Tick-borne diseases in roe deer

Seroprevalence, possible cut-off and effects on health

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Abstract

Understanding the interaction between roe deer and pathogens could potentially help us improve the health in both roe deer and humans. Here, I focused on broadening our basic understanding about three specific tick-borne pathogens, *Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l. and Tick-borne encephalitis virus (TBEV), and their relationship with roe deer (*Capreolus capreolus)*. In this study, for all three pathogens, I aimed to determine roe deer´s capability to function as an indicator species, to establish a seroprevalence, identify factors affecting the seroprevalence in roe deer and, lastly, examine if these pathogens have a negative effect of roe deer's health. Serological data for above mentioned pathogens, haematological parameters and body weight were collected from 51 adult and 49 neonate fawns. Additionally, for neonate fawns also number of ticks, lifespan, and body temperature were examined. Multiple statistical analyses were performed. The seroprevalence in Sweden was determined to be 82%, 43% and 53% respectively for *A. phagocytophilum*, *B. burgdorferi* s.l and TBEV. Antibodies were found for all pathogens in both age groups indicating roe deer as a functional sentinel species for all pathogens in this study. A statistical difference between areas regarding the seroprevalence for *A. phagocytophilum* and TBEV were found with a higher mean in Bogesund. This is discussed in relation to difference in the abundance of roe deer and size of tick population. A correlation between serological data of *B. burgdorferi* s.l and sex were found with findings pointing towards internal factors. Multiple correlations were found between serological data and health parameters. Continued research around these results is needed to make further inference about cause and effect.

Keywords: Anaplasma phagocytophilum, Borrelia burgdorferi, tick-borne encephalitis virus, roe deer, *Capreolus capreolus*, tick-borne pathogens

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Abbreviations

SLU	Swedish University of Agricultural Sciences
TBE	Tick-borne encephalitis
TBEV	Tick-borne encephalitis virus

Definitions

Adult	in this study, an adult is defined as an individual tested during the winter and are therefore older than 6 months. Most likely, these individuals no longer have maternal antibodies but instead can have produced endogenous antibodies reflecting previous infections.
Antibody levels	in this study, the term “antibody levels” refer to the continuous numerical data seen with the serology results of *B. burgdorferi* s.l and TBEV.
Antibody titres	in this study, the term “antibody titres” refer to the semi-quantitative data seen with the serology results of *A. phagocytophilum*.
Amplifying host	“any host (vertebrate or tick) for which infection results in amplification of infection prevalence in the tick population (usually a vertebrate host but may be a tick when transovarial transmission occurs)” (Randolph *et al.* 1996).
Fawn	in this study, a fawn is an individual tested during their first weeks of life.
Reservoir host	“any host (vertebrate or tick) that maintains the pathogen during conditions that preclude active pathogen circulation (e.g., over winter)” (Randolph *et al.* 1996).
Seropositivity	the presence of antibodies in an individual

Seroprevalence	an estimate of the percentage in a population testing positive for antibodies.
Xenodiagnosis	“uninfected ticks (usually larvae) are allowed to feed on hosts that have been exposed to natural or experimental infections, usually without any particular regard to the site of feeding. Infection of the engorged or moulted xenodiagnostic ticks is assessed by appropriate diagnostic techniques e.g. isolation, plaque assay, immunofluorescent antibody test, PCR (polymerase chain reaction)” (Randolph *et al*. 1996).

1. Introduction

If a tree falls in the forest and no one is around to hear it, does it make a sound? Or in this case, if a roe deer falls ill to a tick-borne disease and no one is around to document it, is it sick?

The relevance of tick-borne pathogens in both humans and animals is increasing globally (Madison-Antenucci *et al.* 2020). Roe deer (*Capreolus capreolus)* is an important energy source for ticks (Fabri 2022) and it is therefore likely that they frequently come into contact with pathogens carried by ticks such as those considered in this study: *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu latu, and tick-borne encephalitis virus (TBEV). Exactly what these contacts result in for the roe deer it has not yet been studied. Increasing our understanding on the potential effects of tickborne diseases on wildlife populations is an important aspect of the One-Health concept (World Health Organization 2017). Some aspects regarding these pathogens are well studied, for example their negative effects in humans and their connection to important reservoirs. For roe deer, the studies have been few were most focuses on seroprevalence (Alonso *et al.* 2012; Pato *et al.* 2013; Jahfari *et al.* 2014; Elfving *et al.* 2015; Stigum *et al.* 2019; Silaghi *et al.* 2020; Remesar *et al.* 2021) and the possible effects roe deer might have on the prevalence of the pathogens (Rijpkema *et al.* 1996; Jensen *et al.* 2000; Rizzoli *et al.* 2009; Cagnacci *et al.* 2012; Fabri *et al.* 2021; Gandy *et al.* 2021). The seroprevalence for roe deer in Sweden have been studied for *A. phagocytophilum* meanwhile no studies for *B. burgdorferi* s.l or TBEV have been done. For these two, there are published seroprevalence for a few countries in Europe. To my knowledge, only two studies have been published so far reporting clinical cases in roe deer. In 2006 the only ever published case of clinical anaplasmosis in roe deer (Stuen *et al.* 2006) and in 2022 the first ever case of TBE in roe deer were published (Da Rold *et al.* 2022). Beyond this, studies looking at the possible detrimental effects on roe deer's health have not been performed. Studies on other Cervidae-species are available for *A. phagocytophilum* with mixed results (Stuen *et al.* 2006; Malmsten *et al*. 2014).

The purpose of this study is to expand our knowledge about the adult and neonate roe deer's relationship to the tick-borne pathogens *A. phagocytophilum, B. burgdorferi* s.l and TBEV based on data collected from roe deer in two contrasting areas.

One costal area with a high tick and roe deer population density (Bogesund) and one inland area with very low tick and roe deer densities (Grimsö).

Thus, the study primarily aims to investigate:

- Roe deer´s capability to function as a sentinel for all three pathogens.
- Seroprevalence in roe deer for all three pathogens.
- Factors (sampling area, age, gender) that affect seroprevalence of all three pathogens in roe deer.
- If the examined roe deer show any sign of reduced health linked to any of the three pathogens.

2. Literature review

2.1 Immune system

2.1.1 The inner workings of the immune system

To be able to handle the many threats in the world, organisms have created multiple ways to defend themselves. One of those defensive barriers is called the immune system. The immune system is complex, with multiple pathways, cells, and molecules.

The immune system involves several strategies with the one in focus for this study is the adaptive immune system. This adaptive immunity system, as the name indicates, adapts after having met an intruder for the first time (Tizard 2018). Antibodies have a protective roll by identifying markers of an invader, known as antigens. In some cases, the presence of antibodies after an infection may result in protection (i.e. immunity) against the disease and in other cases antibodies have a minimal protective capacity but they witness that an organism has been infected by or at least in contact with them. The role antibodies take depends on multiple factors such as the type of pathogens where different methods are needed between bacteria, viruses and parasites. It takes approximately one week for the body to produce antibodies the first time which then peaks at day 10-20. The next time the pathogen is encountered, antibodies can be seen rising faster, and to a higher level and for a longer time than the previous infection. This is a progressive improvement dependent on how often the pathogen is exposed to the immune system. The antibodies slowly decline and are therefore measurable after the infection´s end. This makes serology, the detection of antibodies, an excellent indicator of whether the individual has previously been in contact with an antigen (even if antibodies' persistence in the body can vary depending on many factors). How long after an infection, antibodies can be detected is affected by multiple factors such as pathogen and the individual.

Since the adaptive immune system needs to develop by experience, especially young individuals are susceptible to infections (Tizard 2018). Young animals who

have no transfer of antibodies from mother to offspring during the gestation only have the innate immunity system to rely on until their adaptive system has gained more knowledge. They are helped by drinking the colostrum which contains the mother's antibodies. These antibodies obstruct the production of the offspring's own antibodies. In cattle, calves that has received a proper amount of colostrum, will the production on self-produced antibodies commence at week 4 and in piglets at around week 5-6 after birth.

When investigating whether an individual has been in contact with a specific pathogen, it can be tested if the individual has antigen-specific antibodies since this indicates an earlier encounter. The presence of antibodies in newborn or very young animals may not be due to an encounter with a pathogen but rather an acquisition of adaptive immunity with the help of antibodies in the colostrum.

2.1.2 Colostrum

Humans have a haemochorial placenta meanwhile ruminants have an epithelio-chorial placenta where the chorionic villi are localized as cotyledons. This difference results in ruminants and many other animals being born without maternally derived antibodies since they cannot be transferred from the mother to the foetus. There are studies made on different species of ruminants which suggest that not all ruminants have the same exact placenta visible. In an article focusing on Cervidae including roe deer, is the exact term "epithelial-chorial placenta" never used (Hamilton *et al.* 1960). However, roe deer's cotyledons are described in detail and from here it can be hypothesized that roe deer placenta is built in a similar way as in other ruminants. But since there is a lack of more sound experimental data and since the pregnancy in roe deer is not fully studied, it cannot be excluded that some extent of immune transfer could take place via the placenta. some extent. It is very common for twins to occur in roe deer which leads to a fusion of the chorion but no anastomose or other connections between the twins (Hamilton *et al.* 1960).

