The role of diseases in mass mortality of wood lemmings (*Myopus schisticolor)*

Sjukdomars roll i massutdöende av skogslämmel (Myopus schisticolor)

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Master's thesis • 30 credits
Swedish University of Agricultural Sciences, SLU
Department of Wildlife, Fish, and Environmental Studies
Forest Science programme
Examensarbete/Master's thesis, 2021:7
Umeå, Sweden 2021

The role of disease in mass mortality of wood lemming (*Myopus schisticolor)*

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Credits:	30 credits
Level:	Second cycle, A2E
Course title:	Master's thesis in Forest Science, A2E - Wildlife, Fish, and Environmental Studies
Course code:	EX0840
Programme/education:	Forest Science programme
Course coordinating dept:	Department of Wildlife, Fish, and Environmental Studies
Place of publication:	Umeå, Sweden
Year of publication:	2021
Cover picture:	Thomas Secher Jensen
Title of series:	Examensarbete/Master's thesis
Part number:	2021:7
Keywords:	Wood lemming, Myopus schisticolor, Disease, Virus, Pathogens, Mass mortality, Orthohantavirus, Pan-orthohantavirus, Somatic index, Spleen index

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Abstract

Like many other rodent species, the wood lemming (*Myopus schisticolor*) exhibits cycles with large population peaks but unlike most other European rodents, these peaks are frequently followed by mass mortality. It is largely unknown what pathogens wood lemmings carry and if pathogens could affect their population dynamics. Pathogens together with predation, climate and food supply are the main suggested determining factors of population dynamics of small mammals. Here, I tested if pathogens and diseases might be involved in recent mass mortality events of the wood lemming. Therefore, I studied in total 223 wood lemmings from two areas in Värmland and Västerbotten county, Sweden. Samples originated from individuals that were found dead during population outbreaks in 2014 and 2017 together with snap-trapped individuals from 1995-2014. Liver and lung tissue were dissected from each individual and analysed in a laboratory for five pathogens known to be carried by and affecting rodent hosts: Arenavirus (AV), Ljunganvirus (LV), Tick-borne encephalitis virus (TBEV), Cowpox virus, Puumala orthohantavirus (PUUV) and additionally a broad covering Pan-orthohantavirus test. The prevalence of Puumala orthohantavirus in the sampled population was 4.5-9% while the analysis for Pan-orthohantavirus showed positive for 26.9% of the total sample. None of the individuals tested positive for AV, LV, TBEV, or Cowpox. The probability of infection with Pan-orthohantavirus increased with body weight of the individuals but no difference between sexes was found. A spleen-somatic index was used to test the hypothesis that this organ could be used as an indicator of disease, as it is largely connected to the immune system. The probability of infection increased with an increase in spleen index and there was a significantly higher spleen index seen in individuals testing positive for Pan-orthohantavirus. Wood lemmings in Värmland county showed a higher spleen index than animals in Västerbotten and the index was higher in individuals caught in traps than in individuals found dead. The spleen index revealed some interesting patterns, but more research is needed to reveal and understand actual causal relationships between pathogen infections and spleen index. None of the studied pathogens caused the observed mass mortality of wood lemmings. In this study, I was able to study a limited number of pathogens. Hence, I cannot refute the disease hypothesis. Instead, future studies focusing on the disease hypothesis should include other and potentially more fatal pathogens for wood lemmings.

Keywords: wood lemming, *Myopus schisticolor*, disease, virus, pathogens, mass mortality, orthohantavirus, pan-orthohantavirus, somatic index, spleen index

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1. Introduction

1.1. Causes of population dynamics

Small rodents, particularly at northern latitudes exhibit population fluctuations. These population fluctuations demonstrate as high numbers in one year, followed by a sudden drop in population density and almost absence the following year. These types of extreme fluctuations between years may create regular population cycles (Krebs & Myers 1974).

Factors such as climate, food supply, predation and disease have long been suggested to regulate animal populations (Elton 1931). In Europe, cyclic patterns in microtine rodents have initially been ascribed to populations in mainly northern parts of Fennoscandia (Henttonen et al. 1985), while more recent studies have identified cyclicity in large parts of Europe (Cornulier et al. 2013). Studies of rodents in the northern hemisphere have shown that populations often fluctuate regularly in cycles and synchronously (Krebs and Myers 1974; Hörnfeldt 1994). Decades of research have looked at what single or combination of factors can explain the cyclic patterns seen in rodent populations and have suggested different potential causes (Myers 2018). A prerequisite for cyclicity is delayed density dependence (Hörnfeldt 1994). The synchronous fluctuations in Fennoscandian populations of microtine species was seen as a support for the hypothesis of predation as one of the driving factors for population cycles (Korpimäki et al. 2004), but it is still debated if there are general top-down (predation) or bottom-up (e.g., food) drivers of cyclicity (Myers 2018). Also, the early hypothesis that diseases and epidemics are core factors for controlling cyclicity in rodents (Elton 1931; Elton et al. 1935) gets increased attention (Feore et al. 1997; Burthe et al. 2008; Andreassen et al. 2020). Indeed, demography, behaviour, predation and/or habitat can affect the mortality of a species, and these factors have also been shown to affect the prevalence of pathogens in rodent populations (reviewed in Khalil et.al. 2014).

An extended low-phase is characterised by prolonged low population densities for a 1-3 years' period, following a population crash. Hypotheses potentially explaining

such a low phase in population cycles include both extrinsic (environmental) and intrinsic (social) factors (Boonstra et.al. 1998). Extended low phase was observed on Wrangle island, Russia where only 10-18% of the mortality for Arctic lemming (*Dicrostonyx torquatusand*) and Siberian brown lemming (*Lemmus sibiricus*) were caused by predators (Boonstra et al. 1998). The only predators for lemmings found on Wrangle island are arctic fox (*Vulpes lagopus*), snowy owl (*Bubo scandiaca*) and pomarine skua (*Stercorarius pomarinus*) (Menyushina et al 2012). Predators are suggested to be of great importance for the decline of a population along with diseases and the time lag between prey and predator population peaks keeps prey species at low densities (Pearson 1966; MacLean et al. 1974).

1.2. Epizootic and zoonotic diseases

An epizootic is a disease within an animal population while a zoonotic disease is the result of an infectious pathogen transmitted from animals to humans or vice versa. An example of important transmission routes for zoonotic diseases are through pets that we in our modern society bring inside more as a family member rather than keeping them outside as guard dogs and rodent deterring cats (Marshall 2011). Another major factor is the encroachment of humans on wildlife habitats. Each year, approximately 14 million people die around the world because of endoparasitic infectious organisms capable of being pathogenic in humans (Woolhouse 2002). About 150 000 - 200 000 people contract disease caused by zoonotic orthohantaviruses (Schmaljohn and Hjelle 1997).

