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Tick-borne pathogens in Swedish wild boar and their attached ticks: the first detection of Borrelia species in wild boar in northern Europe

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Abstract

Background

Wild boar (*Sus scrofa*) have expanded in northern Europe, yet their role in the ecology of tick-borne pathogens remains poorly understood. This study investigated the occurrence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato in 88 Swedish wild boar and their attached *Ixodes ricinus* ticks by analysing multiple tissues (spleen, heart, urinary bladder and skin) using quantitative PCR.

Results:

A. phagocytophilum was detected in 20 (22.7%) wild boar, with infections (i.e. quantitative PCR positive) more common in juveniles than adults. Infection probability was lowest in spring but markedly higher in summer and autumn. *Borrelia* species DNA was detected in three juvenile males (3.4%), representing the first report of *Borrelia* species in wild boar in northern Europe. Thirty attached ticks were collected from 17 wild boar. Six ticks carried *A. phagocytophilum*, five carried *Borrelia* species, and one was coinfected. Ticks were more likely to be infected when collected from infected wild boar than from uninfected hosts, while feeding duration showed no detectable association with infection in either hosts or ticks.

Conclusions:

These findings provide new insight into the occurrence of tick-borne pathogens in wild boar and highlight the value of multi-tissue sampling and paired host–tick data for understanding pathogen dynamics in wildlife populations.

Background

Tick-borne pathogens such as *Borrelia burgdorferi* sensu lato (s.l.) and *Anaplasma phagocytophilum* are widely distributed across Europe and maintained through enzootic transmission cycles involving *Ixodes* ticks and a diverse range of vertebrate hosts. These pathogens are of considerable medical and veterinary importance and persist in nature through complex interactions between vectors, wildlife hosts, and environmental conditions. While the roles of small mammals and birds as reservoir hosts are well established, and large mammals are recognised as important maintenance hosts for *Ixodes ricinus*, the contribution of many wildlife species to pathogen maintenance and transmission remains insufficiently understood [1–3].

Wild boar (*Sus scrofa*) populations have expanded markedly across Europe in recent decades, including in Sweden, where the wild boar have increased in both abundance and geographic range [4]. As wild boar home ranges increasingly overlap with human-dominated habitats, their potential role in the ecology of

tick-borne pathogens should gain increasing attention. A few earlier studies have examined wild boar as a potential host for *Borrelia* spp. and *A. phagocytophilum*, but previous studies have produced variable and sometimes conflicting results. Moreover, data from northern Europe remains limited, and the role of wild boar in regional transmission cycles is still unclear.

Tick feeding duration influences the likelihood that *B. burgdorferi* s.l. and *A. phagocytophilum* are transmitted, as both pathogens generally require prolonged attachment for successful transfer [5, 6]. Despite this, feeding duration is rarely quantified in field studies, and empirical data on attachment times on large wild mammals, including wild boar, are particularly limited. Previous investigations have also typically focused on a single host tissue or examined either hosts or ticks in isolation, which restricts opportunities to compare infection status across different sample types collected from the same host individual. Low and uneven bacterial loads of both *Borrelia* spp. and *A. phagocytophilum* further complicate detection when only one tissue type is sampled. Together, these factors make it difficult to assess how often infections coincide in hosts and their attached ticks, and how feeding duration may affect these infection patterns.

The aim of this study is to investigate the occurrence of *B. burgdorferi* s.l. and *A. phagocytophilum* in wild boar in Sweden by analysing multiple host tissue types in relation to infection status and feeding duration of attached *I. ricinus*. By combining host- and tick-level data, the study sought to provide a more comprehensive understanding of pathogen detections in wild boar and their ticks in a northern European context.

Methods

Study area and sample collection

In February 2023, local hunting associations across Sweden were invited to participate in a study on tick-borne pathogens in wild boar. This study uses the same wild boar material as Lindell et al. [unpubl.], where spleen tissue was analysed for presence of *Neoehrlichia mikurensis*. In the current study, we extended the approach by including multiple host tissues and attached ticks to investigate the prevalence of *Borrelia* spp. and *A. phagocytophilum*.

A total of 229 hunting associations were contacted, ranging from southern Sweden [Skåne] to Dalarna, which represents the present northern distribution limit of wild boar in Sweden. Thirty-two of the hunting associations agreed to participate in the study. Between March 2023 and March 2025, hunters collected samples from wild boar harvested during regular hunting activities.

Hunters were asked to submit samples of spleen, heart, and urinary bladder from each harvested animal. When present, attached ticks and a small piece of skin from the tick attachment site were also collected. Because tick collection relied on voluntary hunters and was performed under varying field conditions, it is most likely that several attached ticks were not detected or collected. Consequently, the number of wild boar recorded with ticks should be considered as a conservative estimate, and the sampling does

not allow conclusions about overall tick burden in wild boar populations in Sweden. All samples were preserved in 70% ethanol and shipped to the laboratory, where they were stored at − 80°C until further processing

Wild boar were sampled across a broad geographical range in Sweden, covering ten counties: Halland (n = 1), Kronoberg (n = 6), Örebro (n = 3), Östergötland (n = 2), Skåne (n = 4), Södermanland (n = 33)), Stockholm (n = 2), Uppsala (n = 12), Västmanland (n = 3), and Västra Götaland (n = 22) (Fig. 1). The frequency distribution of the wild boar sampled largely reflects the density of wild boar populations in southern and central Sweden estimated from wild boar related traffic collisions, which are commonly used as a proxy for population density [7].

