Anaplasma phagocytophilum in temperate and cold regions of Europe: a review on its prevalence in livestock

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In Europe, Anaplasma phagocytophilum infections in livestock have been reported in several countries, particularly in northern and central Europe, where the climate is temperate or cold. In these regions, the infection is most commonly observed in grazing animals, such as cattle and sheep, during the summer months. The prevalence of A. phagocytophilum infection in livestock can also vary within countries depending on the farming practices and management of the animals. Different studies report varying rates of infection in different countries and regions. In Europe, seroprevalence in livestock has been reported to range from 0% to 55%, with higher rates observed in regions with high tick densities. Molecular methods detect 0% to 85.71% (in animals with clinical symptoms) and 23.94% (using random selection) of A. phagocytophilum genetic material in farm animals. As the infections of Anaplasma spp. bacteria are often asymptomatic or clinical symptoms are not specific in some cases, we hypothesise that there are more anaplasmosis cases in Europe than expected. In this review we analysed scientific data excluding clinical cases, even though there are multiple cases described in different countries in the region of our review.

Keywords: A. phagocytophilum, anaplasmosis, livestock, tick-borne diseases, horse, cattle, sheep

INTRODUCTION

As a result of climatic changes, tick-borne infections are an emerging problem in temperate and cold regions of Europe. Anaplasma spp. is bacteria spread by common ticks. Ixodes ricinus has been identified as the primary vector of this infection in Europe (Kiewra et al., 2014; Ravagnan et al., 2018). Their carriers in Europe are wild rodents, sheep, roe deer, and migrant birds. Although there are several scientific papers regarding the prevalence of A. phagocytophilum in farm animals, the results are inconsistent. Since anaplasmosis infection in livestock can cause significant economic impact, it needs to be researched to raise the accuracy in diagnosis, continue the epidemiological surveillance, and thus implement strategies of control and prevention of this disease. In this review we analyse and summarise scientific work on this issue from 1996 to 2023.
METHODS

In this review article, we collected 25 scientific articles on *A. phagocytophilum* infections in livestock: horses, cows, and sheep. Moreover, we found three articles on *A. phagocytophilum* presence in *Ixodes ricinus* ticks collected in Europe. Most studies (five) were conducted in Slovakia, four in the Czech Republic, three studies in Sweden, France, and Norway each, two studies in Italy, Poland and Germany each, and one from Denmark, Switzerland, Netherlands, and Belgium each. No articles on *A. phagocytophilum* infections in livestock were found in Baltic countries, although there are several research articles on anaplasmosis in companion animals like dogs in the Baltic region (Berzina et al., 2013; Radzijevskaja et al., 2008; Stuen et al., 2005). The earliest article included in this review comes from 1998 (Pusterla et al., 1998) and the latest one from 2023 (Traversa et al., 2023). Case report articles were not included in this review. The information was systematised and visualized graphically using MS Excel 2023.

RESULTS

Out of 25 studies, 11 were conducted using serological methods and 14 using PCR (Table 1); one of the studies used both serology and PCR testing (Drážovská et al., 2021). Positive results by percentage were compared between