1.2.1. Orthohantavirus

Orthohantaviruses are RNA viruses hosted by rodents, bats, shrews and moles (Vaheri et al. 2013) but only a few rodent species within the families of Cricetidae and Muridae seem to carry zoonotic viruses (Olsson et al. 2010). One of the most common transmission routes for orthohantaviruses are through inhaling particles of excrements or secretes from an infected rodent (reviewed by Forbes et al. 2018). Rodents are overrepresented among reservoirs of zoonotic pathogens (Han et al. 2016). An example of such a disease is nephropathia epidemica in humans caused by the Puumala orthohantavirus (PUUV). This virus is known to be carried by the bank vole (*Myodes glareolus*) that can be found almost all over Europe. During the high-density phase of the bank vole, transmission to humans is effective and can therefore cause epidemics of nephropathia epidemica (Khalil et al. 2019). Symptoms of this disease include fever, headaches, nausea, vomiting and can also cause spleen haemorrhage and haemorrhage or necrosis of the pituitary gland (Vapalahti et.al. 2003). The prevalence of orthohantaviruses in rodent populations has been recorded in several studies to be higher in males (Olsson et al. 2002;

Yahnke et al. 2001) and increases with the age of the individual (Kallio et al. 2006). Orthohantavirus infections are suggested to affect winter survival (Kallio et al. 2007), juvenile survival (Douglass et al. 2001) and weight gain (Douglass et al. 2007) in the rodent host; all of which with a potential to affect population dynamics of the reservoir host.

1.2.2. Ljungan virus

The Ljungan virus (LV) is an RNA virus mainly carried by the bank vole. In Fennoscandia, Fevola et.al. 2017 showed that LV was present in 13 of 17 study sites and with an overall mean prevalence of 16%. The study showed that the highest prevalence was found in the intermediate sized individuals rather than light (young) or heavy (old) individuals (Fevola et al. 2017). The zoonotic potential of LV is still debated (Jaaskelainen et al. 2016). However, LV might have a negative impact on the health status of its host (Niklasson et al. 2003).

1.2.3. Arenavirus

Arenaviruses (AV) is a group RNA virus comprising several strains known to cause disease in humans and just like orthohantaviruses, they are host specific with rodents being the primary reservoir (Jay et al. 2005). The Arenaviruses cause a chronic and for a long time seemingly asymptomatic infection in rodents and is spread both within the rodent population and to other species through feces, urine and saliva (Zapata and Salvato 2013). Some studies have shown Arenaviruses to affect survival, weight and fecundity (Vitullo et al. 1987; Vitulllo and Merani 1988; Borremans et al. 2011).

1.2.4. Cowpox virus

Cowpox virus is a DNA virus and found in a wide range of species including rodents. Rodents are considered the reservoir host and the virus can be transmitted to humans where it can be pathogenic (Bennett et al. 1997), even though human cases are rare (Hazel et al 2000). Although cases in humans are rare, it can have a fatal outcome (Czerny et al. 1991; Pelkonen et al. 2003). Cowpox is transmitted through animal contact and can be pathogenic in domestic and zoo animals (Vorou et al 2008). In field voles (*Microtus agrestis*), there is a potential correlation between Cowpox infection and mortality (Burthe et al. 2008). Potential secondary host effects could also be triggered in stressed or malnourished individuals that get weakened or affected by other pathogens (Telfer et al. 2002).

1.2.5. Tick-borne encephalitis virus

Tick-borne encephalitis virus (TBEV) is a vector-borne RNA virus that is commonly spread through the saliva of an infected tick during its bite (Lindquist and Vapiti 2008; Haditsch and Kunze 2013). The disease, tick borne encephalitis (TBE) is of varying severity in humans. Patients can show symptoms ranging from an uncomplicated fever to a severe encephalitis resulting in inflammation of the central nervous system and can in some cases lead to death (Haditsch and Kunze 2013). Tick-borne encephalitis is affecting 2000-3500 people in Europe annually (Gritsun et al. 2003; Beauté et al. 2018).

The virus also has a wide variety of vertebrate hosts in both wild and domestic animals (Haditsch and Kunze 2013). Small mammals such as rodents are important hosts of TBEV (Achazi et al. 2011). Rodents function as reservoir hosts for the virus implying that they maintain and amplify TBEV (Süss 2003). They can maintain the virus through a persistent and latent infection throughout the year depending on the virus strain (Tonteri et al. 2011; Mansfield et al. 2009). Tonteri et al. (2013) highlighted the lack in knowledge of potential secondary effects of infection on mortality in the rodent reservoir.

1.3. Somatic index – a tool for research on disease?

Estimating an animal's resistance, susceptibility and response to pathogens and diseases is challenging. An organ-somatic index (organ weight divided by bodyweight of the animal) could be used as a proxy in such an assessment (Hadidi et al. 2008). Somatic indexes have frequently been used in ecotoxicological studies where changes in relative organ weight have been observed in relation to exposure to environmental contaminants (Bankowska and Hine 1985; Ma 1989; Ecke et al. 2018). The spleen is an organ handling immune system reactions (Davydova et al. 2011). Using a spleen-somatic-index for pathological studies could theoretically be possible as it is also known that pathogens can affect and change the intestinal physiology of an animal with spleen being one of the most commonly infected organs (Baker 1998). Spleen size can be related to the health status of an individual (Blomqvist et.al 2002; Jackson et al. 2011), but not exclusively, as it can also be determined by other factors (Davydova et al. 2011), such as physical exhaustion (Barcroft and Stephens 1927), dietary components (Puangkaew et al. 2005), traumatic shock (Davies and Withringron 1973) and sexual dimorphism (Moller et al. 1998).

1.4. Wood lemming (*Myopus schisticolor*)

Lemmings belong to the subfamily Arvicolinae, microtine rodents (lemmings, voles & muskrats) and are mostly spread across the arctic and subarctic zone but also occur in mountainous areas in the more northern temperate zone (Stenseth & Ims 1993). The wood lemming is predominantly distributed in the taiga biome and recolonized Fennoscandia after the last glaciation. It prefers old-growth spruce dominated forests rich in mosses, especially where *Dicranum* spp., its main preferred food occurs (Eskelinen 2004).

Like other rodent species in Fennoscandia, the wood lemming also exhibits cyclic population fluctuations (Eskelinen et al. 2004; Bobretsov et al. 2017; Wegge and Rolstrad 2018). In parts of Finland and Russia, wood lemming populations have both been seen to exhibit cycles with a periodicity of 3-4 years, as well as no detectable periodicity (Vuorinen and Eskelinen 2005; Bobretsov et al. 2017). Population dynamics of wood lemmings can display fairly regular cycles and/or outbreaks with irregular time intervals (Wegge and Rolstad 2018), often being out of synchrony with other sympatric rodent species (Bondrup-Nielsen & Ims 1987).

Population outbreaks of wood lemmings are often followed by mass mortality where dead wood lemmings can be found even in urban areas, i.e., in usually avoided habitat (Eskelinen 2004). Mass mortality of a species is an event defined by a substantial proportion of a population dying due to increased mortality in all life stages of the species. This occurs often as a consequence of demographic catastrophes, examples of that can be climate events, or diseases (Kock et al. 2018). Because of the differing cycle patterns and non-clear correlation with existing predator densities, it has been discussed that the wood lemming in some areas might be more affected by other factors such as food or disease (Eskelinen et al. 2004). Predation-based mortality was tested by Wallgren (2019) where he found that in Värmland County, Sweden, in the years of 2014 and 2017, only 40% of the population mortality were caused by predation. Hence, there must be other contributing causes triggering mass mortality of wood lemmings.