Ethical approval

was not required for this study because no live animals were handled or culled specifically for research purposes. All wild boars were legally harvested for human consumption in accordance with the Swedish Hunting Act (SFS 1987:259).

DNA extraction

DNA was extracted from wild boar tissues and ticks using two different Qiagen kits. Tissue samples (spleen, heart, urinary bladder, and skin) were processed with the QIAamp Fast DNA Tissue Kit (Qiagen) according to the manufacturer's instructions for animal tissues. Tick DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen), following the manufacturer's supplementary protocol *"Purification of total DNA from ticks using the DNeasy Blood & Tissue Kit for detection of Borrelia DNA"*. All DNA extracts were stored at − 80°C until qPCR analysis.

qPCR

DNA extracts from both wild boar tissues and ticks were analysed using TaqMan qPCR assays targeting the *gltA* gene of *A. phagocytophilum* and the 16S rRNA gene of *B. burgdorferi* s.l. Primer and probe sequences used in both tissue and tick assays are listed in Table 1. The positive control for *A. phagocytophilum* was a plasmid containing the *A. phagocytophilum* gltA sequence (GenBank: U5972). The positive control for *Borrelia* spp. was cultured *Borrelia garinii* DNA provided by Jönköping county hospital Ryhof. The negative control was nuclease-free water.

Table 1
Primers and probes used for qPCR

Target	Primer/probe	Sequence [5'–3']
A phagocytophilum	Forward	TTTTGGGCGCTGAATACGAT
	Reverse	TCTCGAGGGAATGATCTAATAACGT
	Probe	FAM-TGCCTGAACAAGTTATG-MGB
Borrelia spp.	Forward	GCTGAGTCACGAAAGCGTAG
	Reverse	CACTTAACACGTTAGCTTCGGTA
	Probe	FAM-CGCTGTAAACGATGCACACTTGGT-MGB
Sequences are shown in the 5'–3' direction. FAM and MGB denote probe labels		

Tissue extracts (spleen, heart, urinary bladder, and skin) were analysed with a TaqMan Exogenous Internal Positive Control (IPC, Applied Biosystems, Foster City, CA, USA) to monitor potential PCR inhibition. Tissue reactions (50 µl) consisted of 25 µl TaqPath™ BactoPure™ Microbial Detection Master Mix, 5 µl 10× IPC Mix, 1 µl 50× IPC DNA, 2.5 µl of each primer, 1 µl probe, 8 µl nuclease-free water, and 5 µl template DNA. Cycling conditions for tissue samples were 50°C for 2 min, 95°C for 10 min followed by 55 cycles of 95°C for 15 s and 60°C for 1 min. A tissue sample was considered positive when a clear amplification curve with a Cq < 46 was obtained. Only one tissue sample, a urinary bladder sample analysed for *Borrelia*, showed signs of inhibition, which resolved after a 1:5 dilution.

For tick extracts, *A. phagocytophilum* 25 µl reactions was prepared containing 12.5 µl Maxima® Probe qPCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 1.5 µl of each primer, 0.375 µl probe, 7.125 µl nuclease-free water, and 2 µl template DNA. For *Borrelia* spp., 20 µl reactions contained 10 µl Maxima® Probe Master Mix, 0.4 µl of each primer, 0.4 µl probe, 6.8 µl water, and 2 µl template DNA. Cycling conditions for *A. phagocytophilum* were 95°C for 10 min followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. For *Borrelia* spp., reactions were initiated at 95°C for 5 min and followed by 50 cycles of 95°C for 10 s and 60°C for 60 s. Tick sample was considered positive when a clear amplification curve with Cq < 40 was obtained.

Feeding duration

Ticks collected from wild boar were measured under a light microscope equipped with an ocular micrometer at 40× magnification. For each tick, the scutal index (idiosoma length divided by scutal width) and the coxal index (idiosoma length divided by coxal gap between the fourth pair of legs) were calculated (Fig. 2). Feeding duration estimates were then derived using the regression equations published by Gray et al. [9]. Because the accuracy of these indices varies with engorgement stage, both indices were initially calculated for all ticks [8, 9]. The coxal index was used when feeding duration was estimated to be < 24 h or when the scutal index was unreliable due to specimen condition (e.g. damage

or distortion) or in cases of advanced engorgement, where the scutal index equation produced invalid or implausible values due to the rounded body shape. When both indices produced similar values above 24 h, the scutal index was used as the primary estimator.

Statistical analyses

To assess the associations between infection of *A. phagocytophilum* (yes, no) and wild boar life stage (juvenile, adult), gender (male, female) and season (spring, summer, autumn, winter) we used generalized linear models assuming binomial error distribution. The significance of explanatory variables was assessed using likelihood ratio tests. Differences among hunting seasons were analysed with Dunnett's post hoc test using spring as reference season.

Pathogen transmission can occur in both directions (tick-to-host and host-to-tick). Therefore, two different analyses were performed, one with infection risk in hosts as a function of tick status, and with infection risk in ticks as a function of host status. For the host-level analysis, a generalized linear model was used with host infection status (yes, no) as the outcome variable, and with the number of *A. phagocytophilum* infected ticks and total feeding duration summed across all ticks per host as explanatory variables. For the analysis of the risk of infection in ticks we used a generalized linear mixed-effects model. In this model, tick infection status (yes, no) was treated as the outcome, and feeding duration and host infection status (yes, no) were included as fixed predictors. Wild boar ID was incorporated as a random factor to account for the dependence of ticks sampled from the same host.