Colostrum is defined as the milk produced during the first day after parturition. In an article studying red deer's colostrum it could be found that total protein (which includes antibodies) were the highest at five hours after parturition with a decline with over 50% the first 24 hours (de la Vara *et al.* 2020). The same article also looked specifically at both IgG and IgM where IgG is described as more than 4 times higher at <5 hours after parturition than after 24 hours respectively three times higher for IgM.

It is shown in both mule deer and white-tailed deer that fawns with higher antibody levels have a higher chance of surviving compared to fawns with lower levels (Parkinson *et al.* 1982; Evers *et al.* 2017). In contrast, an article studying red deer

saw no correlation in antibody levels and fawn survival but instead observed a correlation between the weight of the fawn and the level of antibodies (Gauzere *et al.* 2021). Gauzere *et al*. (2021) also concluded that "neonatal antibody levels were mainly affected by maternal genes, environmental variation and costs of prior reproductive investment" where, for example, a high-density population were found having a negative effect on both the fawns weight and antibody levels.

Young animals who haven't got enough colostrum can be affected by a so called "failure of passive transfer" (FPT). In calves and foals there are tests and standardised refences values used to assess the status of FPT. There are no similar values for roe deer but they are reported for other species in the Cervidae-family (Rivas *et al.* 2021). However, these results cannot be extrapolated for roe deer due to significant differences between species according to Rivas *et al.* (2021). Calves that do not ingest colostrum, start to produce endogenous IgM in their first week but IgA and IgG are not noticeable until day 16 to 32 days after birth (Chase *et al*. 2008).

Colostral antibodies need to be considered when interpreting serology results. A seropositive newborn may simply have had colostrum, and that provides no information on their disease history. The antibodies, after being absorbed in the intestines, circulate in the fawns for a few weeks.

2.2 Roe deer

The roe deer (*Capreolus capreolus*) is an ungulate in the Cervidae-family, suborder Ruminantia. The roe deer is one of the smaller deer with a height at the withers of around 65-75 cm and a weight of 20-30 kg as adults. They mostly live in woodland and are often spotted in fields. Roe deer only existed in a small part of Scania historical province (Skåne) 150 years ago and has since then had a dramatic increase in population in Sweden (Bergström & Danell 2009) and can now been found spread over whole Sweden below the tree line. The same trend has been observed in Europe (Burbaitė & Csányi 2009).

Roe deer mate in the summer (July-August) and because of delayed implantation parturition doesn't occur until May-June the following year. The first weeks the fawns primarily lay still with the doe leaving and then returning for feeding. Fawns have a high mortality, over 50%, with the main proximate cause of death being predation by foxes (88%) (Jarnemo 2004).

The most common causes of death for adult roe deer in Sweden are trauma, winter starvation and gastritis, which together account for over 50 percent of the deaths

(Aguirre *et al.* 1999). Tick-borne diseases like granulocytic anaplasmosis, borreliosis and TBE were not mentioned in this study, while five cases of babesiosis were diagnosed. Aguirre *et al.* (1999) mentions problems with diagnosis due to "tissue autolysis and overgrowth of unspecific bacterial flora".

2.3 Ticks

There are multiple species of ticks in Sweden but the most common one in most parts of Sweden is *Ixodes ricinus* (Jaenson *et al.* 1994). The populations of *I. ricinus* have spread northwards since the 1990s with a significant increase of the number of ticks and is now believed to be present in every province in Sweden although generally in higher levels on the coastal line and wherever the climate is milder (Jaenson *et al.* 2012b).

Roe deer together with other ungulates are important for tick populations (Jaenson *et al.* 2012a). They play a vital role of in *I. ricinus* life cycle since they are the main feeding host for female *I. ricinus* (Fabri 2022). Ungulates, all though varying depending on species, are also important regarding the spread of tick-borne diseases (Fabri *et al.* 2021).

Roe deer can have a negative effect on the prevalence of tick-borne pathogens in ticks. In a study with red deer the results were varying but pointing towards that a high density of ungulates can result in lower levels of *B. burgdorferi* sensu lato (s.l) in ticks (Gandy *et al.* 2021). This is since ticks feeding of ungulates instead of rodents creates a so called "diluting effect". Gandy *et al*. (2021) argue that even though the prevalence is lower in ticks, the risk of humans getting infected increases due to a rise of tick density since ungulates are such an important feeding host for these parasites.

2.4 Tick-borne pathogens

2.4.1 *Anaplasma phagocytophilum*

Anaplasma phagocytophilum is a bacterium which has been renamed several times (Henry 2019). From Rickettsia to Cytoecetes to Ehrlichia and lastly Anaplasma but all these genus' names had the common denominator of phagocytophilum. According to Henry (2019) the name phagocytophilum originates from the bacteria's "affinity for growing in neutrophils". *A. phagocytophilum* is an obligate gram-negative intracellular bacterium which has adapted to different sets of vertebrates and environments (Jaarsma *et al.* 2019). Genetic typing is challenging

and several approaches are being suggested to cluster different strains of the bacteria (Rar *et al.* 2021). In Europe, four (possibly five (Santos *et al.* 2018)) different strains have been identified; haplotype I and II have been detected in Sweden (Jahfari *et al.* 2014). According to Jahfari *et al.* (2014) humans were only found bearing haplotype I meanwhile both haplotypes I and II were isolated from roe deer. Contrary, a study from Norway only found haplotype II in roe deer (Stigum *et al.* 2019). The exact circulation pattern of different strains of *A. phagocytophilum* between wildlife, ticks and humans in Europe is still not clear. In 2015, 85% of the tested roe deer in Sweden were seropositive for *A. phagocytophilum* adopting a cut-off titre of ≥1:128 using an immunofluorescence antibody test (IFAT) (Elfving *et al.* 2015). A study from 2013 regarding seroprevalence of *A. phagocytophilum* in moose detected a considerable difference in seroprevalence depending on location and year (Malmsten *et al.* 2014). Other studies from Europe show that roe deer and other cervids are often exposed to the bacteria (Stigum *et al.* 2019; Silaghi *et al.* 2020; Remesar *et al.* 2021).

A. phagocytophilum can cause disease in multiple species such as humans, dogs, cats, horses, ruminants, and other mammalian species. In humans the disease is called "human granulocytic anaplasmosis" (HGA) where frequent symptoms are fever, headache and other aches throughout the body; the infection can also lead to leukopenia and granulocytosis (Bakken & Dumler 2015). Regardless of species, the bacteria often infect individuals with the help of ticks but can for example also spread from mother to offspring perinatally in humans (Horowitz *et al.* 1998) and by intrauterine infection in sheep (Stuen *et al.* 2018) and cattle (Henniger *et al.* 2013). There are no signs that humans can develop a chronic illness (Bakken & Dumler 2015) which can be observed in intrauterine infected lambs (Granquist *et al.* 2010; Stuen *et al.* 2018). Infection by *A. phagocytophilum* in ruminants is called tick-borne fever and can be seen in sheep, cattle, goats, deer, reindeer (Constable 2017) and moose (Malmsten *et al.* 2014). The infection is often asymptomatic but can lead to high fever, lethargy, abortion, and a drop in milk production; blood samples analysis can show thrombocytopenia, neutropenia and lymphocytopenia (Constable 2017). In a study inoculating sheep with the bacteria, fever could be seen four days post inoculation (dpi), antibodies specific for *A. phagocytophilum* could be observed 14 dpi and the detection of bacterial DNA by PCR gave positive results in several organs at 14 and 15 dpi (Almazán *et al.* 2020). "Mild splenomegaly and lymphadenomegaly with microscopic evidence of lymphoid hyperplasia" could be seen in another study following an experimental infection of sheep (Kocan *et al.* 2012).

There has been one published case globally of a roe deer with symptoms possibly caused by *A. phagocytophilum* (Stuen *et al.* 2006). The study describes a 3-month-

old roe deer fawn in Norway with over 300 ticks able to move its head but not its limbs. Pathological finds involved petechial subendothelial haemorrhages on the heart and an enlarged spleen (splenomegaly) with subcapsular haemorrhages. Bacterial cultivation from different organs were negative but the DNA of *A. phagocytophilum* was found in multiple tissue samples. Stuen *et al.* (2006) mention possible similar cases (unpublished) in the near vicinity of this case.

A. phagocytophilum and its effects have been studied more thoroughly in other species of cervids. Experimental infection has been done in both red deer (*Cervus elaphus*) and reindeer (*Rangifer tarandus*) where the former were asymptomatic meanwhile the later developed clinical signs (Stuen *et al.* 2006). In a study about the effects of the bacteria on moose no conclusions could be drawn due to a small sample size (Malmsten *et al.* 2014).

