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Relative importance of different ungulate species on the lifecycle of *Anaplasma phagocytophilum*

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Summary

Background: Anaplasmosis is an emerging tick borne disease caused by the bacterium *Anaplasma phagocytophilum*. Ungulate species are an important animal reservoir and therefore they play a central role in the epidemiology. Ungulate management could possibly be used to reduce the amount of infected ticks in the vegetation. To be able to properly implement this management in order to achieve this goal, more knowledge of *Anaplasma* in different ungulate species is needed. Part of this knowledge can be obtained by determining the relative importance. Therefore I determined in this study the relative importance of five different ungulate species with data from previous studies. In addition I looked if there is a difference in infection prevalence of hosts per geographical area. This data could be an important part to determine a different management strategy per area.

Methods: I performed a systematic review with studies that included data on the measurements to calculate the host infection prevalence with *A. phagocytophilum*, and the measurements to calculate the infection prevalence with *A. phagocytophilum* on feeding ticks. I included the following host species: fallow deer (*Dama dama*), moose (*Alces alces*), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*). I analyzed the data from 52 publications.

Results: I found a significant lower infection prevalence in wild boar (6,57%, $p < 0,05$) and in addition, it has been shown that the geographical area is an extra factor in the infection prevalence per host. There is a seems to be relationship between the relative importance in feeding and the relative importance in infecting both nymphs ($t = 28,52$, $p < 0.05$) and larvae ($t = 48,24$, $p < 0,05$).

Conclusion: The significant lower prevalence rate and the low relative importance of wild boar indicates that their role in the lifecycle of *Anaplasma phagocytophilum* is less important, and ungulate management can better be targeted at deer species. The data indicates that there is a relationship between the relative importance in feeding and the relative importance in infection both nymphs and larvae when the data of all host species was combined. As far as management is concerned, this means that if less ticks can feed from the animals, it is likely that the amount of infected ticks in the vegetation can be reduced.

1. Background of the study

Anaplasmosis is an emerging tick-borne disease in both humans and animals, caused by *Anaplasma spp.* (Ismail & McBride, 2017). There are different species of *Anaplasma*, but *Anaplasma phagocytophilum* is considered one of the most important bacteria in the context of public health (Atif, 2015). As ungulate species are an important animal reservoir of *A. phagocytophilum*, they play a central role in the epidemiology (Martin *et al.*, 2011).

Anaplasma spp. are gram-negative obligate intracellular bacteria, belonging to the order of Rickettsiales and the family of Anaplasmataceae. There are six different species: *Anaplasma phagocytophilum, Anaplasma ovis, Anaplasma marginale, Anaplasma bovis, Anaplasma platys and Anaplasma centrale* (Battilani *et al.*, 2017). As mentioned before, *A. phagocytophilum* is one of the most important tick-borne bacteria considering veterinary and public health (Atif, 2015). In humans, *A. phagocytophilum* is the cause of human granulocytic anaplasmosis. This disease shows various clinical signs, varying from subclinical to fatal. In addition to people, *A. phagocytophilum* also causes tick-borne fever in ruminants and equine and canine granulocytic anaplasmosis in horses and dogs respectively. Tick-borne fever in European domestic ruminants has a great impact on the economy (Dugat *et al.*, 2015).

A. phagocytophilum is transmitted by vectors. The main vector in Europe is the *Ixodes ricinus* tick. There are several studies on the prevalence of *A. phagocytophilum* in questing *I. ricinus* in Europe. On average, the prevalence ranges between <1% and 20% (Stuen *et al.*, 2013). The *I. ricinus* tick has a lifecycle consisting of three living stages, larva, nymph and adult. To moult to the next stage, one blood meal from a vertebrate host is required. Ticks can obtain *A. phagocytophilum* through transstadial transmission or from feeding from an infected animal host (Rizzoli *et al.*, 2014). Hosts of *A. phagocytophilum* are wild ruminants, rodents, insectivores, and mammals such as bears, foxes, birds, wild boars and reptiles. DNA from *A. phagocytophilum* has also been isolated in domestic animals, such as dogs, cats and horses, and domestic ruminants, such as goats, cattle and sheep (Stuen *et al.*, 2013).