Wood lemmings and Arctic lemmings (*Dicrostonyx torquatus*) are two species known for their skewed, female-biased sex ratio with a unique genetic system (Gileva & Fedorov 1991). The proportion of males in a wood lemming population varies around 25% (Fredga et al. 1977; Stenseth 1978) but can increase to almost 50% during population decline (Krebs & Myers 1974). Both lemming species have two different forms of X chromosomes, the natural X chromosome and a mutated form, X*, that results in three genotypes of females (XX, X*X and X*Y) and one genotype of males (XY). The mutated X chromosome (X*) has a 100% chance of being passed to the next generation. In wood lemmings, females with X*X have

been recorded to produce 75% females and females with X*Y produces 100% females (Gileva & Fedorov 1991).

1.5. Aims of the project

The aim of my thesis is to increase our understanding of the role of pathogens in mass mortality of wood lemmings. There are many pathogens that potentially can be fatal for wood lemmings. Here, I took the rare opportunity to study the presence and prevalence of Arenavirus, Cowpox virus, Ljungan virus, Tick-borne encephalitis virus and a broad-covering orthohantavirus test, from hereon called Pan-orthohantavirus, including Puumala orthohantavirus. Wood lemmings were opportunistically sampled found-dead during two population outbreaks in Sweden. I compared the found-dead lemmings to a set of individuals caught in snap-traps from the two outbreaks as well as from non-outbreak years.

To assess if pathogen presence is related to physiological effects, I studied the spleen-somatic index of wood lemmings and hypothesized that pathogen infection is associated with an enlarged spleen, and therefore giving a high spleen-to-body-weight ratio. Apart from expecting that spleen size positively correlates to the probability of infection, I also hypothesize that a higher spleen size will be seen in males due to their higher tendency of infection. The probability of infection with at least orthohantavirus will be positively correlated with the weight of the individual as stated in previous studies.

2. Material and methods

2.1. Wood lemming samples

My thesis comprised of 223 wood lemming specimens, caught in traps or found dead and were sampled in various projects, locations (Table 1, Figure 1) and periods. In Värmland County, lemmings included snap-trapped individuals and specimens that were found dead in the outbreak years of 2014 and 2017. In the Västerbotten County, lemmings were snap-trapped in the non-outbreak years 1995, 1997, 1999, 2004, 2005, 2007 and 2014. Of all 223 lemmings used in the study, 57 were trapped and 164 were found dead. The remaining two specimens lacked details on how they were caught.

Table 1. Localities of lemming captures/findings. Lemmings were sampled within the vicinity of these locations. Vindeln in Västerbotten, located in the north of Sweden and remaining locations located in Värmland county, southern Sweden.

Locality	No. of lemmings
Lennartsfors	75
Gustavsfors	61
Vindeln	31
Stöllet, Värnnässjön	19
Filipstad	13
Glaskogen, Arvika	8
Hagfors	4
Geijersholm	3
Rösberget	3
Acksjön	2
Starra	2
Storön	1
Älgsjöberg	1

Figure 1. Map of Sweden showing the areas of sample collection in yellow circles. The northern area is located in Västerbotten County (Vindeln) and comprised snap-trapped animals from 1995, 1997, 1999, 2004, 2005, 2007 and 2014, while the southern area in Värmland County (Lennartsfors, Gustavsfors Stöllet, Värnnässjön, Filipstad Glaskogen, Hagfors, Geijersholm, Rösberget, Acksjön, Starra, Storön, Älgsjöberg) comprised found dead and snap-trapped animals from 2014 and 2017. Map taken and altered from Google maps.

2.2. Dissections

In the laboratory, I dissected 133 wood lemmings and excised spleen, kidneys, liver and lungs and stored the organs in individual tubes at -20ºC. Remaining organs from 90 individuals were available from previous studies.

Dissection was performed with scalpel, scissors and tweezers. To prevent contamination between organs and specimens by potential pathogens, tools were changed before collecting samples from each new individual and separate tools were used in the abdominal cavity and chest cavity. Tools were sterilized after each dissection in a three-stage treatment. First, used tools were put in ethanol until the end of each day, tools were then transferred to a VirkonTM solution overnight. In the morning, VirkonTM-treated tools were washed off in soap water and burned over a candle.

The whole procedure was executed in a biosafety cabinet and a biosafety level 2 (BSL-2) lab, wearing lab coat and powder-free surgical gloves. Gloves were

changed between each individual to not contaminate any samples and each dissection was done on a separate paper sheet. All disposable material used such as gloves and paper sheets were put in a hazardous waste container after each performed dissection.

2.3. Analysis of organs

Liver (≃25 mg) and lung (≃25 mg) samples were mechanically homogenized separately using FastPreps 120 (Q-BIOgene, Irvine, CA, US) at 6.5 m/s for 20 s. Samples were subsequently analyzed with polymerase chain reaction (PCR) methods. For analysis, the 223 sampled individuals were organized in 132 pools comprising 2-4 individual lemming RNA. All analyses were performed in the laboratory at the Department of Clinical Microbiology, Umeå University.

2.3.1. Extraction of viral nucleic acid

Extraction of total viral nucleic acid (TVNA) was performed with the Viral NA Extraction Kit using the magnetic bead-based method (DiaSorin, Dublin, Ireland). The procedure was performed according to the manual (total Viral NA 94, DiaSorin, Ireland), with 250 µL of the homogenate and 10 µL of protein kinase K (Qiagen, Hilden, Germany) in 1.5 mL sterile micro-centrifuge tubes. Afterwards, viral nucleic acid was extracted and collected in an elution volume of 50 µL. The TVNA organized in 132 pools each comprising 2 up to 4 individual lemming TVNA according to sample type.

2.3.2. Arenavirus PCR

RT-PCR detection of Arenaviruses was conducted on TVNA obtained directly from tissue using the SuperScript™ III OneStep RT-PCR Reverse Transcriptase (RT) with Platinum™ Taq DNA Polymerase (Invitrogen, Carlsbad, CA, US). The PCR targeted the L gene of Arenaviruses using primers LVL3359A-plus, LVL3359D-plus, LVL3359G-plus, LVL3754A-minus, and LVL3754D-minus as described by (Vieth et al. 2007).

2.3.3. Cowpox virus PCR

The Cowpox virus PCR was performed according to Sandvik et al. (1998) using the primers: ortho-TK-1 primer (sense) 5′-AAAAGTACAGAATTAATTAG-3′ and the ortho-TK-2 primer (antisense) was 5′-TTCAGATAATGGAATAAGAT-3′ (Hurby et al. 1983).

2.3.4. Ljungan virus PCR

Extracted TVNA was reverse-transcribed into cDNA by using the SuperScript™ III OneStep RT-PCR Reverse Transcriptase (RT) with Platinum™ Taq DNA Polymerase (Invitrogen, Carlsbad, CA, US). Each RNA sample was then run in duplicate in an LV-specific real-time reverse transcriptase PCR, following Donoso Mantke et al. (2007), using the forward primer 5′-GCGGTCCCACTCTTCACAG-3′and the reverse primer 5′-GCCCAGAGGCTAGTGTTACCA-3′ (Mantke et al. 2007).