2.4.2 *Borrelia burgdorferi* s.l

Lyme borreliosis, also called Lyme disease, is a tick-borne disease of humans caused by some species of the complex *Borrelia burgdorferi* sensu lato; the genus was named after Amédée Borrel and the species after Dr Willy Burgdorfer (Johansson 2022). Sensu lato means "in the broad sense" which include multiple species with the most studied being the three genospecies *B. burgdorferi sensu stricto*, *Borrelia afzelii* and *Borrelia garinii* (Cardenas-de la Garza *et al.* 2019). In the Netherlands and in northern Europe the three previous mentioned genospecies were found in *I. ricinus* but also the strain VS116 also known as *Borrelia valaisiana* (Rijpkema *et al.* 1996; Mysterud *et al.* 2019). Not all species in the complex are equally pathogenic (Wang *et al.* 1999) and therefore the symptoms may vary depending on the genospecies responsible for the infection (Cardenas-de la Garza *et al.* 2019).

In humans, Lyme disease develops within months in three steps if untreated (Cardenas-de la Garza *et al.* 2019). The first step in characterized by the well-known skin reaction, a red circle around the tick-bite, which is called "erythema migrans". The two later steps involve more serious effects such as Lyme arthritis, Lyme carditis and Lyme neuroborreliosis affecting respectively 60%, 1-2% and 16-23% of patients (in Europe) infected and left untreated, according to Cardenas-de la Garza *et al.* (2019). Most humans infected by the bacteria develop symptoms compared to dogs where only approximately 5% do (Littman *et al.* 2006). Symptoms which can be seen in dogs are for example pyrexia, lameness and protein losing syndrome though urine. It is hard to prove that these symptoms develop as a consequence of borrelia infection since many diseases can cause similar symptoms and most positive dogs are asymptomatic (Sala & De Faveri 2016).

The relationship that this gram-negative spirochete established with ticks is thought the be a very old one having resulted in the bacteria evolving into becoming dependent on a tick vector and a vertebrate (Estrada-Peña *et al.* 2018). A review article from 2021 found that out of several host species thought to be potential reservoirs only a few were confirmed by xenodiagnosis which the authors deemed as the most reliable method (Wolcott *et al.* 2021). Results from Wolcott *et al.* (2021) led to the conclusion that nineteen animal species worldwide had been confirmed as competent reservoirs including various species of birds, rodents, one reptile and the European hedgehog. Not all these species exist in all continents making the exact transmission cycle depending on multiple factors as location and available hosts and reservoirs, which for northern Europe is not fully mapped out (Mysterud *et al.* 2019). Mysterud *et al.* (2019) observed that in northern Europe different genospecies in the complex might use different hosts, for example *B. burgdorferi sensu stricto* were only found in red squirrel (*Sciurus vulgaris*) meanwhile *B. afzelii* had no preferences and was found in all species of rodents and shrews included in the study. Roe deer are defined as an incompetent host to *B. burgdorferi* s.l since they most likely do not spread the bacteria to the ticks (Jaenson & Tälleklint 1992). Multiple studies have shown roe deer having an effective defence against the pathogen (Bhide *et al.* 2005). Roe deer and specifically their blood are thought to have no adverse effect on the spirochetes in ticks after a blood meal (Rijpkema 1996). Human neuroborreliosis cases are found correlating with a higher tick density depending on a higher roe deer abundance and soil water capacity (Jensen *et al.* 2000).

No data on seroprevalence of *Borrelia* spp. in wild animals are available so far in Sweden. Multiple studies have been done in other countries in Europe for example Spain (68.8%) (Pato *et al.* 2013), and England and Wales where both roe deer and red deer were tested (23%) (Alonso *et al.* 2012)

2.4.3 Tick-borne encephalitis virus

Cases of tick-borne encephalitis caused by TBEV (Tick-borne encephalitis virus) have been reported in Sweden since mid-1980s with a peak 2021 with an almost doubling in cases compared to the year before (Folkhälsomyndigheten 2022). TBEV infection can result in disease in humans, dogs, horses (SVA 2021) and recently it has been described a clinical case in sheep (Böhm *et al.* 2017). Bank voles can develop an encephalitis though most individuals do not display any symptoms, most likely due to limited inflammation (Tonteri *et al.* 2013). As a fact, a lot of species has been proven to be infected, but the infections are usually not leading to the development of any symptom (SVA 2021).

The first described case of TBE in roe deer has been published in 2022 in an endemic area in Italy (Da Rold *et al.* 2022). The roe deer were described symptoms as "ataxia, staggering movements, muscle tremors, wide-base stance of the front limbs, repetitive movements of head, persistent teeth grinding, hypersalivation and prolonged recumbency". The diagnosis was made by using rRT-PCR leading to the finding TBEV in the brain as the sole pathogen. Histological findings confirmed the diagnosis of clinical encephalitis. None of the ticks found on the individual harboured TBEV which is not surprising since the proportion of ticks harbouring TBEV is usually rather low (i.e. usually lower than 1%) even in endemic areas (Brinkley *et al.* 2008). Roe deer were suggested to work as an incomplete but functional sentinel for TBE-infections in humans as early as 1995 (Gerth *et al.* 1995). In the same article there could be seen a higher prevalence of antibodies in male roe deer than in females which could not be explained.

Small mammals, a surrounding environment appealing to the small hosts and a high density of roe deer are found to be influencing the circulation of TBEV (Rizzoli *et al.* 2009). Jaenson *et al.* (2018) agrees with this and has also highlighted the importance of "TBE virus-infected birds, or by birds or migrating mammals infested with TBEV-infected ticks" to create new TBE virus foci (Jaenson *et al.* 2018). Rizzoli *et al.* (2009) also pinpoints the milder and warmer climate as an important factor since this results in a change of vegetation positive for small rodents. Meanwhile Palo (2014) did not find climate change a contributing factor to a rise of TBEV though this article focused on the rise of winter temperature. What all articles agrees on is that host populations and dynamics are complex but probably represent the most important factors. An example of the complexity can be made clear by an article from 2012 where a negative correlation between "deer density and TBE occurrence on a local scale" could be found which was explained due to ticks choosing deer (an incompetent host) instead of rodents, the more competent host (Cagnacci *et al.* 2012).

3. Material and methods

3.1 Data collection

3.1.1 Roe deer capture and sample collection

The adult roe deer were captured using baited traps during winter. The traps were located in different habitats in 15-20 and 10 locations in Grimsö and Bogesund respectively. In summer, either female roe deer equipped with transmitters were located to determine the presence of fawns or the fawns were found by chance, which were then captured and handled (for further details see Davis *et al*. 2016, Bergvall *et al.* 2017 and Bonnot *et al.* 2018).

The data are collected from adults and fawns during captures include blood samples, bodyweight, metatarsal length for both adults and fawns and, additionally for fawns, number of ticks, body temperature and length of life span. Number of individuals is shown in table 1.

The capturing of fawns in Grimsö was performed during May and June in all the years between 2015 – 2018 whereas in Bogesund these captures were done in May and June of 2016. For adults in Grimsö, all but one was captured between January – March 2013 with one in March of 2017. In Bogesund the captured were done between December 2015 to March of 2017.

The roe deer tested inhabited two different areas in Sweden: Bogesund and Grimsö. They differ in animal population where Grimsö has "cyclic population rodents (*Apodemus flavicollis, Apodemus sylvaticus, Clethrionomys glareolus, Microtus agrestis, Myopus schisticolor*) and also the common shrew (*Sorex araneus*)" while Bogesund has "noncyclic rodent populations (*A. flavicollis, A. sylvaticus, C. glareolus*)". They share common mammals such as moose (*Alces alces*), wild boar (*Sus scrofa*), red fox (*Vulpes vulpes*), mountain hare (*Lepus timidus*), European hare (*Lepus europaeus*). Both places are also inhabited by roe deer where Grimsö have a lower density than Bogesund (Bonnot *et al*. 2018). These two places also differ in number of ticks where Bogesund has a substantially higher density. Bogesund is

also deemed a risk area for humans to contract TBE. This information above is a summarization and can be read in more detail in Kjellander *et al.* (2021).

Table 1. Number of individuals and the number of variables.

	Adults (n=51)	Fawns (n=49)
Serology	51	49
Grimsö	26	24
Bogesund	25	25
Females	28	24
Males	23	25
Haematology	34	48
Weight and metatarsal length	30	49
Number of ticks	0	49
Rectal temperature	0	49

3.2 Serological analyses

3.2.1 *Anaplasma phagocytophilum*

The indirect fluorescent antibodies (IFA) method was used for detecting antibodies to *Anaplasma phagocytophilum* in the serum samples obtained from the blood collected from each roe deer. An IFA test kit for human diagnostics was used (Focus Diagnostics (part of DiaSorin Group), product reference IF1450G). The instructtions from the manufacturer were followed but the secondary antibody was replaced with a FITC labelled-anti-Deer IgG (Seracare, cat. no. 5230-0329, 02-31-06). The powder was suspended in 1 ml Phosphate Buffered Saline (PBS) and used without further dilution. No positive control from roe deer were used.