Infected ticks can transmit *A. phagocytophilum* to a new host during a blood meal of its next stage. Only nymphs and adult females can transfer *A. phagocytophilum*, since males do not feed and there is no transovarial transmission (Oechslin *et al.*, 2017). Therefore, the *I. ricinus* tick does not support a persistent infection of *A. phagocytophilum* and is not a reservoir host for this pathogen (Dugat *et al.*, 2015). However*, A. phagocytophilum* does persist in several mammalian hosts. In Europe, the red deer (*Cervus elaphus*) and the roe deer (*Capreolus capreolus*) in particular play an important role as reservoir hosts. Both have a high prevalence *of A. phagocytophilum* infection, up to 87,5% and 98,9% for red and roe deer, respectively (Stuen *et al.*, 2013). Also the fallow deer (*Dama dama*) has a prevalence *of A. phagocytophilum* of up to 72%. The wild boar (*Sus scrofa*) seems to be important as a host for human pathogenic variants of *A. phagocytophilum* (Michalik *et al.*, 2012). Because these hosts play an important part in the transmission and persistence of *A. phagocytophilum* it is important to summarize data on the infection prevalence for hosts and tick-stages.

There are several studies on the prevalence of vector-borne infections in these animals but there is not a quantitative review that integrates the data of a large number of ungulates and looks at the relative importance of these animals. The aim of this study is to determine the relative importance of host species in infecting *I. ricinus* with *A. phagocytophilum*. This data is an important part in determining a new strategy for ungulate management, so that less infected ticks occur in the vegetation. I determined the relative importance by means of the numbers of ticks on the animals and by the infection prevalences of the ticks and the animals themselves.
Besides that, I looked for differences in relative importance between the different host species.

Therefore, I tested for correlations between: (1) the relative importance of host species feeding different stages of *Ixodes ricinus,* (2) the relative importance of host species in infecting *I. ricinus* ticks with *A. phagocytophilum*. In addition I looked if there is a difference in infection prevalence of hosts per geographical area. With this data, a different management strategy could be determined per area.

2. Materials and methods

These materials and methods are based on Hofmeester et al., 2016. (Hofmeester *et al.*, 2016).

Papers are collected through a literature search using PubMed, Web of Science and Scopus with the use of a search string. It concerned papers from January 1945 till December 2018. Only publications with field-derived data were selected, including xenodiagnosis. I included the following European hosts: fallow deer (*Dama dama*), moose (*Alces alces*), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*). Finally, I selected for papers including data on: (1) measurements to calculate the host infection prevalence with *A. phagocytophilum*, and/or (2) measurements to calculate the infection prevalence with *A. phagocytophilum* on feeding ticks.

The following search string is used:
(Anaplasma phagocytophilum OR Ehrlichia phagocytophilum) AND (distribut* OR presen* OR occur* OR report* OR incidence OR prevalence OR spread* OR disper* OR detect* OR diagnos* OR isolate* OR count* OR burden OR infestation) AND (Dama OR Alces OR Capreolus OR Cervus OR Sus)

2.1. Collection of data from the papers

Table 1 shows the extracted variables from each of the selected publications. The variables ecotype of *A. phagocytophilum* and standard deviations, standard errors and confidence intervals, were mentioned in only a small number of articles and were therefore not used in the analysis.

These variables were collected in a database and used for the subsequent calculations and were quality-criteria for data-selection from the database. If there were (1) different hosts species, (2) host collection in different geographical areas, (3) testing for different subtypes of *A. phagocytophilum* ecotypes or (4) multiple sample types from the same animals used, a publication was divided into separate records.

Table 1. Extracted variables from the publications

Extracted variables
Species type
Number of hosts examined
Sample type (blood, tissue or ticks)
Number of positive samples
Ecotype of *A. phagocytophilum*
Host infection prevalence
Infection prevalence for tick stages
Standard deviations, standard errors and confidence intervals
Method of *A. phagocytophilum* detection
Geographical area of the study

2.2. Summarizing of the data

For the summarizing of the data I calculated the infection prevalence of *A. phagocytophilum* per species. This calculation is done with a formula based on formulae from Hofmeester et al. (2016), adjusted for my purpose. Equation (1) shows the calculation of the infection prevalence P_i of the host species with *A. phagocytophilum*.

$$P_i = \frac{\sum_{s=1}^{n} I_{i_s}}{\sum_{s=1}^{n} H_{i_s}} \quad (1)$$

$P_i$is the infection prevalence of species *i*, with *A. phagocytophilum*. I_{i_s} is the total number of individual animals of species *i*, in study *s* infected with *A. phagocytophilum*. H_{i_s} is the total number of host individuals of species *i* sampled in study *s*. In these studies there were two ways to test whether the animals were positive for *Anaplasma*, a PCR on tissue or on blood. If the same animals were tested both in blood and tissue, I use the results of the tissue sample for the calculation. To make this decision I checked if there was a significant difference in infection prevalence between tissue and blood if both were used in the same study and I compared the infection prevalence of all the studies were blood was used to all the studies were tissue was used. Neither showed a significant difference.

