The Role of Rodents in the Transmission of *Echinococcus multilocularis* and Other Tapeworms in a Low Endemic Area

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Abstract

Echinococcus multilocularis is zoonotic tapeworm in the *Taeniidae* family with a two part lifecycle involving a canid definitive host and a rodent intermediate host. The work of this thesis followed the first identification *E. multilocularis* in Sweden in 2011 in a red fox (*Vulpes vulpes*). The main purpose was to describe the importance of the rodents for *E. multilocularis* transmission in Sweden.

Echinococcus multilocularis was identified in both the water vole (*Arvicola amphibius*) and the field vole (*Microtus agrestis*), but not the bank vole (*Myodes glareolus*) or mice (*Apodemus* spp). As the number of *E. multilocularis* positive rodents was low (n=9), the examination of other taeniid parasites was used to investigate overall parasite transmission patterns. Rodents caught in field habitat (field voles and water voles) were ten times more likely to be parasitized than rodents caught in forest habitat (bank voles and mice). These results provide further support for the importance of field- and water voles found in field habitat for cestode transmission. Still, these rodent species differ from the most common rodent intermediate hosts in central Europe, and metacestode development within these species may be limited. Thus, the presence of *E. multilocularis* in Sweden could be constrained by the lack of an ideal intermediate host.

The distribution *E. multilocularis* was found to be highly aggregated with localized areas of high parasite egg contamination. Despite an extremely low national prevalence, multiple positive rodents and feces were identified in areas with known and unknown *E. multilocularis* status. This success is credited to the targeted sampling strategy, which was designed to focus collection efforts in areas where risk for parasite presence was estimated to be highest. This sampling strategy could be used as a basis for future risk-based sampling to detect *E. multilocularis* in areas where parasite prevalence is low or unknown.

Keywords: Echinococcus multilocularis, tapeworm, fox, rodent, intermediate host, transmission ecology, risk-based sampling, targeted sampling

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Dedication

To my parents for always encouraging me to be what I want to be and to Grandma and Grandpa Fischer for encouraging me to do it with a healthy sense of adventure

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Miller, A. L., Olsson, G. E., Walburg, M. R., Sollenberg, S., Skarin, M., Ley, C., Wahlström, H., and Höglund, J. (2016). First identification of *Echinococcus multilocularis* in rodent intermediate hosts in Sweden. *International Journal of Parasitology: Parasites and Wildlife* 5(1), 56-63. doi: 10.1016/j.ijppaw.2016.03.001

II Miller, A. L., Olsson, G. E., Sollenberg, S., Walburg, M. R., Skarin, M. S., and Höglund, J. Transmission ecology of taeniid liver parasites in rodents in Sweden, a low endemic area for *Echinococcus multilocularis*. (Manuscript).

III Miller, A. L., Olsson, G. E., Sollenberg, S., Skarin, M., Wahlström, H., and Höglund, J. Support for targeted sampling of red fox (*Vulpes vulpes*) feces in Sweden: a method to improve the probability of finding *Echinococcus multilocularis* (*Parasites & Vectors*, In Press)

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The contribution of ALM to the papers included in this thesis was as follows:

I Determined field and laboratory design in cooperation with co-authors and as part of the EMIRO group. Performed fieldwork with some support from student volunteers and co-authors. Performed the majority of the rodent dissection and some molecular labwork. Mainly responsible for data interpretation. Drafted the manuscript and handled correspondence with the journal.

II Determined field and laboratory design in cooperation with co-authors and as part of the EMIRO group. Performed fieldwork with some support from student volunteers and co-authors. Performed the majority of the rodent dissection and some molecular labwork. Mainly responsible for data interpretation in collaboration with main supervisor and statistician. Drafted the manuscript.

III Determined field and laboratory design in cooperation with co-authors and as part of the EMIRO group. Performed fieldwork with some support from student volunteers and co-authors. Performed the majority of fecal egg collections and some molecular labwork. Mainly responsible for data interpretation in collaboration with supervisors and statistician. Drafted the manuscript and handled correspondence with the journal.

Abbreviations Used

BLAST	Basic Local Alignment Search Tool
EES	European Economic Area
EFSA	European Food Safety Authority
ELISA	Enzyme Linked Immunosorbant Assay
EMIDA	Coordination of European Research on Emerging and Major Infectious Diseases of Livestock
EMIRO	*Echinococcus Multilocularis* in ROdents
ERA	European Research Area
EU	European Union
FOHM	Public Health Agency of Sweden
FoMA	National environmental and wildlife monitoring assessment program (Sweden)
FORMAS	Swedish Research Council
G/N	Gnesta/Nyköping
K	Katrineholm
MC-PCR	Magnetic capture polymerase chain reaction
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain reaction
SJV	Swedish Board of Agriculture
SLV	National Food Agency, Sweden
SVA	National Veterinary Institute (Sweden)
U	Uddevalla
V/V	Vetlanda/Växjö

1 Background

Echinococcus multilocularis, the red fox tapeworm, is part of the parasite family, *Taeniidae*. It is the shortest of the tapeworms reaching only a maximum length of 4.5 mm as an adult (Thompson and McManus, 2001) and, thus, has also been termed the dwarf fox tapeworm. Despite its size, it causes one of the most severe parasitic diseases known to man (Torgerson *et al.*, 2008). This tapeworm has a lifecycle which involves many hosts across the northern hemisphere, and, in recent years, has been suggested to be a zoonotic parasite of increasing concern (Davidson et *al.*, 2012).

Figure 1. Example of a newspaper article (front page photo) after the first finding of *E. multilocularis* in Sweden. Main headline: “*I am worried about the children*” *Affected landowner fears the tapeworm*. Top box, lower right: *Voles give clues in the hunt for infection*. Lower box, lower right: *The Minister: “It can change our outdoor life”*, Swedish translation by author. Newspaper: Göteborgs-Posten (The Göteborg Post), Nr 101, Week 15, April 13, 2011. Photographer: Elisabeth Alvenby. Used with permission.

With the first report of *E. multilocularis* in 2011 (Osterman Lind *et al*., 2011), Sweden became part of the northernmost border for *E. multilocularis* in Europe (except for Svalbard, Henttonen *et al*., 2001). Fueled by media reports, such as the ones in Figure 1, the public reacted with not only fear for personal safety, but also concern for the preservation of the Scandinavian cultural concept of "friluftsliv". Directly translated as "outdoor life", this concept is an intrinsic need to connect to nature though, for example, outdoor activities (for a review of this idea see Beery (2013). Connected to this concept is the strong tradition of collecting and eating fresh berries, the practice of which has been suggested as a route of human exposure for *E. multilocularis* (e.g Kern *et al.*, 2004).

The Public Health Agency of Sweden (FOHM) and National Food Safety Agency (SLV) immediately began work to answer questions about the safety of the outdoors (Wahlström *et al*., 2012). Furthermore, the Swedish Board of Agriculture (SJV) decided to take action, in collaboration with the National Veterinary Institute (SVA), to clarify the geographical distribution of the parasite. An intense monitoring effort was undertaken to analyze a national collection of almost 3000 foxes and a regional collection of nearly 800 fox feces by the end of 2011, at a cost of nearly 4.7 million SEK (~466,000 €) (Wahlström *et al.*, 2015). Although positive results were few (see also Section 2.3.1), it was concluded that *E. multilocularis* was established in the country, that eradication was not possible, and that risk for public health was low (Wahlström *et al.*, 2012).

Soon after the first finding in Sweden, funding was obtained for a project investigating the role of the rodent in the *E. multilocularis* lifecycle across Europe (see Section 2.3.2). Sweden's involvement, in the form of this thesis, couldn't have been more timely. Many questions still remained about the presence of *E. multilocularis* in Sweden—the most important of which was the identification of the rodent link (which was still unknown). Furthermore, from a research perspective, studying this parasite at the northern border of its range provided a unique opportunity to understand what factors, if any, limit parasite prevalence.

2 Introduction

2.1 *Echinococcus multilocularis*

2.1.1 Lifecycle

The cestode *Echinococcus multilocularis* has an indirect lifecycle involving a canid definitive host and a rodent intermediate host (Figure 2). The adult tapeworm lives within the small intestine of the definitive host. The prepatent period is about a month but has been reported as less than 25 days (Kapel *et al.*, 2006). When reproductively mature, the adult worm sheds eggs into the environment with the definitive host's feces. The shedding period has been shown to be up to three months in young foxes (Kapel *et al.*, 2006). When an intermediate host ingests these eggs, the shells are digested in the stomach to release the oncospheres (parasite larvae). From the small intestine, the

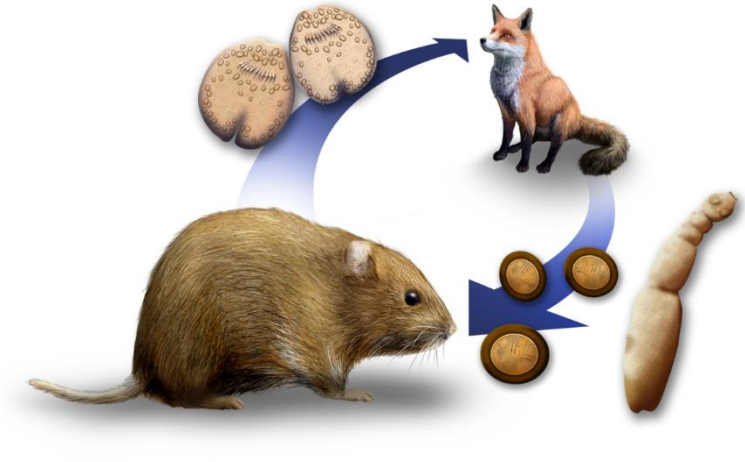

Figure 2. The lifecycle of *E. multilocularis* with focus on the rodent intermediate host. The adult worm (lower right) lives in the fox and sheds eggs (lower right) into the environment. After a rodent ingests the eggs, a metacestode develops in the rodent liver. Only metacestodes which contain protoscolices (upper left) are infectious to the fox after eating the rodent. © Diogo Guerra. Used with permission within the EMIRO Project.

oncospheres migrate though the bloodstream and lymphatic system to the liver and develop into a metacestode (Thompson, 1995) (Figure 3A). The metacestode is a multivesicular structure that has an outer laminar layer and an

inner germinal layer (Thompson, 1995). The inner germinal layer gives rise to brood capsules which produce protoscolices (Thompson, 1995) (Figure 3B). This maturation can take 6-10 weeks in highly susceptible hosts (Woolsey *et al.*, 2015b). Without protoscolices the metacestode is considered non-infectious. The lifecycle is only completed when a definitive host ingests an infectious intermediate host (i.e. one that contains a metacestode with protoscolices).

