

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

Donata Mikalauskiėnė\*,

Zoja Mikniėnė

Large Animal Clinic,  
Faculty of Veterinary Medicine,  
Veterinary Academy, Lithuanian University  
of Health Sciences, Kaunas, Lithuania

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. bacterium 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.

\* Corresponding author. Email: donata.mikalauskiene@lsmu.lt

## 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; Radzijeuskaja 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 (Dražovská et al., 2021). Positive results by percentage were compared between

Table 1. Scientific articles on the subject *A. phagocytophilum* in livestock, in chronological order

Author	Species	Method	Year	Test group	Positive	Positive %	Country
	horse	PCR	1996	26	18	69.23%	Sweden
	cattle			7	6	85.71%	
Pusterla et al., 1998	cattle	PCR	1996–1997	70	12	17.14%	Switzerland
Egenvall et al., 1998	horse	serology	1997–1998	2018	336	16.70%	Sweden
Stuen et al., 2002	lambs	serology	2001	361	129	35.70%	Norway
	sheep	serology	2001	388	141	36.30%	
Hulínská et al., 2004	horse	PCR	2004	40	2	5.00%	Czech Republic
	cattle			55	3	5.45%	
Zeman & Pecha, 2008	sheep	serology	2004	41	13	31.70%	Czech Republic
Leblond et al., 2005	horse	serology	2005	424	48	11.30%	France
Stuen et al., 2005	cattle	PCR	2005	12	1	8.33%	Norway
Víchová et al., 2014	cattle	PCR	2006–2009	178	0	0.00%	Slovakia
	sheep	PCR		147	0	0.00%	
Maurizi et al., 2009	horse	serology	2009	408	55	13.50%	France
Hansen et al., 2010	horse	serology	2010	390	87	22.30%	Denmark
Teodorowski et al., 2021	horse	PCR	2013	512	9	1.80%	Poland
Grøva et al., 2011	sheep	serology	2011	1208	664	55.00%	Norway
				76	2	2.63%	Poland
Slivinska et al., 2016	horse	PCR	2013	39	1	2.56%	Slovakia
				100	0	0.00%	Ukraine
Ebani, 2019	horse	serology	2013	479	109	22.75%	Italy
Dražovská et al., 2021	horse	PCR	2014	200	0	0.00%	Slovakia
		serology		200	85	42.50%	

Table 1. (Continued)

Author	Species	Method	Year	Test group	Positive	Positive %	Country
Andersson et al., 2017	cattle	PCR	2017	71	17	23.94%	Sweden
Lagrée et al., 2018	cattle	PCR	2018	289	57	20.00%	France, Germany
Schäfer et al., 2022	horse	serology	2022	3849	1036	26.90%	Germany
		PCR		1246	190	15.20%	
Traversa et al., 2023	horse	serology	2023	150	12	8.00%	Italy

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.