2.3. Host infection prevalence per geographical area

For the determination of the host infection prevalence per geographical area I divided Europe into four different areas based on the number of studies conducted in each country and which hosts were studied. As displayed in figure 1 the following areas were formed: (1) Northern Europe: Norway and Sweden, (2) West/Central Europe: England, Belgium, Germany and Austria, (3) Southern Europe: Portugal, Spain, France and Italy, (4) Eastern Europe: Poland, Slovakia, Slovenia, Hungary, Czech Republic and Romania.

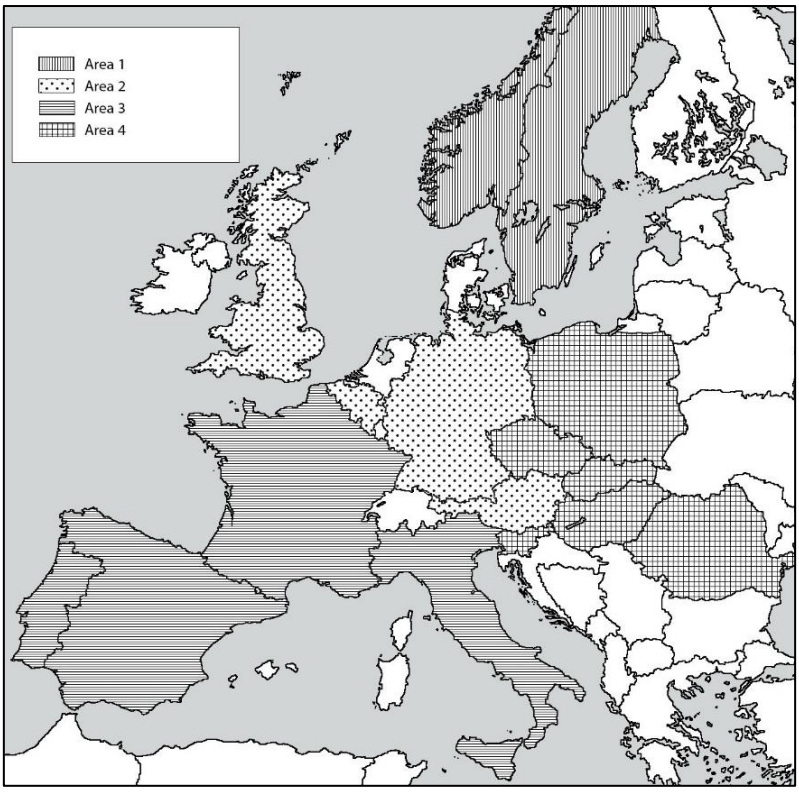


Figure 1. Selected geographical areas for determination of host infection prevalence per geographical area.

2.4. Quantifying the role of ungulate species

The role of ungulate species is quantified by calculation of the relative importance of host species in infecting *I. ricinus* ticks with *A. phagocytophilum*. I used the formulae from Hofmeester et al. (2016) for this, however since the life-cycle of *A. phagocytophilum* and its transmission is different from *Borrelia burgdorferi* I adjusted the formulae. An important difference is the fact that the main pathway of transmission of *B. burgdorferi* is from nymphs to hosts to larvae, while the main pathway for *A. phagocytophilum* is from adults to hosts to nymphs. But also larvae can be infected by the hosts. For the calculation of the relative importance of host species in infecting *I. ricinus* nymphs and larvae with *A. phagocytophilum* I calculated the nymphal burden and larval burden (equation 2) and the reservoir competence (equation 3) first.

$$B_{n_i} = \frac{\sum_{s=1}^{n} N_{i_S}}{\sum_{s=1}^{n} H_{i_S}} \quad (2)$$

B_{n_i} is the mean nymphal burden per individual of host species i. N_{i_S} is the total number of nymphs counted on host species i in study s. H_{i_S} is the total number of individual animals of species i in study s. N_{i_S}can be replaced by the total number of larvae counted on species i in study s in order to calculate the mean larval burden per individual. For this calculation I used data from Hofmeester et al. (2016) combined with my own data to create a more reliable outcome.

$$RC_i = \frac{\sum_{s=1}^{n} IL_{i_S}}{\sum_{s=1}^{n} L_{i_S}} \quad (3)$$

RC_i is the realized reservoir competence of species i for *A. phagocytophilum*. IL_{i_S} is the total number of *A. phagocytophilum* infected larvae, sampled of species i in study s. L_{i_S} is the total number of larvae tested of species i in study s. For nymphs the same equation is used, except it is not called realized reservoir competence since it is not clear if the nymphs were already infected. A name for this is not known yet and therefore I call it α. So RC_i is substituted with α (equation 4).

$$\alpha_i = \frac{\sum_{s=1}^{n} IN_{i_S}}{\sum_{s=1}^{n} N_{i_S}} \quad (4)$$

With this data I was able to calculate the relative importance of different species using the following equations (equation 5, 6, 7, 8).