3.2.2 *Borrelia burgdorferi* s.l

Antibodies to *Borrelia burgdorferi* sensu lato were analysed using an in-house ELISA developed at Statens Serum Institut in Denmark as described by Hansen *et al.* (1988). Each serum sample was diluted 1:100. The secondary antibody was replaced by a Peroxidase labelled-anti-Deer IgG (Seracare, cat. no. 5230-0298). The powder was rehydrated in 1 ml PBS and used at 1:500 dilution. No positive control from roe deer were used.

3.2.3 Tick-borne encephalitis virus

The analysis of TBEV antibodies was made with Euroimmun ELISA for TBE virus (art. No. EI 2661-9601 G, Euroimmun, Lübeck, Germany). The sera were diluted 1:100 in the kit buffer. As secondary antibody peroxidase labelled-anti-Deer IgG (Seracare, cat. no. 5230-0298) were used and diluted 1:100 in the kit buffer. The test followed the kit's instructions except that the incubation with substrate was increased from 15 to 20 minutes.

Both of the TBE antibody detection kits were controlled by using positive control sera from moose provided by Jon Valbjørn Hagelin, Veterinærinstituttet in Norway. The control sera from moose were previously found positive by the ELISA test-kit Enzygnost anti-TBE virus (IgM) by Siemens.

3.3 Haematological data

All analyses regarding haematological data were performed by Universitetsdjur-sjukhuset (University Teaching Hospital of the Swedish University of Agricultural Sciences, SLU) in Uppsala, Sweden.

3.4 Statistical analysis

All statistical analysis has been performed in Minitab® 19.2020.1 (64-bit), except calculations to define cut-offs in seroprevalence, that were performed in R.

3.4.1 Under-weight individuals

There is a correlation between metatarsal length and body weight (Kjellander *et al.* 2006). Therefore, I used a regression analysis to model changes in the body weight of roe deer in relation to metatarsal length in Minitab. The equation used is the following:

$$Y = m + k * X$$

Where Y is the dependent variable (body weight), m is a constant representing where the function crosses the Y-axis, k is the coefficient of X representing the slope and X is the independent variable (metatarsal length).

I then used this regression equation to estimate whether roe deer individuals were underweight or not. I defined underweight as any roe deer whose weight is below the predicted weight according to the length of the metatarsal. I made one equation for fawns and one for adults.

3.4.2 Cut-off for seropositive and seronegative individuals

A cut-off between the positive and the negative populations were calculated with the help by my collaborator, Dr. Romain Garnier for *B. burgdorferi* s.l. and TBEV. The calculations were done using the method described by Garnier *et al.* (2017). Briefly, using R, two normal distributions were fitted to the antibody levels of the adult animals. One of the normal distributions corresponds to the seropositive and the other to the seronegative part of the population. The cut-off is calculated as the fitted mean of the seronegative distribution plus two standard deviations.

This method is not applicable for *A. phagocytophilum* because the results are expressed as titres and, as a result, are semi-quantitative. In this case, a cut-off found in previous literature was used, see "3.3.3 Seroprevalence".

3.4.3 Seroprevalence

For *B. burgdorferi* s.l. and TBEV, all individuals above the cut-off level determined by above-mentioned method were counted as seropositive. A cut-off at 1:128 was used for *A. phagocytophilum* since this was used in other studies, i.e. Skarphédinsson *et al.* 2005 and Elfving *et al.* 2015.

Only adults were included since the fawns have not yet developed antibodies of their own at the time of the testing and the values might be biased by the presence of maternal antibodies.

3.4.4 Antibody levels in fawns

Basic descriptive statistics were calculated using Minitab for all three pathogens.

3.4.5 Serological status, environmental and individual factors

The different sampling areas, month of testing, ages, and sex were individually compared to all pathogen´s seroprevalence for adult and antibody levels for fawns. For the adult individual's seroprevalence in TBEV and *B. burgdorferi* s.l. a Pearson chi-square test was used when comparing sampling areas, sex and month of testing and a binary logistic regression comparing age in year and seroprevalence. Months were divided to December and January respectively February and March.

For fawns and adults, regarding *A. phagocytophilum* Pearson chi-square tests were conducted for determining possible association between sampling area, sex, and month of testing versus antibody titres and an ordinal logistic regression for comparing age in days for fawns and age in years for adult and titre levels. For fawns and *B. burgdorferi* s.l. and TBEV a t-test was used comparing the different sampling areas, month of testing, and sex to antibody levels and linear regression

analysis comparing age in days and antibody levels. Months are divided in May and June.

3.4.6 Effect on health in adults

The effects on health for adults were statistically analysed by comparing the positive and negative seroprevalence individuals. The roe deer included in this test were the individuals with either a haematological test or classified as underweight or normal weight. Each individual blood parameter was analysed with two-sample t-tests comparing the positive and negative population. A Pearson chi-square test was conducted comparing the individuals defined as underweight and normal weight to the positive and negative seroprevalence group.

3.4.7 Effect on health in fawns

Due to time limitations, a cut-off for fawns was not possible to calculate. Instead, all individuals were included by looking at differences in absorbance/OD values (for *B. burgdorferi* s.l. and TBEV) or antibody titre (for *A. phagocytophilum)*. For TBEV and *B. burgdorferi* s.l. the antibody levels were handled as numerical variables and the titres levels for *A. phagocytophilum* were handled as categorical.

The antibody levels for TBEV and *B. burgdorferi* s.l. were compared to the results of the haematological tests, number of ticks and rectal temperature with a simple regression. Antibody levels were also compared with a binary logistic regression analysis comparing the individuals defined as underweight and normal weight to the antibody levels of the different pathogens respectively.

For *A. phagocytophilum* a one-way ANOVA was used looking at the antibody titres versus the haematological tests, number of ticks respectively body temperature. Using a Pearson chi-square test the antibody titres were compared to individuals defined as underweight.

Due to the fawns being attached with a tracking collar, if the fawn died during the summer the exact date is known. The fawns have, depending on lifespan, been divided into four groups. This groups were with an ordinal logistic regression compared to antibody levels for *B. burgdorferi* s.l. and TBEV and a MANOVA for *A. phagocytophilum*. The four groups are “dead before 1 month old”, “dead between 1–2-month-old”, “dead between 2-3 months old” and “survived to at least 3 months old”. Fawns in this study who were found dead were in some cases sent to SVA (Statens veterinärmedicinska anstalt) for necropsy.

4. Results

4.1 Underweight individuals

4.1.1 Adult

A regression analysis with 30 adult roe deer was made finding a p-value <0.001. The regression equation was determined to:
Weight = -46.7 + 1.924 * length of metatarsal

15 individuals were found as underweight.

4.1.2 Fawns

A regression analysis with 49 individuals was made finding a p-value of <0.001. The regression equation was determined to:
Weight = -6.634 + 0.4321 * length of metatarsal

26 individuals were found as underweight.

4.2 Determining a cut-off for seropositive and seronegative individuals

4.2.1 *B. burgdorferi* s.l

Using Akaike information criterion (AIC) two normal distributions were found better fitted than one indicating a seropositive and a seronegative group. The green normal distribution being the seronegative and the red normal distribution bring the seropositive group, see figure 1. Above-mentioned method was used finding a cut-off at 0.1624 OD.

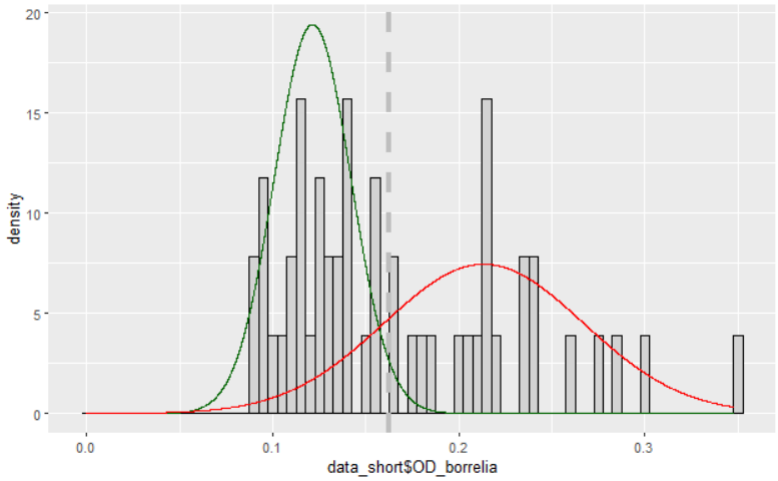

Figure 1. The green normal distribution representing the seronegative population and the red normal distribution representing the seropositive population. The grey line in between represents the calculated cut-off for B. burgdorferi *s.l.*

4.2.2 TBEV

Using Akaike information criterion (AIC) two normal distributions were found better fitted than one indicating a seropositive and a seronegative group. The green normal distribution being the seronegative and the red normal distribution bring the seropositive group, see figure 2. The figure is log-transformed due a very broad range of antibody levels. One individual was removed due to an antibody level of 0. Above-mentioned method was used finding a cut-off at 0.1286 RU/ml.

