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Seroprevalence of *Anaplasma phagocytophilum* in domestic and wild animals from central Italy

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SUMMARY

From January 2004 to July 2007, 2455 sera were collected from domestic and wild animals living in central Italy and tested by indirect immunofluorescence assay to detect antibodies against *Anaplasma phagocytophilum*. Considering sera with 1:40 antibody titers as positive, 336 (13.68%) animals scored positive. The percentages of seropositivity were: 46.26% (31/67) in fallow deer, 46.15% (24/52) in red deer, 16.89% (134/793) in horses, 16.78% (23/137) in cattle, 12.74% (13/102) in sheep, 8.76% (108/1232) in dogs, 4.16% (3/72) in goats. These data confirm the presence of *A. phagocytophilum* in wild ruminants and domestic animals, including pets, in central Italy.

KEY WORDS: Anaplasma phagocytophilum, Horses, Dogs, Domestic and wild ruminants

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INTRODUCTION

Anaplasma phagocytophilum is an obligate intracellular bacterium belonging to the family *Anaplasmataceae*, order *Rickettsiales*. It resides within the granulocytes, particularly neutrophilic, of its host. It is transmitted by *Ixodes* ticks. *Ixodes ricinus* is the most important vector in Europe, in which rates of anaplasma infection range from 1.9% to 34% (Lillini *et al.,* 2006).

Anaplasma phagocytophilum was originally referred to as the Human Granulocytic Ehrlichiosis (HGE) agent, first identified in 1990. Horses, dogs, cats, domestic and wild ruminants are known to be susceptible.

Wild ruminants are important hosts for the vector *I. ricinus*; usually deer do not develop clinical signs, but may serve in monitoring of granulocytic anaplasmosis and other tick-borne infections in a given area. Results of previous serological and molecular investigations disclosed *A. phagocytophilum* in wild ruminants living in the USA and European countries. Recently, this etiologic agent was detected by PCR in blood samples of fallow deer living in a natural preserve of central Italy (Ebani *et al.,* 2007).

Other animal species do not seem to play the reservoir role, but in some cases the infection may cause clinical signs, particularly in dogs and horses.

Anaplasma phagocytophilum in dogs causes a disease termed canine granulocytotropic anaplasmosis. Reported clinical findings are almost exclusively from acute disease during the bacteremic phase. Chronic anaplasmosis has not been documented. Given that the dog is an unnatural host for this infection, self-limiting infection would be expected. The majority of dogs with *A. phagocytophilum* infections have nonspecific signs of illness: fever, depression, anorexia, musculoskeletal pain. Signs of bleeding disorder have not been observed (Greig and Armstrong, 2006).

Anaplasma phagocytophilum in horses, originally referred to *Ehrlichia equi*, is responsible for

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equine granulocytic ehrlichiosis. The disease is characterized by fever, anorexia, depression, reluctance to move, limb edema, petechiation, icterus, leukopenia, thrombocytopenia and anemia (Pusterla and Madigan, 2007). It is well known in the United States, but in the last years several cases have also been reported in Europe (Hermann *et al.,* 1985; Bjorersdorff *et al.,* 1990; Pusterla *et al.,* 1998; Shaw *et al.,* 2001; Bermann *et al.,* 2002; Von Loewenich *et al.,* 2003; Scarpulla *et al.,* 2003; Alberti *et al.,* 2005; Lillini *et al.,* 2006). The horse is considered an aberrant host, and it seems unlikely that infected horses could serve as effective reservoirs of *A. phagocytophilum* because the presence of the organism in an affected animal is limited to the acute phase of the disease (Pusterla and Madigan, 2007).

This microorganism is also known as the etiologic agent of tick-borne fever (TBF) or pasture fever of ruminants, observed in the United States and Europe.

The infection causes a disease characterized by high fever, recurrent bacteremia, neutropenia, lymphocytopenia, thrombocytopenia, general immunosuppression, resulting in more severe secondary infections such as tick pyemia, pneumonic pasteurellosis, listeriosis and enterotoxemia (Woldehiwet, 2006).