$$RI_{Il_i} = \frac{B_{l_i} D_i RC_i}{\sum_{j=1}^{n} B_{l_j} D_j RC_j} \quad (5)$$

RI_{Il_i} is the relative importance of host species i in infecting larvae with *A. phagocytophilum*. B_{l_i} is the mean larval burden per individual of host species i. D_i is the density in which species i occurs. $\sum_{j=1}^{n} B_{n_j} D_j RC_j$ is the total number of nymphs infected by all host species in the assemblage, as determined by their mean nymphal burden, density and realized reservoir competence.

$$RI_{In_i} = \frac{B_{n_i} D_i \alpha_i}{\sum_{j=1}^{n} B_{n_j} D_j \alpha_j} \quad (6)$$

RI_{In_i} is the relative importance of host species i in infecting nymphs with *A. phagocytophilum*. B_{n_i} is the mean nymphal burden per individual of host species i. D_i is the density in which species i occurs. $\sum_{j=1}^{n} B_{n_j} D_j \alpha_j$ is the total number of nymphs infected by all host species in the assemblage, as determined by their mean nymphal burden, density and amount of infected nymphs divided by the total number of nymphs tested.

$$RI_{Fl_i} = \frac{B_{l_i} D_i}{\sum_{j=1}^{n} B_{l_j} D_j} \quad (7)$$

RI_{Fl_i} is the relative importance of host species i in feeding larvae. B_{l_i} is the mean larval burden per individual of host species i. D_i is the density in which species i occurs. $\sum_{j=1}^{n} B_{l_j} D_j$ is the total number of larvae feeding from all host species in the assemblage, as determined by their mean larval burden and density.

For nymphs the same equation can be used to calculate the relative importance of host species in feeding them (equation 8).

$$RI_{Fn_i} = \frac{B_{n_i} D_i}{\sum_{j=1}^{n} B_{n_j} D_j} \quad (8)$$

RI_{Fn_i} is the relative importance of host species i in feeding nymphs. B_{n_i} is the mean nymphal burden per individual of host species i. D_i is the density in which species i occurs. $\sum_{j=1}^{n} B_{n_j} D_j$ is the total number of nymphs feeding from all host species in the assemblage, as determined by their mean larval burden and density.

2.5. Statistical analysis

To test if there is a difference in infection prevalence of different hosts species I used a generalized linear model with a gaussian distribution. The data shows that hosts and geographical area are correlated so I used area as an extra factor. I used the number of hosts infected and the total number of hosts tested, using data obtained by PCR testing, to test for a correlation between the host species, geographical area and the infection prevalence. Visual inspection of the residues of the model showed that they were normally distributed and homogenous.

To test for correlations between the relative importance of host species feeding different stages of *Ixodes ricinus* (using data from Hofmeester et al. (2016)) and the relative importance of host species in infecting *I. ricinus* ticks with *A. phagocytophilum* I used a simple linear regression. The scatterplot showed that there is a strong positive linear relationship between the relative importance in feeding and infecting nymphs and larvae, which is confirmed with a Pearson's correlation coefficient of 1,00 in both nymphs and larvae.

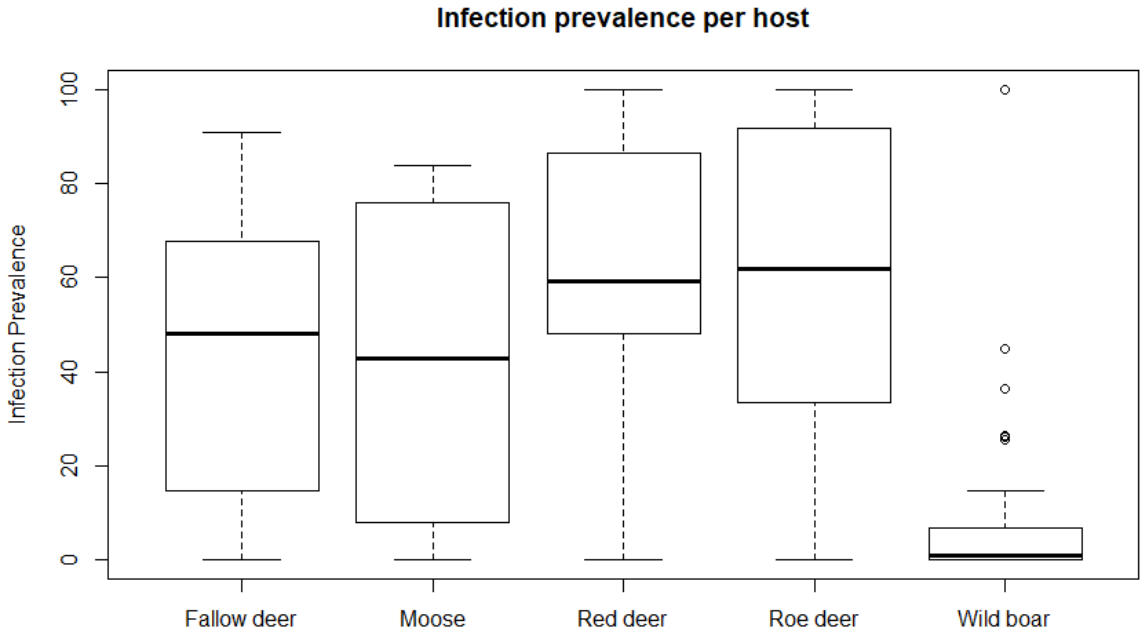
All statistical analyses were performed in RStudio version 1.1.463.