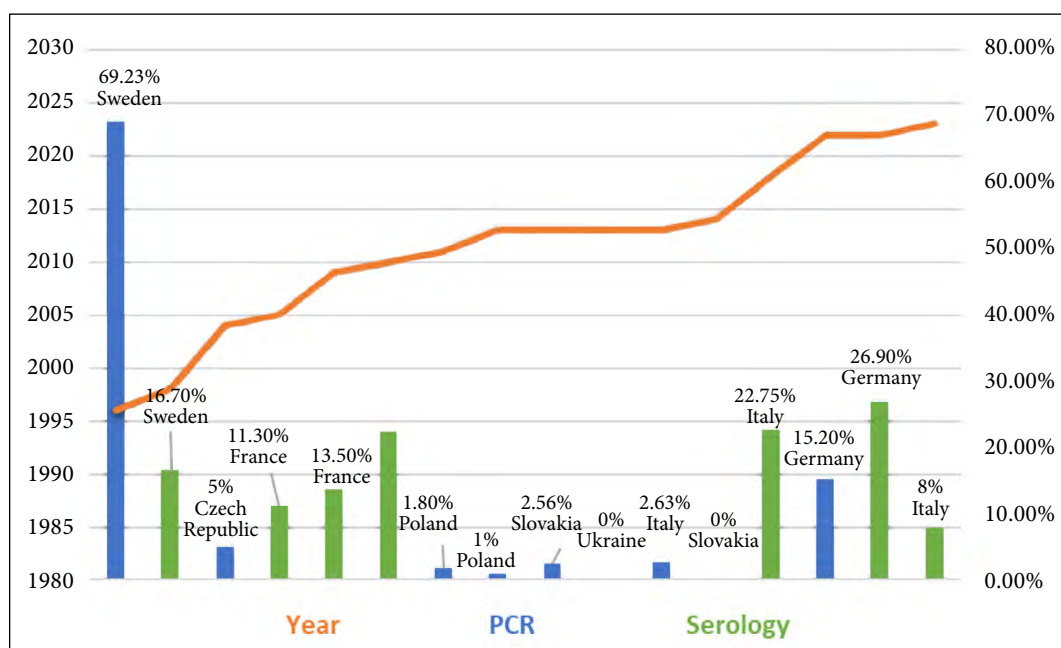
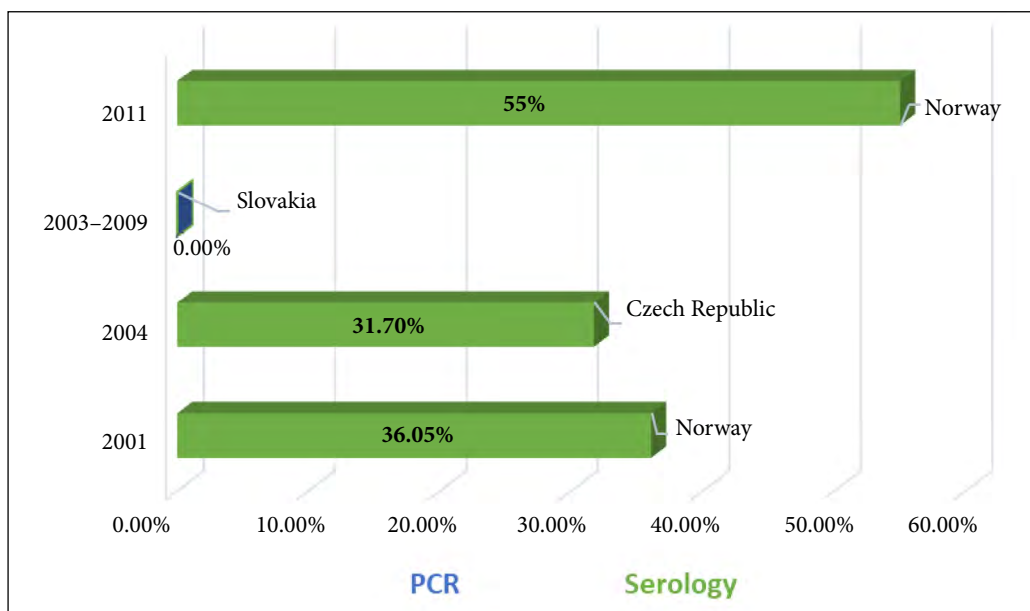
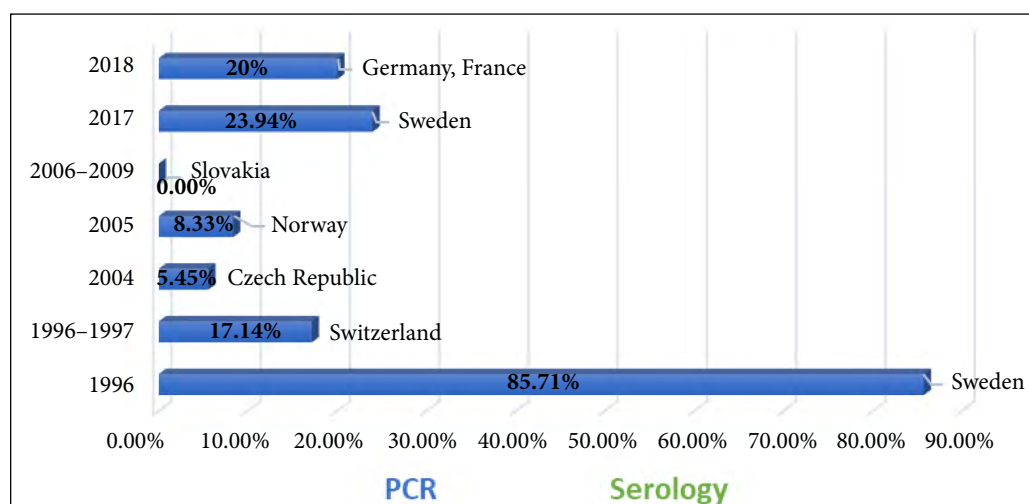


Fig. 1. *A. phagocytophilum* equine infections detected between 1996 and 2023, detected using serological and molecular testing methods



**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 signifi-

cant 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 (Grøva 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%).