TBF has been described in cattle, sheep and goats. After patent bacteremia, these animals become persistently infected carriers, perhaps playing an important role in the maintenance of infection in the flock or herd (Woldehiwet, 2006). The reservoir competency of goats for *A. phagocytophilum* has been demonstrated by experimental infection (Massung *et al.,* 2006).

The seroprevalence varies widely on the basis of the geographic area considered, ranging from about 0.5% to 13.3% (Amusategui *et al.,* 2006; Lillini *et al.,* 2006).

In Italy some cases of disease due to *A. phagocytophilum* were observed in humans, horses and dogs (Ruscio and Cinco, 2003; Alberti *et al.,* 2005; De la Fuente *et al.,* 2005).

The etiologic agent has been detected by molecular investigations in these cases, but also in *Ixodes* ticks and wild ruminants (Cinco *et al.,* 1998; Ebani *et al.,* 2007). However, the spread of this microorganism in Italy is not completely clear, because few data are available on the seroprevalence of *A. phagocytophilum* infection among domestic and wild animals (Lillini *et al.,* 2006).

The purpose of the present research was to evaluate the seroprevalence of *A. phagocytophilum* on the basis of the data obtained by indirect immunofluorescence assay carried out on sera of domestic and wild animals living in central Italy.

MATERIAL AND METHODS

Samples

In the period from January 2004 to July 2007, 2455 blood serum samples of different animal species were examined by the Serology Laboratory at the Department of Animal Pathology, Prophylaxis and Food Hygiene of University of Pisa, Italy. In particular, the sera were collected from 1232 dogs, 793 horses, 137 cattle, 102 sheep, 72 goats and 67 fallow deer (*Dama dama*), 52 red deer (*Cervus elaphus*). Sera were separated by centrifugation and stored at -20°C until serological tests were executed. All sera were examined by indirect immunofluorescence assay (IFA) to detect antibodies to *Anaplasma phagocytophilum*. All the animals were from different areas of central Italy.

Serological analysis

The assay was executed employing specific IFA slides prepared with cells infected by *Anaplasma phagocytophilum* (Fuller Laboratories Fullerton, California, USA).

Blood sera were diluted 1:40 in phosphate-buffered saline (PBS, pH 7.2) and incubated on wells of the slides in a humidified chamber at 37°C for 30 min. The slides were rinsed three times in PBST (PBS + 0.4% Tween 80 - Sigma, St. Louis, Missouri, USA) and once in distilled water and air-dried. Each well of the slides was probed with fluorescein isothiocyanate - conjugated (FITC) secondary antibodies, specific for each animal species tested.

In particular, the secondary antibodies employed and their working dilutions in Evans Blue solution are reported in Table 1. The slides were incubated in a humidified chamber at 37°C for 30 min, then washed and dried as described, and examined with a fluorescent microscope. Positive samples were subsequently tested at 1:80 and 1:160 dilutions.

Table 1 - Secondary antibodies employed in the immunofluorescence test.

Animal species	*FITC secondary antibodies*	*Working dilution*	*Manufacturer*
Dogs	Rabbit anti-Dog IgG	1:30	Sigma, St. Louis, Missouri, USA
Equine	Rabbit anti-Horse IgG	1:32	Sigma
Cattle	Rabbit anti-Bovine IgG	1:300	Sigma
Ovine	Rabbit anti-Sheep IgG	1:100	Sigma
Goats	Rabbit anti-Goat IgG	1:400	Sigma
Fallow deer Red deer	Rabbit anti-Deer IgG	1:10	Kirkegaard and Perry Laboratories,Inc. Gaithersburg, MD, USA

RESULTS

Among the 2455 sera examined, 336 (13.68%) were positive. In particular, considering the animal species, the sera scored positive were: 108 (8.76%) among dogs, 134 (16.89%) among horses, 23 (16.78%) among cattle, 13 (12.74%) among sheep, 3 (4.16%) among goats, 31 (46.26%) among fallow deer and 24 (46.15%) among red deer. Eighty-nine (3.62%) sera of the 336 total positive samples showed 1:40 antibody titre, 144 (5.87%) 1:80 titre, 103 (4.19%) ≥1:160 titre. The results about the number of samples for each animal species scored positive at different antibody titres are reported in Table 2.

