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Detection of *Anaplasma phagocytophilum* in wild boar in Slovenia

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Human granulocytic anaplasmosis (HGA) comprises a group of emerging tick-borne infectious diseases and it is caused by the intracellular bacteria, *Anaplasma phagocytophilum*. Infections with *Anaplasma* spp. have been described in humans and animals. Many studies have been performed to elucidate the natural cycle of *A. phagocytophilum*, but a competent reservoir host has not been clearly established in Europe [1].

Roe and red deer represent a very important host for the adult tick *I. ricinus*, a vector of *A. phagocytophilum*, and could therefore also serve as a natural reservoir for *A. phagocytophilum* in enzootic transmission of this bacterium [1]. Two genetic lineages of *groESL* operon were described among isolates of *A. phagocytophilum* from deer in Slovenia. However, both genetic lineages differed from a single variant, found in all Slovenian HGA patients [2]. PCR screening of different wild animals from Austria and the Czech Republic showed that wild boar (*Sus scrofa*) were infected with *A. phagocytophilum* [3]. Nucleotide sequences of *groESL* operon of *A. phagocytophilum* obtained from these infected animals were identical to the sequences from Slovenian patients [3]. Interestingly, a German horse with equine granulocytic ehrlichiosis was also infected with an identical variant of *A. phagocytophilum* to that detected in Slovenian patients [4].

In the years 2002 and 2007, 248 wild boars (*Sus scrofa*) from different locations in Slovenia were shot by professional hunters. Blood and spleen samples collected by hunters were analysed for infection with *Anaplasma* species in our laboratory. DNA was extracted from 135 spleen (year 2002) and 113 blood (year 2007) samples as described previously [1]. For the initial screening of all samples a segment of the *16S rDNA* of *A. phagocytophilum* was used [1]. All positive samples were additionally amplified with a nested PCR targeting *groESL* operon of variants of *A. phagocytophilum* [1]. The gene for 16S rDNA is very conservative, therefore only the amplicons of the *groESL* operon were further analysed by sequencing. To obtain the genetic variation of *A. phagocytophilum*, all amplicons of *groESL* operon were further sequenced on both strands and analysed by using TREECON software. A phylogenetic tree was constructed with the neighbour-joining method. Support for the tree nodes was calculated with 1000 bootstrap replicates.

Of 135 spleen samples (2002) and 113 blood (2007) samples, six (4.4%) and four (3.5%) tested positive for the presence of *Anaplasmae* spp. DNA, respectively. The *groESL* gene was subsequently amplified from all positive samples. The homology search and the alignment of *groESL* sequences showed 100% identity with *A. phagocytophilum* from a human patient (acc. no. AF033101) and from the *Ixodes ricinus* tick (acc. no. EU246961) from Slovenia (Fig. 1). Genetic sequences from wild boar were also 100% identical among each other.

Our study confirms that wild boar is naturally infected with *A. phagocytophilum*. The *Anaplasmae* spp. DNA was detected in 10 of 248 (4.0%) samples, collected from different locations in Slovenia in two different years. *Anaplasma phagocytophilum* was previously detected in wild boar samples. Interestingly, the wild boar were hunted in a different geographical area (Czech Republic) but the results were in accordance with our findings [3]. Scandinavian *groESL* sequences from dogs and horses differ from Slovenian in six nucleotides, but are identical among each other. These differences might suggest geographical variability of *A. phagocytophilum* and contribute