For most of Europe, the most important definitive host is the red fox (*Vulpes vulpes*) (Eckert & Deplazes, 2004). Prevalence of *E. multilocularis* in the red fox has been reported over 60% in some regions (Raoul *et al.*, 2001; Hofer *et al.*, 2000). Other hosts in Europe may include the arctic fox (*V. lagopus*) (important on Svalbard, Fuglei *et al.*, 2008), raccoon dog (*Nyctereutes procyonoides*) (Kapel *et al.*, 2006) and other canids. Pet dogs can host the parasite, but, even in high endemic areas, prevalence is usually low (<10%) (see also section 2.1.2) (Deplazes *et al.*, 2011). Although cats can serve as hosts, Kapel *et al.* (2006) noted that parasite development within the cat is limited.

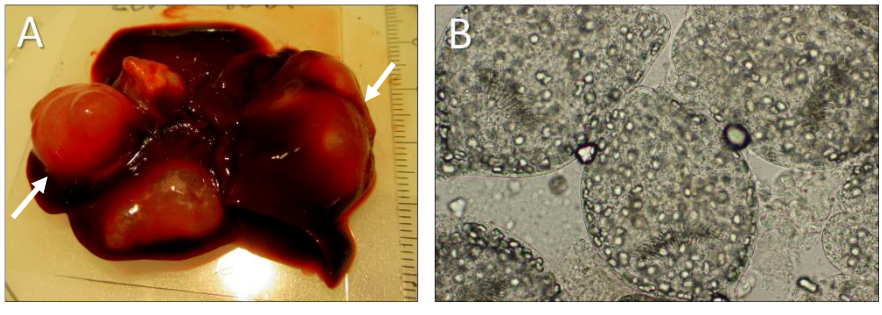

Figure 3. Echinococcus multilocularis A) Multiple mestacestodes (white arrows) in a water vole liver B) Protoscolices (larvae) from a metacestode Photos: Andrea Miller

In Europe, the most important rodent intermediate hosts are within the *Arvicolinae* subfamily, which includes voles, lemmings, and muskrats (Eckert & Deplazes, 2004). Of these, the common vole (*Microtus arvalis*) and the water vole (*Arvicola scherman*) are considered the most important rodent intermediate hosts within central Europe due to reports of high prevalence of infection (e.g. 39% in *Arvicola* spp. Gottstein et al., 2001) and importance as prey items for the red fox (Raoul et al., 2010). The bank vole (*Myodes glareolus*) may also be an important intermediate host in some areas (Reperant et al., 2009; Barábasi, S.S. *et al.*, 2011); however, overall this species is considered of lesser importance than the common vole or water vole in central Europe due to typically low reports of prevalence (Hanosset *et al.*, 2008). Furthermore, a recent laboratory study has indicated a low susceptibility of bank voles to *E. multilocularis*

(Woolsey *et al.*, 2016). Other sympatric rodents, e.g. mice (*Apodemus* spp.), are believed to be of even less importance to the transmission of *E. multilocularis* given the rare observations of infection (e.g. Barabási et al., 2011; Stieger et al., 2002).

2.1.2 Zoonotic Potential

Humans can become infected by *E. multilocularis* by ingestion of eggs from the environment. Like in the rodent intermediate host, a metacestode develops most commonly in the liver but can metastasize to bones and other organs (Ammann & Eckert, 1995). Development in the liver is usually very slow (5-15 years before obvious symptoms arise) and is characterized by a proliferative lesion(s) which invades the surrounding liver tissue much like cancer (Ammann & Eckert, 1995). Advocated treatment is often long-term use of anthelminthics, such as menbendazole or albendazole, but may also include invasive procedures, such as liver resection and transplants (Brunetti *et al.*, 2010). Without such treatment, case prognosis is very poor (Ammann & Eckert, 1995).

Risk factors for exposure are difficult to explore due to long incubation period. Factors such as lifestyle (e.g. farming, hunting), consumption of contaminated forest berries or unwashed garden vegetables, and drinking contaminated water have all been suggested as routes of contamination (Kern *et al.*, 2004; Schantz *et al.*, 2003; Yamamoto *et al.*, 2001). Due to close contact with humans, infected dogs may be one of the most important sources of infection. Dog ownership (or simply presence of dogs) which have access to infected rodents has been shown to be a risk factor for alveolar echinococcosis in both Alaska and China (Craig *et al.*, 2000; Rausch *et al.*, 1990).

Occurrence in humans is rare even in high endemic areas, and recent studies have suggested natural immunity may protect a large proportion of infected/exposed humans from actually developing the disease (Gottstein *et al.*, 2015; Vuitton & Gottstein, 2010). However, in Europe, it has been suggested that the risk for exposure may be increasing as the geographic range of the parasite expands and numbers of definitive and intermediate hosts increase (Davidson *et al.*, 2012). This suggestion is supported by studies, such as Schweiger *et al.* (2007) which reported nearly doubled incidence of human infection, from ~0.15/100000 to 0.26/100,000, in Switzerland in the early 2000s. This increase was positively correlated with the increase in fox population which followed the European rabies vaccine campaign in the 1980s (Schweiger *et al.*, 2007). However, outside of the *E. multilocularis* high endemic countries of Europe (e.g. Germany, Switzerland, France), it is more difficult to analyze trends. In many other European countries, the parasite has only recently been

recognized in the past decades and reports of human cases may be lacking due to poor reporting or simply misdiagnosis (Vuitton *et al.*, 2015).

2.1.3 Transmission Dynamics and Micro-Foci

As an indirectly transmitted parasite with a free-living egg stage and multiple potential final and intermediate hosts, the transmission of *E. multilocularis* is very complex (for a recent review see Raoul *et al.* 2015). Factors such as predator-prey dynamics, host density and susceptibility as well as seasonal, yearly, and environmental conditions interact to influence each stage of the parasite's lifecycle (Giraudoux *et al.*, 2003). In addition, movements of the red fox definitive host can carry the parasite over large distances. Although typical movements are within several kilometers, red fox dispersal has been reported up to 60-65 km in Sweden (Englund, 1980). Still, actual parasite transmission occurs within only 100s of meters in the more localized home ranges of the rodent intermediate host (e.g. Erlinge *et al.*, 1990). When optimal conditions for all the factors listed above exist in these geographically limited areas, transmission is enhanced and "hotspots" or "micro-foci" of parasite presence are formed (Giraudoux *et al.*, 2002).

In central Europe, micro-foci have been most closely associated with field habitats where both common voles and water voles live (Giraudoux *et al.*, 2003). These voles are highly susceptible to *E. multilocularis* (e.g. Burlet *et al.*, 2011; Woolsey *et al.*, 2015b) and, within a micro-focus, prevalence in these species can be high (e.g. 39% of 28 examined water voles, Gottstein *et al.* 2001). These rodent species live in dense populations and exhibit both seasonal and interannual peaks in population numbers (Duhamel *et al.*, 2000b; Delattre *et al.*, 1992). Although the red fox appears to have a dietary preference for *Microtus* spp (Raoul *et al.*, 2010; Guislain *et al.*, 2008), it also feeds on water voles, particularly during times of high population densities (Raoul *et al.*, 2010). Increased presence of foxes in these rodent habitats is marked by increased fecal densities (Robardet *et al.*, 2011; Guislain *et al.*, 2007). Thus, the basic components needed for parasite transmission (definitive and intermediate hosts, feces) are focused in these areas. Transmission is further enhanced if micro-habitat conditions, such as a cool, moist environment, or seasonal conditions, such as cold winters, exist to increase parasite egg survivability (Veit *et al.*, 1995).

Micro-foci are important not only for understanding parasite transmission between wildlife hosts, but also for understanding transmission to humans. Although the specific risk for human exposure in these areas yet unknown, micro-foci are areas of focused parasite egg contamination. Viel *et al.* (1999) showed that areas with high densities water voles were correlated to a high

incidence of human cases in eastern France. As discussed above and in Viel *et al.* (1999), intense feeding of foxes on water voles during periodic outbreaks likely increased parasite transmission and environmental contamination and, thus, human infection years later. In addition, it has been suggested that clusters of human infection demonstrated in high endemic areas may be linked to occurrence of micro-foci (Said-Ali *et al.*, 2013; Danson *et al.*, 2004; Giraudoux *et al.*, 2002).

A better understanding of the relationship between human and wildlife infection will provide insight into limiting or even controlling risk of infection risk humans. However, the identification of micro-foci in the environment remains challenging. The occurrence and relative importance (i.e. *E. multilocularis* prevalence of hosts present) of these areas varies in both space and time for many reasons, some of which are still unknown (Giraudoux *et al.*, 2002). For instance, as discussed above, fluctuations in rodent population densities create variation in fox feeding patterns and, thus, changes in levels of transmission and environmental contamination. In addition, landscape changes due to deforestation or agricultural practices may create or destroy habitat opportunities for suitable rodent intermediate hosts (Giraudoux *et al.*, 2013). Due to the potential link to public health, this is an area of much needed research.

2.2 Monitoring/Surveillance Considerations

2.2.1 Monitoring/Surveillance in the EU

Due to the zoonotic potential of *E. multilocularis* and its status as an emerging disease in Europe (Eckert *et al.*, 2000), *E. multilocularis* was added to the list of zoonotic diseases for which monitoring and yearly reporting is required for EU member states in 2003 (European Parliament & Council of the European Union, 2003). Because both the diagnostic and sampling techniques to be used are not well described, member states have the freedom to choose how to implement this directive. However, without standardized sampling and diagnostic techniques, comparisons between countries are difficult perform (Conraths & Deplazes, 2015). In addition to these difficulties, an underlying issue for successful monitoring/surveillance programs in all countries is the difficulty and expense of obtaining representative wildlife samples (Mörner *et al.*, 2002).