All statistical analyses were conducted in R version 4.4.2 (R Core Team 2024) and Rstudio [10] using packages car [11] and lme4 [12]. GLM models were evaluated using basic diagnostic plots and model check with AIC. For the mixed-effects tick model, residual diagnostics were performed using package DHARMa [13].

Results

In total, 88 wild boar were included in the study. Heart tissue was available from all animals (n = 88), while spleen (n = 87) and urinary bladder samples (n = 82) were available from slightly fewer individuals. Attached ticks (n = 30) and skin samples from the corresponding attachment sites (n = 28) were obtained from 17 wild boar, with some individuals carrying more than one tick. Of the 88 wild boar, 45 (51.1%) were males and 43 (48.9%) were females. Of these, 47 were juveniles (4–12 months; 53.4%) and 41 were adults (> 12 months; 46.6%). No piglets (< 4 months) were obtained during wild boar sampling. Animals were collected across all seasons, with most samples obtained in winter (n = 40), followed by spring (n = 21), summer (n = 16), and autumn (n = 11).

Anaplasma phagocytophilum

A. phagocytophilum was detected in 20 of 88 wild boar, corresponding to an overall prevalence of 22.7%. Among positive animals, 12 (60%) were males and 8 (40%) were females, corresponding to a prevalences of 26.7% (12/45) and 18.6% (8/43), respectively. Regarding age, 15 of the 20 positive wild boar (75%) were juveniles and five (25%) were adults, with corresponding prevalences of 31.9% (15/47) and 12.2% (5/41), respectively (Table 2). Of the 20 positive animals, one was collected in spring (5%), seven in summer (35%), five in autumn (25%), and seven in winter (35%). When related to the total number of animals sampled in each season, this corresponded to prevalences of 4.8% (1/21) in spring, 43.8% (7/16) in summer, 45.5% (5/11) in autumn, and 17.5% (7/40) in winter (Table 2). *A. phagocytophilum* was detected throughout the study area (Fig. 3).

Table 2
Prevalence of *Anaplasma phagocytophilum* in wild boar by gender, age class, and hunting season

Category	**Positive for *A. phagocytophilum* n (%)**	**Total n**	**Prevalence within category**
Gender			
Male	12 (60)	45	26.7%
Female	8 (40)	43	18.6%
Total	20 (100)	88	22.7%
Age			
Piglet: < 4 months	0	0	0%
Juvenile: 4–12 months	15(75)	47	31.9%
Adult: >12 months	5 (25)	41	12.2%
Total	20 (100)	88	22.7%
Hunting season			
Spring (March-May)	1 (5)	21	4.8%
Summer (June-August)	7 (35)	16	43.8%
Autum (September-November)	5 (25)	11	45.5%
Winter (December-February)	7 (35)	39	17.9%
Total	20 (100)	88	22.7%

The column “Positive for *A. phagocytophilum* n (%)” shows the number of PCR-positive animals in each category, with percentages indicating the proportion of all qPCR-positive animals (n = 20) represented by that category. The column “Prevalence in each category” shows the prevalence within each category, calculated as the proportion of qPCR-positive animals relative to the total number of sampled animals in that category (overall n = 88)

Infection in wild boar with *A. phagocytophilum* was significantly associated with age and hunting season. Juveniles had significantly higher odds of infection than adults (OR = 3.19, p = 0.048). Compared with spring, the odds for infection were significantly higher in summer (OR = 14.42, p = 0.046) and autumn (OR = 16.26, p = 0.048). Gender was not associated with host infection status (p = 0.57).

In most cases (n = 14), *A. phagocytophilum* was detected in a single organ (spleen n = 8, heart n = 3, urinary bladder n = 2, and skin n = 1). Of the remaining six animals, three showed infection in two organs (spleen and skin n = 2; spleen and heart n = 1), and three were positive in three organs [spleen, heart and skin n = 2; spleen, heart and urinary bladder n = 1). Overall, *A. phagocytophilum* was detected in 14 spleen samples (70% of the positive animals), 7 heart samples (35%), 3 urinary bladder samples (15%), and 5 skin samples (25%).

Borrelia spp.

Borrelia spp. DNA was detected in three male wild boar, corresponding to a prevalence of 3.4% among the 88 sampled individuals. All positive animals were young and originated from Kronoberg (n = 1) and Sörmland (n = 2). The three infected individuals tested positive in different organs: one in the skin, one in both the urinary bladder and spleen, and one in the heart. Two *Borrelia* detections occurred during the winter hunting season, while one was recorded in summer. Two of the three *Borrelia* spp. positive animals were also co-infected with *A. phagocytophilum*, one sampled in Sörmland and one in Kronoberg. Because of the low number of positive cases, no statistical analyses were performed with *Borrelia*.

Ticks and feeding duration

Ticks were collected from 17 wild boar, yielding a total of 30 attached ticks. The majority were adult females (n = 26), while the remainder were nymphs (n = 4). Overall, 10 of the 30 ticks (33.3%) were infected with at least one pathogen. *A. phagocytophilum* was detected in 6 (20%) ticks and *Borrelia* spp. in 5 (16.7%) ticks, with coinfection observed in one tick (3.3%).