2.3.5. Pan-orthohantavirus PCR

TVNA was screened for orthohantavirus RNA with a nested 264 PCR as described by (Meheretu et al. 2019) targeting a 347 nucleotide-long part of the polymerase (L) gene of the orthohantavirus 265 genomes using primers
Han-L-F1: 5'-ATGTAYGTBAGTGCWGATGC-3';
Han-L-R1: 5'-266AACCADTCWGTYCCRTCATC-3';
Han-L-F2: 5'-TGCWGATGCHACIAARTGGTC-3';
Han267 L-R2: 5'-GCRTCRTCWGARTGRTGDGCAA-3' (Klempa et al. 2006).

2.3.6. Puumala orthohantavirus PCR

TVNA was subjected to a real-time reverse transcriptase (RT) PCR method for detection of PUUV RNA as described by Evander et al. (2007). The real-time RT-PCR targeted the S-gene of the Puumala virus.

2.3.7. Tick-borne encephalitis virus PCR

TVNA was screened for Tick-borne encephalitis virus (TBEV) RT-PCR using the the QuantiTect SYBR green RT-PCR kit (Qiagen) on a LightCycler 1.5 instrument (Roche) with Primers and TaqMan probe for the TBEV RNA as previously described by Schwaiger and Cassinotti (2003).

2.4. Spleen-somatic index

For each specimen, I calculated the spleen-somatic index, from here on called spleen index, by dividing the weight of the spleen by the full body weight of each individual and expressed as percentage of total bodyweight. The spleen index therefore gives the relative weight of the organ to the weight of the animal.

2.5. Statistical analysis

Neither the raw nor transformed spleen index met the assumption of normal distribution necessary for parametric tests. Instead of transformations and for simplicity, I used the non-parametric Mann Whitney U, also known as Wilcoxon rank sum test (McKnight and Najab 2010), for group comparisons of the spleen index. The Mann Whitney U test was used to analyze differences in spleen size between sexes, counties, cause of death (trapped vs. found dead) and between infected and non-infected individuals.

To analyze what affects the probability of pan-orthohantavirus infection, I used fixed generalized linear models (glm) where the response variable was a factor with 2 levels (Positive and Negative).

Microsoft Excel was used for data storage and creating tables. For all Statistical analysis, R and RStudio software was used (R Core Team, 2018). I used the package "ggplot2"to create graphics, "arm" for checking Pearson correlation coefficients and the incorporated glm() and wilcox.test() functions for statistical analysis.

3. Results

3.1. Pathogen prevalence

Of the 132 analyzed pools, 35 tested positive for Pan-orthohantavirus by nested PCR. Within these 35 positive pools, 60 individuals (26.9% of total sample) tested positive using Pan-orthohantavirus nested PCR. Of the 35 Pan-orthohantavirus positive pools, five pools recorded CT values from a Puumala orthohantavirus (PUUV) specific qRTPCT. Hence, PUUV was present in 4.5-9% of the sampled wood lemming population. All 132 pools tested negative for Arenavirus, Cowpox virus and Tick-borne encephalitis virus (TBEV).

3.1.1. Probability of Pan-orthohantavirus infection

The probability of infection increased with increased body weight (Figure 2, Table 2). Sex did not affect infection probability (Figure 3, Table 3).

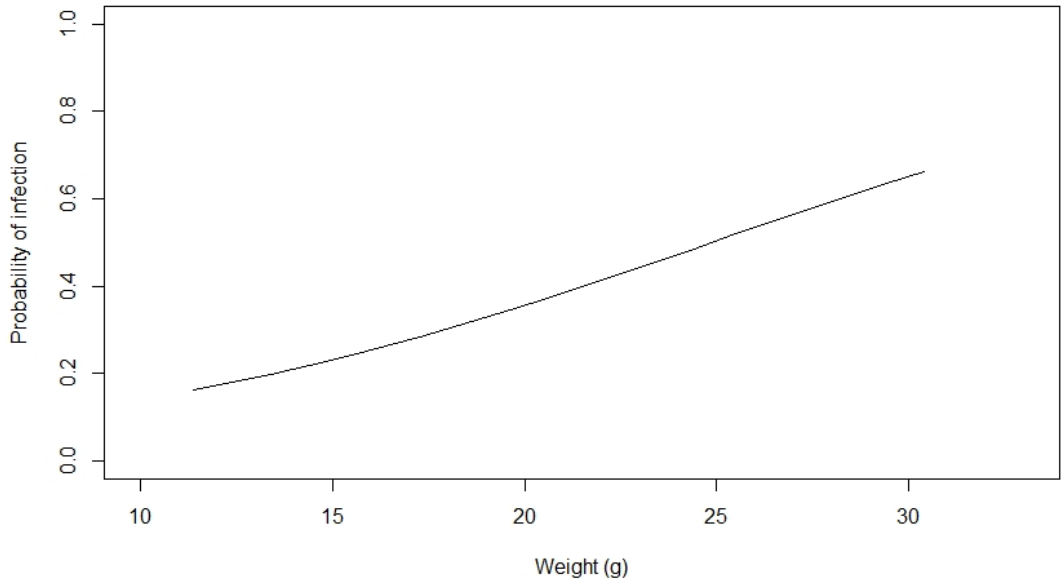

Figure 2. Predicted probability of Pan-orthohantavirus infection in wood lemmings as a function of body weight including male and female wood lemmings, found-dead and trapped in the county of Värmland in the years of 2014 and 2017 (n = 132).

Table 2. Modeling result from a generalized linear model for the effect of weight on the probability of testing positive for Pan-orthohantavirus. The value of Pr(>z) showing a value of <0.05 suggests the relevance of the variable and it being significant to explaining the data.

N	Variable	Estimate	SE	Z	Pr(>z)
132	Weight (g)	0.122	0.049	2.489	0.013

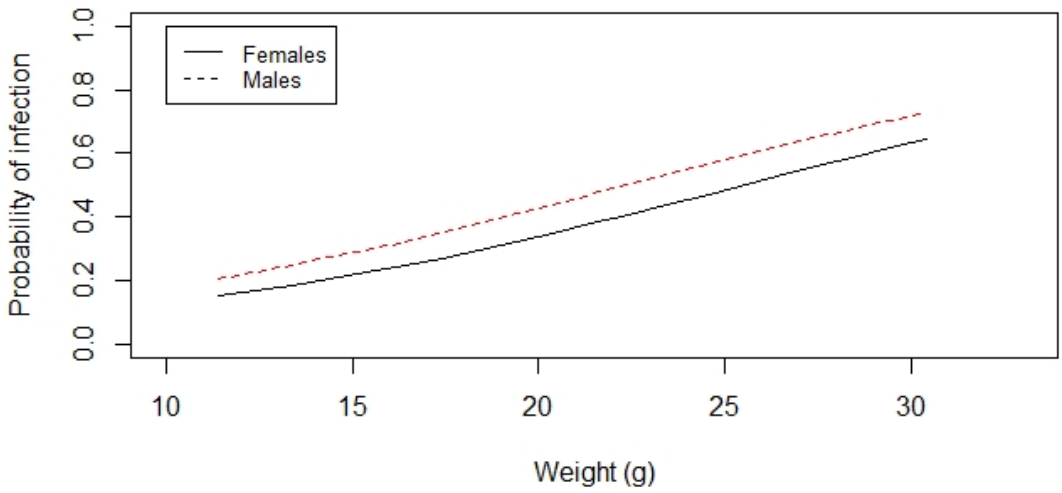

Figure 3. Predicted probability of Pan-orthohantavirus infection in wood lemmings found dead and trapped in the county of Värmland (males n =22, females n =108) as a function of body weight and sex.