Most monitoring efforts for *E. multilocularis* in Europe focus on the fox rather than the rodent. Reasons for this may include that prevalence in foxes is often observed to be higher than in rodents (e.g Hanosset *et al.*, 2008; Hofer *et al.*, 2000) and samples can be collected over large areas (e.g. Raoul *et al.*, 2001). In addition, fox samples are perceived to be more easily obtained (i.e. through hunter collaboration or other existing wildlife programs) (Conraths & Deplazes,

2015). However, hunter collected samples are often biased both spatially and temporally, as hunters usually concentrate efforts only in certain regions or during certain time periods (Conraths *et al.*, 2003). Furthermore, by focusing on the fox, very little or no information is gained about the rodent intermediate host. As demonstrated in this thesis, the evaluation of rodent samples can, for instance, give insight into the localized distribution of the parasite and potential limitations to parasite transmission.

2.2.2 Freedom from Disease

Monitoring/surveillance of *E. multilocularis* is, for obvious reasons, most important for countries trying to maintain or declare *E. multilocularis* disease-free status. According to the EU directive (No. 1152/2011), a country can declare freedom from *E. multilocularis* if a monitoring program is designed to show with 95% confidence that 1% or less of foxes in the country are infected with the parasite (European Commission, 2011). To maintain disease-free status, this must be reconfirmed on a yearly basis (European Commission, 2011). Countries with disease free status are allowed to enact deworming regulations to restrict the importation of dogs from *E. multilocularis* endemic countries (European Commission, 2011). Within the EU/EES, only Finland, the United Kingdom, Ireland, Malta, and mainland Norway are considered disease free (European Commission, 2011). Sweden lost disease-free status in 2011 (see Section 2.3.1).

2.2.3 Sample Collection

As discussed in Section 2.2.1, most monitoring focuses on the fox definitive host. Identification of positive foxes is typically done either through examination of intestines (from fox carcasses) or collected feces (from environment or fox carcasses). For biosafety reasons, these samples are typically frozen at -80 C for some time (≥1 week, carcass; ≥3 days, feces) to kill parasite eggs (Eckert *et al.*, 2001b). The gold standard for diagnosis in the fox is the sedimentation and counting technique (SCT), whereby worms are counted from subfractions of intestinal content (Eckert *et al.*, 2001a). However, this method is time consuming and, thus, costly to perform (Conraths & Deplazes, 2015). In addition, there are significant biosafety concerns in handling the carcass before freezing (Eckert, 2001). In contrast, diagnosis based on fecal examination can provide a safer, more cost efficient method of parasite detection (Conraths & Deplazes, 2015). Methods for fecal evaluation focus on detection of either copro-antigen (e.g. Raoul *et al.*, 2001) or taeniid egg DNA (e.g. Mathis *et al.*, 1996). For a recent review and comparison of commonly used diagnostic techniques for each of these samples see Conraths and Deplazes (2015).

2.2.4 Risk-based Sampling

Although the statistical concepts behind risk-based sampling are beyond the scope of this thesis, the overall purpose of risk-based sampling is to increase the probability of disease detection (Stärk *et al.*, 2006). This technique uses prior knowledge of factors affecting disease transmission to focus sample collection in, for example, a habitat or species known to most likely to harbor the disease (Stärk *et al.*, 2006). By concentrating sampling in high-risk areas or populations, this technique is considered a more efficient method (both in time and costs) for disease detection as compared to systematic techniques (Stärk *et al.*, 2006; Paisley, 2001). As stated by Cameron (2012):

> The increase in efficiency gained through risk-based sampling is due to the simple concept that one is more likely to find something if one looks where it is most likely to be.

Risk-based sampling may be particularly relevant for detecting disease in low prevalence areas and for documenting freedom from disease (Hadorn *et al.*, 2002). However, the difficulty with risk-based sampling is that the selection of the high risk areas may be biased by lack of knowledge of certain risk factors. Thus, preliminary studies are needed to clearly define risk factors for the specific species and regions in question (Stärk *et al.*, 2006). Risk factors for the detection of *E. multilocularis* in wildlife have not been specifically defined. However, a recent EFSA scientific opinion supports research into this area (European Food Safety Authority Panel on Animal Health and Welfare, 2015).

2.3 *Echinococcus multilocularis* in Sweden

2.3.1 History of *E. multilocularis* in Sweden

Monitoring for *E. multilocularis* in Sweden began in 2000 as a response to the parasite's expanding range (Osterman Lind *et al.*, 2011). Of particular concern was the first report of the parasite in a red fox (*Vulpes vulpes*) in 2000 in the neighboring country of Denmark (Saeed *et al.*, 2006). In addition, a risk assessment performed in 2006, estimated that the highest risk for the introduction of *E. multilocularis* was from importation of infected dogs (Vågsholm, 2006). Therefore, initial monitoring efforts by the Swedish National Veterinary Institute (SVA) were focused in the southern part of the country. This part of the country contains popular tourist areas, particularly along the west coast, and, most importantly, is connected to Denmark by the Øresund bridge (Malmö-Copenhagen). From 2000-2010 nearly 3000 foxes (~300/year) were examined for *E. multilocularis* with the first positive finding reported from a fox

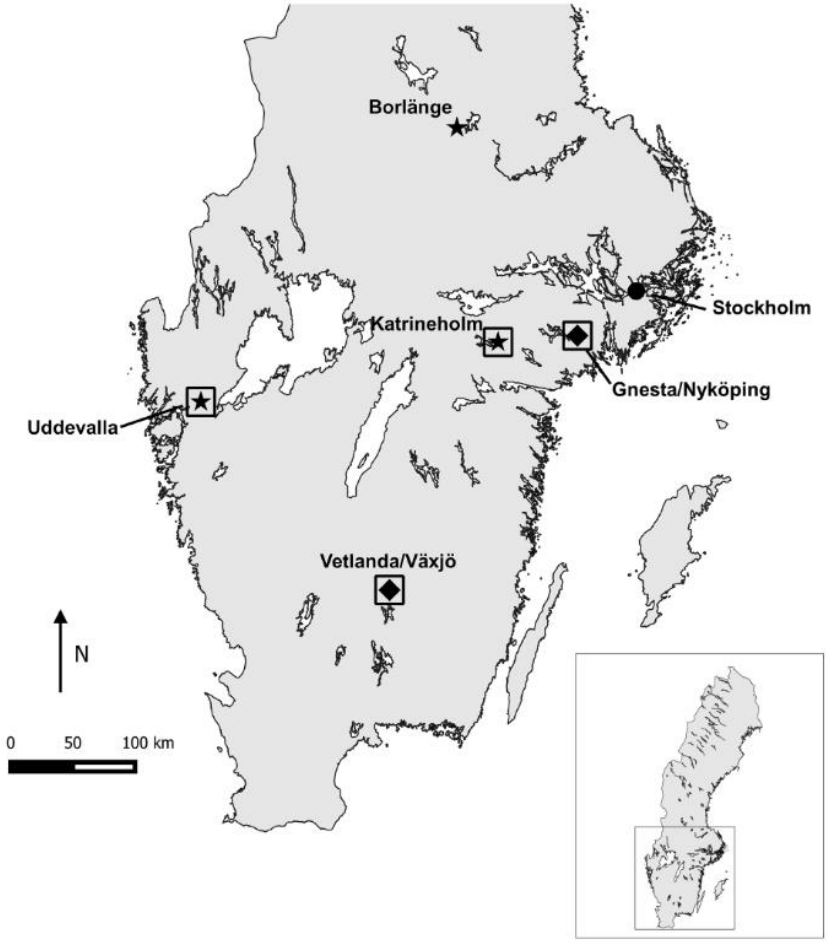

Figure 4. Map of the southern half of Sweden and study regions (boxes) (*see also Table 1*). Black stars indicate areas where intestinal samples from shot foxes were identified as positive for *Echinococcus multilocularis* through national monitoring (2011) before this thesis began (2013) (Wahlström et al. 2012). Black diamonds indicate additional areas identified positive for *E. multilocularis* by the conclusion of this thesis work (2015). Map created in QGIS v2.12.3. (Basemap: Sweden 1000plus 6.0, SWEREF 99 TM, 2008, © Lantmäteriet). Modified from Fig 1 in Miller et al. (2016). Used with permission from Miller *et al.* (in press) (Paper III).

shot December 2010 near the city of Uddevalla on the west coast (Osterman Lind *et al.*, 2011) (Figure 4).

Following this first finding, monitoring efforts were expanded with the goal to determine the geographic spread of the parasite within the country. By the conclusion of 2011, nearly 3000 intestines from hunter-shot foxes had been examined and three additional positive foxes had been identified (Wahlström *et al.*, 2012). This included a second positive fox shot near Uddevalla and one fox in each of two new areas near the cities of Katrineholm and Borlänge (Wahlström *et al.*, 2012) (Figure 4). During 2011, a regional baseline monitoring of collected fox feces was performed in a 50 km diameter area near Katrineholm (Wahlström *et al.*, 2015). Here 6/790 (0.8%) feces were found *E. multilocularis* positive (Wahlström *et al.*, 2015).

During the completion of this thesis project, a second nation-wide monitoring was performed between 2012 and 2014. Within this monitoring, nearly 3000 fox feces collected by hunters as well as ~20-30 samples collected as part of this thesis work were analyzed by the newly developed magnetic capture PCR (MC-PCR) technique for detecting *E. multilocularis* DNA in fox feces (National Veterinary Institute, 2016; Isaksson *et al.*, 2014). Only three feces were found to be *E. multilocularis* positive (National Veterinary Institute, 2016). One of these feces was a positive collected within this thesis work near Katrineholm (Paper III). Another feces was found the region of Gnesta/Nyköping, where the first rodent finding was reported earlier as part of this thesis work in 2013 (Paper I). The final feces was found near Uddevalla.