<table>
<thead>
<tr>
<th>Author</th>
<th>Species</th>
<th>Method</th>
<th>Year</th>
<th>Test group</th>
<th>Positive</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pusterla et al., 1998</td>
<td>cattle</td>
<td>PCR</td>
<td>1996</td>
<td>26</td>
<td>18</td>
<td>69.23%</td>
</tr>
<tr>
<td></td>
<td>cow</td>
<td></td>
<td></td>
<td>7</td>
<td>6</td>
<td>85.71%</td>
</tr>
<tr>
<td>Egenvall et al., 1998</td>
<td>horse</td>
<td>PCR</td>
<td>1996–1997</td>
<td>70</td>
<td>12</td>
<td>17.14%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stuen et al., 2002</td>
<td>lambs</td>
<td>serology</td>
<td>2001</td>
<td>361</td>
<td>129</td>
<td>35.70%</td>
</tr>
<tr>
<td></td>
<td>sheep</td>
<td>serology</td>
<td>2001</td>
<td>388</td>
<td>141</td>
<td>36.30%</td>
</tr>
<tr>
<td>Hulínská et al., 2004</td>
<td>horse</td>
<td>PCR</td>
<td>2004</td>
<td>40</td>
<td>2</td>
<td>5.00%</td>
</tr>
<tr>
<td></td>
<td>cattle</td>
<td></td>
<td></td>
<td>55</td>
<td>3</td>
<td>5.45%</td>
</tr>
<tr>
<td>Zeman &amp; Pecha, 2008</td>
<td>sheep</td>
<td>serology</td>
<td>2004</td>
<td>41</td>
<td>13</td>
<td>31.70%</td>
</tr>
<tr>
<td>Leblond et al., 2005</td>
<td>horse</td>
<td>serology</td>
<td>2005</td>
<td>424</td>
<td>48</td>
<td>11.30%</td>
</tr>
<tr>
<td>Stuen et al., 2005</td>
<td>cattle</td>
<td>PCR</td>
<td>2005</td>
<td>12</td>
<td>1</td>
<td>8.33%</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>55</td>
<td>3</td>
<td>5.45%</td>
</tr>
<tr>
<td>Víchová et al., 2014</td>
<td>cattle</td>
<td>PCR</td>
<td>2006–2009</td>
<td>178</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>sheep</td>
<td>PCR</td>
<td></td>
<td>147</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>Maurizi et al., 2009</td>
<td>horse</td>
<td>serology</td>
<td>2009</td>
<td>408</td>
<td>55</td>
<td>13.50%</td>
</tr>
<tr>
<td>Hansen et al., 2010</td>
<td>horse</td>
<td>serology</td>
<td>2010</td>
<td>390</td>
<td>87</td>
<td>22.30%</td>
</tr>
<tr>
<td>Teodorowski et al., 2021</td>
<td>horse</td>
<td>PCR</td>
<td>2013</td>
<td>512</td>
<td>9</td>
<td>1.80%</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Grova et al., 2011</td>
<td>sheep</td>
<td>serology</td>
<td>2011</td>
<td>1208</td>
<td>664</td>
<td>55.00%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>76</td>
<td>2</td>
<td>2.63%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39</td>
<td>1</td>
<td>2.56%</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>Slivinska et al., 2016</td>
<td>horse</td>
<td>PCR</td>
<td>2013</td>
<td>479</td>
<td>109</td>
<td>22.75%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>0</td>
<td>0.00%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>85</td>
<td>42.50%</td>
</tr>
<tr>
<td>Ebani, 2019</td>
<td>horse</td>
<td>serology</td>
<td>2013</td>
<td>76</td>
<td>2</td>
<td>2.63%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39</td>
<td>1</td>
<td>2.56%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>Drážovská et al., 2021</td>
<td>horse</td>
<td>PCR</td>
<td>2014</td>
<td>200</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>85</td>
<td>42.50%</td>
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</tbody>
</table>
serologically- and PCR-tested groups. Furthermore, the results in different animal groups – equine, bovine, and ovine – were analysed separately. Most studies (13) were conducted on horse anaplasmosis (Fig. 1), seven on bovine (Fig. 2), and four on sheep anaplasmosis (Fig. 3). In the horse group, seven studies used the PCR method and seven serology method. In the ovine group, only one study used the PCR method, and in the bovine group all seven studies used the PCR method.

The largest test groups were studied by Schäfer and colleagues (Schäfer et al., 2022) and this study also compares PCR (190/1246 positive) and serological (1036/3849 positive) methods in A. phagocytophilum diagnostic in horses from Germany. The second largest test group was collected by Egenvall et al. (Egenvall et al., 2001). In 1996–1997 in Sweden, horses were tested for anaplasmosis serologically (33/2018 positive). Another large study, which was conducted in Norway (Grøva et al., 2011) and consisted of 1208 samples taken from sheep, shows 55% seropositivity.

The smallest test groups were collected by Egenvall et al. (Engvall et al., 1996) in Sweden and consisted of seven cows with clinical symptoms, and six out of seven were proven to be A. phagocytophilum positive by PCR testing. Another smaller research consisted of 12 samples from cows in Norway (Stuen et al., 2002) where only one sample was PCR positive.

![Fig. 1. A. phagocytophilum equine infections detected between 1996 and 2023, detected using serological and molecular testing methods](image-url)
Fig. 2. *A. phagocytophilum* ovine infections detected between 2001 and 2011 using serological and molecular testing methods

Fig. 3. *A. phagocytophilum* bovine infections detected between 1996 and 2018 using molecular testing

To evaluate the difference between the two methods (serology and PCR), articles with fewer than 50 test samples were eliminated and the remaining 19 studies were compared. The average PCR positive sample percentage is 7.92%, while serologically positive samples reach 21.35% on average in all animal groups combined. This comparison, together with Schäfer et al. (2022) research, shows a significant difference between the two methods: serology test groups consist of more positive samples than PCR test groups in general.

To compare the results of different studies across the timeline from 1996 until 2023 by animal species and testing method, we analysed each animal group individually. The equine group was the most diverse between the two testing methods (Fig. 1).
The largest number of PCR-positive horse samples were collected in 1996 in Sweden (Engvall et al., 1996), where 26 horses with clinical symptoms were tested. This study does not convey infection rates in Sweden: the testing group was not random as animals had clinical symptoms. The second largest PCR-positive group was described in Germany in 2022 (Schäfer et al., 2022), where 190 out of 1246 horses were PCR positive (15.20%). Most of serologically positive samples were collected also in Germany (Schäfer et al., 2022), where 1036 samples out of 3849 were positive. Negative samples were detected in Ukraine (Slivinska et al., 2016) – 0/100 in 2013 by PCR, and in Slovakia (Drážovská et al., 2021), but in the same study, 85/200 (42.50%) samples were seropositive.

