoan parasite affecting several domestic species as intermediate-hosts. It is a recognised major cause of abortion in cattle. Dogs and coyotes (*Canis latrans*) are considered as both definitive and intermediate hosts (DUBEY, 1999a; GONDIM et al., 2004). Red fox (*Vulpes vulpes*) and mustelids have been demonstrated to be intermediate hosts (MCALLISTER et al., 1999; ALMERIA et al., 2002; SCHARES et al., 2002). Only recent reports suggest the red fox as a definitive host (WAPENAAR et al.,

* Contact address:

Dr. Marco Bregoli, Istituto Zooprofilattico Sperimentale delle Venezie – AT5 Trento, Via Lavisotto, 129 38100 Trento Italy, Phone: + 39-0461-822458; Fax: + 39-0461-829065; E-mail: as.fauna.at5@izsvenezie.it

2005). Naturally infected wildlife have been detected by discovering *N. caninum* in the tissues of white-tailed deer (*Odocoileus virginianus*) (VIANNA et al., 2005), black-tailed deer (*Odocoileus hemionus columbianus*) (WOODS et al., 1994), red foxes (ALMERIA et al., 2002), camelids (SERRANO-MARTINEZ et al., 2004) or demonstrating antibodies in the sera of moose (*Alces alces*) (GONDIM et al., 2004), water buffalos (DUBEY, 1999a), chamois (*Rupicapra rupicapra*), roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) (FERROGLIO and ROSSI, 2001), alpine ibex (*Capra ibex*) (FERROGLIO et al., 2001), coyotes (GONDIM et al., 2004), red foxes (JAKUBEK et al., 2001), brown hares (FERROGLIO and TRISCIUOGLIO, 2003) and several wild African mammals (FERROGLIO et al., 2003). Diagnostic tools for *N. caninum* include isolation from tissues, serological tests such as indirect fluorescent antibody test (IFAT), direct agglutination test (DAT), enzyme-linked immunoadsorbent assay (ELISA) and immunoblot, and polymerase chain reaction (PCR) methods for phylogenetic analysis (DUBEY, 1999a; MUGRIDGE et al., 1999; SONDGREN et al., 2001; CANADA et al., 2004).

The unclear role of wild carnivores in the epidemiology, the possible connection between domestic and sylvatic cycles and the still unknown pathogenic effects of *N. caninum* on wild ruminant populations make this parasite of major interest in wildlife surveys.

The objective of this study was to obtain preliminary data on the spread of *N. caninum* within alpine wild ruminants in an area not monitored before.

Materials and methods

A retrospective serological survey was conducted on free-ranging wild ruminants hunted in the period 2000-2003, from free-ranging wild ruminants in the Trentino region (north-eastern Italy). A total of 694 blood samples were collected, 503 originating from alpine chamois, 66 from roe deer and 125 from red deer. These species are the most abundant wild ruminants living in the area, while alpine ibex and moufflon (*Ovis musimon*) are less represented. Wild carnivores present in the area are red fox, European badger (*Meles meles*), stoat (*Mustela erminea*), pine marten (*Martes martes*), stone marten (*Martes foina*) and brown bear (*Ursus arctos*).

A commercial competitive enzyme-linked immunoadsorbent assay (cELISA-VMRD® Inc., WA, USA) was used to detect antibodies against *N. caninum* following the manufacturer's instructions. The results were expressed as the percentage of inhibition. Sera were considered negative if the sample caused <30% inhibition (cut-off) and positive if the sample caused ≥30% inhibition, as indicated by the manufacturer. Differences of prevalence were evaluated by means of Yates' corrected χ^2 test (EpInfo 6.0).

Results and discussion

Seroreactors were found in all the three species examined (Table 1). Seroprevalence were 1.4% in alpine chamois, 7.6% in roe deer and 3.2% in red deer. Prevalence in roe deer was significantly higher than in chamois ($\chi^2 = 8.02$; $p = 0.0046$).

Table 1. cELISA to *Neospora caninum* antibodies in sera of wild ruminants

	Examined	Positives (%)	95% confidence interval
Roe deer	66	5 (7.6)	1.23 – 13.97
Red deer	125	4 (3.2)	0.15 – 6.25
Chamois	503	7 (1.4)	0.41 – 2.39

The geographical distribution of cases showed the presence of seroreactors in all the subareas considered. The results indicate for the first time in this area that wild ruminant populations under survey are exposed to *N. caninum*. The prevalence recorded in wild ruminants was comparable to the findings in roe deer, red deer, alpine chamois and alpine ibex in the western Alps (FERROGLIO and ROSSI, 2001; FERROGLIO et al., 2003), but much lower than the prevalence in white-tailed deer in Illinois (DUBEY et al., 1999b) where the values are consistent with a sylvatic cycle. However, it has to be considered that the cELISA used in this study may lack sensitivity and/or specificity in wildlife species; even if competitive assays allow, theoretically, many species to be tested, this test has only been validated in cattle (BLASZLER et al., 2001).

Our findings could be consistent with contact with a domestic cycle. On the other hand, the highest prevalence was found in roe deer, a species that has major contact with urban areas compared to alpine chamois and red deer. The presence of domestic animals, mainly dogs and cattle has recently been considered responsible for the higher prevalence in cervids populations in Brazil (TIEMANN et al., 2005).

Further studies on wildlife and domestic livestock are needed to improve knowledge of the presence of *N. caninum* in the area and the possible life-cycles. Perspectives include the investigation of *N. caninum* in red foxes in order to evaluate the role of this species in the epidemiology of neosporosis in local wildlife populations and the study of effects on the health status and population dynamics of free ranging wild ruminants.

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SAŽETAK

Neospora caninum protozojski je parazit koji između ostaloga kao posredne domaćine iskorištava i neke domaće životinje. Tako je primjerice *N. caninum* jedan od glavnih uzroka pobačaja goveda diljem svijeta. Konačni i posredni nositelji toga nametnika mogu biti i divlje životinje. Retrospektivnom pretragom krvnih seruma (kompetitivni imunoenzimni test – cELISA, VMRD®) 694 divokoze (*Rupicapra rupicapra*), jelena običnih (*Cervus elaphus*) i srna običnih (*Capreolus capreolus*), odstrijeljenih u razdoblju od 2000. do 2003. godine, dokazivana je prisutnost protutijela na *N. caninum*. Prvo pretraživanje divljih preživača na prisutnost ovoga parazita u regiji Trentino (sjeveroistočna Italija) otkrilo je nazočnost protutijela u 1,4% divokoza, 3,2% jelena običnih i 7,6% srna. Ovakvi rezultati potvrđuju izloženost divljih preživača invaziji praživotinjom *N. caninum* u pretraživanom području.

Ključne riječi: *Neospora caninum*, divlji preživači, cELISA, sjeveroistočna Italija