Feeding duration was successfully estimated for 28 ticks. Both scutal and coxal indices were obtained for 26 ticks, while only one index could be calculated for two ticks due to damage. Feeding duration ranged from 6.4 to 189 hours (h). For *A. phagocytophilum*, simultaneous infection in the tick, the skin at the attachment site, and the host was observed in five cases, with feeding durations ranging from 38.1 to 159.9 h. In one case, the tick was positive while the host was negative (64.6 h). Conversely, in five cases the host tested positive, but the tick did not, with feeding durations ranging from 17.6 to 95.5 h. For *Borrelia*, one of the five positive ticks was associated with a positive skin sample, with a feeding duration of 38.1 h. The remaining three ticks were attached to non-infected wild boar, with feeding durations ranging from 45.2 to 82.6 h. Because only three wild boar tested PCR-positive for *Borrelia* spp., the host-level and tick-level analyses were conducted for *A. phagocytophilum* only.

In the host-level model, wild boar infection status with *A. phagocytophilum* was used as the response variable, and the number of attached ticks infected with *A. phagocytophilum* and cumulative tick feeding

duration were included as explanatory variables. Among the 17 wild boar included in this analysis, 5 carried at least one tick infected with *A. phagocytophilum*, while 12 carried only uninfected ticks. Infection in wild boar increased substantially with the number of attached ticks infected with *A. phagocytophilum* (OR = 23.3, p = 0.009), whereas total tick feeding duration showed no association with infection status (p = 0.503).

In the tick-level mixed-effects model, tick infection was used as the response variable, and host infection status and feeding duration were included as explanatory variables. Host ID was added as a random effect to account for multiple ticks originating from the same animal. Feeding duration was not associated with infection in ticks (p = 0.841). Ticks collected from infected hosts were more likely to be infected (p = 0.013). Estimates for the host-infection effect were very large due to the small sample size and close to total separation, so the emphasis is on the direction and statistical significance rather than the numerical magnitude of the odds ratio.

Discussion

A recent 15-year systematic review of *A. phagocytophilum* in European wild boar reported substantial geographic differences in prevalence across Europe [14]. The highest prevalence values were reported from Slovakia (28.2%) and Poland (20.34%), whereas markedly lower rates were observed in France (2%) and Portugal (3.1%). In addition, several studies from Austria and northern Spain detected no PCR-positive wild boar. Within this European context, the prevalence observed in the present study in Sweden (22.7%) falls within the upper range reported for the European continent.

The only previous Swedish investigation of *A. phagocytophilum* in wild boar reported a much higher prevalence (70.6%) than observed in our present study. Several factors may account for the discrepancy between our study and former study by Fabri et al. [15]. The earlier Swedish study was conducted within a restricted geographic area in south-central Sweden (Södermanland, Southern Stockholm, and Östergötland), which may represent a local hotspot both for *A. phagocytophilum*, ticks and wild boar. In contrast, the present study included samples collected across a much broader geographic range, likely capturing greater spatial heterogeneity in tick abundance, host community composition, and pathogen transmission dynamics. Furthermore, methodological differences between the studies, including sample type and PCR protocols, may also contribute to variation in reported prevalence estimates. Despite these differences in study design and spatial scale, both Swedish studies demonstrate that *A. phagocytophilum* occurs frequently in wild boar, supporting the notion that this species may be involved in the local epidemiology of the pathogen. Compared with many other European regions where prevalence in wild boar tends to be low or sporadic, the relatively high prevalence observed in Sweden suggests that wild boar could play a more significant role in local transmission cycles, potentially acting as important hosts contributing to pathogen maintenance within tick populations.

Another important factor to consider is the diversity of *A. phagocytophilum* ecotypes maintained by different reservoir hosts. Roe deer predominantly sustain ecotype 2, which is generally considered non-

zoonotic. In contrast, wild boar have repeatedly been shown to harbour ecotype 1, the ecotype responsible for all confirmed cases of human granulocytic anaplasmosis in Europe [16–18]. This distinction highlights an important epidemiological difference between these host species. While roe deer are often regarded as important hosts for maintaining *A. phagocytophilum* in enzootic cycles, their contribution to zoonotic transmission appears limited. However, wild boar may play a more relevant role in the maintenance and circulation of clinically relevant zoonotic strains. Consequently, the relatively high prevalence observed in Swedish wild boar populations may have important implications for the epidemiology and potential public and animal health risk associated with *A. phagocytophilum* in the region, suggesting a greater public health relevance than previously believed.

In addition to geographic variation, individual-level factors also appear to influence the prevalence of *A. phagocytophilum* in wild boar. The strong age effect observed in the present study, where juveniles showed approximately threefold higher odds (OR:3.16) of infection than adults, may be explained by several mechanisms. Younger animals generally have lower cumulative exposure and limited acquired immunity, potentially making them more susceptible to infection. Younger animals may also have a higher exposure risk due to higher exposure risk due to behavioural differences, movement patterns, habitat use or reduced grooming efficiency. Infection rates were lowest in spring, with much higher odds for infection observed during summer (OR:14.42) and autumn [OR:16.26]. This pattern is consistent with the seasonal ecology of *I. ricinus*, whose nymphal activity typically follows a bimodal distribution, with a second peak in late summer and early autumn [19], thereby increasing host exposure during these periods. In addition, cumulative exposure of hosts to tick bites over the season may increase the likelihood that hosts encountered in autumn are already infected, further contributing to the elevated infection risk.