These monitoring efforts are summarized in Table 1. Overall, the estimated prevalence of *E. multilocularis* in foxes is very low (<0.1%) nation-wide and slightly higher regionally (0.8%) (National Veterinary Institute, 2016; Wahlström *et al.*, 2015). Still, as a result of these findings Sweden lost its *E. multilocularis* disease free status and the consent to require deworming of incoming dogs (Wahlström *et al.*, 2015).

Despite the intensity of these monitoring efforts, very few positives were found and individual positive foxes and fox feces were generally found 100s of kilometers apart (Figure 4). In addition, very little attention was given to the rodent intermediate host. Following the first fox finding in 2011, only 236 rodents, mainly water voles, captured near Uddevalla were examined for *E. multilocularis* and found negative (Wahlström *et al.*, 2012). As such, very little was known about the local presence and transmission dynamics of the parasite

Table 1. Summary of major investigations undertaken in Sweden to examine for *Echinococcus multilocularis* in red foxes *(Vulpes vulpes*) and in rodents. (Used with permission from Miller *et al*., (In Press))

	Investigation	Duration	Species/sample	n	Pos. (%)	Year	Place of positive finding	Reference***
SVA								
	Yearly monitoring	2000-2010	Fox intestines	3,266	1 (<0.01)	2010	U	Osterman Lind et al. [3]
	First nation-wide screening after positive finding	2011	Fox intestines	2,985	3 (0.1)	2011	B, K, U	Wahlström et al. [4]
	Regional survey[a]	2011	Rodent livers	236	0 (0)	2011		Wahlström et al. [4]
	Regional survey[b]	2011	Fox feces[c]	790	6 (0.8)	2011	K	Wahlström et al. [38]
	Second nation-wide screening	2012-2014	Fox feces[c]	2,779	3 (0.1)	2012–2014	G/N, K, U	National Veterinary Institute, www.sva.se [39]
SLU								
	EMIRO project[d]	2013-2015	Rodent livers	1,566	9 (0.6)	2013–2015	G/N, K	Miller et al. [5]
		2013-2015	Fox feces[c]	714	41 (5.7)	2013–2015	G/N, K, U, V/V	This paper

Abbreviations: *n* total samples; *Pos. (%)* number and percent positive; *SVA* National Veterinary Institute; *SLU* Swedish University of Agricultural Sciences; *EMIRO Echinococcus Multilocularis* in ROdents-this research project; *B* Borlänge; *K* Katrineholm; *G/N* Gnesta/Nyköping; *U* Uddevalla; *V/V* Vetlanda/Växjö

[a]Samples collected near Uddevalla

[b]Samples collected from a localized region (50km diameter) near Katrineholm

[c]Feces collected from environment

[d]Samples collected from four regions (10x10km or 20x20km) in Sweden

***Reference numbers correspond to years [3=2011, 4=2012, 38=2015, 39=2016, 5=2016]

in Sweden at the beginning of this thesis work in 2013, and, most importantly, the rodent intermediate host was yet unknown.

2.3.2 The EMIRO Project

This thesis was performed within the framework of an European wide EMIDA/ERA net funded project by Formas (www.formas.se) entitled ***E**chinococcus **M**ultilocularis* in **RO**dents (EMIRO) (Figure 5). This project was a collaboration between five different countries (Denmark, Finland, Lithuania, Switzerland, and Sweden). The main work for this project was completed between 2012 and 2015. The overall goal of this project was to describe and compare the role of the rodent in the lifecycle of *E. multilocularis* in both high and low endemic regions in different European countries. To do this, field investigations were performed in Lithuania and Switzerland (high endemic) as well as in Sweden (low endemic). Two workshops were performed early in the project to harmonize both field and laboratory techniques between these countries and, thus, streamline results for later comparison. Field results were supported by laboratory experiments investigating infection dynamics in wild rodent intermediate hosts in Denmark.

Figure 5. EMIRO logo. Designed by Diogo Guerra for use by the EMIRO Project.

This thesis is a summary of the work performed within Sweden. At the time of writing, finalized results are pending from some countries within the EMIRO project.

2.3.3 Definitive Hosts in Sweden

To date, *E. multilocularis* has only been identified in the red fox in Sweden. Although other potential definitive hosts (e.g. raccoon dogs, e.g. wolves) have been examined, none have been found positive (Osterman Lind *et al.*, 2011; Wahlström *et al.*, 2011). Taeniid eggs have been identified in arctic fox (*Vulpes lagopus*) feces from northern Sweden, but these eggs were not identified to species (Meijer *et al.*, 2011). Furthermore, population numbers of the red fox likely far outnumber that other wild canids in Sweden, as these species are highly managed for conservation (arctic fox, wolves) (Dalén *et al.*, 2006; Wabakken *et al.*, 2001) or hunted for eradication (raccoon dogs) (Mårhundprojektet, https://jagareforbundet.se/vilt/Mardhundsprojektet/). In 2011, 119 hunting dogs were examined in the area near the finding of the first positive red fox in Uddevalla; all were negative (Wahlström *et al.*, 2012). Similarly, no dogs (n=16)

examined from the Uddevalla and Katrineholm study regions in this thesis were found positive for *E. multilocularis* (data not shown). Therefore, for Sweden, as in central Europe, the red fox is the most important definitive host for *E. multilocularis* transmission.

The red fox is a generalist predator which inhabits a variety of habitats. Rodents, particularly field voles (*M. agrestis*), have been shown to be a significant proportion of the fox diet in Fenno-Scandinavia (as elsewhere) (e.g. Dell'Arte *et al.*, 2007; Lindström, 1989). In times of low rodent densities, the red fox may even switch to other more abundant non-rodent prey, such as roe deer fawns (Kjellander & Nordström, 2003). However, the relative proportion of rodents in the diet may change according to changes in vole densities (Dell'Arte *et al.*, 2007). This may be explained in part by optimal foraging theory (reviewed in Pyke, 1984), which is based on the idea that an animal will choose food resources that are energetically most beneficial (e.g. abundant and easy to catch). In reality, fox feeding patterns can be more complex, particularly when multiple species are considered (Raoul *et al.*, 2010). Still, understanding the different feeding behaviors of the fox may help explain the variability observed in *E. multilocularis* transmission. For instance, Saitoh and Takahashi (1998) found that, in some regions of Japan, prevalence of *E. multilocularis* infected foxes varied according to both the density of the main intermediate host, the gray-sided vole (*Clethrionomys (Myodes) rufocanus*) and the degree of fox predation.

2.3.4 Proposed Rodent Intermediate Hosts in Sweden

At the beginning of this thesis work, the rodent intermediate host was still unknown in Sweden. Rodent species that were present in the country and that could be considered included the water vole (*A. amphibius*), the field vole (*Microtus agrestis*), the bank vole (*Myodes glareolus*), the yellow necked mouse (*Apodemus flavicollis*) and the wood mouse (*Apodemus sylvaticus*) (Wilson & Reeder, 2005). Of these, the water vole, field vole, and bank vole were hypothesized to be the most likely to fulfill the role of intermediate host in Sweden based on observed prevalence of these species (or their close relatives) in central Europe (see Section 2.1.1).

One of the most commonly reported rodent intermediate hosts in central Europe, the common vole (*M. arvalis*), is not present in Sweden (Wilson & Reeder, 2005). The most closely related species present is the field vole (*M. agrestis*). In south-central Sweden, the field vole occupies open field, agricultural, and regenerating (e.g. clear-cut) forests (Hansson, 1977; Hansson, 1968) and has been shown to be common prey of foxes (Lindström, 1982). Although the field vole is susceptible to *E. multilocularis* (Woolsey *et al.*,

2015a), prevalence in this species in central Europe is difficult to determine as most studies report findings as *Microtus* spp.

The other most commonly reported rodent intermediate host in central Europe is the water vole (*Arvicola terrestris*). Recently, this species has been reclassified into two separate species based on ecology and genetics—the semi-aquatic *A. amphibius* and the fossorial *A. scherman* (Wilson & Reeder, 2005). *Arvicola scherman* is a smaller vole which burrows in grasslands of higher elevation of in central Europe, while *A. amphibius* is a larger vole normally associated with water and distribute across most of mainland Europe and Scandinavia (Piras et al., 2012; Wilson & Reeder, 2005). Of these two, only *A. amphibius* exists in Sweden; however, very little is known about its ecology in this country. There is little mention of water voles in Swedish diet studies, except for Englund (1965) which reported water vole presence in the stomach of foxes from southern half of the country. Until recent years, most studies reported water voles as *A. terrestris*, making it difficult to know what the prevalence of *E. multilocularis* is specifically for *A. amphibius* in central Europe. However, recent studies (e.g. Raoul et al., 2015) indicate that most reports (at least for France and Switzerland) have been for *A. scherman*.

As discussed in Section 2.1.1, although susceptible, bank voles are considered of lesser importance than *Microtus* spp. and water voles for *E. multilocularis* transmission (Romig et al., 2006; Stieger et al., 2002). Bank voles are typically found in forest and shrubland, but will disperse into bordering field or regenerating forested areas (Hansson, 1979; Hansson, 1968). Bank voles are not considered main prey of foxes in southern Sweden (Lindström, 1982).

Sylvan mice (*Apodemus* spp.) were considered to be the least likely of the species present to host *E. multilocularis* (see Section 2.1.1). Both *Apodemus* species present in these study regions prefer forested habitat (Bergstedt, 1965). However, the yellow-necked mouse (*A. flavicollis*) is more likely to restrict populations to forest, whereas the wood mouse (*A. sylvaticus*) has been shown to also inhabit edge habitat or fields in low densities (Hansson, 1968; Bergstedt, 1965). Furthermore, these species do not appear to be heavily preyed upon by foxes in Sweden (Erlinge et al., 1983).

2.4 Other Taeniid Parasites

2.4.1 As a Proxy

The family *Taeniidae* is composed of several genera, such as *Echinococcus*, *Taenia*, *Versteria*, and *Hydatigera* (Nakao *et al.*, 2013). Members of this family have an indirect lifecycle generally between a carnivore definitive host (e.g. canids, felids) and an herbivore intermediate host (e.g. rodent, lagomorph,

ungulate) (Deplazes *et al.*, 2016). In an area, such as Sweden, where *E. multilocularis* is relatively rare, transmission dynamics for other related taeniid species may provide a representative model (i.e. proxy) for understanding *E. multilocularis* transmission.