The largest number of seropositive samples were collected in Norway in 2011 (Grova et al., 2011) – 664/1028 (55%), while the only study that used the PCR method, in Slovakia, did not find any positive sheep samples. As for seropositive samples, 31.05% were collected in the Czech Republic in 2004, and 36.05% in Norway (Stuen et al., 2002).

In the bovine group, no serological testing data was found in the selected region. The largest percentage of PCR-positive animals were detected in Sweden in 1996 (Engvall et al., 1996) in a study with only seven cows with clinical symptoms, where six of them tested positive. More relevant data was collected also in Sweden in 2017 (Andersson et al., 2017) where 17 cows out of 71 tested were PCR-positive (23.94%).

As for the information on *A. phagocytophilum* molecular testing on tick vectors of this bacteria in European regions, it is insufficient. There are three studies from the period of 2004–2014 (Table 2).

The largest amount of testing material was collected in a 2014 study in Netherlands and Belgium (Jahfari et al., 2014), where 3879 *Ixodes ricinus* ticks of different life stages were tested using PCR analysis, and only 95 ticks (2.45%) were PCR-positive for *A. phagocytophilum*. Between 2006 and 2009, in Slovakia, 1075 *I. ricinus* ticks were screened and the overall prevalence of the infection ranged from 1.4% to 5.5% depending on the region of the country (Vichová et al., 2014). A smaller study was conducted in the Czech Republic, where 82 ticks were screened and eight were positive (9.76%) (Hulinská et al., 2004).

Based on these studies, *A. phagocytophilum* in *Ixodes ricinus* population varies from 1.3% to 9.76% depending on the country. In 2004, in the Czech Republic, Hulinská et al. (2004) tested horses and cows using PCR. They found that 5% of all samples in the horse group and 5.45% in the cow group were PCR confirmed for *A. phagocytophilum*; in comparison, ticks were 9.76% positive. Similarly, another study conducted in Slovakia between 2006 and 2009 (Vichová et al., 2014) found 0% PCR-confirmed cases in sheep and in cow test groups, while 1.4% to 5.5% samples were confirmed in the tick test group. In both cases, a higher percentage of *A. phagocytophilum* infected ticks than host animals was observed.

### Table 2. Scientific articles on the subject *A. phagocytophilum* in detected *I. ricinus* ticks using PCR testing, in chronological order

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th><em>I. ricinus</em> life stage</th>
<th>Test group</th>
<th>Positive</th>
<th>Positive %</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hulinska et al.</td>
<td>2004</td>
<td>not specified</td>
<td>82</td>
<td>8</td>
<td>9.8%</td>
<td>Czech Republic</td>
</tr>
<tr>
<td>Vickova et al.</td>
<td>2006–2009</td>
<td>not specified</td>
<td>1075</td>
<td></td>
<td>1.4–5.5%</td>
<td>Slovakia</td>
</tr>
<tr>
<td>Jahfari et al.</td>
<td>2014</td>
<td>mature and nymphs</td>
<td>3493</td>
<td>90</td>
<td>2.6%</td>
<td>Netherlands, Belgium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>larva</td>
<td>386</td>
<td>5</td>
<td>1.3%</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

*Anaplasma phagocytophilum* infections are relatively common in companion animals in temperate and cold regions of Europe, such as Scandinavia, the Baltic countries, and parts of Central Europe (Berzina et al., 2013; Radzijevskaja et al., 2008; Stuen, 2007). *A. phagocytophilum* infections have also been reported in various livestock species, including horses, cattle, sheep, and goats (Stuen, 2007). Factors that may contribute to the variation in prevalence include differences in tick populations and management practices, as well as differences in the diagnostic methods used to detect the infection.

Definitive confirmation of anaplasmosis is problematic, and currently diagnosis is based on finding a positive antibody titre. This has several limitations: firstly, with standard test methods, it may take up to three months following infection for animals to seroconvert, which means that many early cases will be false negatives on serology; secondly, animals can seroconvert without showing any clinical symptoms, and therefore no diagnostic measures are taken; thirdly, even successfully treated animals may still remain seropositive for a very long time thereafter, thus complicating interpretation of successful resolution (Laamari et al., 2020; Russell et al., 2021).