3. Results

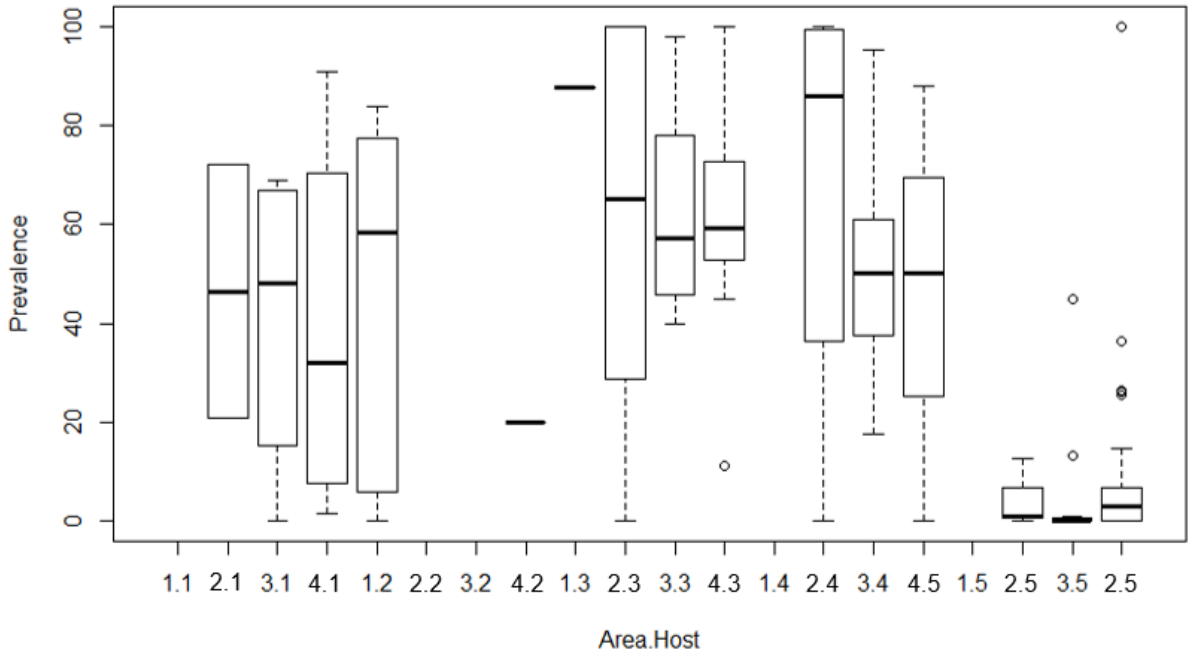
The results of the literature search are shown in appendix 1.

3.1. Infection prevalence per host

The infection prevalence is 41,38% in fallow deer, 42,72% in moose, 62,93% in red deer, 58,89% in roe deer and 6,57% in wild boar (graph 2). Only the prevalence in wild boar is significant lower than in the other host species. There is a correlation between area and host species (graph 2, table 2 and 3), therefore area was included as an extra factor in the generalized linear model. There is an overview of the data shown in table 4.



Graph 1. Infection prevalence per host.



the host species (1 = fallow deer, 2 = moose, 3 = red deer, 4 = roe deer, 5 = wild boar). For example in area 1 there are only moose and red deer.

Table 2. Overview of the number of animals per species per geographical area in the included studies

	Fallow deer	**Moose**	**Red deer**	**Roe deer**	**Wild boar**
Northern Europe	0	1236	8	0	0
Central Europe	101	0	218	975	716
Southern Europe	248	0	293	346	461
Eastern Europe	280	5	491	849	2180

Table 3. Relation between infection prevalence per host with geographical area as an extra factor

I group – J group	**Mean difference (I – J)**	**Std. Error**	**95% confidence interval**	
			Lower bound	Upper bound
Roe deer – Fallow deer	-15,19	8,60	-33,35	0,41
Roe deer – Moose	-15,73	9,32	-32,39	1,17
Roe deer – Red deer	5,75	5,91	-6,73	16,58
Roe deer – Wild boar	-48,81	5,17	-60,91	-39,56

Table 4. Host species, their taxonomic class, infection prevalence with A. phagocytophilum, average tick burden and realized reservoir competence for *A. phagocytophilum*.