This concept was also used in a study by Al-Sabi *et al.* (2013b) which, similar to the investigations in this thesis, examined liver taeniid parasites in rodents in a low endemic area for *E. multilocularis* (Denmark). Although no *E. multilocularis* infections were detected, the high prevalence of *V. mustelae* and *T. polyacantha* in both urban and rural forests together with prior knowledge of location of taeniid infected foxes led these authors to conclude that risk for *E. multilocularis* risk was higher in forested areas as compared to residential and farm gardens. However, it should be noted that few field voles and water voles (n=37), the species most likely to host *E. multilocularis* (Section 2.3.3), were examined as compared to bank voles (n=403) (Al-Sabi *et al.*, 2013b).

2.4.2 Other Parasites

Recent molecular studies have classified *Versteria* (formerly *Taenia*) *mustelae* as most closely related *Echinococcus* (Nakao *et al.*, 2013; Knapp *et al.*, 2011). In contrast to *E. multilocularis*, the definitive hosts for *V. mustelae* are typically mustelids (Iwaki *et al.*, 1996; Hoberg *et al.*, 1990). In Sweden, this may include the least weasel (*Mustela nivalis*) and the stoat (*Mustela erminea*) (Bang *et al.*, 2001; Iwaki *et al.*, 1995; Hoberg *et al.*, 1990). Metacestodes (cysticerci) of this parasite are usually multiple and occur in the liver of the intermediate host (Freeman, 1956) (Figure 6). *Versteria mustelae* is commonly reported in *M. glareolus* (Behnke *et al.*, 2008; Pétavy *et al.*, 2003; Le Pesteur *et al.*, 1992), but is also reported in other voles such as *M. agrestis* (Soveri *et al.*, 2000) and *A. terrestris* (Chechulin *et al.*, 2010). An experimental study showed limited development of *V. mustelae* in laboratory mice (Iwaki *et al.*, 1996).

Figure 6. Liver from a bank vole with three visible cystercerci (white arrows) of *V. mustelae* (2-3mm diameter) Photo: Andrea Miller

Hydatigera (formerly *Taenia*) *taeniaeformis* most commonly occurs in felids (e.g. domestic cat), but also in the red fox (Deplazes *et al.*, 2016; Saeed *et al.*,

2006). Like several other taeniids, the metacestodes (strobilocerci) develop in the liver. Mestacestodes can be one or many, and mature metacestodes contain a characteristic larvae (strobilocercus fasciolaris) (Deplazes *et al.*, 2016) (Figure 7). The intermediate hosts for *H. taeniaeformis* are broad and include both voles (Burlet *et al.*, 2011; Fichet-Calvet *et al.*, 2003; Tenora *et al.*, 1979) and mice (Montgomery & Montgomery, 1988).

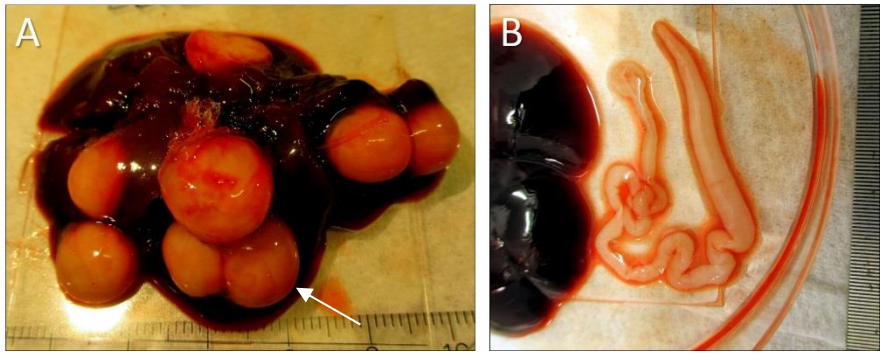

Figure 7. A) Liver from a water vole containing multiple strobilocerci (9-12mm diameter) (white arrow) of *H. taeniaeformis*. B) Strobilocercus fasciolaris extracted from a strobilocercus of *H. taeniaeformis* found in a water vole liver. Ruler seen to the right of the picture is in millimetres. Photos: Andrea Miller

Taenia polyacantha most commonly occurs in foxes (both red and arctic) but it can also infect other canids (Deplazes *et al.*, 2016; Rausch & Fay, 1988b). The experimental study of Rausch and Fay (1988a) noted early development of *T. polyacantha* metacestodes in the liver of *M. oeconomus* which later migrated to become free-floating in the abdominal cavity (Figure 8). However, an experimental study by *Myodes* (formerly *Clethrionomys*) *rufocanus bedfordiae* found that early development occurs in the intestinal wall and that then larvae migrate to the abdominal cavity but rarely to the liver. This suggests that development varies between species. *Taenia polyacantha* is commonly reported in voles, in particular bank voles (Haukisalmi & Henttonen, 1993; Wiger *et al.*, 1974), but rarely in mice (Goüy de Bellocq *et al.*, 2003; Ihama *et al.*, 2000).

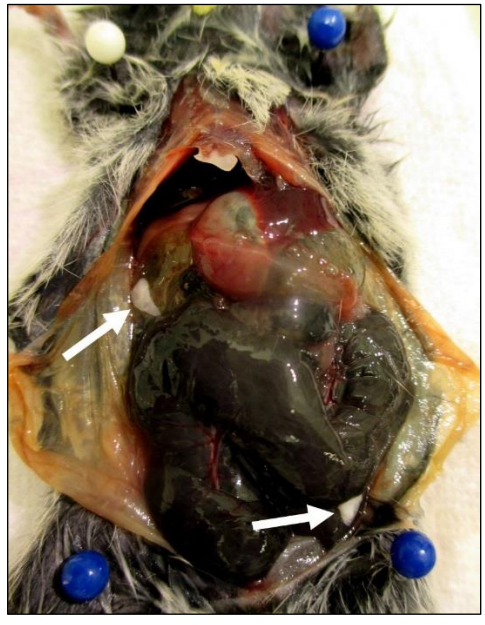

Figure 8. Dissected bank vole specimen showing an open abdomen. For orientation the head is to the top of the picture and tail is to the bottom. Free-floating metacestodes of *T. polyacantha* (3-4mm in length) in the abdominal cavity indicated by white arrows. Photo: Andrea Miller

Mesocestoides spp are in the same order (*Cyclophyllida*) as *Taenidae*, but are in a separate family, *Mesocestoididae* (Deplazes *et al.*, 2016). The lifecycle of these parasites are poorly understood, but are thought to involve two intermediate hosts. The definitive hosts are varied but includes canids and mustelids (Deplazes *et al.*, 2016). In Europe, the red fox is a common definitive host for *Mesocestoides* spp, particularly *M. litteratus* (Al-Sabi *et al.*, 2013a; Hrčkova *et al.*, 2011). The first intermediate host is thought to be an invertebrate (e.g. orbatid mite), but this is still under debate (Deplazes *et al.*, 2016; Loos-Frank, 1991). The second intermediate host is a variety of small vertebrates, including rodents (Deplazes *et al.*, 2016). Loos-Frank (1980) suggested that *Mesocestoides* spp are more commonly reported in bank voles and *Apodemus* species than common voles due to the fact that bank voles and *Apodemus* are more likely to include insects in their diet than common voles. Larvae of *Mesocestoides* spp. (tetrathyridia) are most commonly found free-floating in the abdomen of rodent hosts, but can occasionally invade other organs (Deplazes et al., 2016) (Figure 9). *Mesocestoides* spp are included as part of this thesis work (Paper II), because, similar to *E. multilocularis*, the lifecycle includes at least a partial fox-rodent transmission pattern and the larval stage can be present in the liver.

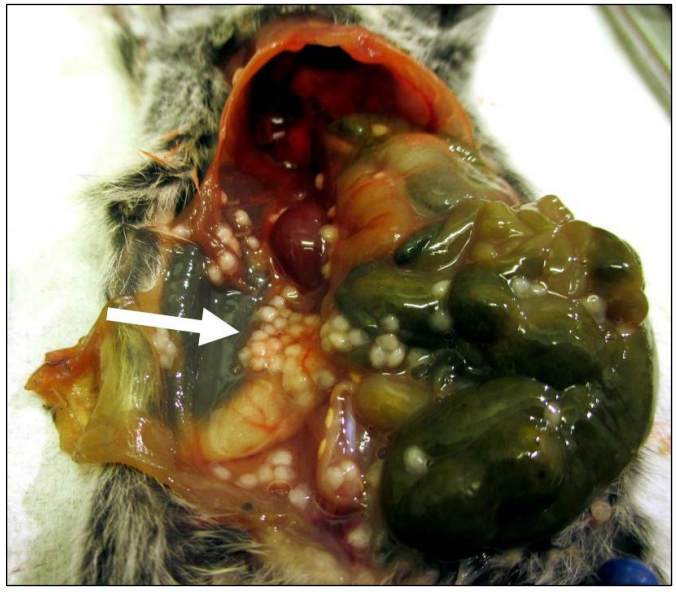

Figure 9. Dissected bank vole specimen showing an open abdomen. For orientation the head is to the top of the picture and tail is to the bottom. Intestines are pulled to the right. Free-floating tetrathryidia of *Mesocestoides* spp. (2-3 mm in length) in the abdominal cavity indicated by white arrow. Photo: Andrea Miller

3 Aims of the Thesis

The overall aim of this thesis was to investigate the role of the rodent intermediate host for the *E. multilocularis* lifecycle in a low endemic environment (Sweden) and to use this knowledge to make suggestions for future monitoring.