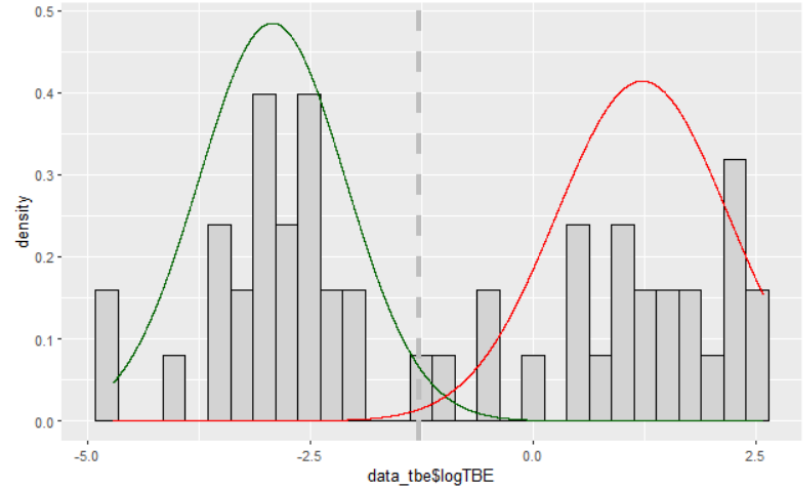

Figure 2. The green normal distribution representing the seronegative population and the red normal distribution representing the seropositive population. The grey line in between represents the calculated cut-off for TBEV.

4.3 Seroprevalence in adults

4.3.1 *A. phagocytophilum*

18 individuals in Grimsö and 24 individuals in Bogesund were defined as seropositive according to the above-mentioned cut-off (1:128). Resulting in a 69.2% (18/26) seroprevalence in Grimsö, 96% (24/25) seroprevalence in Bogesund with an overall seroprevalence of 82.3% (42/51). The information above is summarized in table 2.

4.3.2 *B. burgdorferi* s.l.

The cut-off for *B. burgdorferi* s.l. was determined to 0.1624 OD as described above, meaning that all individuals with 0.1624 OD or above is determined as seropositive. 9 individuals in Grimsö and 13 individuals in Bogesund are defined as seropositive. Resulting in a 34.6% (9/26) seroprevalence in Grimsö, 52% (13/25) seroprevalence in Bogesund with an overall seroprevalence of 43.1% (22/51). The information above is summarized in table 2.

4.3.3 TBEV

The cut-off for TBEV was determined to 0.1286 RU/ml as described above meaning that all individuals with 0.1286 RU/ml or above is determined as seropositive. Resulting in a 11.5% (3/26) seroprevalence in Grimsö, 96% (24/25) seroprevalence in Bogesund with an overall seroprevalence of 52.9% (27/51). The information above is summarized in table 2.

Table 2. An overview of seropositive, seronegative and seroprevalence in adult roe deer in Sweden for A. phagocytophilum, B. burgdorferi *s.l. and TBEV.*

	Grimsö (n=25)	Bogesund (n=26)	Total (n=51)
Anaplasma phagocytophilum	18 (69%)	24 (96%)	42 (82%)
Borrelia burgdorferi s.l	9 (35%)	13 (52%)	22 (43%)
TBEV	3 (12%)	24 (96%)	27 (53%)

4.4 Antibody levels in fawns

4.4.1 *Anaplasma phagocytophilum*

49 fawns had antibody titre levels with a minimum value of 0 and a maximum value of 8000. Q1 being 128, median of 256 and Q3 being 512. A histogram of the results can be seen in figure 3.

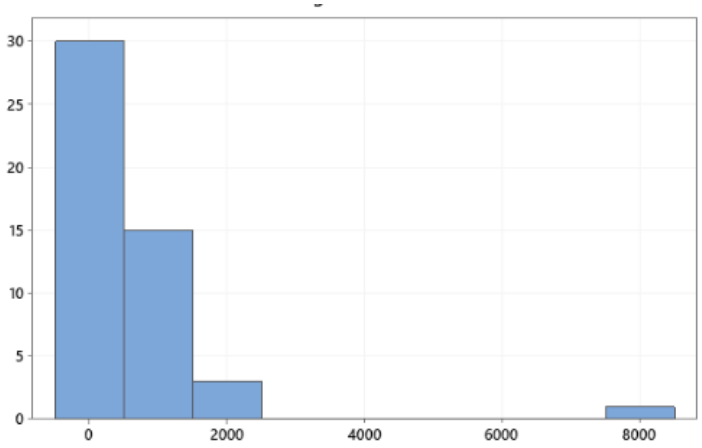

Figure 3. Histogram describing antibody titres of A. phagocytophilum *in fawns. X-axis showing titre levels and Y-axis showing number of individuals.*

4.4.2 *Borrelia burgdorferi* s.l.

49 fawns had antibody levels with a minimum value of 0.0940 and a maximum value of 0.6090. Q1 being 0.1430, a mean of 0.2325, median of 0.2230 and Q3 being 0.2735. A histogram of the results can be seen in figure 4.

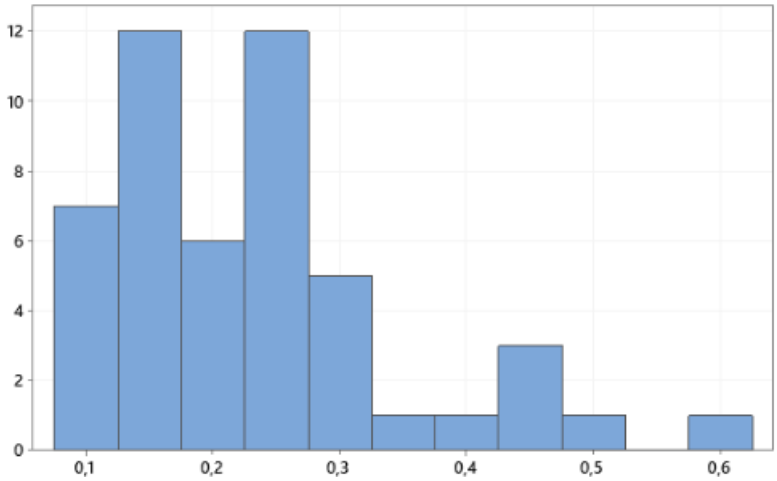

Figure 4. Histogram describing antibody levels of B. burgdorferi *s.l.in fawns. X-axis showing antibody levels in OD and Y-axis showing number of individuals.*

4.4.3 TBEV

49 fawns had antibody levels with a minimum value of 0.02 RU/ml and a maximum value of 40.75 RU/ml. Q1 being 0.06 RU/ml, a mean of 9.63 RU/ml, median of 4.47 RU/ml and Q3 being 17.62 RU/ml. A histogram of the results can be seen in figure 5.

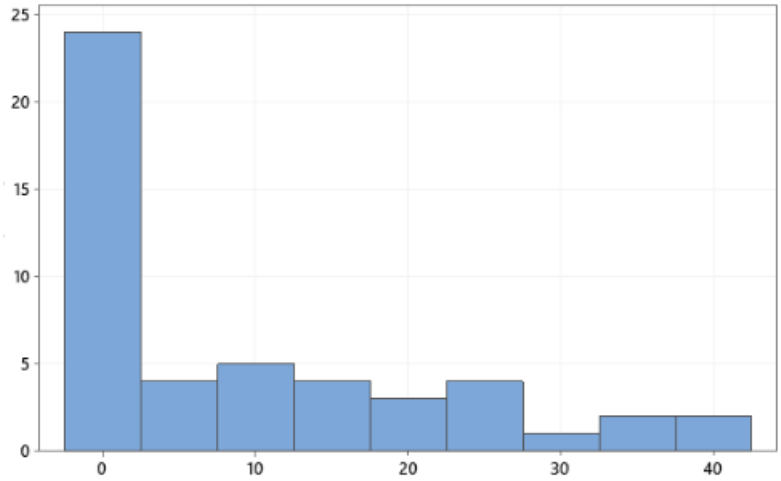

Figure 5. Histogram describing antibody levels of TBEV in fawns. X-axis showing antibody levels in RU/ml and Y-axis showing number of individuals.

4.5 Serological status, environmental and individual factors

4.5.1 Adult individuals

A. phagocytophilum

No statistically significant association could be found between seropositivity and sex (p=0.487) respectively time of season (p=0.527). A statistically significant association were found between seropositive individuals and sampling areas (p=0.012) indicating a higher chance of seroprevalence in Bogesund (observed count 24, expected 20.588). A binary logistic regression performed to investigate association between age and seroprevalence indicated no statistically significant relationship (p=0.698). The information above is summarized in table 3.