Species	**Taxonomic class**	**Infection prevalence with *A. phagocytophilum* (%)**	**Average tick burden (larvae/nymphs /adults)**	**Realized reservoir competence for *A. phagocytophilum* (larvae/nymphs)**	**References**
Dama dama	Mammal	41,38	57,18/13,58/5,5	0,75/0,61	(Adaszek *et al.*, 2012; Di Domenico *et al.*, 2016; Ebani *et al.*, 2016; Ebani *et al.*, 2008; García-Pérez *et al.*, 2016; Hapunik *et al.*, 2011; Kazimírová *et al.*, 2018; Pereira *et al.*, 2016; Robinson *et al.*, 2009; Zeman & Pecha, 2008)
Alces alces	Mammal	42,72	290,29/173,29/ 122,29	-/-	(Karbowiak *et al.*, 2015; Malmsten *et al.*, 2014; Milner & van Beest, 2013; Puraite *et al.*, 2015)
Cervus elaphus	Mammal	62,93	10,80/5,86/14,1 7	0,83/0,80	(Adamska, 2010; Cézanne *et al.*, 2017; Di Domenico *et al.*, 2016; Dugat *et al.*, 2016; Ebani *et*

					al., 2016; Ebani *et al.*, 2008; García-Pérez *et al.*, 2016; Kazimírová *et al.*, 2018; Petrovec *et al.*, 2002; Polin *et al.*, 2004; Portillo *et al.*, 2011; Robinson *et al.*, 2009; Rymaszewska, 2014; Silaghi *et al.*, 2011; Skotarczak *et al.*, 2008; Stefanidesova *et al.*, 2008; Štefanidesová *et al.*, 2011; Stuen *et al.*, 2013; Zeman & Pecha, 2008)
Capreolus capreolus	Mammal	58,89	42,04/18,00/17,7	0,81/0,29	(de la Fuente *et al.*, 2008; Di Domenico *et al.*, 2016; Dugat *et al.*, 2016; García-Pérez *et al.*, 2016; Han *et al.*, 2017; Hapunik *et al.*, 2011; Jouglin *et al.*, 2017; Kauffmann *et al.*, 2017; Kazimírová *et al.*, 2018; Mogl *et al.*, 2011; Oporto *et al.*, 2003; Overzier *et al.*, 2013; Petrovec *et al.*, 2002; Robinson *et al.*, 2009; Rymaszewska, 2014; Scharf *et al.*, 2011; Silaghi, Hamel *et al.*, 2011; Silaghi, Kauffmann *et al.*, 2011; Skotarczak *et al.*, 2008; Stefanidesova *et al.*, 2008; Štefanidesová *et al.*, 2011; Szekeres *et al.*, 2019; Tavernier *et al.*, 2015; Torina *et al.*, 2008; Welc-Falęciak *et al.*, 2013;

					Žele *et al.*, 2012; Zeman & Pecha, 2008)
Sus scrofa	Mammal	6,57	2,72/2,43/0,56	0,00/0,052	(Adamska, 2010; Dugat *et al.*, 2016; Kazimírová *et al.*, 2018; Kiss, 2014; Masuzawa *et al.*, 2011; Michalik *et al.*, 2012; Pereira *et al.*, 2016; Polin *et al.*, 2004; Reiterová *et al.*, 2016; Silaghi *et al.*, 2014; Skotarczak *et al.*, 2008; Stefanidesova *et al.*, 2011; Stefanidesova *et al.*, 2008; Zele *et al.*, 2012; Zeman & Pecha, 2008)