The specific aims were as follows:

- To identify rodent intermediate host(s) for *E. multilocularis* in Sweden
- To describe *E. multilocularis* infections within the individual rodent intermediate hosts and to describe the prevalence for each rodent species
- To describe the prevalence of other taeniid parasites within the livers of rodent intermediate hosts and to relate these findings to *E. multilocularis* transmission
- To investigate the parasite background (environmental) contamination from fox feces within rodent habitat and to relate this to *E. multilocularis* and other taeniid parasite transmission
- To use both rodent and fox feces results to comment on methods for future monitoring of *E. multilocularis* in Sweden

4 Materials and Methods

Decisions for field study design, sample collection methods, and sample analysis in this thesis were based initially on the framework outlined within the EMIRO project and then adjusted for use within Sweden and within the aims/limits of this thesis. Analysis of samples collected from fieldwork, 2013-2015, formed the database used for Papers I-III.

4.1 Study Regions (Papers I-III)

At the beginning of this project (2013), very little was known about *E. multilocularis* presence in Sweden. Study regions were chosen based mostly on results from the first nation-wide monitoring for *E. multilocularis* in hunter-shot foxes (2011) (Wahlström et al., 2012), but also on the regional study performed near Katrineholm (Wahlström et al., 2015) (Table 1, Figure 4). From these results, it appeared that most positives were identified in the southern half of the country and near the municipalities of Uddevalla and Katrineholm. Therefore, to implement the EMIRO design and optimize the possibility of finding positive samples, two study regions (10x10km) were chosen near Katrineholm and Uddevalla.

To investigate regions with an unknown status, two additional regions (20x20km) were chosen near the municipalities of Gnesta/Nyköping and Vetlanda/Växjö (Figure 4). This was done as part of a collaboration with Sweden's National Environmental and Wildlife Monitoring and Assessment program (FoMA, http://www.slu.se/en/environment). Since 2012, rodents in these regions have been collected and examined for presence of viral and bacterial zoonotic diseases.

4.2 Rodent Trapping (Papers I-II)

Rodent trapping was performed 2013-2015 under ethical permits from the Swedish Environmental Protection Agency (NV-02939-11) and the Swedish Board of Agriculture (A-135-12). Trapping design is described in detail in Paper I. Some additional details can be found in Paper II and III (in reference to targeted/risk-based sampling). Trapping was performed in the spring and autumn to capture the seasonal variation in rodent populations. Rodent populations were assumed to be at their lowest in the spring and the highest in autumn (Haukisalmi *et al*., 1988; Myllymäki, 1977b; Bergstedt, 1965). Sampling continued only for the FoMA regions (Gnesta/Nykjöping and Vetlanda/Växjö) spring 2015 for logistical reasons.

4.2.1 Snap Trapping (Papers I-II)

Two trapping designs, the small quadrat method and the line transect, were considered for rodent collections (specifically, field voles, bank voles, and mice). As described in Myllymäki *et al.* (1971), the small quadrat method is designed to encompass the home range of the resident rodents within a defined habitat. Thus, it is can be used to estimate relative rodent densities (Hansson, 1972) and has been used extensively in Scandinavia for both short and long term rodent population monitoring (e.g. Oksanen & Oksanen, 1992; Christiansen, 1983; Myllymäki *et al.*, 1971). In contrast, line transects cross multiple habitats and potentially sample multiple rodent home ranges. In comparison to grid methods, such as the small quadrat, line transects have been proposed to increase sample numbers and provide a better representation of microhabitat differences (Pearson & Ruggiero, 2003). However, accurate density estimates may involve additional trapping (Hansson, 1967). Furthermore, line transect design is highly variable (Hansson, 1972; Hansson, 1967).

According to EMIRO discussions, the small quadrat design was chosen over the line transect due to the interest in obtaining comparable rodent densities for specific habitats in each country. Furthermore, in the interest of Swedish sampling, results from quadrats using snap traps were then comparable to FoMA sites and potentially other projects in Scandinavia in the future. Snap trap sites consisted of multiple quadrats (2-4) in an effort to obtain a sample representative for the habitat of that area. In addition, quadrats were placed at least 50m apart to lessen the likelihood of sampling rodents from the same home range (Erlinge *et al.*, 1990; Mironov, 1990; Hansson, 1969) (see also Figure 11). As indicated in (Myllymäki *et al.*, 1971), catches for the first two nights are highest and are most likely to capture resident breeding animals. Therefore, it was also decided within the EMIRO project to trap for two trap nights.

4.2.2 Topcat Trapping (Papers I-II)

Water voles (primarily) and field voles were trapped using topcat (Andermatt Biocontrol AC, Grossdietwil, Switzerland) traps. Topcat traps do not require bait, but must be set into water vole tunnels. Therefore, these traps could only be used in fields where clear signs (e.g. tunnels, tumuli) of water voles were present (Figure 10). Although permanent sites were chosen for topcat trapping, the actual location of the traps changed based on the movements of the voles. Occasionally, anthropogenic influences (plowing) eliminated the ability to trap entirely. This was less commonly noted for snap trap sites.

In contrast to the small quadrat method, topcat traps are set in an unsystematic manner. Therefore, these traps can not be used to create density estimates for water voles. In France, methods for estimating densities of both *Microtus* spp.

and *Arvicola scherman* based on observed signs (tunnels, runways, tumuli) (Figure 11) has been developed (Giraudoux *et al.*, 1995; Quéré *et al.*, 2000). However, these methods have not been validated for the habitat in Sweden. In addition, *Arvicola scherman* has a differing ecology than *Arvicola amphibius* (see Section 2.3.3). Furthermore, Hansson (1979) found that occurrence of grass runways was not well correlated with *M. agrestis* abundance in southern Sweden, possibly due to the permanence of tunnels and holes even after the rodents had migrated from the area. Because of the difference in traps and lack of a validated surface index method, rodent density estimates could not be calculated within this project.

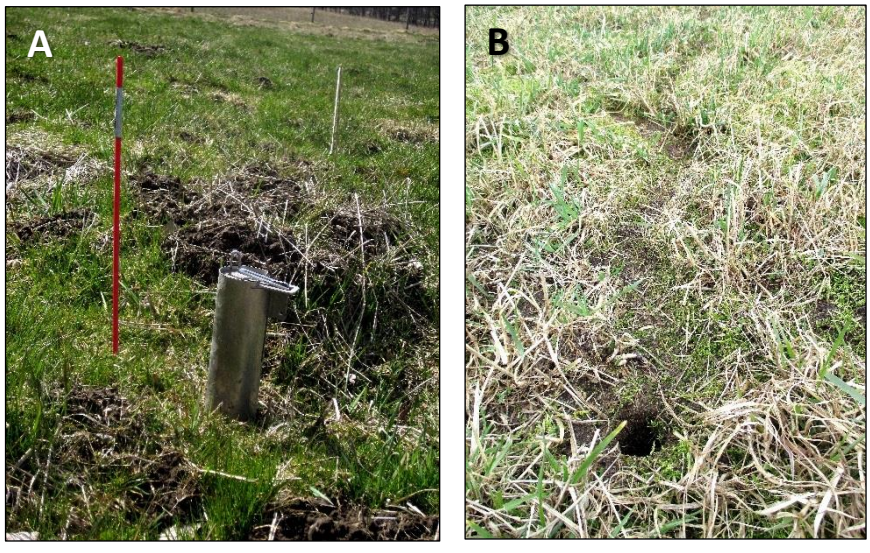

Figure 10. Examples of water and field vole signs. A) Topcat trop placed between mounds of dirt indicative of water vole tumuli. B) Grass trail of either field or water voles leading to hole in the ground. Photos: Andrea Miller

4.2.3 Rodent Trapping Site Placement (Papers I-III)

In Paper II a **rodent trapping site** is defined as either a set of 2-4 small quadrats (i.e. snap trap site) (Figure 11) or a collection of topcat traps (i.e. water vole field). The placement of these traps are described in detail in Paper I. Please note corrections made here to the sizes of the EMIRO study regions (i.e. 10x10) and fox home ranges (i.e. 2x2) from those reported in Paper 1.

The placement of rodent trapping sites in the 10x10km study regions of Uddevalla and Katrineholm was guided according to the EMIRO design (Figure 12). The purpose of the EMIRO field studies was to investigate the transmission ecology of *E. multilocularis* at the localized level of the rodent intermediate host

(i.e. micro-foci). Therefore, the size of the larger study region was of less importance than the placement of the rodent trapping sites. To help guide trap placement within the larger study regions, smaller areas representative of a fox home range for each country were chosen. Rodent trapping sites within these smaller regions were assumed to represent feeding opportunities for one fox. In Sweden, 2x2 km areas encompassing both field and forest habitat were designated based on estimates of fox home ranges in southern Sweden (e.g. von Schantz, 1981).

Within the 20x20 km regions of Gnesta/Nyköping and Vetlanda/Växjö, the placement of snap trap sites was guided by sampling points previously defined for the FoMA monitoring design. FoMA monitoring is spaced according to the the Swedish National Grid (1 km x 1 km). Whenever possible, topcat traps were set in fields near snap trap sites, and other fields observed with signs of water vole activity.

Regardless of EMIRO or FoMA rodent trapping design, final placement of trapping sites was based on the criteria specifically outlined in Paper III and listed below. Emphasis was placed on selected habitats known to be suitable for the targeted rodent species. In addition, to increase the likelihood of catches and diversity of species obtained, rodent trapping areas were placed on or near ecotones (Lidicker Jr, 1999). As described in Paper II rodent trapping sites were broadly classified as "field", "mix", or "forest" based on vegetation type.