B. burgdorferi s.l.

No statistically significant association could be found between seropositivity and sampling areas (p=0.210) or time of season (p=0.651). A statistically significant association was found between seropositive individuals and sex (p=0.026) indica-

ting a higher chance of seroprevalence in females (observed count 16, expected 12.08). A binary logistic regression performed to found association between age and seroprevalence indicated no statistically significant relationship (p=0.077). The information above is summarized in table 3.

TBEV

No statistically significant association could be found between seropositivity and sex (p=0.921) respectively time of season (p=0.756). A statistically significant association were found between seropositive individuals and sampling area (p<0.001) indicating a higher chance of seroprevalence in Bogesund (observed count 24, expected 13.24). A binary logistic regression performed to found association between age and seroprevalence indicated no statistically significant relationship (p=0.171). The information above is summarized in table 3.

Table 3. Summary of p-values for the difference in means between seropositive and seronegative adult roe deer and various factors relating to effect on seroprevalence. Based on samples collected from 51 free ranging roe deer in south central Sweden, 2013-2017. Significant tests are indicated in bold.A p = A. phagocytophilum*; B b* = B. burgdorferi *s.l.*

	A p adult	B b adult	TBEV adult
Sex	0.487	**0.026**	0.921
Time of season	0.527	0.651	0.756
Area	**0.012**	0.210	<**0.001**
Age	0.698	0.077	0.171

4.5.2 Fawns

A. phagocytophilum

No statistically significant association could be found between sampling area (p=0.501) or sex (p=0.643) and titre levels. For this test titre level 8000 were included with the group 2048 otherwise the expected count was below 1. No statistically significant association was seen with month of testing or titre levels (p=0.392). In this test titre level 8000 were included with 2048 and titre level 64 were included with titre level 0 otherwise the expected count was below 1. An ordinal logistic regression was performed to look for association between age and titre levels and indicated no statistically significant relationship (p=0.418). The information above is summarized in table 4.

B. burgdorferi s.l.

No statistically significant difference could be found between antibody levels and area (p=0.249) or month of testing (p=0.428). A statistically significant variation

was found between antibody levels and sex (p=0.003) indicating a higher mean of antibody levels in females (females mean 0.283, males mean 0.1838). The linear regression analysis performed to found association between age and antibody levels indicated no statistically significant relationship (p=0.100). The information above is summarized in table 4.

TBEV

No statistically significant difference could be found between antibody levels and sex (p=0.110) or month of testing (p=0.254). A statistically significant variation was found between antibody levels and sampling area (p<0.001) indicating a higher mean of antibody levels in Bogesund (Bogesund mean 17.8, Grimsö mean 1.10). The linear regression analysis performed to found association between age and antibody levels indicated no statistically significant relationship (p=0.633). The information above is summarized in table 4.

Table 4. Summary of p-values for the difference in means between serological data in fawns and various factors relating to effect on seroprevalence. Based on samples collected from 49 free ranging fawns in south central Sweden, 2015-2018. Significant tests are indicated in bold. A p = A. phagocytophilum; *B b =* B. burgdorferi *s.l.*

	A p fawn	B b fawn	TBEV fawn
Sex	0.643	**0.003**	0.110
Month of testing	0.392	0.428	0.254
Area	0.501	0.249	**<0.001**
Age	0.418	0.100	0.633

4.6 Effect on health in adults

4.6.1 *A. phagocytophilum*

2 seronegative and 33 seropositive adult individuals were included. No statistical significance could be found between the positive respectively negative group and haemoglobin (p=0.377), haematocrit (p=0.141), leukocytes (p=0.261), segmented neutrophils (p=0.298), or monocytes (p=0.555). Band neutrophils, basophils and eosinophils were not included in the analysis due to all values are identical.

Lymphocytes were found having a p-value of <0.001. The seronegative group have a mean of 4.550 and the seropositive a mean of 2.59. With a 95% confidence the population mean for the difference is between 1.349 and 2.569.

When comparing individuals defined as underweight with seropositive and seronegative individuals with chi-square test no association could be found (p=0.143).

The information above is summarized in table 5.

4.6.2 *B. burgdorferi* s.l.

16 seronegative and 19 seropositive adult individuals were included. No statistical significance could be found between the positive respectively negative group and haemoglobin (p=0.335), haematocrit (p=0.156), leukocytes (p=0.320), segmented neutrophils (p=0.094), eosinophils (p=0.280), basophils (p=0.249), lymphocytes (p=0.545) or monocytes (p=0.471). Band neutrophils were not included in the analysis due to all values are identical (0).

When comparing individuals defined as underweight with seropositive and seronegative individuals with chi-square test no association could be found (p=0.464).

The information above is summarized in table 5.

4.6.3 TBEV

10 seronegative and 25 seropositive adult individuals were included. No statistical significance could be found between the positive respectively negative group and haematocrit (p=0.313), leukocytes (p=0.060), segmented neutrophils (p=0.141), basophils (p=0.246), lymphocytes (p=0.513) or monocytes (p=0.619). Band neutrophils and eosinophils were not included in the analysis due to all values are identical.

Haemoglobin was found having a p-value of 0.001. The seronegative group have a mean of 187.1 and the seropositive a mean of 166.5. With a 95% confidence the population mean for the difference is between 9.35 and 31.89.

When comparing individuals defined as underweight with seropositive and seronegative individuals with chi-square test no association could be found (p=0.068).

The information above is summarized in table 5.

Table 5. Summary of p-values for the difference in means between seropositive and seronegative adult and various factors relating to health. Based on samples collected from free ranging roe deer in south central Sweden, 2013-2017. A p = A. phagocytophilum*; B b =* B. burgdorferi *s.l. Significant tests are indicated in bold.*

	A p adult	B b adult	TBEV adult
Haemoglobin (n = 34)	0.377	0.335	**0.001**
Haematocrit (n = 34)	0.141	0.156	0.313
Leukocytes (n = 34)	0.261	0.320	0.060
Segmented neutrophils (n = 34)	0.298	0.094	0.141
Banded neutrophils (n = 34)	-	-	-
Eosinophils (n = 34)	-	0.280	-
Basophils (n = 34)	-	0.249	0.246
Lymphocytes (n = 34)	<**0.001**	0.545	0.513
Monocytes (n = 34)	0.555	0.471	0.619
Underweight (n = 30)	0.143	0.464	0.068

4.7 Effect on health in fawns

Only 48 of the 49 included fawns had available haematological parameters. In all statistical analyses where the haematological parameters are compared to antibody levels only 48 fawns are included. In the other cases, body temperature, under-weight, number of ticks and life, all 49 fawns are included. This applies to all pathogens.

4.7.1 *A. phagocytophilum*

48 individuals were included in the analysis where haematological parameters were available; for the analysis of all the other parameters all 49 individuals were included (see previous paragraph).

A one-way ANOVA was made looking for a correlation between titre levels and haematological parameters. No statistically significant association was found between titre levels and the following parameters: haemoglobin (p=0.463), haematocrit (p=0.226), leukocytes (p=0.337), banded neutrophils (p=0.950), eosinophils (p=0.354), basophils (p=0.925), lymphocytes (p=0.723) or monocytes (p=0.207).

For segmented neutrophils the p-value were 0.039 indicating a titre of 0 results in a higher mean of segmented neutrophils.

A one-way ANOVA test was made between mean number of ticks and titres finding no statistical significance (p=0.062). No statistical significance was found between antibody levels and temperature (p=0.450).

Chi-square test comparing antibody titres and underweight found no statistical significance (p=0.403). One individual was excluded since being the only individual with that antibody titre (8000) made resulted in an expected count below one and therefor making the test invalid. Using a MANOVA looking at survival rates and titre levels no statistically significant finding was found (p=0.862).

The information above is summarized in table 6.

4.7.2 *B. burgdorferi* s.l.

48 individuals were included in the analysis where haematological parameters were available; for the analysis of all the other parameters all 49 individuals were included (see paragraph 4.7).

A regression analysis was made looking for a correlation between antibody levels and haematological parameters. No statistically significant association was found between titre levels and the following parameters: leukocytes (p=0.732), banded neutrophils (p=0.577). segmented neutrophils (p=0.988), eosinophils (p=0.462), basophils (p=0.563), lymphocytes (p=0.239) or monocytes (p=0.321).

Statistical significance was found between antibody levels and haemoglobin (p=0.031) with a coefficient of -43.1 suggesting lower antibody levels resulting in higher haemoglobin levels. A similar association can be seen regarding haematocrit (p=0.027), where lower antibody levels indicate a higher haematocrit (coefficient -0.1033).

A regression analysis was made between mean number of ticks and antibody levels finding no statistical significance (p=0.658). No statistical significance was found between antibody levels and temperature (p=0.416). Binary logistic regression analysis comparing antibody titres and underweight found no statistical signify-cance (p=0.822).

