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Chlamydiosis: Seroepidemiologic Survey in a Red Deer (*Cervus elaphus*) Population in Italy

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ABSTRACT: Chlamydiae are obligate, intracellular, gram-negative bacteria that are responsible for important diseases in humans, other mammals, and birds. Studies have shown that chlamydiae could be present in wild ruminants, but the serodiagnostic method most commonly used did not allow identification of chlamydial species. We determined the prevalence of antibodies to *Chlamydia pecorum*, *Chlamydia suis*, *Chlamydia abortus*, and *Chlamydia psittaci* in 271 red deer (*Cervus elaphus*) of a central Italian population, by using the microimmunofluorescence test that shows antibody response against genus-specific and species-specific antigens. No sera had detectable antibodies to *C. pecorum* and *C. abortus*. Antibodies were detected against *C. psittaci* (9.6%) and *C. suis* (3.3%). Antibody response could be related to contact of the red deer with birds and wild boars (*Sus scrofa*), respectively, and confirm an extended host range of individual *Chlamydia* species. In view of the potential zoonotic risk related to exposition of *C. psittaci*, our findings suggest surveillance of wild ruminants as potential reservoirs for chlamydiae.

Key words: Antibody prevalence, Chlamydiae, Italy, microimmunofluorescence test, red deer.

According to the latest revisions (Everett et al., 1999; Kuo et al., 2011), the *Chlamydiaceae* family contains one genus (*Chlamydia*) and nine species that are responsible for important diseases in animals and humans. In domestic ruminants, chlamydiosis is associated with enteritis, keratoconjunctivitis, mastitis, pneumonia, hepatitis, abortion, reproductive disorders, polyarthritis, and meningoencephalitis as well as inapparent enteric infections (Reinhold et al., 2011). The *Chlamydia* species most frequently occurring in domestic ruminants are *Chlamydia abortus* (formerly *Chlamydia psittaci* ovine serovar), *Chlamydia pecorum*, *Chlamydia psittaci*, and rarely *Chlamydia suis* (formerly *Chlamydia trachomatis*-like swine isolates; Pantchev et al., 2009). Among these species, *C. abortus* and *C. psittaci* cause zoonotic infections in humans, resulting in spontaneous abortion and severe respiratory disease, respectively (Longbottom and Coulter, 2003).

Little is known about chlamydiosis in wild ruminants, although serologic investigations in Europe suggest a role for wild ruminants as a chlamydial wildlife reservoir (Andreani et al., 1986; Giovannini et al., 1988; Cordier, 1991; Dedek et al., 1991; Cubero-Pablo et al., 2000; Gaffuri et al., 2006). Most studies detected genus-specific antibody response because investigators usually used the complement fixation test (CFT). This test is recommended by the Office Internationale des Epizooties for diagnosing chlamydiosis, but it is not species-specific because the antigen used (lipopolysaccharide from the chlamydial cell wall) is common to all *Chlamydia* species. Using two in-house blocking enzyme–linked immunosorbent assays, Salinas et al. (2009) differentiated the antibody response to *C. abortus* from that to other *Chlamydiaceae* in wildlife hosts, suggesting frequent contacts with *Chlamydiaceae* other than *C. abortus*, such as *C. pecorum*, *C. suis*, and *C. psittaci*. We evaluated the prevalence of antibody against chlamydiae in a red deer (*Cervus elaphus*) population in Italy by the microimmunofluorescence (MIF) test; MIF is more specific than CFT (Saikku, 1982) because it shows antibody reactivity against genus-specific and species-specific antigens, by using purified elementary bodies as antigen.

The sampled red deer sera belonged to a wild population living in Tuscan-Emilian

TABLE 1. Prevalence of antibodies to *Chlamydia psittaci* and *Chlamydia suis* in the red deer (*Cervus elaphus*) population tested.

	Antibody titer				
Chlamydiaceae	<32	32	64	128	256
C. psittaci	245	11	14	1	0
C. suis	262	2	4	2	1

Apennines, along the Apenninic ridge (44°06′N, 11°00′E) between Tuscany and the Emilia-Romagna region. The deer population in this area is estimated to be 2,500 (Mattioli and Nicoloso, 2003). Each year in the province of Pistoia, approximately 200 red deer are harvested and 140 hunters handle approximately 20 tons of raw deer meat.

We studied 271 sera from deer collected during three hunting seasons—2007–2008 (54 sera), 2008–2009 (108 sera), and 2009–2010 (109 sera)—from Pistoia province, Tuscany (44°00′N, 11°00′E; 80–1,200 m above sea level). The deer sampled were 109 males and 162 females, aged ≥7 mo and were divided into three age classes: calf (<1 yr old; $n=87$), yearling (1–2 yr old; $n=31$), and adult (>2 yr old; $n=153$). Pregnancy and evidence of nursing (signs of suckling) were recorded for each female.