Although several studies have investigated the presence of *Borrelia* spp. in wild boar, detections have generally been rare. *Borrelia* DNA has previously only been reported in wild boar in Portugal and Italy, with prevalences of 3.3% and 3.0%, respectively [20, 21]. The present study therefore represents the first report of *Borrelia* spp. in wild boar in northern Europe. The prevalence observed (3.4%) closely matches those documented in the two earlier studies.

Members of the *B. burgdorferi* s. l. complex are notoriously difficult to detect in host tissues. Two biological characteristics contribute to this challenge: rapid clearance of spirochetes from the bloodstream and the typically low bacterial loads present in infected hosts. Experimental infections have shown that intravenously injected *B. burgdorferi* is cleared from the bloodstream within one hour in rats, while dissemination to organs such as heart, kidneys, brain, and spleen occurs within 1–6 h post-infection [22]. Although individual ticks may contain several thousand spirochetes, bacterial loads in clinical blood samples often fall below 50 genomes per milliliter, particularly during later stages of infection [23]. When selecting tissues for pathogen detection, it is therefore important to consider both the dissemination pathway of the pathogen within the host and the practical feasibility of collecting samples in wildlife studies. This is an especially relevant consideration in studies relying on hunter participation.

Most previous studies have primarily used blood or spleen as sample material [15, 20, 21, 24–28]. However, experimental infection studies suggest that these tissues may not always be optimal for detecting *Borrelia spp*. In non-human primates, spirochetes have been detected months after infection in cerebrospinal fluid, peripheral nerves, skeletal muscle, heart, and urinary bladder, with the highest bacterial loads in bladder tissue [29]. Similar patterns have been observed in experimentally infected white-footed mice, where *Borrelia* was isolated most frequently from urinary bladder (94%), followed by kidneys (75%), spleen (61%), and blood (13%), with immunofluorescence confirming spirochetes within the bladder wall [30]. Comparable findings in Syrian hamsters demonstrated persistent infection in heart and urinary bladder tissues for up to 84 days, whereas persistence in spleen, liver, and kidneys was shorter and less consistent [31]. Together, these studies indicate that tissue selection strongly influences the probability of detecting *Borrelia* in hosts and that organs such as bladder and heart may provide higher detection sensitivity than blood or spleen alone. As an example, had the present study relied solely on spleen or blood, only one of the three *Borrelia*-positive wild boar would have been detected. The inclusion of multiple tissues therefore substantially increased detection probability and enabled a more complete assessment of *B. burgdorferi* s.l. occurrence in this host species.

Including multiple tissue types in the analysis may also increase the detection probability for *A. phagocytophilum*, although for somewhat different biological reasons than for *Borrelia* spp. In general, blood or spleen samples are considered suitable materials for determining the prevalence of this obligate intracellular bacterium. In humans, the pathogen can remain detectable in the bloodstream for up to 28 days, and persistent infections have been documented in several animal species, including sheep, horses, dogs, cattle, and red deer [32]. Consequently, PCR-based diagnostics are routinely used for detecting *Anaplasma* spp. infections in both humans and domestic animals [33, 34]. However, when bacterial loads are very low, infected neutrophils may be unevenly distributed within host tissues, which can reduce the probability of detection when only a single tissue type is analysed.

This pattern was demonstrated by Myczka et al. [35], who examined paired liver and spleen samples from 59 wild boar. Of the 12 individuals that tested positive, only one contained *A. phagocytophilum* DNA in both organs, whereas all other positives were detected in just one tissue. A similar pattern was observed in the present study. If we had analysed only spleen samples, 14 positive cases would have been detected, compared with the 20 identified when multiple tissues were included. Together, these findings demonstrate that analysing more than one tissue type can substantially improve detection sensitivity for *A. phagocytophilum*, particularly when pathogen loads are low or unevenly distributed within the host.

Tick numbers reported in this study should be interpreted with caution, as tick collection was not conducted systematically or with the specific aim of quantifying tick burden. One commonly stated reason for a limited consideration of wild boar in the epidemiology of tick-borne diseases is the perception that this species carries relatively few ticks. However, in the present study, a notable number of ticks were detected on wild boar despite the absence of systematic or exhaustive tick searches. This indicates that the tick burden documented here likely represents a conservative estimate of true

exposure. Together with the relatively high prevalence of tick-borne pathogens detected in wild boar, these findings suggest frequent contact with ticks and highlight that wild boar may be more involved in tick-borne disease ecology than is often recognized.

The strong association between infection in ticks and infection in wild boar is biologically reasonable, as ticks acquire pathogens from their hosts during feeding. However, because both tick and host infection were measured at a single time point, the direction of transmission cannot be determined. An infected tick may have acquired the pathogen from the host, the host may already have been infected prior to tick attachment, or both may have been infected independently. Even though transmission direction cannot be resolved, the close correspondence between host and tick infection nevertheless indicates that wild boar are involved in the epidemiology of *A. phagocytophilum*, at least as exposed and potentially infectious hosts within the local transmission system.

Feeding duration was not associated with infection in either ticks or hosts. Most collected ticks were already late-stage or heavily engorged, resulting in limited variation in feeding - only three ticks had attachment times shorter than 24 h. The low sample size of this study unavoidably results in low statistical power and restricts the possibilities to study underlaying relationship. Therefore, the absence of a detectable association in this study should not be taken as evidence for feeding duration to be biologically unimportant. The question is left open for future studies.