DISCUSSION

Anaplasma phagocytophilum causes an emerging tick-borne infection in several animal species and is sometimes responsible for a serious disease in humans.

Data obtained in the present research showed the highest value of seroprevalence in the wild ruminants population. Similar percentages of positive responses were observed among the domestic animal species.

8.76% of canine blood samples scored positive to *A. phagocytophilum*, showing low antibody titers. These results could mean that dogs are not commonly infected with *A. phagocytophilum* and are

TABLE 2 - *Data obtained by indirect immunofluorescence test with sera from domestic and wild animals.*

Species	*Number of examined sera*	*Number of positive sera*	*Antibody titers*		
			1:40	*1:80*	*≥1:160*
DOGS	1232	108 (8.76%)	34 (2.76%)	57 (4.62%)	17 (1.38%)
EQUINE	793	134 (16.89%)	38 (4.79%)	47 (5.92%)	49 (6.18%)
CATTLE	137	23 (16.78%)	0	12 (8.76%)	11 (8.02%)
OVINE	102	13 (12.74%)	2 (1.96%)	7 (6.86%)	4 (3.92%)
GOATS	72	3 (4.16%)	0	1 (1.39%)	2 (2.77%)
FALLOW DEER	67	31 (46.26%)	10 (14.92%)	9 (13.43%)	12 (17.91%)
RED DEER	52	24 (46.15%)	5 (9.62%)	11 (21.15%)	8 (15.38%)
TOTAL	2455	336 (13.68%)	89 (3.62%)	144 (5.87%)	103 (4.19%)

thus unlikely to serve as maintenance hosts for this microorganism.

Among the equine sera tested, 16.89% scored positive. Differences between percentages of animals positive at different antibody titers were not relevant. The results suggest that this infection is not widespread among horses living in central Italy. However, the animals studied belonged to private stables under optimal conditions of breeding. Different results could be found among horses living under more uncomfortable conditions and in areas shared by other animals which serve as reservoirs.

Among the domestic ruminants, the highest value (16.78%) of prevalence was detected within the bovine population, followed by the value (12.74%) within the ovine population. Only 4.16% of goats was positive, even if at not very low antibody titers. *Anaplasma phagocytophilum* and *Anaplasma marginale*, which causes bovine anaplasmosis, often co-exist in geographic areas causing concurrent infections in ruminants and ticks.

Serosurveys may detect false values of prevalence because of cross-reactions between the two *Anaplasma* species (De La Fuente *et al.,* 2005; Dreher *et al.,* 2005; Carelli *et al.,* 2007).

Our results confirm the diffusion of anaplasmosis among fallow deer and suggest the presence of this infection also in red deer. The percentages of positive results in fallow deer (46.26%) and red deer (46.15%) were very similar suggesting that there are no differences between the two animal species.

In conclusion, the data obtained in the present research confirmed that wild ruminants are the main hosts for *A. phagocytophilum*, representing important reservoirs of the microorganism in a geographic area.

Ixodes ricinus, the main vector of *A. phagocytophilum*, is less widespread among domestic animals. For this reason it is reasonable that the seroprevalence values observed in the domestic species considered were lower than those of the deer population.

The seroprevalence detected in cattle and horses was quite similar, whereas a low value was observed among goats.

The percentage of positive responses detected in the canine population was not very high, but it suggests that another member of the *Anaplasmataceae* family, other than *Ehrlichia canis*, etiologic agent of the canine monocytic ehrlichiosis, are able to infect dogs. Data on the seroprevalence in canine population may be useful, because the dogs live in close contact with humans, sharing environments and habits of life.

Prevalence results might have been higher if samples had been collected from areas where anaplasma is naturally more common. However, it is possible that domestic animals are not very susceptible to the strain circulating in our regions.

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