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Molecular Investigation for Bacterial and Protozoan Tick-Borne Pathogens in Wild Boars (*Sus scrofa*) from Southern Germany

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Abstract

Wild boars (*Sus scrofa*) have been suggested to be involved in the enzootic cycle of the tick-borne pathogen *Anaplasma phagocytophilum*. This observation raises the question whether they serve as reservoir hosts for *A. phagocytophilum* and potentially for other tick-borne pathogens of public health relevance. The aim of this study was to investigate wild boars and their ticks from a forest site in southern Germany for the presence of *A. phagocytophilum, Candidatus* Neoehrlichia mikurensis, *Rickettsia* spp., *Borrelia burgdorferi* sensu lato (s.l.), *Borrelia* spp. of the relapsing fever group, and *Babesia* spp. Therefore, 24 wild boars collected from October, 2010, to February, 2013, were investigated by molecular methods. DNA of *A. phagocytophilum* was detected in three out of 24 (12.5%) wild boars and in four out of 16 (25%) ticks. DNA of none of the other pathogens was found in any wild boar, but *Rickettsi*a spp., *B. burgdorferi* s.l., and *Cand.* N. mikurensis were found in one of the investigated ticks each. Sequences of the partial *16S rRNA* gene of *A. phagocytophilum* from one spleen and two ticks showed 100% similarity to GenBank entries from human anaplasmosis cases (accession nos. U02521 and AY886761). The sequence from the third tick was 100% similar to sequences obtained from *Ixodes ricinus* and roe deer from the same study area previously. Detecting a potentially human pathogenic *A. phagocytophilum* variant in wild boar confirms previous findings and is of public health interest. To our knowledge, this is the first report of *A. phagocytophilum* in wild boars in Germany. Whether wild boars support the enzootic cycle of *A. phagocytophilum* variants involved in human disease requires further attention in future systematic studies.

Key Words: *Sus scrofa*—*Anaplasma phagocytophilum*—*Ixodes ricinus*—*Rickettsia* spp.—*Borrelia* spp.—*Babesia* spp.—*Candidatus* Neoehrlichia mikurensis—Germany.

Introduction

Wild boars (*Sus scrofa*) have been suggested as being involved in the enzootic cycle of the tick-borne pathogen *Anaplasma phagocytophilum*, the causative agent of granulocytic anaplasmosis in several mammalian species (Michalik et al. 2012). Thereby, the prototype variant of *A. phagocytophilum*, known to be present in human anaplasmosis cases (GenBank accession no. U02521), has been identified in wild boar (accession no. GU391320) (Michalik et al. 2012). This observation raises the question whether wild boars serve as reservoir hosts for *A. phagocytophilum* and potentially for other tick-borne pathogens of public health relevance. To our knowledge, there is no information available on tick-borne pathogens in wild boars in Germany. Thus, the aim of the present study was to investigate wild boars and their ticks for the presence of *A. phagocytophilum*, *Candidatus* Neoehrlichia mikurensis, *Rickettsia* spp., *Borrelia burgdorferi* sensu lato (s.l.), *Borrelia* spp. of the relapsing fever group (aiming at potential detection of *B. miyamotoi*), and *Babesia* spp.

Materials and Methods

From October, 2010, to February, 2013, a total of 24 wild boars (*Sus scrofa*) were sampled. The animals have been professionally hunted in the Angelberger Forst/South Germany. The study site has previously been described in detail (Overzier et al. 2013). Altogether 24 spleen and 21 blood samples (obtained from October, 2010, to January, 2013) as well as 12 skin samples (obtained from May, 2011, to January, 2013) were collected. Additionally, 16 engorged *Ixodes ricinus* ticks were collected from two of the 24 wild boars (8.3%). No ticks were found on the remaining wild boars. DNA was isolated from all tissue samples and ticks. Samples

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were investigated with molecular methods for the presence of *A. phagocytophilum*, *Cand.* N.mikurensis, *B. burgdorferi* s.l., *B. miyamotoi* (all materials), *Rickettsia* spp. (spleen, skin, ticks), and *Babesia* spp. (blood, spleen, ticks). Additionally, in samples positive for *A. phagocytophilum*, a part of the *16S rRNA* gene was amplified and sequenced. Details of the DNA extraction, PCR conditions of *A. phagocytophilum*, *Cand*. N. mikurensis, *Rickettsia* spp., and *Babesia* spp. as well as the sequencing process have been published elsewhere (Silaghi et al. 2012, Overzier et al. 2013).

B. burgdorferi s.l. were detected with a conventional PCR targeting the *rrfA–rrlB* (5S 23S rDNA) intergenic spacer (Derdakova et al. 2003) using primers IgsA 5′-CGA CCT TCT TCG CCT TAA AGC-3′, and IgsB 5′-AGC TCT TAT TCG CTG ATG GTA-5′ followed by agarose gel electrophoresis. *Borrelia* spp. of the relapsing fever group were targeted with a probe-based real-time PCR aiming at the 23S DNA with primers RF23sF 5′-CGGTACTCTTCACTATCG GTAGCTT-3′ and RF23sR TGGAAAAGTTAGCCARAG AAGG, as well as probe RF23sP 6FAM-TCCCGTCCTAC TTAGGAACATC-TAMRA (Subramanian et al. 2012), in an AB7500fast using the TaqMan® Fast Universal PCR Mastermix (2×) (Applied Biosystems, Darmstadt, Germany).