Conclusions

This study provides updated information on the occurrence of *A. phagocytophilum* and *Borrelia* spp. in Swedish wild boar and their attached ticks. The relatively high prevalence of *A. phagocytophilum* adds to growing evidence that this pathogen occurs regularly in wild boar populations. Because wild boar can carry *A. phagocytophilum* lineages belonging to ecotype I, the ecotype most often associated with human-pathogenic strains in Europe, this host species is relevant when assessing local transmission dynamics. We also report the first detection of *Borrelia* spp. in wild boar in northern Europe. The use of multiple tissue types increased detection sensitivity for low-level infections, and matching host–tick samples provided additional context for interpreting infection patterns. Larger and more complete datasets, ideally including systematic tick collection and consistent sampling of key organs, will be important for clarifying the role of wild boar in the circulation of tick-borne pathogens in northern Europe.

Declarations

Authors’ contributions

H.L. contributed to conceptualization, methodology, investigation, data curation, statistical analyses, and formal analysis, and drafted the original manuscript as well as contributed to review and editing.

K.L. contributed to supervision, statistical analyses, and review and editing of the manuscript.

P.D. contributed to supervision, funding acquisition, statistical analyses, and review and editing of the manuscript.

All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable

Ethics approval

This study did not require official or institutional ethical approval. The animals were handled according to high ethical standards and national legislation.

Prior publication

Data have not been published previously.

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References

1. Gassner F, Takken W, Lombaers-van der Plas C, Kastelein P, Hoetmer AJ, Holdinga M, et al. Rodent species as natural reservoirs of *Borrelia burgdorferi* sensu lato in different habitats of *Ixodes ricinus* in the Netherlands. Ticks Tick Borne Dis. 2013;4:452–8.
2. Humair PF. Birds and *Borrelia*. Int J Med Microbiol. 2002;291:70–4.

3. Mysterud A, Hügli C, Viljugrein H. Tick infestation on medium–large-sized mammalian hosts: are all equally suitable to *Ixodes ricinus* adults? Parasites Vectors. 2021;14:254.
4. Bergqvist G, Kindberg J, Elmhagen B. From virtually extinct to superabundant in 35 years: establishment, population growth and shifts in management focus of the Swedish wild boar (*Sus scrofa*) population. BMC Zool. 2024;9:14.
5. Katavolos P, Armstrong P, Dawson J, Telford S. Duration of tick attachment required for transmission of granulocytic ehrlichiosis. J Infect Dis. 1998;177:355–7.
6. Piesman J, Mather TN, Sinsky RJ, Spielman A. Duration of tick attachment and Borrelia burgdorferi transmission. J Clin Microbiol. 1987;25:557–8.
7. Viltolyckor för respektive viltslag [Internet]. [cited 2025 Oct 15]. Available from: https://statistik.viltolycka.se/statistik/antal-genomforda-uppdrag/
8. Falco RC, Daniels TJ, Vinci V, McKenna D, Scavarda C, Wormser GP. Assessment of duration of tick feeding by the scutal index reduces need for antibiotic prophylaxis after *Ixodes scapularis* tick bites. Clin Infect Dis. 2018;67:614–6.
9. Gray J, Stanek G, Kundi M, Kocianova E. Dimensions of engorging *Ixodes ricinus* as a measure of feeding duration. Int J Med Microbiol. 2005;295:567–72.
10. Rstudio team. RStudio: Integrated Development Environment for R [Internet]. RStudio, PBC. 2024. Available from: https://posit.co/products/open-source/rstudio/
11. Fox J, Weisberg S. An R companion to applied regression. Sage Publ; 2018.
12. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Softw. 2015;67:1–48.
13. Hartig F. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models [Internet]. 2024. Available from: https://CRAN.R-project.org/package=DHARMa
14. Dreghiciu IC, Hoffman D, Florea T, Oprescu I, Dumitru S, Imre M, et al. A systematic review on the occurrence of *Babesia* spp. and *Anaplasma* spp. in ticks and wild boar from Europe—a 15-year retrospective study. Pathogens. 2025;14:612.
15. Fabri ND, Sprong H, Hofmeester TR, Heesterbeek H, Donnars BF, Widemo F, et al. Wild ungulate species differ in their contribution to the transmission of *Ixodes ricinus*-borne pathogens. Parasites Vectors. 2021;14:360.
16. Jahfari S, Coipan EC, Fonville M, van Leeuwen AD, Hengeveld P, Heylen D, et al. Circulation of four *Anaplasma phagocytophilum* ecotypes in Europe. Parasites Vectors. 2014;7:365.
17. Lesiczka PM, Hrazdilova K, Hönig V, Modrý D, Zurek L. Distant genetic variants of *Anaplasma phagocytophilum* from *Ixodes ricinus* attached to people. Parasites Vectors. 2023;16:80.
18. Smrdel KS, Bidovec A, Malovrh T, Petrovec M, Duh D, Zupanc TA. Detection of *Anaplasma phagocytophilum* in wild boar in Slovenia. Clin Microbiol Infect. 2009;15:50–2.
19. Rymaszewska A, Grenda SJ. Bacteria of the genus *Anaplasma*: characteristics of *Anaplasma* and their vectors: a review. Vet Med (Praha). 2008;53:573–84.