The MIF test was performed using purified elementary bodies (EBs) of four chlamydial species as antigens: the Italian *C. pecorum* PV5268 isolate obtained from a bovine cervical swab and the Italian *C. suis* MS04 isolate obtained from a pig conjunctival swab (both characterized by molecular analysis), and *C. abortus* S26/3 and *C. psittaci* 6BC reference strains. The EBs were purified by sucrose density-gradient ultracentrifugation (Fukushi and Hirai, 1988). The presence of chlamydial antibodies was assessed using a fluorescein-conjugated goat anti-deer IgG serum (Euroclone Milan, Italy). Sera were screened at 1:16 and 1:32 dilutions in phosphate-buffered saline supplemented with 2% fetal calf serum according to the method of Wang and Grayston (1986). Sera that fluoresced apple green at 1:32 dilution were considered positive and were tested by serial dilution. The reciprocal of the highest serum dilution that was positive was considered to be the antibody titer of the sample.

Prevalence by sex, age, hunting season, pregnant females, and nursing females was analyzed by chi-square analysis. We used $P \leq 0.05$ to indicate significance of statistical tests.

No sera had detectable antibodies to *C. abortus* and *C. pecorum*; 26 sera (9.6%; 95% confidence interval [CI]=6.6–13.7%) were positive for antibodies to *C. psittaci*, with titers of 32–128 (median=64). Nine sera (3.3%; CI=1.7–6.3%) had antibodies to *C. suis*, with titers of 32–256 (median=64). We detected no coinfection (Table 1).

Antibody prevalences for *C. psittaci* varied among hunting seasons: nine of 54 (17%; 95% CI=8.8–28.8%) were positive in 2007–2008; 11/108 (10.2%; 95% CI=5.6–17.5%) in 2008–2009, and 6/109 (5.5%; 95% CI=2.3–11.7%) in 2009–2010. No sex- or age-related differences in prevalence were detected, but differences ($P<0.05$) were observed among the three hunting seasons: the prevalences in calves were significantly different between hunting seasons 2007–2008 and 2008–2009 ($P=0.03$), in adult males during 2007–2008 and 2009–2010 ($P=0.005$), and in positive animals during 2007–2008 and 2009–2010 ($P=0.03$). There were no differences in pregnant and nursing females among positive and negative animals. Antibody prevalences among hunting seasons for *C. suis* were as follows: 2007–2008, two positive of 54 (4%; 95% CI=0.3–13.3%); 2008–2009, 3/108 (2.8%; 95% CI=0.6–8.2%); and 2009–2010, 4/109 (3.7%; 95% CI=1.1–9.4%). There were no significant differences for any of the variables analyzed.

The finding of antibody to *C. psittaci* is not surprising because red deer have been shown to be susceptible to experimental infection with a pathogenic *Chlamydia* isolate from ovine pneumonia (McMartin et al., 1979). Several investigators have detected *C. psittaci* in domestic ruminant

hosts (Reinhold et al., 2011), although it is unclear whether these are strains acquired from birds or autochthonous nonavian strains, and their zoonotic potential is unknown (Pantchev et al., 2010). We found a decreasing prevalence over the years that was statistically significant between hunting seasons 2007–2008 and 2009–2010. No data were available for density or migration of wild birds or number of grazing livestock in the area during the study.

The finding of antibody to *C. suis* is consistent with molecular studies in domestic ruminants. Teankum et al. (2007) detected DNA of chlamydiae sharing 98% identity to the 16S rRNA sequence of *C. suis* in semen of two bulls. Pantchev et al. (2010) identified *C. suis* in cattle ($n=9$) by species-specific real-time PCR assays and suggested an extended host range of individual *Chlamydia* species. Antibody reactivity to *C. suis* could be related to possible contacts between red deer and wild boar (*Sus scrofa*); there were more than five wild boar/100 ha in the area tested (Provincia di Pistoia, 2009). In addition, a recent seroepidemiologic study provided some evidence of *C. suis* circulation in Italian wild boar populations (Di Francesco et al., 2011). The absence of antibody to *C. abortus* and *C. pecorum* may be related to the lack of contact between the tested red deer population and domestic ruminants, because transmission of *Chlamydia* probably occurs by grazing on the same pastures (Andreani et al., 1986; Gourreau et al., 1993).

Our results confirm the role of wild ruminants as a reservoir for chlamydiae and show the circulation of a zoonotic pathogen (*C. psittaci*) among red deer. In view of the potential zoonotic risk for hunters and other personnel handling carcasses and raw game meat, further studies focused on the direct evidence of the infectious agents by isolation or molecular analysis are needed to assess the hazards to human health.

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