Sequences from this study were deposited in GenBank: *A. phagocytophilum* wild boar (accession no. KC833754), engorged *I. ricinus* (KC833751–KC833753), *Rickettsia* spp. engorged *I. ricinus* (KC833755), *B. burgdorferi* sensu stricto (s.s.) engorged *I. ricinus* (KF859737).

Results

DNA of *A. phagocytophilum* was detected in three out of 24 (12.5%) wild boars and in four out of 16 (25%) ticks. The four *A. phagocytophilum*–positive ticks were from one of the *A. phagocytophilum*–positive wild boars (Table 1). Sequences of the *16S rRNA* gene (497 bp) of *A. phagocytophilum* were

Table 1. Results of the PCR Screening for Tick-Borne Pathogens in Wild Boar in Southern Germany, 2010–2013

	Wild boar positive for							
Wild boar ID no.	Anaplasma phagocytophilum	Candidatus *Neoehrlichia mikurensis*	Rickettsia *spp.*	Borrelia burgdorferi *sensu lato*	*Relapsing fever group* Borrelia	Babesia *spp.*	*No. of ticks from wild boar*	*No. of ticks positive for pathogens*
TW22	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW23	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW25	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW32	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 40	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 42	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 43	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 46	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 47	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 48	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 50	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 51	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 71	Positive[a]	Neg	Neg	Neg	Neg	Neg	15	4 x *A. phagocytophilum* 1 x *Rickettsia* spp. 1 x *Cand.* Neoehrlichia mikurensis
TW 89	Positive[b]	Neg	Neg	Neg	Neg	Neg	1	1 x *Borrelia burgdorferi* sensu strictu
TW 99	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 106	Positive[c]	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 114	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 122	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 133	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 137	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 152	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 156	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 157	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
TW 158	Neg	Neg	Neg	Neg	Neg	Neg	n.d.	
All wild boar	**3/24**	**0/24**	**0/24**	**0/24**	**0/24**	**0/24**	**16**	

[a]Positive in spleen and skin, negative in blood.
[b]Positive in spleen and blood, negative in skin.
[c]Positive in spleen, negative in blood and skin
n.d., not detected.

obtained from one positive spleen and three ticks. Sequences from the spleen and two ticks showed 100% similarity to GenBank entries from human anaplasmosis cases in the United States and Slovenia (accession nos.U02521 and AY886761) and from wild boars and their engorged *I. ricinus* ticks in Poland (accesssion nos. GU391320 and GU391319) as well as from questing *I. ricinus* previously investigated from the same study area in southern Germany (Overzier et al. 2013; accession no. JX627369). The partial *16S rRNA* gene sequence of *A. phagocytophilum* from the third tick was 100% similar to sequences obtained from *I. ricinus*, from roe deer, and from engorged *I. ricinus* from roe deer also previously investigated from the same study area in Southern Germany (Overzier et al. 2013; accession nos. JX627371, JX627364, and JX627377). DNA of none of the other pathogens was found in any wild boar tissue samples. However, *Rickettsi*a spp. were found in one out of 16 (6.3%) of the investigated ticks (Table 1). Sequencing of the amplified *gltA* gene sequence revealed 100% identity to uncultured *Rickettsia* sp. clone (accession no. JN849396), whereas *omp B* gene sequencing failed. One further tick each was positive for *Cand*. N. mikurensis (6.3%) and *B. burgdorferi* s.l. (6.3%). Sequencing for species identification revealed *B. burgdorferi* s.s.

Discussion

No evidence for the involvement of wild boars and their ticks in the natural cycle of *Cand.* N. mikurensis, *B. burgdorferi* s.l., *B. miyamotoi*, *Rickettsia* spp., and *Babesia* spp. was obtained in this study. However, due to the low sample size, their potential involvement in the life cycles of these pathogens cannot be excluded. *A. phagocytophilum* has previously been detected in wild boars from several countries (*e.g*., Czech Republic, Poland, and Slovenia) with prevalence ranging from 6.2% to 14.3% (Petrovec et al. 2003, Michalik et al. 2012, Žele et al. 2012). Therefore, our detected prevalence lies within the range of the previously detected rates.

To our knowledge, we have detected *A. phagocytophilum* for the first time in wild boars in Germany. We identified a potentially human pathogenic *A. phagocytophilum* variant in wild boar that is of public health interest. The presence of *A. phagocytophilum* in 12.5% of wild boars and 25% of their ticks compared to 99% of roe deer and >85% of their ticks from the same study area (Overzier et al. 2013) is in line with the theory of a lower impact of wild boars as reservoir hosts for *A. phagocytophilum* (de la Fuente and Gortazar 2012, Galindo et al. 2012). On the contrary, a stable cycle of *A. phagocytophilum* involving wild boars was previously discussed, because a similar prevalence of *A. phagocytophilum* was detected in wild boars in Slovenia over several years (Strasek Smrdel et al. 2009, Žele et al. 2012). A similar stable cycle may develop in Germany because the *A. phagocytophilum* positive ticks were collected from *A. phagocytophilum*–positive wild boar and as such the pathogen may be disseminated further. So far, only the human pathogenic prototype variant was detected in wild boar and it is thus far not known if wild boars are susceptible to only this one *A. phagocytophilum* variant. It will be necessary to find out if wild boars develop clinical signs of granulocytic anaplasmosis and how long the infection persists in these animals, which are occurring more and more close to or in urban settlements. Whether wild boars support this enzootic cycle of *A. phagocytophilum* variants involved in human disease, and whether they consequently pose a threat to human health requires further attention in future systematic studies.

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Author Disclosure Statement

No competing financial interests exist.

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