Table 3. Modeling result from generalized linear model for the effect of weight and sex on the probability of testing positive for Pan-orthohantavirus. The Pr(>z) value for sex here showing >0.05 suggests the irrelevance of the variable and it being non-significant to explaining the data.

N	Variable	Estimate	SE	Z	Pr(>z)
132	Weight (g)	0.122	0.049	2.489	0.013
	Sex. Male	0.379	0.500	0.758	0.448

3.1.2. Spleen-somatic index

The probability of Pan-orthohantavirus infection increased with increased spleen index (Figure 4, Table 4).

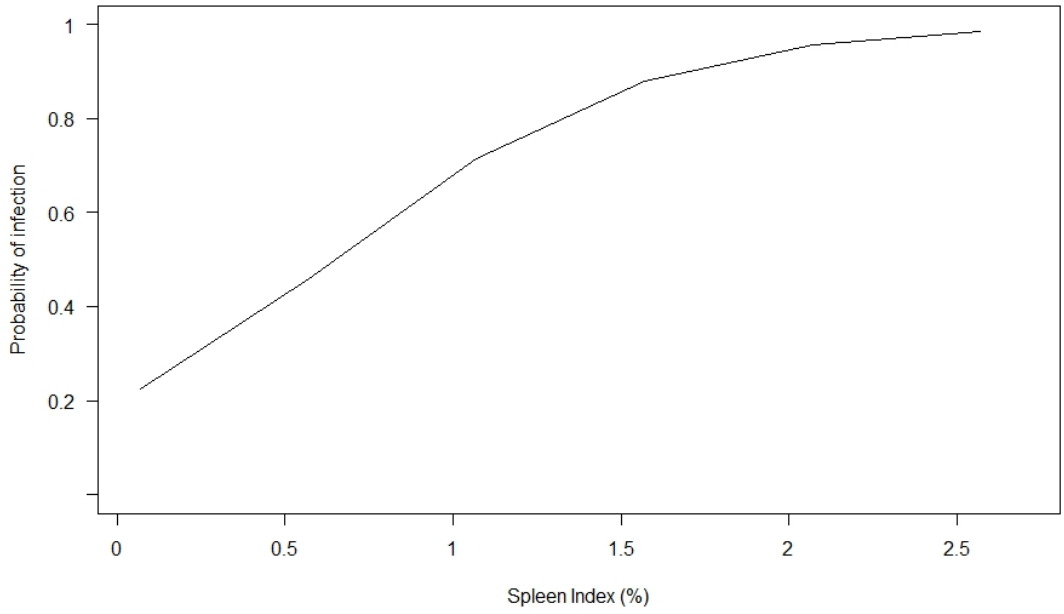

Figure 4. Predicted probability of Pan-orthohantavirus infection in wood lemmings (males and females, found-dead and trapped in the county of Värmland; n = 132) as a function of spleen index (organ-to-body-weight ratio).

Table 4. Generalized linear model predicting the probability of wood lemmings testing positive for Pan-orthohantavirus as a function of spleen index. The value of Pr(>z) showing a value of <0.05 suggests the relevance of the variable and it being significant to explaining the data.

N	Variable	Estimate	SE	Z	Pr(>z)
132	Spleen index	215.705	84.217	2.561	0.010

Modeling both spleen index and county revealed significantly higher probability of infection in Värmland than in Västerbotten (P=0.035, Figure 5, Table 5). Adding county to the model made the spleen index non-significant (P=0.079, Figure 5, Table 5).

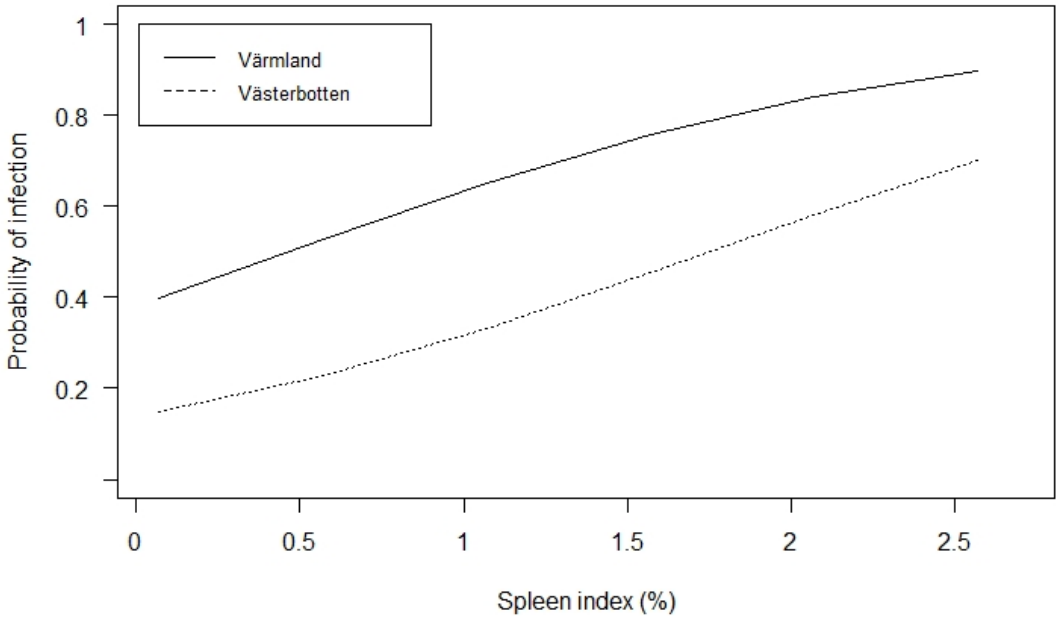

Figure 5. Predicted probability of Pan-orthohantavirus infection in wood lemmings caught in traps as a function of spleen index and county (Värmland n =26, Västerbotten n =29).

Table 5. Modeling result from generalized linear model for the effect of spleen index and county on the probability of testing positive for Pan-orthohantavirus. The Pr(>z) value for county here showing <0.05 suggests the relevance of the variable and it being significant to explaining the data.

N	Variable	Estimate	SE	Z	Pr(>z)
55	County Västerbotten	-1.331	0.630	-2.113	0.035
	Spleen Index	103.521	58.971	1.755	0.079

Body weight and spleen index were correlated (r_s=0.29, P<0.001, Figure 6).