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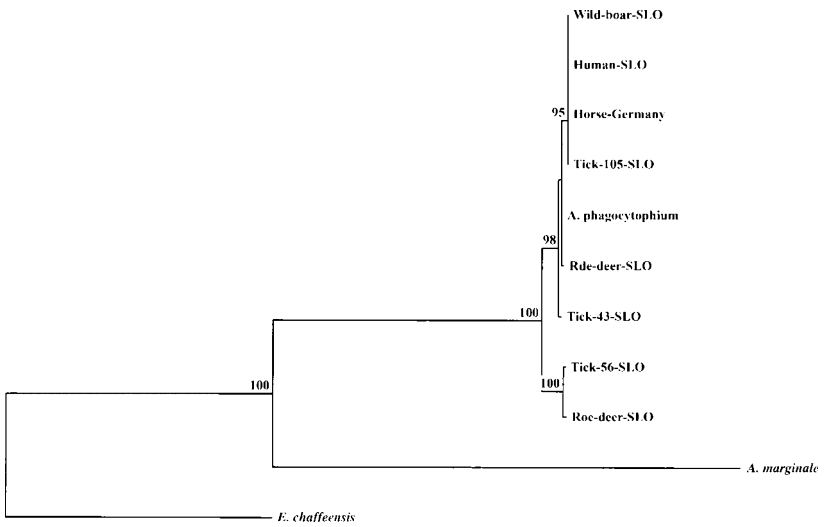

Fig. 1. Phylogenetic relationship of representative anaplasmae deposited in GenBank and detected in this study in wild boar samples (because of 100% similarity only one sequence of *Anaplasma phagocytophilum* from wild boar is shown). Accession numbers: *A. phagocytophilum* from a human patient from Slovenia, AF033101; *A. phagocytophilum* from wild boar from Slovenia, EU184703; *A. phagocytophilum* from a horse from Germany, AF482760; *A. phagocytophilum* isolate X7 from *I. ricinus* from Germany, AY281847; *A. phagocytophilum* from red deer from Slovenia, AF478562; *A. phagocytophilum* from roe deer from Slovenia, AF478564; *A. phagocytophilum* from ticks from Slovenia: tick 105, EU246961; tick 43, EU246959; tick 56, EU246960; *A. marginale*, AF414865; *E. chaffeensis*, L10917. The number on each branch shows the per cent occurence in 1000 bootstrap replicates.

to difficulties associated with finding an appropriate potential reservoir.

The prevalence of *A. phagocytophilum* infection in wild boar in two distant time intervals (2002 and 2007) was approximately the same, implying that the bacterium has established a stable cycle in nature. Detection of *A. phagocytophilum* in the spleen and blood samples of Slovenian wild boar also suggests the active infection of animals. It is well documented that cervids serve as a natural reservoir host for several *A. phagocytophilum* genetic variants. It has been shown before that roe and red deer can be chronically infected with *A. phagocytophilum* as well [1]. However, the genetic variants of the *groESL* gene from deer have not been detected in humans in Europe [1,2]. According to Massung *et al.* [5], non-pathogenic variant, AP-variant 1, is restricted to ruminant species and represents a lineage distinct from the human variant of *A. phagocytophilum*, which infects humans and numerous other mammals in the USA. A similar distinction between ruminant and non-ruminant lineages in Europe based on the *msp4* gene was also suggested by de la Fuente *et al.* [6]. The finding of a wild boar as a potential reservoir host would complete the zoonotic cycle of a human variant of *A. phagocytophilum* in reservoir and vector species in Europe. An interesting observation of this study is the fact that sequences of *A. phagocytophilum* were identical in all wild boar tested in Slovenia as well as in other European countries [3]. Given the high genetic variability of *A. phagocytophilum* in general, those results additionaly suggest that wild boar may represent a potential reservoir of a variant of *A. phagocytophilum* found in Slovenian patients.

In conclusion, our results represent substantial molecular evidence of the potential zoonotic cycle of *A. phagocytophilum* between the *I. ricinus* tick, wild boar and humans in Europe. The identity of sequences of *A. phagocytophilum* from wild boar and human patients is consistent with the definition of reservoir–target transmission [7]. In addition, experimental studies with wild boar and human variants of *A. phagocytophilum* are needed to determine the persistence of infection in wild boar and to define the pathogenicity of *A. phagocytophilum* for wild boar.

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