

## Asian Pacific Journal of Tropical Disease

journal homepage: <http://www.apjtdm.com>Short communication <https://doi.org/10.12980/apjtd.7.2017D6-412> ©2017 by the Asian Pacific Journal of Tropical Disease. All rights reserved.Zoonotic tick-borne bacteria among wild boars (*Sus scrofa*) in Central Italy

Valentina Virginia Ebani, Fabrizio Bertelloni, Gaia Cecconi, Micaela Sgorbini, Domenico Cerri

Department of Veterinary Science, University of Pisa, Viale delle Piagge 2, 56124, Pisa, Italy

## ARTICLE INFO

## Article history:

Received 11 Nov 2016

Received in revised form 22 Nov, 2nd revised form 29 Nov 2016

Accepted 19 Dec 2016

Available online 16 Jan 2017

## Keywords:

Wild boars (*Sus scrofa*)

Tick-borne pathogens

*Anaplasma phagocytophilum**Borrelia burgdorferi* s.l.*Coxiella burnetii*

## ABSTRACT

The objective 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 *Coxiella 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.

## 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 three zoonotic bacteria, *Anaplasma phagocytophilum* (*A. phagocytophilum*), *Borrelia burgdorferi* sensu lato (*B. burgdorferi* s.l.) and *Coxiella burnetii* (*C. burnetii*), in wild boars hunted in Central Italy.

The first two pathogens have been chosen because they are largely present in this geographic area. *A. phagocytophilum* is an intracellular bacterium transmitted by ixodid ticks, causing a disease called granulocytic anaplasmosis in humans and animals, mainly dogs, horses and ruminants[9].

*B. burgdorferi* s.l. complex includes the spirochetes that cause Lyme borreliosis that is currently considered the most common tick-borne zoonosis in Europe and North America[10,11], mainly transmitted by *Ixodes ricinus*[12].

*C. burnetii*, agent of the Q fever, has been investigated because it is an emerging microorganism related to reproduction disorders of ruminants and severe cases of human infections. *Coxiella* is

\*Corresponding author: Valentina Virginia Ebani, DVM, PhD, Department of Veterinary Science, viale delle Piagge 2, 56124 Pisa, Italy.

Tel: +39 50 2216968

E-mail: [valentina.virginia.ebani@unipi.it](mailto:valentina.virginia.ebani@unipi.it)

The journal implements double-blind peer review practiced by specially invited international editorial board members.

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  $\mu$ L containing EconoTaq PLUS 2X Master Mix (Lucigen Corporation, Middleton, Wisconsin, USA), 0.5  $\mu$ mol/L of forward and reverse primers and 1  $\mu$ L 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 1**

Primers sequences, investigated amplicons and PCR conditions of the PCR assays employed to detect each pathogen.

Pathogens	Amplicons (target gene)	Primers sequence (5'–3')	PCR conditions	References
<i>A. phagocytophilum</i>	932 bp (16S rRNA)	*GE3a (CACATGCAAGTCGAACGGATTATTC) GE10r (TTCCGTAAAGAAGGATCTAATCTCC)	95 °C, 30 s 55 °C, 30 s 72 °C, 1 min	[15]
	546 bp (16S rRNA)	**GE9f (AACGGATTATTCCTTATAGCTTGCT) GE2 (GGCAGTATTAAGCAGCTCCAGG)	95 °C, 30 s 55 °C, 30 s 72 °C, 1 min	
<i>B. burgdorferi</i> s.l.	261 bp (23S rRNA)	JS1 (AGAAGTGCTGGAGTCGA) JS2 (TAGTGCTCTACCTCTATTAA)	95 °C, 1 min 39 °C, 1 min 72 °C, 2 min	[16]
<i>C. burnetii</i>	687 bp (IS1111a)	Trans-1 (TATGTATCCACCGTAGCCAGT) Trans-2 (CCAACAACACCTCCTTATTC)	95 °C, 30 s 64 °C, 1 min 72 °C, 1 min	[13]

\*: 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

This study was performed with funds from the University of Pisa.

