Zoonotic tick-borne bacteria among wild boars (Sus scrofa) in Central Italy

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1. Introduction

In the last few years, hematophagous arthropod populations, in particular ticks, have been significantly increased mainly resulting from the climate change, especially global warming. Moreover, the increasing movements of production and companion animals allow the circulation of arthropods and pathogens, and changes of landscape such as the creation of recreational parks and the development of suburban areas facilitate the spread of tick populations and the possible contact with domestic animals and humans[1]. Consequently, the tick-borne pathogens have become a severe treat for animal and human health.

Wildlife, including wild boars, that can harbor numerous ticks, also belonging to different species, act as vectors for pathogens of veterinary and human concern.

Several surveys carried out in ticks, animals and humans have detected these pathogens worldwide, including Italy. Even though serological and molecular investigations have been carried out to estimate the spreading of tick-borne pathogens among wildlife, data about the epidemiological role of wild boars (Sus scrofa) in these infections are very scant[2-8].

The Eurasian wild boar population has been increased in Italy, as in other European countries, raising concerns regarding diseases transmission.

The aim of this investigation was to estimate the occurrence of infections by the three zoonotic bacteria Anaplasma phagocytophilum (A. phagocytophilum), Borrelia burgdorferi sensu lato (B. burgdorferi s.l.) and Coxiella burnetii in wild boars (Sus scrofa) in Central Italy. The spleen samples from 100 hunted wild boars were submitted to DNA extraction and PCR assays were carried out to detect the three agents. One (1%) animal was positive for A. phagocytophilum, and three (3%) for B. burgdorferi s.l. No positive reactions were observed for Coxiiella burnetii. Wild boars did not seem to play an important role in the epidemiology of the three investigated agents. However, the detection of A. phagocytophilum and B. burgdorferi s.l. in the spleen of the tested animals showed that wild boars can harbor these pathogens, thus ticked that feeding on infected wild boars are likely to become infected, too, which represents a source of infection for other animals and humans. This is the first detection of A. phagocytophilum in wild boars in Italy.
largely shed in placentas and feces of infected mammals. Humans and animals usually acquire the infection through inhalation or ingestion[13]. However, *C. burnetii*-infected ticks may be a source of the microorganisms and transmit them to wild animals that can concur to the maintenance of this pathogen in the environment[14].

2. Materials and methods

Spleen samples were collected from 100 wild boars (*Sus scrofa*) killed during the hunting seasons November 2013–December 2014, November 2014–December 2015, in forested areas of Tuscany.

Genomic DNA was extracted from each sample using the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany) and following the manufacturer’s protocol. DNA samples were submitted to three different PCR assays to detect *A. phagocytophilum*, *B. burgdorferi* s.l. and *C. burnetii*.

PCR amplifications were performed in a total volume of 25 µL containing EconoTaq PLUS 2X Master Mix (Lucigen Corporation, Middleton, Wisconsin, USA), 0.5 µmol/L of forward and reverse primers and 1 µL of DNA. All primers sequences and PCR conditions were reported in Table 1. All PCRs were performed in an automated thermal cycler (Gene-Amp PCR System 2700, Perkin Elmer, Norwalk, Connecticut, USA).

PCR products were run on 1.5% agarose gel at 100 V for 45 min, and gel was stained with ethidium bromide and observed. SharpMass™ 100 Plus Ladder (Euroclone, Milano, Italy) was added as DNA marker in each gel.

3. Results and discussion

Among the 100 wild boars tested in the present investigation, one (1%) was positive for *A. phagocytophilum*, 3 (3%) for *B. burgdorferi* s.l., whereas no positive reactions were observed for *C. burnetii*.

Wild boars have been previously suggested to be involved in the enzootic cycle of *A. phagocytophilum*. In fact, some studies detected DNA of this tick-borne pathogen in tissue samples collected from wild boars with prevalence values ranging from 2.7% to 12.5%[5,9], and *A. phagocytophilum*-infected ticks have been found among wild boar populations[5]. However, the studies about the presence of *A. phagocytophilum* in wild boars are very few and they are carried out on reduced number of subjects, thus it is not possible to determine if these animals can be considered as reservoir hosts of the pathogen.

At the best of our knowledge, this is the first report of *A. phagocytophilum* infection in wild boars in Italy, thus even though the detected low prevalence suggests that these animals are not important reservoir hosts for *A. phagocytophilum*, wild boars result to be susceptible to the agent.