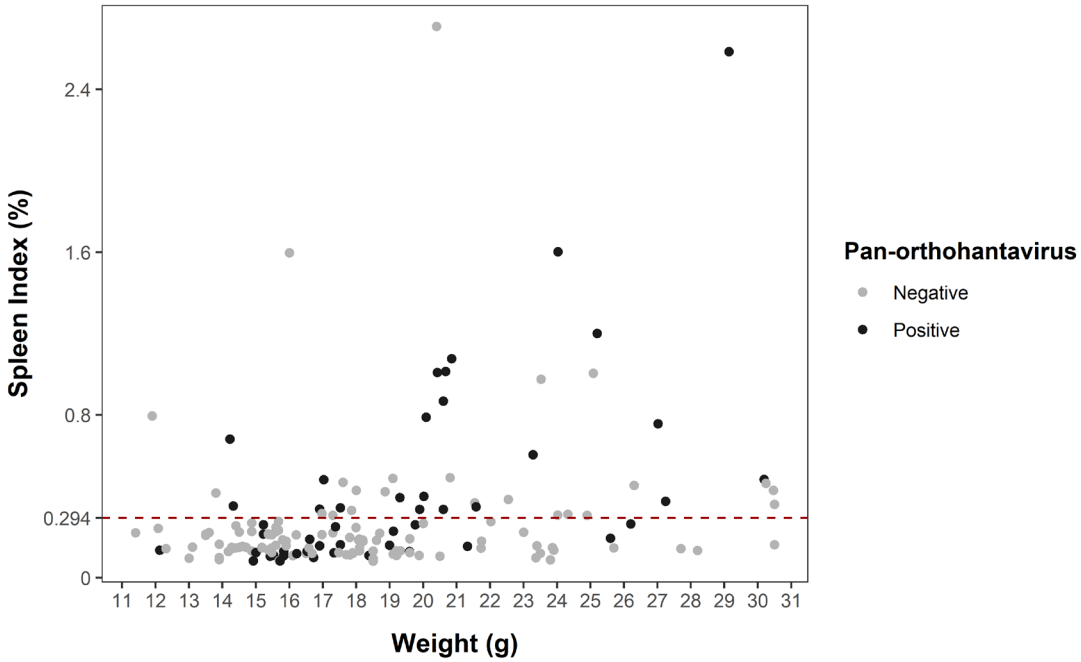

Figure 6. Relationship between spleen index (organ-to-body-weight ratio) and body weight for male and female wood lemmings (found-dead and trapped) in Värmland and Västerbotten County (n = 161). The dashed horizontal line shows the mean spleen index. Above the mean spleen index: n = 22 positive and n = 23 negative. Below the mean spleen index: n = 26 positive and n = 90 negative.

The spleen index was higher in Pan-orthohantavirus positive than in negative individuals (P=0.019, Figure 7).

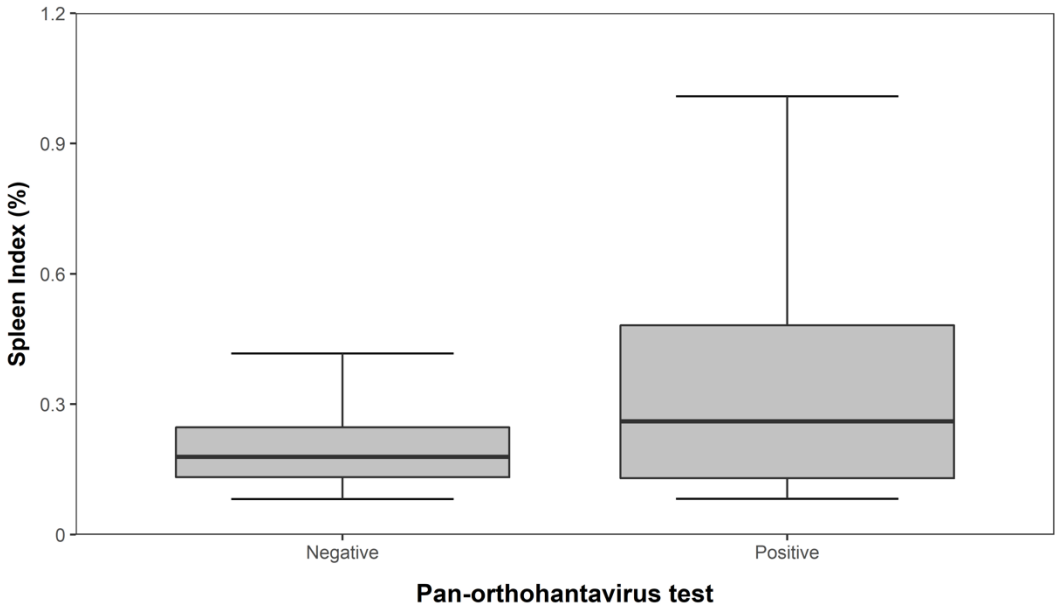

Figure 7. Median spleen index (Organ-to-body-weight ratio) (-/+ 25th and 75th percentiles and minimum respectively maximum observation) for male and female wood lemmings, trapped and

found dead that tested negative (n = 113) and positive (n = 48), respectively, for Pan-orthohantavirus.

At county level, the somatic index did not differ between Pan-orthohantavirus positive and negative specimens (Figure 8). Pan-orthohantavirus negative individuals in Värmland had a significantly higher spleen index compared to the negative individuals collected in Västerbotten (P-value=0.026, Figure 8). There was no difference in the somatic index between Pan-orthohantavirus positive individuals in Värmland and positive individuals in Västerbotten (P-value=0.320, Figure 8).

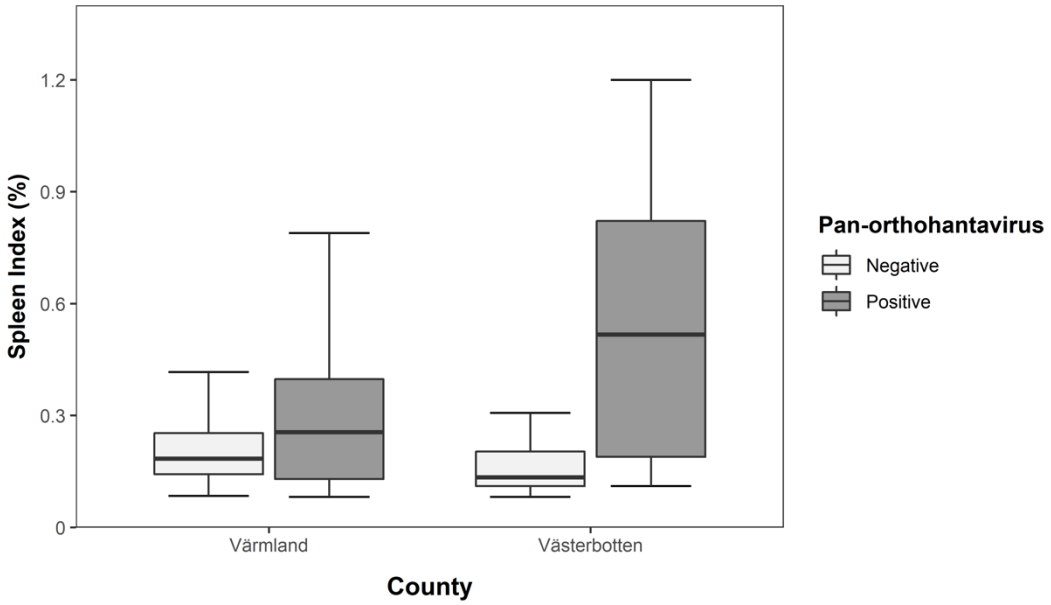

Figure 8. Median spleen index (organ-to-body-weight ratio) (-/+ 25th and 75th percentiles and minimum respectively maximum observation) for male and female wood lemmings, found dead and trapped in the counties of Värmland and Västerbotten. Värmland: positive n = 42, negative n = 90 Västerbotten: positive = 6, Negative n = 23.

