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BRIEF REPORT

Anaplasmataceae in wild rodents and roe deer from Trento Province (northern Italy)

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In recent decades, a number of intracellular bacterial strains within the family *Anaplasmataceae* have been identified around the globe. These bacteria include *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis and *Anaplasma marginale*, which causes disease in ruminants. Bacteria from this family often have a wide range of hosts, infecting both vertebrates and invertebrates. *A. phagocytophilum* is an obligate intracellular pathogen that parasitises the granulocytes of humans and animals, such as domesticated dogs, sheep, cows and horses, as well as wildlife species, such as deer and rodents [1]. Various strains of *A. phagocytophilum* have been identified, but only some are considered human pathogens [2].

Different studies have demonstrated the role of the tick *Ixodes ricinus* as a potential vector for the transmission of *A. phagocytophilum* [1, 3, 4]. Since transovarial transmission of *Anaplasma* species appears to be inefficient in ticks, mammalian hosts are presumed to play an important role in the maintenance and propagation of *Anaplasma* species in nature [5]. However, the reservoir species of the human pathogenic strains of *A. phagocytophilum* in Europe is not known [2]. In Italy, the presence of members of the *Anaplasmataceae* family is known from screening studies of ticks; however, there are few data on their presence in vertebrate hosts. Human granulocytic anaplasmosis was first described in the USA in 1994 and is emerging in Europe [5, 6]. Although only three possible human cases have been reported in Italy [7, 8], serological and molecular studies have respectively shown *A. phagocytophilum* infections in domesticated animals and in *I. ricinus* ticks (see for example [3, 9]).

To check the potential role of wild animals as reservoirs for these bacteria, we performed a PCR screening of either spleen or blood of roe deer (*Capreolus capreolus*) and bank voles (*Clethrionomys glareolus*) collected in Trento province, northern Italy. A total of 96 spleen samples collected in 2001 from roe deer that had either been hunted or found dead in Trento province were examined. Following DNA extraction using a DNeasy Tissue Kit (Qiagen, Hilden, Germany), PCR analysis with the primer pair PER1/PER2, which amplifies a 452-bp portion of the 16S rRNA of the *Ehrlichia-Anaplasma* group, was performed as reported previously [10]. PCR bands from the positive products were then sequenced directly. A total of 19 (19.8%) positive samples were found and then sequenced; the 452-bp sequences obtained from all samples were identical, revealing 100% identity with the 16S rRNA sequence of *A. phagocytophilum* (AF481853) previously reported in red deer, ticks and sheep. Following an identical procedure, 34 blood samples collected in 2002 from the bank vole *C. glareolus* were examined by PCR using the PER1/PER2 primer set. One positive result was found, and the sequence obtained revealed 100% identity with the 16S rRNA sequence of *Candidatus* Neoehrlichia mikurensis (AB213021) [11].

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Our study reports, for the first time in Italy, the presence of *A. phagocytophilum* in samples from wild roe deer (*C. capreolus*) collected in Trento province (northern Italy). In this area, roe deer are abundant and serve as hosts for all stages of *I. ricinus* ticks. Tick-transmitted diseases, such as Lyme borreliosis or tick-borne encephalitis, are endemic in this region, and wild ruminants play an important role in the ecology of these human pathogens [12]. The present study suggests that roe deer are competent reservoirs of a variant of *A. phagocytophilum* in Italy. The potential role of this variant as a human pathogen has yet to be confirmed.

The presence of a member of a novel clade within the family *Anaplasmataceae*, *Candidatus* Neoehrlichia mikurensis, was demonstrated by PCR in one *C. glareolus* blood sample (prevalence 2.9%). In Europe, *Clethrionomys* voles are important hosts for larval *I. ricinus* [4]. Unlike other rickettsial agents, *Ehrlichia*/*Anaplasma* spp. appear not to be maintained through transovarial transmission in ticks; therefore, the prevalence of granulocytic ehrlichial infection in small mammals could be expected to be low. *Candidatus* Neoehrlichia mikurensis, previously detected in ticks in Europe and in ticks and wild mammals worldwide (see for example [13, 14]), has hitherto never been reported in mammals from Europe.

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