*B. burgdorferi* s.l. is currently considered the most common tick-borne pathogen in the northern hemisphere and it has been frequently detected in ticks and wild animals in Italy[17-22]. Several vertebrates are involved in the cycle of this agent, but the role of wild boars is currently not defined[7]. Our results are strongly in agreement with those obtained in a recent molecular investigation which found *B. burgdorferi* s.l. in wild boars blood samples with a 3.3% prevalence[7]. A serological survey carried out in Czech Republic on 642 wild boars sera found an overall seroprevalence rate of 12.8%, suggesting the exposure of these animals to ticks infected with *B. burgdorferi* s.l.[2]. Other studies detected both wild boar DNA and *Borrelia* DNA in the blood meal of ticks[4], but borrelia DNA was not found in wild boars samples (blood and tissues) during recent surveys[5,8].

In the last few years, the presence of *C. burnetii* was reported in Central Italy in ticks and cervids[21,22], suggesting that *Coxiella* is circulating in the wild environments of this geographic area where their transmission is mainly due to infected ticks.

Even though wild mammals are considered susceptible to *C. burnetii* infections, data about the spreading of this pathogen among wildlife and in particular wild boars are very scant. Some authors, thinking that the demographic explosion of wild boars in Europe could influence *C. burnetii* ecology, carried out two surveys in Spain finding the 4.3% and 1% of wild boars infected by *C. burnetii*, respectively[3,6]. However, nothing is known about infection and shedding pathways in these suids to date.

Our negative results could be related to the absence or a very low circulation of *C. burnetii* among wildlife of the considered geographic area at the sampling time.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Primers sequences, investigated amplicons and PCR conditions of the PCR assays employed to detect each pathogen.</th>
<th>PCR conditions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pathogens</strong></td>
<td><strong>Amplicons (target gene)</strong></td>
<td><strong>Primers sequence (5′–3′)</strong></td>
<td><strong>PCR conditions</strong></td>
</tr>
<tr>
<td><em>A. phagocytophilum</em></td>
<td>932 bp (16S rRNA)</td>
<td>GE3a (CAGATCGAAGTCGAAACGGATTATTC)</td>
<td>95 °C, 30 s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GE10r (TTCGTTAAGAGGATCTAATCCTC)</td>
<td>55 °C, 30 s</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72 °C, 1 min</td>
</tr>
<tr>
<td></td>
<td>546 bp (16S rRNA)</td>
<td>GE9f (AACGGATTATCTTTTADAGCTTGCT)</td>
<td>95 °C, 30 s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GE2 (GGCAGTATTAAAAGCAGCTCCAGG)</td>
<td>55 °C, 30 s</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72 °C, 1 min</td>
</tr>
<tr>
<td><strong>B. burgdorferi</strong> s.l.</td>
<td>261 bp (23S rRNA)</td>
<td>JS1 (AGAGGTGCTGGAGTCTGA)</td>
<td>95 °C, 1 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JS2 (TAGGTCTCTACCTTATATA)</td>
<td>39 °C, 1 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72 °C, 2 min</td>
</tr>
<tr>
<td><strong>C. burnetii</strong></td>
<td>687 bp (JS1111a)</td>
<td>Trans-1 (TATGATCCACCGTAGGCAG)</td>
<td>95 °C, 1 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trans-2 (CCCCGAAACACCTTCATTAC)</td>
<td>64 °C, 1 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72 °C, 1 min</td>
</tr>
</tbody>
</table>

*: Primary amplification; **: Secondary amplification.
4. Conclusion

The demographic increasing of wild boar population has drawn attention to the risk of transmission of pathogens from these animals to livestock and humans. The role of wild boars in the epidemiological cycle of the tick-borne agents is poorly investigated, so final conclusions are currently not possible.

According to the findings reported herein wild boars do not seem to play an important role in the cycle of *A. phagocytophilum*, *B. burgdorferi* s.l. and *C. burnetii* in Central Italy.

Considering that the analyzed animals lived in geographic areas where these tick-borne agents have been previously reported, the obtained results could be related to a low circulation of the pathogens at the sampling time. However, the detection of *A. phagocytophilum* and *B. burgdorferi* s.l. in the spleen of the tested animals shows that wild boars can harbor these pathogens, thus they can be involved in their epidemiological cycle, even though marginally.

Ticks, sucking the blood of infected wild boars, may acquire the pathogens and thus become a source of infection for other wild animals, such as for hunters and their dogs.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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References