20. Ebani VV, Bertelloni F, Cecconi G, Sgorbini M, Cerri D. Zoonotic tick-borne bacteria among wild boars (*Sus scrofa*) in central Italy. Asian Pac J Trop Dis. 2017;7:141–3.
21. 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.
22. Galbe JL, Guy E, Zapatero JM, Peerschke EI, Benach JL. Vascular clearance of *Borrelia burgdorferi* in rats. Microb Pathog. 1993;14:187–201.
23. Schmidt BL. PCR in laboratory diagnosis of human *Borrelia burgdorferi* infections. Clin Microbiol Rev. 1997;10:185–201.
24. Díaz-Cao JM, Adaszek Ł, Dzięgiel B, Paniagua J, Caballero-Gómez J, Winiarczyk S, et al. Prevalence of selected tick-borne pathogens in wild ungulates and ticks in southern Spain. Transbound Emerg Dis. 2022;69:1084–94.
25. Kazimírová M, Hamšíková Z, Špitalská E, Minichová L, Mahríková L, Caban R, et al. Diverse tick-borne microorganisms identified in free-living ungulates in Slovakia. Parasites Vectors. 2018;11:495.
26. 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. Parasites Vectors. 2016;9:251.
27. 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.
28. Skotarczak B, Adamska M, Sawczuk M, Maciejewska A, Wodecka B, Rymaszewska A. Coexistence of tick-borne pathogens in game animals and ticks in western Poland. Vet Med (Praha). 2008;53:668–75.
29. Cadavid D, O'Neill T, Schaefer H, Pachner AR. Localization of *Borrelia burgdorferi* in the nervous system and other organs in a nonhuman primate model of Lyme disease. Lab Invest. 2000;80:1043–54.
30. Schwan TG, Burgdorfer W, Schrumpf ME, Karstens RH. The urinary bladder, a consistent source of *Borrelia burgdorferi* in experimentally infected white-footed mice (*Peromyscus leucopus*). J Clin Microbiol. 1988;26:893–5.
31. Goodman JL, Jurkovich P, Kodner C, Johnson RC. Persistent cardiac and urinary tract infections with *Borrelia burgdorferi* in experimentally infected Syrian hamsters. J Clin Microbiol. 1991;29:894–6.
32. Bakken JS, Dumler JS. Human granulocytic anaplasmosis. Infect Dis Clin North Am. 2015;29:341–55.
33. Silaghi C, Santos AS, Gomes J, Christova I, Matei IA, Walder G, et al. Guidelines for the direct detection of *Anaplasma* spp. in diagnosis and epidemiological studies. Vector Borne Zoonotic Dis. 2017;17:12–22.
34. Centers for Disease Control and Prevention. Clinical and laboratory diagnosis of anaplasmosis [Internet]. 2021 [cited 2022 Nov 2]. Available from: https://www.cdc.gov/anaplasmosis/healthcare-providers/clinical-lab-diagnosis.html

35. Myczka AW, Szewczyk T, Laskowski Z. The occurrence of zoonotic *Anaplasma phagocytophilum* strains in the spleen and liver of wild boars from north-west and central parts of Poland. Acta Parasitol. 2021;66:1082–5.