#### References

- [1] Beugnet F, Marié JL. Emerging arthropod-borne diseases of companion animals in Europe. *Vet Parasitol* 2009; **163**: 298-305.
- [2] Juricová Z, Hubálek Z. Serologic survey of the wild boar (*Sus scrofa*) for *Borrelia burgdorferi* sensu lato. *Vector Borne Zoonotic Dis* 2009; **9**: 479-82.
- [3] Astobiza I, Barral M, Ruiz-Fons F, Barandika JF, Gerrickagoitia X, Hurtado A, et al. Molecular investigation of the occurrence of *Coxiella burnetii* in wildlife and ticks in an endemic area. *Vet Microbiol* 2011; **147**: 190-4.
- [4] Wodecka B, Rymaszewska A, Skotarczak B. Host and pathogen DNA identification in blood meals of nymphal *Ixodes ricinus* ticks from forest parks and rural forests of Poland. *Exp Appl Acarol* 2014; **62**: 543-55.
- [5] Silaghi C, Pfister K, Overzier E. Molecular investigation for bacterial and protozoan tick-borne pathogens in wild boars (*Sus scrofa*) from Southern Germany. *Vector Borne Zoonotic Dis* 2014; **14**: 371-3.
- [6] González-Barrío D, Martín-Hernando MP, Ruiz-Fons F. Shedding patterns of endemic Eurasian wild boar (*Sus scrofa*) pathogens. *Res Vet Sci* 2015; **102**: 206-11.
- [7] Faria AS, Paiva-Cardoso Md, Nunes M, Carreira T, Vale-Gonçalves HM, Veloso O, et al. First detection of *Borrelia burgdorferi* sensu lato DNA in serum of the wild boar (*Sus scrofa*) in northern Portugal by nested-PCR. *Ecohealth* 2015; **12**: 183-7.
- [8] Pereira A, Parreira R, Nunes M, Casadinho A, Vieira ML, Campino L, et al. Molecular detection of tick-borne bacteria and protozoa in cervids and wild boars from Portugal. *Parasit Vectors* 2016; **9**: 251.
- [9] Stuen S, Granquist EG, Silaghi C. *Anaplasma phagocytophilum* – a wide spread multi-host pathogen with highly adaptive strategies. *Front Cell Infect Microbiol* 2013; **3**: 33.
- [10] Rizzoli A, Hauffe HC, Carpi G, Vourec'h GI, Neteler M, Rosà R. Lyme borreliosis in Europe. *Euro Surveill* 2011; **16**(27): 19906.
- [11] O'Connell S. European concerted action on Lyme borreliosis (EUCALB). *Euro Surveill* 1996; **1**(3): 23-4.
- [12] Gern L. Life cycle of *Borrelia burgdorferi* sensu lato and transmission to humans. *Curr Probl Dermatol* 2009; **37**: 18-30.
- [13] Berri M, Rekiki A, Boumedine KS, Rodolakis A. Simultaneous differential detection of *Chlamydomydia abortus*, *Chlamydomydia pecorum*, and *Coxiella burnetii* from aborted ruminant's clinical samples using multiplex PCR. *BMC Microbiol* 2009; **9**: 130.
- [14] Ruiz-Fons F, Rodríguez O, Torina A, Naranjo V, Gortázar C, De la Fuente J. Prevalence of *Coxiella burnetii* infection in wild and farmed ungulates. *Vet Microbiol* 2008; **126**: 282-6.
- [15] Massung RF, Slater K, Owens JH, Nicholson WL, Mather TN, Solberg VB, et al. Nested PCR assay for detection of granulocytic ehrlichiae. *J Clin Microbiol* 1998; **36**: 1090-5.
- [16] Chang YF, Novosol V, McDonough SP, Chang CF, Jacobson RH, Divers T, et al. Experimental infection of ponies with *Borrelia burgdorferi* by exposure to ixodid ticks. *Vet Pathol* 2000; **37**: 68-76.
- [17] Santino I, Iori A, Nicoletti M, Valletta S, Cimmino C, Scoarughi GL, Santapaola D, et al. Prevalence of *Borrelia burgdorferi* sensu lato genospecies and of the human granulocytic ehrlichiosis (HGE) agent in *Ixodes ricinus* ticks collected in the area of Monti Lepini, Italy. *Int J Immunopathol Pharmacol* 2003; **16**: 105-8.
- [18] Lorusso V, Lia RP, Dantas-Torres F, Mallia E, Ravagnan S, Capelli G, et al. Ixodid ticks of road-killed wildlife species in Southern Italy: new tick-host associations and locality records. *Exp Appl Acarol* 2011; **55**: 293-300.
- [19] Pascucci I, Di Domenico M, Dall'Acqua F, Sozio G, Cammà C. Detection of Lyme disease and Q fever agents in wild rodents in Central Italy. *Vector Borne Zoonotic Dis* 2015; **15**: 404-11.
- [20] Pintore MD, Ceballos L, Iulini B, Tomassone L, Pautasso A, Corbellini D, et al. Detection of invasive *Borrelia burgdorferi* strains in north-eastern Piedmont, Italy. *Zoonoses Public Health* 2015; **62**: 365-74.
- [21] Ebani VV, Bertelloni F, Turchi B, Filogari D, Cerri D. Molecular survey of tick-borne pathogens in ixodid ticks collected from hunted wild animals in Tuscany, Italy. *Asian Pac J Trop Med* 2015; **8**: 714-7.
- [22] Ebani VV, Rocchigiani G, Bertelloni F, Nardoni S, Leoni A, Nicoloso S, et al. Molecular survey on the presence of zoonotic arthropod-borne pathogens in wild red deer (*Cervus elaphus*). *Comp Immunol Microbiol Infect Dis* 2016; **47**: 77-80.