3.2. Relative importance in infecting larvae and nymphs per host species

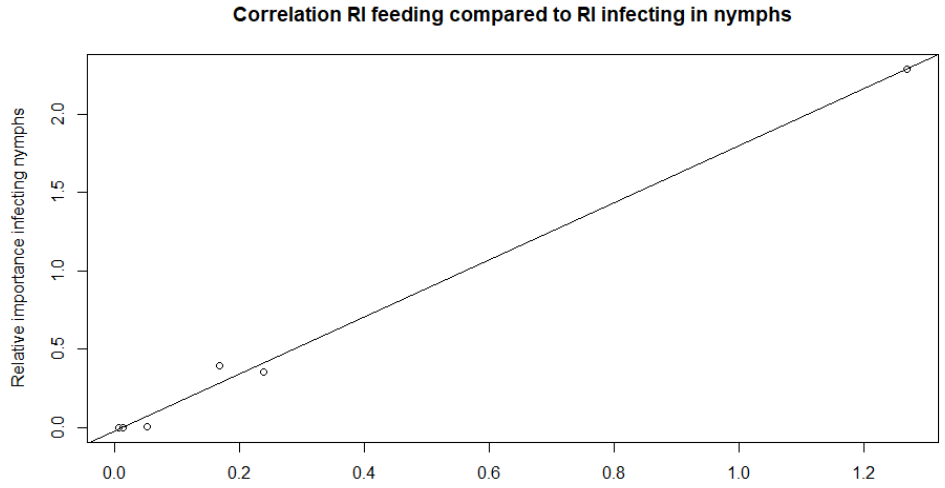
The relative importance in infecting larvae with *A. phagocytophilum* is 3,35% in fallow deer, 0,20% in red deer, 1,35% in roe deer and 0% in wild boar. There is no data available to calculate the relative importance in infecting larvae in moose. The one-way ANOVA between the host species showed that there is not enough data available to determine the differences between the host species.
The relative importance in infecting nymphs with *A. phagocytophilum* is 2,29% in fallow deer, 0,20% in red deer, 0,18% in roe deer and 0,0079% in wild boar. There is no data available to calculate the relative importance in infecting nymphs in moose. The one-way ANOVA between the host species showed that there are no significant differences between the host species [$F(3,2) = 18,28$, $p = 0,0523$].

3.3. Relative importance in feeding larvae and nymphs per host species

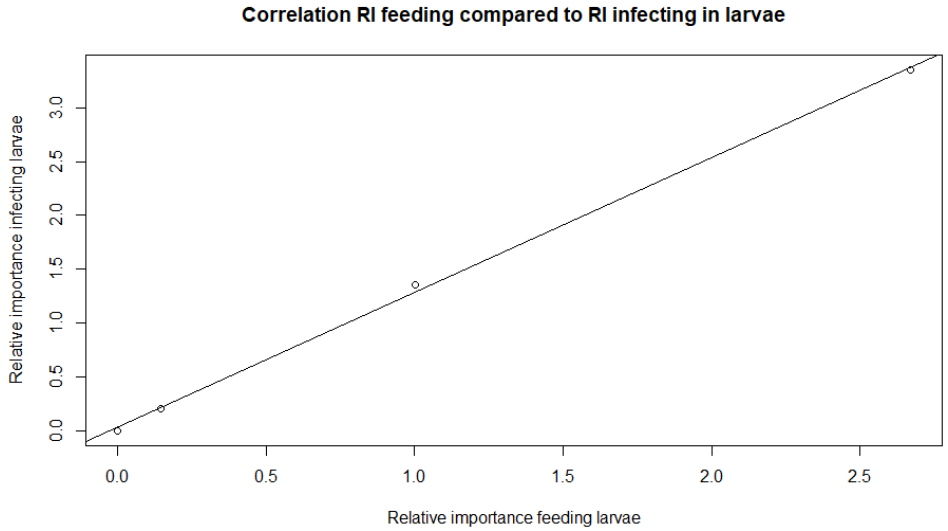
The relative importance in feeding larvae is 2,36% in fallow deer, 4,80% in moose, 0,22% in red deer, 0,96% in roe deer and 0,056% in wild boar. The one-way ANOVA between the host species showed that there are no significant differences between the host species [$F(4,13) = 2,478$, $p = 0,0958$].
The relative importance in feeding nymphs is 1,27% in fallow deer, 0,87% in red deer, 0,13% in roe deer and 0,026% in wild boar. There is no data available to calculate the relative importance in feeding nymphs in moose. The one-way ANOVA between the host species showed that there are no significant differences between the host species [$F(3,3) = 1,049$, $p = 0,485$].

3.4. Correlation between the relative importance in feeding and infecting

Simple linear regression shows a relationship between the relative importance in feeding and the relative importance in infecting both nymphs ($t = 28,52$, $p < 0.05$) and larvae ($t = 48,24$, $p < 0,05$). The slope coefficient for relative importance in infecting nymphs is 0,55 (graph 3), so the relative importance in infecting increases by 0,55 when the relative importance in feeding increases with 1. The slope coefficient for relative importance in infecting larvae is 0,80 (graph 4). The R^2 value shows that respectively 99,5% and 99,9% of the variation in relative importance in infecting nymphs and larvae can be explained by the model containing only the relative importance in feeding.



Graph 3. Correlation between the relative importance in feeding and infecting nymphs



Graph 4. Correlation between the relative importance in feeding and infecting larvae

4. Discussion

In this study I collected data from previous studies to get an idea of the role of ungulates in the lifecycle of *Anaplasma phagocytophilum* and to see if ungulate management could make a difference.