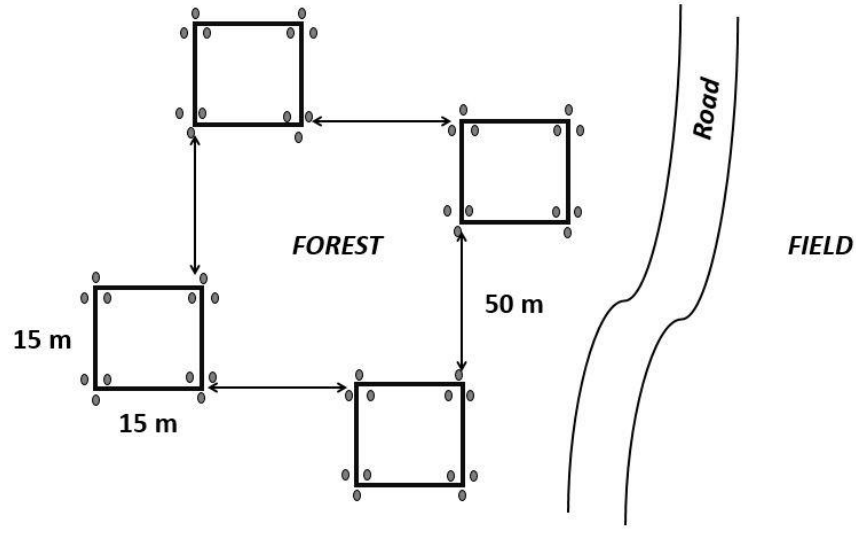

Figure 11. Example of rodent trapping sites. Two to four quadrats (15x15m) are positioned at least 50m apart in a habitat (forest). Three snap traps (small gray ovals) are placed at the corners of each quadrat. Distances not to scale.

Figure 12. Example of EMIRO rodent trapping design. The large square outline (10x10km) shows the Katrineholm study region. The smaller squares outlines (2x2km) were designed after the idea of fox home ranges and were used to guide placement of rodent trap sites. Approximate location of snap trap sites (solid white square) and water vole/field vole trap sites (solid white triangle) are shown. Scale to 2 km. Map created in QGIS v2.12.3 with a background satellite image (WMS ortofoto årsvis 2015, SWEREF99, © Lantmäteriet).

4.3 Fox Fecal Collections (Papers II-III)

Methods for fecal collection and the definition of a fecal collection site are specifically outlined in Paper III. As discussed in Paper II, the purpose of fecal collection was to estimate parasite background contamination from fox feces in rodent habitats. This concept was also used by Stieger *et al.* (2002) to compare *E. multilocularis* presence between three different zones surrounding Zürich, Switzerland and to relate these percentages to infected rodents trapped in these same zones. Although fox fecal analysis can be used to estimate the parasite prevalence in the fox population of an area, collections must occur on a much broader scale than was performed for this thesis to avoid collecting feces from the same individual fox (Raoul *et al.*, 2001). Therefore, results for fecal analysis

performed in this manner should be considered rather as an "index of contamination" (Conraths & Deplazes, 2015).

Knowledge of fox movements and marking behavior were used to optimize fox fecal collection efforts. For instance, studies have observed a predilection for foxes to mark edges (Giraudoux *et al.*, 2002), carrion sites (Goszczyński, 1990) and the tops of water vole tumuli (Stieger *et al.*, 2002) (Figure 13). A description and an example of the search pattern used is provided in Paper III. Although molecular techniques have been shown to be a more accurate form of fecal identification (Monterroso *et al.*, 2013, Knapp *et al.*, 2016), feces collected for this thesis were identified as fox based on morphology and environmental location. While this is commonly done within *E. multilocularis* studies (e.g. (Stieger *et al.*, 2002, Robardet *et al.*, 2011, Guislain *et al.*, 2007), it is important to remember that estimates for species specific background contamination (or fecal densities) could be somewhat underestimated due to misidentified species.

Figure 13. Fox feces (white shapes) on top of a water vole mound. Photo: Andrea Miller

Fox fecal samples are less subject to degradation in the winter months when rainfall is typically less, temperatures are low, and snow may be present (Cavallini, 1994, Lucchini *et al.*, 2002). Shorter vegetation height also increases the visibility of deposited feces. In addition, some studies have observed higher numbers of infected foxes in autumn months and have suggested that, due to the ~3 month patent period (Kapel *et al.*, 2006), infected feces could be continued to be deposited into the winter months (Hegglin *et al.*, 2007, Stieger *et al.*, 2002). For these reasons, two winter fecal collections were performed in addition to

regular collections during rodent trapping. Due to time constraints associated with the logistics of trapping, winter collections also allowed for more focused fecal sampling.

In Paper III, a fox fecal collection site is specifically defined as an area where at least one fox feces was collected within 500-600m of a rodent trapping site. A fecal collection site could contain more than one rodent trapping site, but was usually limited by habitat. That is, search efforts, and thus collections sites, were usually were restricted to either the field or forest (but could occasionally incorporate a rodent trapping site set on a mix of these two habitats). As such, feces were identified as being collected in "field habitat", "field/forest edge" or "forest habitat".

4.4 Laboratory Methods

4.4.1 Rodent Dissection (Papers I, II)

Rodent dissection methods are outlined in Paper I with some supporting details concerning breeding status in Paper II. Particular focus was put on liver examination as *E. multilocularis* has a predilection for this organ in the rodent intermediate host (Eckert, 1998). In addition to macroscopic examination, livers were held over a strong light and palpated to search for parasitic lesions within the liver parenchyma (Figure 14). Still, it was accepted that early infections (lesions <1mm) may have been missed. Similarly, although intestines were removed and the abdominal cavity investigated, early or low intensity infections for *Mesocestoides* spp. and *T. polyacantha* within the abdomen may have been missed.

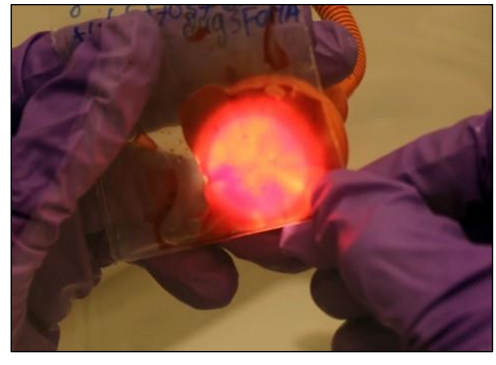

Figure 14. Examining a liver over a strong light. Photo: Andrea Miller

Although the functional group was identified for *E. multilocularis* positive rodents in Paper I, this was not performed for all rodents in Paper II. Rodent functional groups refer to the age cohort, size, and breeding status of an individual within a community of rodents (Haukisalmi *et al.*, 1988, Myllymäki, 1977a). For instance, subadult *M. agrestis* (i.e. those born later in the year) experience reduced growth and opportunity to breed (Myllymäki, 1977a). Parasite prevalence within these cohorts has been shown to significantly differ.

In particular, over-wintered (breeding) animals are often more likely to be parasitized (Haukisalmi *et al.*, 1988, Haukisalmi and Henttonen, 1993). However, the purpose of Paper II was to examine the parasitization patterns across species. Therefore, these cohort differences (with the exception of breeding status) were largely ignored.

Due to logistical constraint, only *Apodemus* spp from 2014 were examined. It was decided to dissect fewer *Apodemus* spp as these species were considered the least likely to host *E. multilocularis* (Stieger *et al.*, 2002; Barabási *et al.*, 2011). Still, *Apodemus* spp. from 2014 were chosen as that year had the largest sample of these rodents.

4.4.2 Fecal Egg Isolation (Papers II, III)

Fecal analysis is specifically described in Paper III. For both Paper II and III, only results for feces collected within fecal collection sites (Section 4.3) are reported.

To analyze feces, both coproantigen ELISA and PCR methods for extracted eggs were considered. Fecal analysis for environmental contamination is often performed using the copro-antigen ELISA test (Raoul *et al.*, 2001, Stieger *et al.*, 2002). This method has the advantage of detecting both patent and pre-patent infections and is less labor intensive than fecal egg extraction (Deplazes *et al.*, 1999, Mathis *et al.*, 1996). However, the specificity of the copro-antigen ELISA is lower than PCR methods (Conraths & Deplazes, 2015). As the interest of the EMIRO project was to assess level of parasite egg contamination in rodent habitats, prepatent infections were of less interest. Furthermore, for Sweden, where the parasite was newly identified and any new findings were reportable, a method with high specificity was needed. Therefore, PCR methods, which have specificities of nearly 100%, were preferable (Conraths & Deplazes, 2015). In addition, egg isolation allowed identification of multiple parasite species (Mathis *et al.*, 1996, Trachsel *et al.*, 2007).

4.5 Parasite Identification (Papers I-III)

4.5.1 Morphologic and Histologic Methods (Paper I-II)

In Paper I, *E. multilocularis* lesions in rodent livers were described grossly and histologically. The combination of these findings in addition to molecular methods (Section 4.5.2) were used to confirm *E. multilocularis* diagnosis. Furthermore, to assess the infectivity of the rodents, metacestode lesions were examined for presence of protoscolices through microscopic examination of metacestode fluid, histologic sections, or both. In Paper II mature *H. taeniaeformis* lesions were identified by extracting the stobilocercus fasciolaris (Figure 7B).

4.5.2 Molecular Methods (Papers I-III)

Specific methods for DNA extraction, PCR, and sequencing are detailed in Papers I-III. Nomenclature in those papers was based on Nakao *et al.* (2013) and Lavikainen *et al.* (2016).

Except for mature *H. taeniaeformis* lesions (Paper II), all parasitic lesions from rodents (Paper I and Paper II) were tested using the multiplex PCR described in Trachsel *et al.* (2007). This PCR method identifies both *E. multilocularis* and *E. granulosus* specifically, but other taeniids only to genus (*Taenia*). In addition, the primers used for *Taenia* spp. also detect *Mesocestoides* spp. (Trachsel *et al.*, 2007).

Although the multiplex PCR in Trachsel *et al.* (2007) was originally designed for testing fecal parasite eggs, it has also been used for identification of liver parasites (Beiromvand *et al.*, 2013). Similar to Beiromvand *et al.* (2013), samples negative for *E. multilocularis* in the multiplex PCR were also tested using an additional single PCR specific for *E. multilocularis* (Stieger *et al.*, 2002) (Paper I). In Paper III, fecal parasite eggs were only tested using the multiplex PCR (Trachsel *et al.*, 2007).

Sequencing for both parasite lesions and for parasite eggs was performed for diagnostic purposes only. For *E. multilocularis*, sequencing only provided further confirmation of morphologic (Paper I) and PCR results (Paper I, III). For other taeniids and *Mesocestoides* spp., sequencing was needed for identification to species or genus level (Paper II, III).

4.6 Statistical Analyses (Papers II-III)

The statistical analyses performed are detailed in Paper II and III.