Using an ordinal logistic regression looking at survival rates and antibody levels no statistically significant findings was observed (p=0.565).

The information above is summarized in table 6.

4.7.3 TBEV

48 individuals were included in the analysis where haematological parameters were available; for the analysis of all the other parameters all 49 individuals were included (see paragraph 4.7).

A regression analysis was made looking for a correlation between antibody levels and haematological parameters. No statistically significant association was found between titre levels and the following parameters: haemoglobin (p=0.161), haematocrit (p=0.345), leukocytes (p=0.495), banded neutrophils (p=0.303). segmented neutrophils (p=0.845), eosinophils (p=0.124), basophils (p=0.707), lymphocytes (p=0.042) or monocytes (p=0.385).

Statistical significance was found between antibody levels and lymphocytes (p=0.042) with a coefficient of approximately 0.015 suggesting higher antibody levels resulting in higher lymphocyte levels.

A regression analysis was made between mean number of ticks and titres finding no statistical significance (p=0.699). No statistical significance was found between antibody levels and temperature (p=0.578). Binary logistic regression analysis comparing antibody titres and underweight found a statistical significance (p=0.024) with a coefficient at 0.0688 indicating higher antibody levels are associated with a lower bodyweight.

Using an ordinal logistic regression looking at survival rates and titre levels statistically significant findings was observed (p=0.001). The coefficient being approximately 0.103 suggests higher antibody levels results in a higher chance of survival.

The information above is summarized in table 6.

Table 6. Summary of p-values for the difference in means between serological data in fawns and various factors relating to health. Based on samples collected from free ranging fawns in south central Sweden, 2015-2018. Significant tests are indicated in bold. A p = A. phagocytophilum*; B b =* B. burgdorferi *s.l.*

	A p fawn	B b fawn	TBEV fawn
Haemoglobin (n = 48)	0.463	**0.031**	0.161
Haematocrit (n = 48)	0.226	**0.027**	0.345
Leukocytes (n = 48)	0.337	0.732	0.495
Segmented neutrophils (n = 48)	**0.039**	0.988	0.845
Banded neutrophils (n = 48)	0.950	0.577	0.303
Eosinophils (n = 48)	0.354	0.462	0.124
Basophils (n = 48)	0.925	0.563	0.707
Lymphocytes (n = 48)	0.723	0.239	**0.042**
Monocytes (n = 48)	0.207	0.321	0.385
Number of ticks (n = 49)	0.062	0.658	0.699
Rectal temperature (n = 49)	0.450	0.416	0.578
Underweight (n = 49)	0.403	0.822	**0.024**
Lifespan (n = 49)	0.862	0.565	**0.001**

5. Discussion

5.1 Roe deer as a sentinel

This is the first contemporary study assessing the presence of antibodies against three major tick-borne pathogens in roe deer, to my knowledge; (*A. phagocytophilum, Borrelia burgdorferi* s.l., tick-borne encephalitis virus). Since all samples from both adults and fawns show antibodies against these pathogens it is a possible to state that roe deer is a good candidate for being used as a sentinel for the three of them. This is strengthened by earlier studies discussing (Gerth *et al.* 1995) and using (Skarphédinsson *et al.* 2005) roe deer as a sentinel for tick-borne diseases in Germany and Denmark, respectively.

5.2 Seroprevalence

The seroprevalence for *A. phagocytophilum*, *B. burgdorferi* s.l. and TBEV were determined in adult roe deer as 82.3%, 43.1% and 52.9%, respectively. For *A. phagocytophilum* this number is well matching previous studies suggesting a seroprevalence of 85% (Elfving *et al.* 2015). For *B. burgdorferi* s.l no previous studies have been published based on Swedish data. Still, compared to other studies throughout Europe our results are not unique and rather in the middle with Spain in the top at 68.8% (Pato *et al.* 2013) and England and Wales at 23% (Alonso *et al.* 2012). The study in Spain used indirect immunofluorescence and a cut-off at titre $\geq$ 1:64 meanwhile the study for England and Wales used ELISA (same as this study) but with a cut-off at 0.55 OD. These differences in methodology make it hard or impossible to further compare these results to the one obtained in the present study. For TBEV, my finding are uniquely high with the only comparable published article having found a seroprevalence of 2% in Netherlands (Jahfari *et al.* 2017). Jahfari *et al.* (2017) describes the number of serologically positive roe deer as "striking" making the impression of 2% being an unexpectedly high number and thus making 52.9% even more unanticipated.

5.3 Factors effecting serological data

5.3.1 Area

The relationship between tick-borne pathogens, ticks and ungulates is a complicated one. A higher prevalence of roe deer is in accordance with a larger population of ticks (Jaenson *et al.* 2012a). It is shown that red deer can result in lower levels of *B. burgdorferi* s.l. in ticks (Gandy *et al.* 2021). This due to a diluting effect where ticks choose ungulates over rodents. It is reasonable to believe this could also be the case for roe deer and other tick-borne pathogens since it is the same principle behind the diluting effect. Gandy *et al.* (2021) discusses that this would still lead to a higher prevalence of infection in humans due to the abundance of ticks. This is strengthened by Rizzoli *et al.* (2009) finding correlation between, among other, a high density of roe deer and circulation of TBEV. This is in accordance with our findings since Bogesund have both a higher tick density (Kjellander *et al.* 2021) and a higher roe deer density (Bonnot *et al.* 2018) than Grimsö but are also seen as a risk area for humans to contract TBE (Kjellander *et al.* 2021*).* A statistically significant correlation could be seen in between area and serological data regarding TBEV in adults and fawns and *A. phagocytophilum* in adults. All were found being higher in Bogesund in comparison to Grimsö. This can partially be because of the higher abundance of ticks in Bogesund compared to Grimsö (Kjellander *et al.* 2021). A higher prevalence of ticks would lead to a higher risk of infection in roe deer since roe deer are an important food source for ticks (Jaenson *et al.* 2012a). It would be reasonable to expect the same results for *B. burgdorferi* s.l but could possibly have to do with roe deer´s effective defence against the pathogen (Bhide *et al.* 2005) among other factors. The exceptionally high prevalence in Bogesund could also be affected by TBEV often condense to specific foci due to birds and migrating mammals (Jaenson *et al.* 2018).

5.3.2 Gender

In both adults and fawns a statistically significant difference was found between sex and serological data regarding *B. burgdorferi* s.l. Females were found having a higher number of mean antibodies. Since the difference is seen in both adults and fawns, environmental factors can be ruled out since any environmental factors should not be noticeable in fawns. It is more likely a difference regarding internal factors such as their immune system. A similar finding has been found in males regarding TBEV (Gerth *et al.* 1995) but could not be explained.

5.3.3 Time for the testing

In adults, measured antibodies are their own and can therefore directly reflect their previous contacts with a given pathogen. However, we cannot determine if the infection is ongoing or not, by only looking at the antibodies. Different diseases result in antibodies remining in the blood stream for different periods of time, so it is not even possible to know when a roe deer encountered the pathogen. Furthermore, if no correlations, between antibody levels and health status (as evaluated through the health parameters), are found this could either mean that the pathogens have no effect or that the individuals were infected earlier, and any effects are now no longer noticeable. This complicates the interpretation of the results since multiple factors can affect the levels of antibodies. One of these factors – according to my hypothesis – is the exposure level to a given pathogen. Since during winter the density of ticks and tick exposure should go down, so should also do the amount of newly infected roe deer. If the antibodies would decrease at a high rate a difference should be seen from the roe deer tested in early winter (December and January) compared to the roe deer tested in late winter (February and March). No statistical significance could be seen between these different groups and their antibody levels indicating either that roe deer are getting infected at a similar rate as in the summer or that the decrease in antibodies during these months are too small and slow for us to be able to distinguish newly infected versus roe infected at an earlier time.

A similar test was performed for the fawns looking at the circulating number of antibodies and time of birth with the two groups being fawns born in April and May. This was done to investigate if being born later would lead to a higher number of antibodies since the later date would mean a longer time the doe has been able to be infected and increased its concentration of antibodies. No statistically significant differences could be found.

5.4 Serological data and health parameters

No previous studies, to my knowledge, have been found looking at the levels of antibodies in fawns compared to their mother in any comparable species. This means that no precise correlation, between the fawn's antibody levels, and their mother's antibody levels can be expected when analysing results in this study. Therefore, all calculations in this study have been done for fawns and adult animals separately. This does not mean that the measured antibodies in fawns are without benefit seeing as it is plausible thinking the fawns' antibodies gives us an indication of the doe's antibody levels.