In addition, the spleen index did not differ between male and female wood lemmings (P= 0.951, Figure 9). The difference between the sexes remained unsignificant also when only looking at negative or positive individuals (only negative giving P=0.713 and only positive giving P=0.427, Figure 9). Within sexes, comparisons showed that Pan-orthohantavirus positive females had a higher spleen index than negative females (P=0.018, Figure 9), while the spleen index did not differ between positive and negative males (P=0.941, Figure 9).

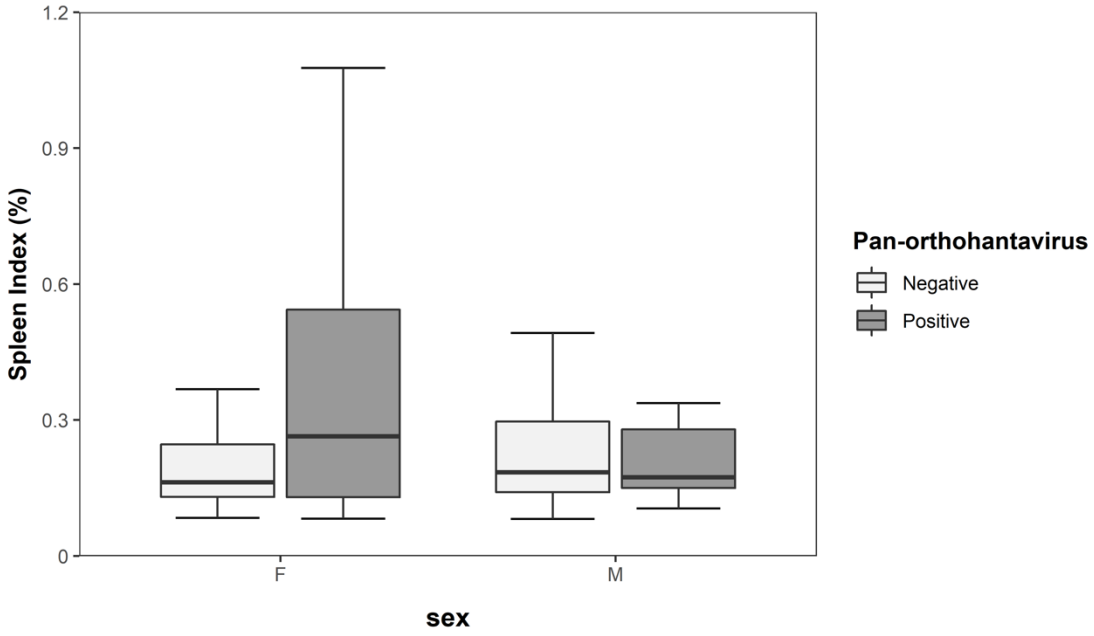

Figure 9. Median spleen index (Organ-to-body-weight ratio) (-/+ 25th and 75th percentiles and minimum respectively maximum observation)) for female (F) and male (M) wood lemmings, trapped and found dead that tested negative and positive, respectively, for Pan-orthohantavirus. Females: n=39 positive and n=92 negative, Males: n = 8 positive and n = 20 negative.

Trapped individuals had a higher spleen index than found dead individuals (P<0.001, Figure 10). Comparing trapped individuals between the two counties showed a higher spleen index in the Värmland County (P<0.001, Figure 10).

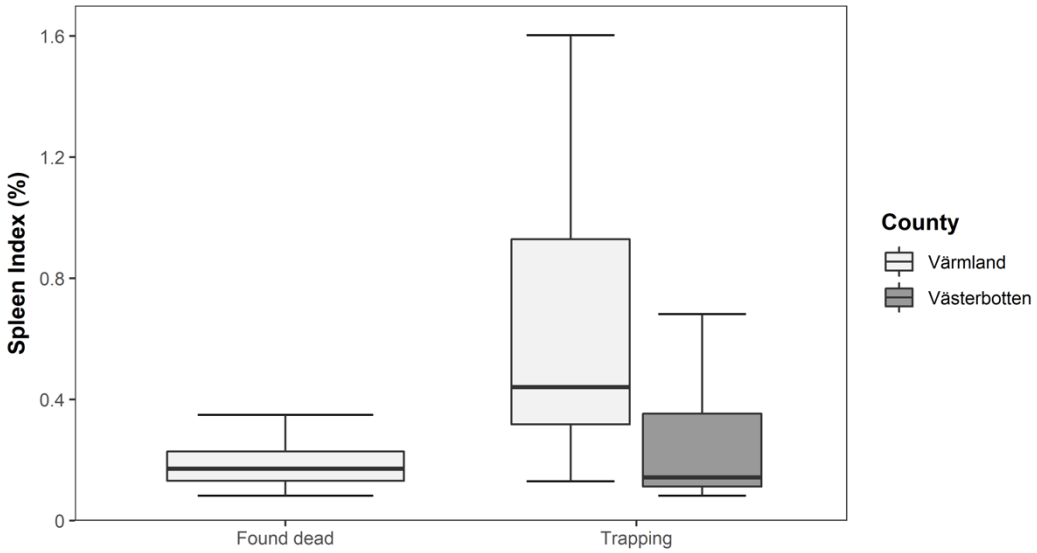

Figure 10. Median spleen index (organ-to-body-weight ratio) (-/+ 25th and 75th percentiles and minimum respectively maximum observation) for male and female wood lemmings in Värmland and

Västerbotten County. In Värmland, lemmings were trapped and collected if found dead while specimens were trapped, only, in Västerbotten. Värmland: found dead n = 117, trapping n = 26, Västerbotten: found dead = no sampling, Trapping n = 29

The spleen index did overall not differ between the outbreak years 2014 and 2017 (P=0.134, Figure 11) and there was no year effect either when accounting for infection status (Pan-orthohantavirus positive: P=0.751, Pan-orthohantavirus negative: P=0.139; Figure 11).

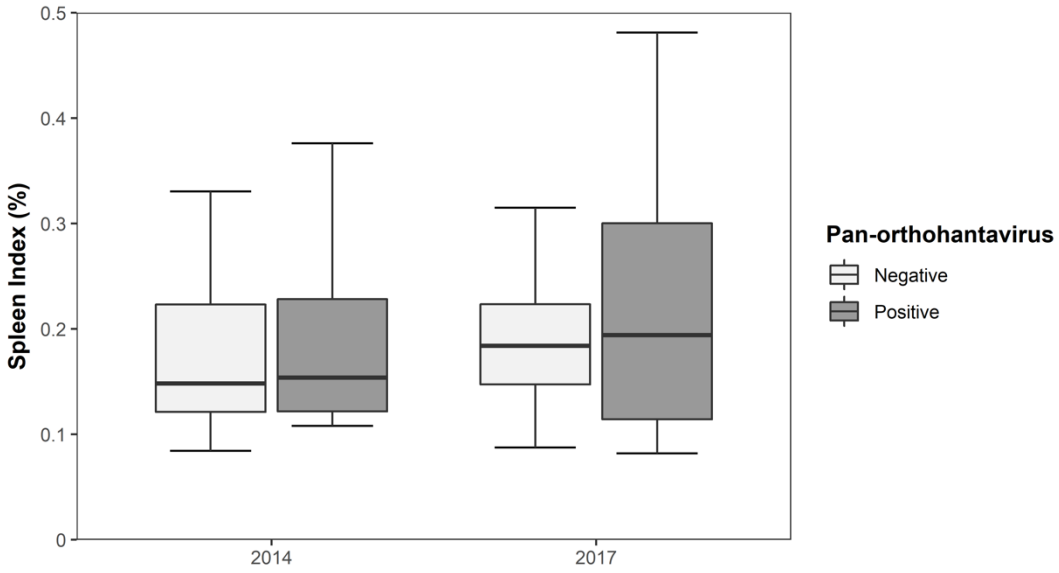

Figure 11. Median spleen index (organ-to-body-weight ratio) (-/+ 25th and 75th percentiles and minimum respectively maximum observation) for male and female wood lemmings found dead in Värmland County in 2014 and 2017. 2014: positive n = 13, negative n = 31, 2017: positive n = 15, Negative n = 47