Figures

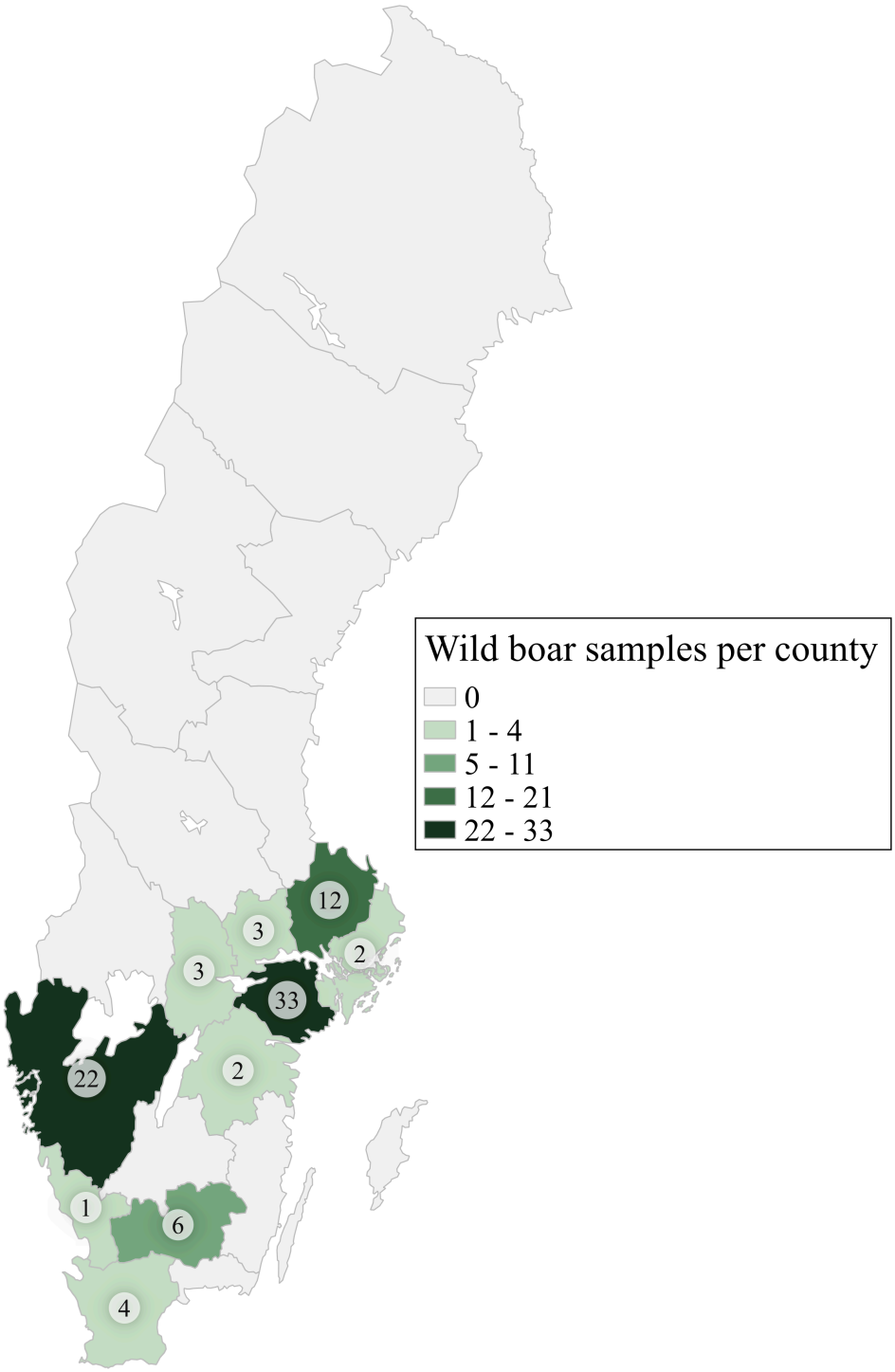


Figure 1

Map of Sweden showing the counties from which wild boar were sampled between 2023 and 2025. Darker shading indicates counties with a higher number of submitted animals. Numbers represent the sample size for each county.



Figure 2

Measurements for feeding duration: Scutal index: idiosoma length (A) divided by scutal width (B) and the coxal index: idiosoma length (A) divided by coxal gap between the fourth pair of legs (C). Original artwork by Emma Lindbom.

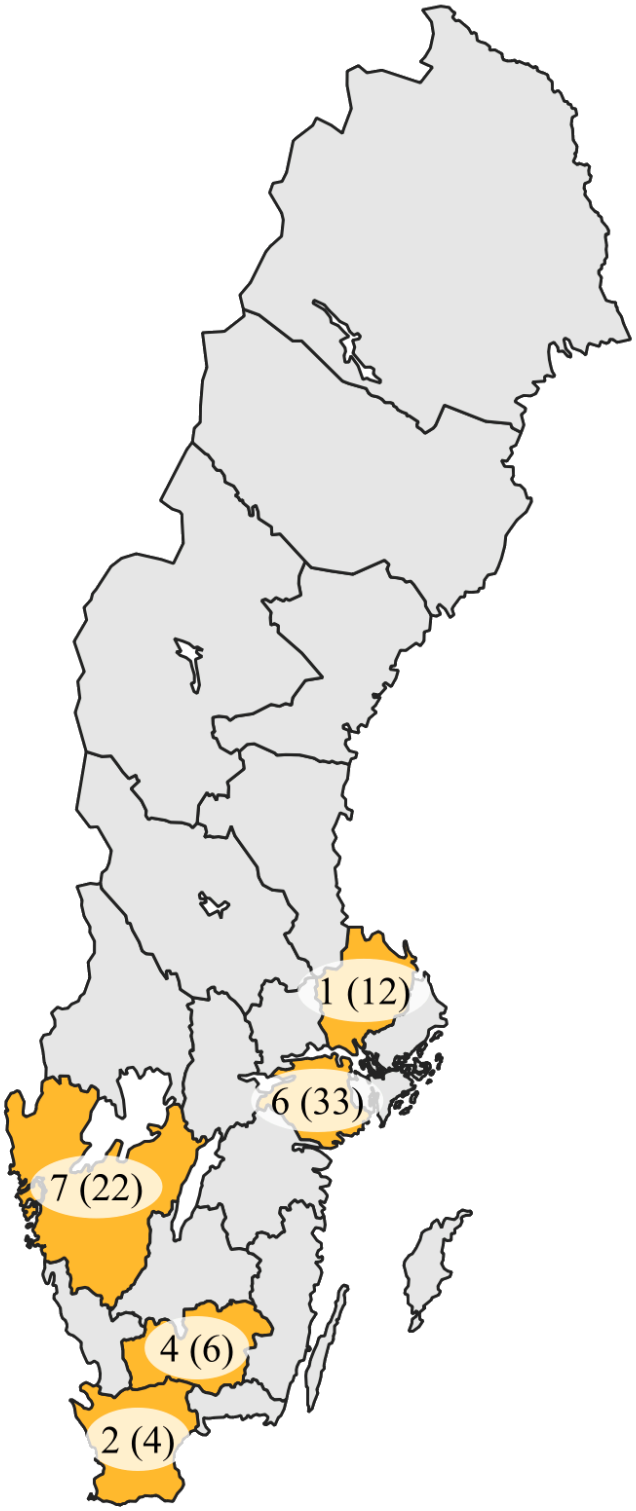


Figure 3

Number of *Anaplasma phagocytophilum* positive wild boar detected in different counties in southern Sweden. The number of tested animals is shown in parentheses.
Västra Götaland: 7 (22), Södermanland: 6 (33), Kronoberg: 4 (6), Skåne: 2(4), Uppsala: 1 (12). Counts rather than prevalence is presented to avoid misleading impressions in counties with small sample sizes.