5.4.1 *A. phagocytophilum*

It was found that a titre of 0 resulted in a higher mean of segmented neutrophils in fawns. A titre of 0 would mean that the fawn has no passive innate immunity for the disease resulting in a higher chance of being infected and affected by the pathogen. The fawn having no immunoglobins for this specific pathogen could also mean a lower number of antibodies overall (a failure of passive transfer (FPT)) resulting in a higher chance of being infected by any pathogen which would then explain the higher number of segmented neutrophils. For *A. phagocytophilum* and adults, a statistically significant correlation were found regarding lymphocytes where the seropositive group had a lower mean. Lymphocytopenia along with thrombocytopenia and neutropenia are characteristic of an infection with *A. phagocytophilum* (Constable 2017).

5.4.2 *B. burgdorferi* s.l

For *B. burgdorferi* s.l. in fawns, a statistically significant difference could be seen in both lower haemoglobin levels and with a lesser haematocrit with higher antibody levels. This difference cannot be seen in adults which makes it possible to believe that these changes are only beheld in younger animals since they could be more easily affected due to their underdeveloped immune system. There is no discernible reason why higher antibody levels should lead to elevated levels of haemoglobin and haematocrit. The opposite relationship is more easily explained. Low levels of antibodies would mean less protection against the disease. Being infected could lead to the fawn being less inclined to ingest milk making it dehydrated which would then lead to the previous mentioned parameters increase.

5.4.3 TBEV

Among fawns, a statistically significant correlation was found between multiple parameters: lymphocytes, lifespan, and weight (discussed later). The correlation between lymphocytes and antibody levels were found positive. This could be explained by the fact that higher antibody levels come from a recently infected mother; this can in turn indicate the circulation of the pathogen in that area and therefore a higher infection risk for the fawn. When the fawn is infected the lymphocyte levels rise but infections can lead to both lymphocytosis and lymphopenia. If the correlation is rather that a low level of antibodies results in lymphopenia, this could be explained by the fawn's lack of protection leading to a lack of lymphocytes. TBEV was the only pathogen showing a statistically significant correlation between antibody levels and lifespan suggesting that higher antibody levels result in a higher chance of survival. This indicates that antibodies in fawns are protective against TBE and that the pathogen, in fawns with lower levels of antibodies, can have a more severe impact on fawns' health. It could be

discussed that more parameters such as pyrexia and dehydration should have been in effect in such a case. The only published case of TBEV in roe deer (Da Rold *et al.* 2022), a clinical encephalitis, also strengthen this thought. One possible explanation could be that the ongoing infection is leading to a higher need for food making the fawn more active and searching for its mother subsequently making it an easier target for predators. This correlation between infection and elevated mobility have been discussed as a possible association by Escutenaire *et al.* (2002) in bank voles (*Clethrionomys glareolus*) infected by Puumela virus meanwhile the opposite was found for bare-nosed wombat (*Vombatus ursinus*) with sarcoptic mange (Martin *et al.* 2018). In adult roe deer a statistically significant difference was found between the seropositive and seronegative group for haemoglobin where the seronegative group were found having a mean of 187.1 g/L and the seropositive a mean of 166.5 g/L. Normally, haemoglobin and the haematocrit follow each other which is not the case in this study and no good explanation can be found for this one-sided difference.

5.4.4 Weight

No significant relationship between body weight and antibodies could be seen for either *A. phagocytophilum* or *B. burgdorferi* s.l. but was found in fawns and TBEV where a higher antibody level indicates a lower body weight. High antibody levels in the fawns could indicate high levels in the mother suggesting a more recent, more often or more severe infection affecting the female's production of milk. This has not, to my knowledge, been proven in any studies for TBEV but can be seen in other diseases, for example *A. phagocytophilum* in different mammals (Constable 2017). This is strengthened by the fact that fawns solely drink milk during the first two weeks in life (Adams 2015) making them completely dependent on the doe's producing a sufficient amount. All tests of fawns were made up to day 15 of age. A possible negative effect on the doe's health, which is indicated by the possibly lower weight in fawns, is in part supported by a nearly significant relationship among the adults regarding antibody levels and weight ($p=0.068$). This indicates a negative effect on the roe deer's health when infected by TBEV. The correlation between underweight and higher antibody levels could also by connected with the theory that higher levels in the fawns indicate a higher risk of infection meaning that the underweight is caused by an active infection.

5.5 Limitations

This study is a master's thesis and the study were thus limited by finances and time. If more time were available more calculations could have been performed such as finding a cut-off for fawns. It would also have been possible to look at additional

parameters regarding the pathogens, such as how it affects an individual to be seropositive for multiple pathogens.

Since the projects have been ongoing over multiple years, there is a possibility that unknown changes in the environment have occurred that affect the results. For example, the levels of pathogens might have changed in the two areas during this time. This could lead to important consequences for the comparison between the different areas. There is also a possibility of sampling bias in the adult roe deer. Sick or weaker roe deer might be more motivated to use the food in traps since it is easily accessible and simultaneously, they might have a bigger need for food. The captured deer might therefore not be representative of the population but instead represent a higher level of sick animals. This might affect the seroprevalence.

A weight classified as underweight was determined for both adults and fawns. The reasoning behind this method was to investigate if a high antibody level, perhaps indicating a recent infection result in a weight loss in roe deer. Since individual under- or overweight is determined in relation to the other animals in the study they might not actually be underweight and simply at a weight that is below average for their body size (metatarsal length) compared to the other animals. This could mean that we are comparing the food availability in different years with antibody levels. A year with a lot of food would lead to a higher weight for a given body size compared to the years with low food availability.

Furthermore, we are dealing with a wild population and the limitations that comes with it. To be able to, with accuracy, determine the effects of these pathogens on roe deer, a more controlled approach would be needed. For example, experimental infections with *A. phagocytophilum* have been performed resulting in data relating both symptoms and diagnostic test results (Almazán *et al.* 2020) but also post-mortem findings (Kocan *et al*. 2012). The results of this study should therefore be seen for what they are: indications that make it impossible to completely rule out that roe deer are not affected by these pathogens.

Finally, the idea from the very beginning was, as part of this study, to look at the relationship between mother and fawn. It was decided not to proceed with this due to too few of these relationships and the fawns being too old when the blood tests were taken. Since these tests were performed with the fawns being over 6 months old, all antibodies gotten through the colostrum from the mother would no longer be present. A comparison between the mother´s and fawn´s serological data would therefore not have any correlation anymore.

6. Conclusions

Ticks work as vectors for multiple pathogens such as *A. phagocytophilum*, *B. burgdorferi* s.l and TBEV affecting both humans and animals negatively world-wide. Roe deer and previous mentioned pathogens are connected, as demonstrated by the fact that seropositive individuals were identified for all pathogens and could therefore work as a sentinel species. This relationship is complex where the proportion of seropositive individuals is affected by factors such as area and gender and indications point to seropositive individuals being negatively affected by the pathogens. Further research is needed to continue the work started by this study to fully map the relationship between deer, ticks, and tick-borne pathogens.

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Popular Science Summary

Ticks are found all over the world. Ticks are not dangerous in themselves but can spread diseases. The number of ticks and the number of human cases of disease caused by tick-borne diseases are increasing. Examples of such diseases, which were also investigated in this study, are anaplasmosis, Lyme disease and Tick-borne encephalitis. The disease-causing agent (pathogen) for these diseases are *Anaplasma phagocytophilum* (bacteria), *Borrelia burgdorferi* sensu lato (bacteria) and Tick-borne encephalitis virus (virus), respectively. These diseases can cause disease in different species such as humans. Ticks feed on blood and an important source of blood is roe deer. This means that roe deer often encounter ticks and the diseases the ticks carry and pick up from previous hosts. Roe deer thus play a role in terms of ticks and tick-borne pathogens, but exactly what this role is, is not yet fully mapped out. This study therefore focused on finding out more about this relationship.

After the immune system encounters a pathogen, it creates antibodies that remember that pathogen. By taking a blood sample, you can check if an individual has antibodies against a certain pathogen. This then show if an individual has encountered the pathogen or not. This was done on 51 adult deer in Grimsö and Bogesund, tested for antibodies against *A. phagocytophilum, B. burgdorferi* s.l and TBEV. It was found that 82%, 43% and 53%, respectively of the roe deer were found carrying antibodies against the pathogens. However, many more of the roe deer had antibodies against *A. phagocytophilum* and TBEV in Bogesund than in Grimsö. This may be because there are more roe deer and more ticks in Bogesund than Grimsö. In addition, more females were found to have antibodies to *B. burgdorferi* s.l than males but it is difficult to say what this was caused by.

In addition to the 51 adult roe deer, 49 fawns were also tested. Antibodies against all pathogens could be found in both age groups. This shows that deer could function as a sentinel species. Sentinel species are organisms used to find various hazards for other living species and the environment. This can be dangerous substances such as metals or as in this case signs of pathogens. By finding out how many and which deer have carried the pathogen, you can find out where the pathogen is and how much of it there is. Not all bacteria and viruses cause disease.

Sometimes bacteria and viruses cause disease in some species but not in others. It is not fully determined whether roe deer can get sick from these diseases. In this study, signs were found that deer may become ill, but further research is needed to confirm this.

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