4. Discussion

From the sampled population of wood lemmings, 26.9% was found to carry hantavirus. Only 4.5-9% of these individuals were carrying PUUV, meaning that a great number of individuals likely carried other hantaviruses.

Modeling the probability of infection in wood lemmings as a function of weight showed a significant, positive effect as weight increased. This same relationship (Figure 1) has earlier been seen in bank voles and PUUV (Khalil et al. 2016). This strengthens the hypothesis that weight, and potentially age, are important factors for wood lemmings to be infected with an orthohantavirus. The results show that sex had no significant effect on probability of infection (Figure 3). However, non-significance between the sexes has also been seen in comparisons of PUUV infected bank voles (Tersago et al. 2008). Other studies of disease in rodents have contrarily shown higher infections in male individuals than females (Bernshtein et al. 1999; Olsson et al. 2003; Kallio et al. 2010).

Individuals testing positive for Pan-orthohantavirus showed a significantly higher spleen index than those testing negative (Figure 4 & 7). Changes in spleen size/morphological characteristics during an infection is nothing new and has earlier been seen in both humans infected with PUUV (Koskela et al. 2014) and rodents infected with Sin Nombre virus (SNV) (Netski et al. 1999). This makes evident, the degree of physical change that can occur when an individual is affected by pathogens or other stress factors. More importantly, body weight and spleen weight showed weak correlation, (Pearson correlation test, Coefficient = 0.29, $P<0.01$), which illustrates why both are important factors in explaining probability of infection. Sex was found to have no significant effect on the spleen index. Contrarily to this, several studies have found a higher risk of disease in males (Bernshtein et al. 1999; Olsson et al. 2003; Kallio et al. 2010).

To find dead wood lemmings is almost impossible during non-outbreak years (Ecke, personal communication) and during non-outbreak years, sample sizes for wood lemmings are generally low (Ecke et al. 2017). Hence, to use the rare opportunity of mass mortality is one of the few options to increase insight into the

potential of pathogens and diseases to drive population dynamics including mass mortality of wood lemmings.

The spleen index amongst negative individuals showed to be significantly higher in Värmland. As these did not test positive for the pan-hantavirus, this group of southern lemmings might have been affected by something else impacting their health. Further studies should be made to investigate if this could be due to another pathogen or other unknown factors.

The spleen index was significantly higher in trapped animals than in individuals found dead. This comparison was only made within the county of Värmland. Of the individuals that were found dead, 26% (30 positives of n=114) tested positive, while 54% of trapped (14 positives of n=26) individuals tested positive. The large difference in total number of samples between the groups could be a bias in our results. Nevertheless, this is an interesting find, as it could potentially mean that the virus-carrying lemmings differ in terms of behavior and are therefore more likely to be caught in traps. The fact that sick rodents could be more prone to trapping and therefore also affect the statistical outcome has been studied before, especially when having a low number of samples (Stryjek et al. 2019).

There was no difference in spleen index between the peak years, which was expected as the number of diseased individuals should be similar between these years compared to the non-peak years. No comparisons between peak years and non-peak years were made, again due to very low sample numbers. The non-peak years had only one to five individuals collected.

When adding county in the model to predict the probability of infection (Figure 5), spleen index became non-significant (Table 5). This would suggest that county is more important than spleen index and the result would not be in line with the hypothesis of spleen being used as an indicator for disease. This change in significance could be due the fact that in this comparison I excluded lemmings that were found dead, and only compared trapped individuals, therefore also affecting the impact that spleen index might have on the probability of infection.

Sex was not a significant factor for predicting disease. This result should be interpreted with caution, as the number of individuals included in this test were largely uneven (22 males and 108 females). A more even sex representation in my samples would have been needed to draw any definite conclusions. The result here is probably affected by the low number of males included in the test, 40% of males (8 of n=28) and 42% of females (39 of n=131) testing positive.

Age is a very important factor when looking at survival rates. However, using weight exclusively as an index for age should be done with caution. It has been seen to be much less reliable and more variable with environmental conditions than other methods of age determination (Fuller 1988; Janova et al. 2007). In further studies of disease in wood lemmings, true age could be another area of focus to investigate.

The idea of using a spleen-somatic index to predict disease has been explored before. Davydova et al. (2011) stated that there is too little known about what exactly determines the changes in spleen size and more scientific trials are needed before spleen size can be used as an indicator for diseases. These results should therefore be considered, but they are not robust enough for us to draw conclusions on the ability of spleen enlargement to predict disease. More research needs to be done, as many other factors could be causing this response.

I found a higher probability of infection in Värmland, which suggests the necessity for testing wood lemmings for different orthohantaviruses than PUUV. Further research should aim to evaluate what different strains of hantavirus are present in the population of wood lemmings in Sweden. Today, there are over 40 known hantaviruses in the world. In 2013, Seoul hantavirus was found in imported pet rats in Sweden (Lundkvist et al. 2013). Knowledge of historical introductions of foreign hantaviruses greatly supports the need-to-know which hantaviruses are affecting the wood lemming population in Sweden.

In this study, I was able to shed further light on pathogens hosted by wood lemmings, but I did not find any support for the disease hypothesis, i.e. that pathogens and subsequent diseases might cause mass mortality in wood lemmings. Future studies should focus on potentially more fatal pathogens for wood lemmings including *Listeria monocytogenes* (Skarén 1981) or *Toxoplasma gondii* (Ingram et al 2013). Focus should also be in gathering enough material for comparing years of population outbreaks with years of low density. This would allow one to look at how the prevalence of harmful pathogens shifts before and after mass mortality events.

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6. Acknowledgements

I would like to thank my supervisor Frauke Ecke and assistant supervisor Magnus Magnusson for helping me and guiding me through this thesis. A big thanks to Mark Jamieson for all the invaluable help, both in pushing me through R and through my writing process. Thanks so much to Laura Juvany Canovas for supporting me all the way, keeping me positive, motivating and helping greatly with my writing. I would also like to thank Björn Wallgren, my lab partner for sharing the interesting and fun experience in the lab and Richard Larsson for great discussions and help. Also, a big thanks to my family for always being supportive from start to finish.

An extra special, thank you to Cau for sitting by my side at home every single day, reminding/forcing me to take breaks and go outside for a walk and some fresh air.

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