The results show that the infection prevalence is significant lower in wild boar compared to roe deer, fallow deer, moose and red deer. With such a low infection prevalence there is a small chance that a wild boar will infect nymphs or larvae. This suggests that in regard to ungulate management the wild boar is less relevant. This contradicts the results of the previously mentioned study by Michalik et al. (Michalik *et al.*, 2012). They found a prevalence of 9% -20% which was described as "compelling evidence for the involvement of wild boars in the enzootic cycle or A. phagocytophilum". In my study, with all the available data considered, it can be concluded that the average prevalence in wild boar is lower, namely 6,57%. This fits with the results from a study by Galindo et al. (Galindo *et al.*, 2012). In this study the immune system of the wild boar was examined. It showed that the wild boar can control an infection with *A. phagocytophilum*, which can result in an infection that is not detectable with PCR. This explains the low prevalence of detected infections.

In contrast to infection prevalence, there are no significant differences between the host species in the relative importance in infecting and feeding nymphs and larvae. It is to be expected that if the host species has a low infection prevalence, the relative importance is also low. This is because the relative importance depends on the tick burden, reservoir competence and density of the host. The higher these values are, the greater the relative importance, and the greater the chance that a host is infected with *A. phagocytophilum*. That this difference in relative importance is not visible between the hosts can be explained by the small amount of available data. Only four studies (Kazimírová *et al.*, 2018; Michalik *et al.*, 2012; Pacilly *et al.*, 2014; Wegner *et al.*, 1997) contained data on the tick burden in wild boar, two of these studies (Kazimírová *et al.*, 2018; Michalik *et al.*, 2012) also examined the infection prevalence and in these studies the average infection prevalence was 25,8%. This is considerably higher than the average I determined across all studies included, which also explains the higher relative importance in infecting the ticks. The results regarding the relative importance are based on a small amount of data, so no firm conclusion can be drawn on this basis.

Linear regression seems to show a relationship between the relative importance of hosts in feeding and infecting nymphs and larvae, with the data of all the host species combined. The higher the relative importance of the host species in feeding the ticks, the higher the relative importance of the host species in infecting the ticks. This result was to be expected since this can be explained by the fact that if there are relatively more ticks that feed from a host (high relative importance in feeding), the chance of getting infected is higher (high relative importance in infecting), so the chance of testing positive on *A. phagocytophilum* in the ticks is higher. However, these results are based on too little data to be able to draw a firm conclusion. Another study, into the influence of the number of deer on the amount of ticks, has been conducted. This study shows that if deer are excluded from an area, the number of questing ticks are reduced 12-fold. This suggests that reducing the density of the host species can also reduce the relative importance in infecting the ticks, because if there are fewer ticks, less ticks can be infected (Bown *et al.*, 2008). These results fit with the relationship between the relative importance of hosts in feeding and infecting nymphs and larvae.

In this study I selected five different host types to determine their role in the life cycle of *Anaplasma phagocytophilum*. To get a clear view of how the risk of infection in an area can be reduced, the

relative importance of all mammals in that area should be determined. Because the research would be too extensive in this way, I decided to look first at these five ungulate species to see what the influence is. In this study I chose for ungulates because it is suggested that adult ticks preferentially feed from large mammals, like deer (Dugat *et al.*, 2015). In addition, several studies have shown that the prevalence of *A. phagocytophilum* is high in fallow deer (Ebani *et al.*, 2008; Kauffmann *et al.*, 2017), moose (Malmsten *et al.*, 2014), red deer (García-Pérez *et al.*, 2016; Petrovec *et al.*, 2002), roe deer (Kauffmann *et al.*, 2017; Rymaszewska, 2014; Scharf *et al.*, 2011; Silaghi *et al.*, 2011; Žele *et al.*, 2012) and wild boar (Michalik *et al.*, 2012; Stefanidesova *et al.*, 2011; Strasek Smrdel *et al.*, 2009) and in addition, management measures can be applied easier to these species than to rodents and rabbits for example. In a follow-up study there can be looked at multiple mammalian species and the mammals with the greatest relative importance can be selected.

I used the data from 52 studies to determine the relative importance of the five host species in this study. Due to the small amount of data, the results are less reliable. There are more studies needed which collects data on the prevalence of *A. phagocytophilum* in both the hosts and ticks. A better view can be obtained when there is more available data. From this study it can be concluded that ungulates do play a role in the lifecycle of *Anaplasma phagocytophilum*. This study indicates that the relative importance of wild boar is less relevant than the other host species. In addition, there seems to be a relationship between the relative importance in feeding and infecting in both nymphs and larvae when the data of all host species was combined. As far as management is concerned, this means that if less ticks can feed from the animals, it is likely that the amount of infected ticks in the vegetation can be reduced.

5. Literature

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Appendix 1 PRISMA flow diagram of included studies