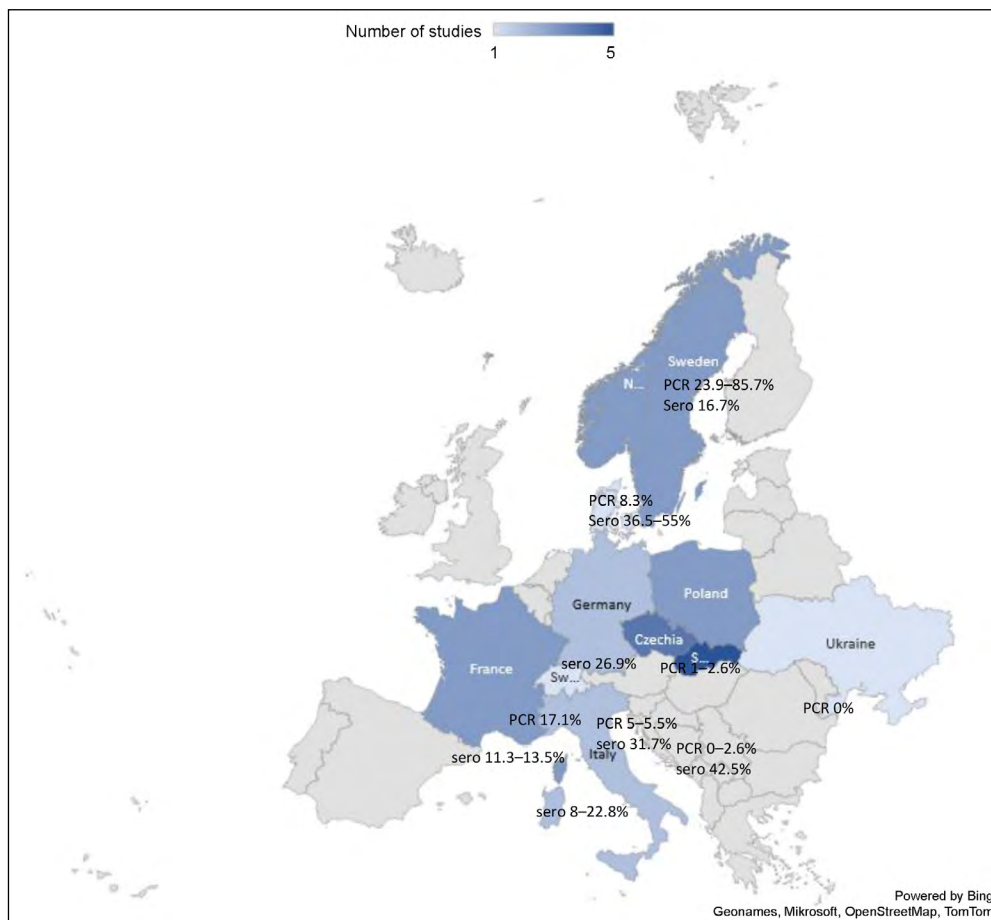
As for the information on *A. phagocytophilum* molecular testing on tick vectors of this bacteria in European regions, it is insufficient. There are three studies from the period of 2004–2014 (Table 2).

The largest amount of testing material was collected in a 2014 study in Netherlands and Belgium (Jahfari et al., 2014), where 3879 *Ixoides 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 (Víchová et al., 2014). A smaller study was conducted in the Czech Republic, where 82 ticks were screened and eight were positive (9.76%) (Hulínská et al., 2004).

Based on these studies, *A. phagocytophilum* in *Ixoides ricinus* population varies from 1.3% to 9.76% depending on the country. In 2004, in the Czech Republic, Hulínská 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 (Víchová 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

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



**Fig. 4.** A map showing the numbers of scientific articles related to the topic of *A. phagocytophilum* prevalence in livestock in Europe were published from 1996 to 2023 and positive samples recorded

## 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; Radzijeuskaja 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; Stuenkel 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 (Gotić 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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**Donata Mikalauskienė, Zoja Miknienė**

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

#### *Santrauka*

*Anaplasma phagocytophilum* infekcijos dokumentuotos daugelyje Europos šalių, daugiausia Šiaurės ir Vidurio Europoje, kur klimatas yra vidutinis arba šaltas. Šiuose regionuose *A. phagocytophilum* infekcijos daugiausia aprašomos vasaros laikotarpiu tiriant ganyklinius gyvūnus, tokius kaip galvijai bei avys. Infekcijos dažnis tarp ūkinių gyvūnų gali priklausyti nuo ūkininkavimo metodikos ir bandos valdymo. Skirtingose šalyse atliktais tyrimais nustatyti įvairūs ligos paplitimo dažniai. Serologinis *A. phagocytophilum* paplitimas Europoje siekia iki 55 %. Didesni paplitimo rodikliai nustatomi regionuose, kuriuose didesnis ir erkių paplitimo tankis. Molekuliniiais metodais pagrįstais moksliniais tyrimais nustatytas iki 85,71 % (tiriant gyvūnus su klinikiniais simptomais) ir 23,94 % (taikant atsitiktinę atranką) užsikrėtimo dažnis. *Anaplasma* spp. infekcijos dažnai būna besimptomės arba klinikiniai simptomai yra nespecifiniai, todėl keliame hipotezę, jog Europoje anaplazmozės atvejų gali būti daugiau, nei šiuo metu yra įvertinta. Šioje apžvalgoje į mokslinių tyrimų duomenų analizę neįtraukėme kliniki- nių atvejų, nors mūsų tyrimo regione apie šios ligos atvejus pranešama net keliose skirtingose valstybėse.

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