Previous studies reported that anti-*A. phagocytophilum* IgG antibodies persist for approximately 300 days after experimental infection (Ristic et al., 1991) and may persist up to two years after natural infection (Sellon, Long, 2013). In the acute stage of the disease, PCR is
recommended as the most sensitive diagnostic tool in routine diagnostics (Engvall et al., 1996). Some studies reported that after experimental infection, *A. phagocytophilum* DNA in horses can be detected over a period of approximately four months (at least 129 days) in the blood (Franzén et al., 2009). There is a lack of information about seroconversion timelines of different livestock animal species and on PCR detection intervals.

The summarised information about *A. phagocytophilum* infection rates in temperate and cold regions of Europe varies significantly and also depends on the testing method used; in general, there are more serologically positive samples than there are PCR confirmed cases.

Moreover, results of different studies depend on the test group sizes, and in controlled selection, smaller test groups tend to result in more anaplasmosis-positive samples than large and randomly selected ones (Engvall et al., 1996; Stuen et al., 2005), which represent epidemiological situation more accurately. Average results change significantly when smaller-scale studies (up to 50 test samples) are eliminated from the calculations. Comparing data from all the articles (Table 1), PCR positive samples account for 16.2% on average, while serologically positive samples add up to 24% on average. However, only 7.92% PCR-positive and 21.35% seropositive samples were confirmed in farm animals that were tested in larger than 50 groups per study. For this reason, it is necessary to continue large-scale research to obtain a better perspective on the actual epidemiological situation.

Even though some countries in Europe make a big effort to investigate tick-borne diseases, including anaplasmosis (Drážovská et al., 2021; Slivinska et al., 2016; Vichová et al., 2014), there is little to no information about epidemiological situation of *A. phagocytophilum* infections in livestock in the Baltic region, the United Kingdom, and Ireland, as well as in Spain, Portugal, and all the south-east region of Europe, although clinical cases are described in most countries (Gotic et al., 2017; Gussmann et al., 2014).

In addition to both serological and molecular tests in livestock animals, it is also very important to evaluate *A. phagocytophilum* provenance in its most common vector, *Ixodes ricinus* ticks. Even though there is a lack of information from different countries in Europe to evaluate the actual situation, the available data shows that *A. phagocytophilum* infection rates detected in tick population using the PCR method are relatively higher than infection rates in farm animals (Hulínská et al., 2004; Vichová et al., 2014).

**CONCLUSIONS**

The prevalence of *A. phagocytophilum* infection in livestock varies depending on various factors such as the geographical location, the density of tick populations, the animal husbandry practices, and the testing methods used. In general, there are more serologically positive samples detected than PCR-confirmed ones.

*A. phagocytophilum* infection in livestock is often asymptomatic or results in mild clinical symptoms, and therefore the true prevalence of the disease may be underestimated. Additionally, tick-borne diseases are expected to become more prevalent in the future due to climate change and other environmental factors, highlighting the need for increased surveillance and control measures.

*A. phagocytophilum* bacteria circulating in the vector population (*I. ricinus*) may be higher than in the host population. There is limited data on this subject, and further investigation is needed to determine if this difference is significant and related.

Overall, while *A. phagocytophilum* infections in livestock are present in temperate and cold regions of Europe, the prevalence can vary widely, and further research is needed to fully understand the epidemiology of this bacterium in livestock populations.

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References


30. Teodorowski O, Kalinowski M, Winiarczyk D,  
Janecki R, Winiarczyk S, Adaszek Ł. Molecular  
surveillance of tick-borne diseases affecting  
horses in Poland–Own observations. Vet Med  

31. Traversa D, Milillo P, Maggi R, Simonato G,  
Di Cesare A, Pezzuto C, Grillini M, Morelli S,  
Colombo M, Passarelli A, Grassano A, Serio P,  
Lorusdo M, Brueckmann R. Seroexposure to  
zoonotic *Anaplasma* and *Borrelia* in dogs and  
horses that are in contact with vulnerable peo-  
doi.org/10.3390/pathogens12030470

32. Víchová B, Majláthová V, Novákova M,  
Stanko M, Hviščová I, Pangrácová L, Chru-  
dimský T, Čurlík J, Peťko B. *Anaplasma* in-  
fec tions in ticks and reservoir host from Slovakia.  
doi.org/10.1016/j.meegid.2013.06.003

33. Zeman P, Pecha M. Segregation of genetic var-  
ants of *Anaplasma phagocytophilum* circulat-  
ing among wild ruminants within a Bohemian  
forest (Czech Republic). Int J Med Microbiol.  
ijmm.2008.03.003

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**ANAPLASMA PHAGOCYTOPHILUM PAPLITIMO TARP ŪKINIŲ GYVŪNŲ VIDUTINIO IR ŠALTO KLIMATO EUROPOS REGIONUOSE APŽVALGA**

**Santrauka**


**Raktažodžiai:** *A. phagocytophilum*, anaplazmozė, gyvūnai, erkių platinamos ligos, arkliai, galvijai, avys