In Paper II, a multiple logistic regression model was used to model the relationship between multiple variables and proportion of rodents parasitized. Logistic regression models are particularly useful for multiple explanatory variables, both continuous and categorical, and for a binomial response variable (Hosmer *et al.*, 2013). In addition, the results of this model can be used to calculate odds ratios (Hosmer *et al.*, 2013). Odds ratios are a calculated measure of risk for exposure to, in this case, taeniid parasites (Giesecke, 2002).

Multivariable modeling was considered for Paper III. However, as discussed in Paper III, preliminary analysis demonstrated that the unbalanced dataset produced poorly fitted models. Resulting p-values were, therefore, highly uncertain. For these reasons, complex modeling was abandoned and proportions were compared using only the Fisher's exact test of independence with a Bonferroni correction for multiple comparisons when appropriate (McDonald, 2014).

5 Results and Discussion

The results of this thesis are based on rodents collected (n=1566) and examined for taeniid larval cestodes from over five trapping seasons and fox feces (n=714) collected during seven collection periods from four different regions of Sweden, 2013-2015. These results are discussed in detail in Papers I-III in relation to the importance of the rodent intermediate hosts and implications for future monitoring of this parasite in Sweden. Highlights from these results and discussions are summarized below.

5.1 Role of Rodents for *E. multilocularis* Transmission in Sweden

5.1.1 The Importance of Field Voles (*M. agrestis*) and Water Voles (*A. amphibius*)

In Paper I, *E. multilocularis* was identified for the first time in Sweden in eight water voles (n=439) and one field vole (n=187). It was not identified in any of the bank voles (n=655) or mice (n=285) examined (Paper I). These results were not surprising given that the most common rodent intermediate hosts in central Europe are the closely related common vole (*M. arvalis*) and water vole (*A. scherman)* (Raoul *et al.*, 2015). Furthermore, as described in the introduction, the prevalence of *E. multilocularis* is usually low in bank voles (*M. glareolus*) (Romig *et al.*, 2006; Stieger *et al.*, 2002) and only very rarely reported in mice (*Apodemus* spp.) (Barabási *et al.*, 2011; Stieger *et al.*, 2002).

The results of Paper I indicated that field voles and water voles were important for *E. multilocularis* transmission. The results of Paper II provided even stronger support for this conclusion. In addition to *E. multilocularis*, field voles and water voles were also found to be more highly parasitized with other larval taeniid cestodes than bank voles and mice (Figure 15). The ecology of the field and water vole, such as living in close, overlapping home ranges and lack of anti-predatory behavior (Henttonen, 1987), appear to predispose these species to predation by definitive hosts for both *E. multilocularis* and other taeniid species (foxes, cats, mustelids) (Liberg, 1984; Lindström, 1982; Erlinge, 1981) (Paper II). Focus of these predators in a small area increases fecal density, which, in turn, increases opportunities for parasite egg exposure (see Section 2.1.3). Together the results of Paper I and II suggest that field voles and water voles are rodent species at high risk for taeniid infections in Sweden and, in particular, *E. multilocularis*.

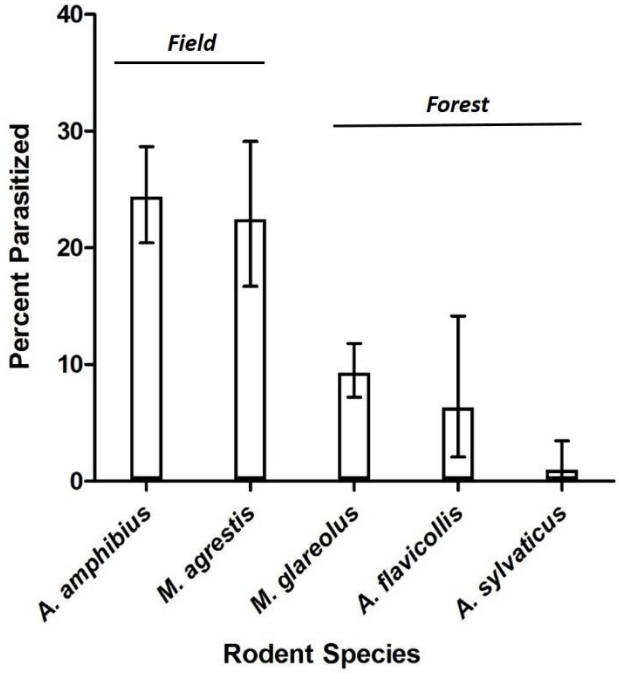

Figure 15. Number of parasitized rodents (in percent) for each species examined 2013-2015. Bars indicate binomial exact 95% confidence intervals. Rodent species found mostly in the field (water vole-*A. amphibius*; field vole-*M. agrestis*) and forest (bank vole-*M. glareolus*; mice-*Apodemus* spp.) are labelled. Mice species (*A. flavicollis, A. sylvaticus*) only examined for 2014.

5.1.2 Presence and Susceptibility of Rodents as a Limiting Factor

As indicated in Section (2.1.3), there are many factors which influence parasite transmission. Ultimately, however, both hosts need to be present for transmission to take place. Of the two hosts for *E. multilocularis*, the rodent intermediate host is the most spatially limited with home ranges of only hundreds of meters (Erlinge *et al*., 1990; Mironov, 1990). Therefore, transmission happens at a localized scale and, thus, may be strongly influenced by both rodent susceptibility and rodent ecology.

Sweden is missing one of the most important intermediate hosts in central Europe, the common vole (Wilson & Reeder, 2005). The closely related field vole is present and one individual field vole was found to be infected with an infectious metacestode (i.e. contained protoscolices) (Paper I). However, as discussed in Paper I, the relative importance of this species to *E. multilocularis* transmission in Sweden may also be limited. It has recently been suggested that metacestode development is reduced in the field vole as compared to the common vole (Woolsey *et al.*, 2015a). In addition, the results of Paper II and

other studies (Myllymäki, 1977b, Hansson, 1977) showed that the field vole is not strictly confined to the field habitat, which was the habitat found to have the highest likelihood for parasite infection in Paper II (see also Section 5.2.4). Still, as only one infected individual was found, further work is needed to make strong conclusions about the relative role of this species in the *E. multilocularis* lifecycle in Sweden.

Sweden is also missing one of the other most important intermediate hosts in central Europe, the water vole (*A. scherman*). The results of Paper I found infections in the closely related *A. amphibius* in Sweden. However, not all infected individuals (3/8) were considered infectious (i.e. contained protoscolices) (Paper I). Reasons for this may include immaturity of either the water vole or the metacestode (Burlet *et al.*, 2011), but may also be that water voles have a reduced susceptibility for metacestode development. Results from other field studies have also shown a low proportion of infectious metacestodes in water voles (e.g. 2/31 infected water voles in Reperant *et al.*, 2009 and 2/19 infected water voles in Hofer *et al.*, 2000). Experimental studies are needed to fully address the question of susceptibility of water voles and potential differences between these two water vole species. However, based on this discussion, it could be suggested that, of the two identified intermediate hosts in Sweden, the field vole may be the species with the highest susceptibility in Sweden.

As discussed in Section 2.3.3, the recent reclassification of *Arvicola terrestris* has made interpretation of previous literature relating water voles to *E. multilocularis* transmission difficult. While it seems that the role of A. *scherman* in the *E. multilocularis* lifecycle has been well established (e.g. review by Raoul *et al.*, 2015), less is known about role of *A. amphibius*. Another challenge for interpreting Swedish results is the lack of knowledge about the ecology of water voles in this country. Only one study (as part of a larger thesis) was found describing water vole ecology in Sweden (Jeppsson, 1990). This author found that populations of *A. amphibius* were split into two groups. One group had a permanent fossorial lifestyle (much like *A. scherman*) in fields near water sources. However, the other population lived much more above ground and migrated seasonally between field habitat and the banks of nearby water sources. Furthermore, peaks in population density like those noted for *A. scherman* e.g. (Duhamel *et al.*, 2000a; Viel *et al.*, 1999) were not reported (Jeppsson, 1990) and have, in general, not been reported for rodent species in southern or central Sweden (Hansson, 1989). A relatively stable population density, migration between habitats (which could be more or less contaminated with parasite eggs), and an above ground lifestyle may limit the role of water voles for *E.*

multilocularis transmission in Sweden. However, further research is needed to investigate these hypotheses.

A better understanding of species interactions between field and water voles could also provide some answers as to why *E. multilocularis* prevalence is so low in both rodents and foxes in Sweden. Although field voles were mostly captured above ground in snap traps (n=139), they could also be captured by topcats (n=48). However, this was most common in the spring (43/48, 90%). It may be that low water vole density in the spring allowed for cohabitation of the species, or that water voles overwintered in entirely different habitats leaving these tunnels vacated (Jeppsson, 1990). In Paper II it is suggested that underground behavior may increase risk of parasite transmission in rodents. If field voles are forced to vary their underground habits through the year, this could limit opportunities for parasite exposure for this species and may explain the single finding reported in Paper I. Similarly, if foxes prefer to eat larger water voles rather than smaller field voles in areas where the two rodent species co-exist, parasite transmission involving field voles could be could be limited (see also Section 2.3.3). If field voles were indeed found to be the more highly susceptible species in Sweden (and, thus, theoretically more important for parasite transmission), limited opportunities for exposure and predation may restrict transmission of *E. multilocularis* and, therefore, overall prevalence in foxes. Further work is needed to investigate rodent movements, fox diet, and species interactions in Sweden to fully address these questions.

5.2 Spatial and Temporal Parasite Distribution and Transmission Factors

5.2.1 Micro-foci

The results of Paper I and Paper III show a highly aggregated distribution of *E. multilocularis* in the Swedish environment. Forty-one positive feces were found in 8/57 collections sites (all fields) and nine positive rodents in 4/107 trapping sites (all fields). These areas are indicative of micro-foci (see Section 2.1.3). The proportion of positive feces in some of these fields was surprisingly high—up to 52% (Paper II). As discussed in Section 2.1.3, these areas may be considered areas of high potential for egg exposure and, therefore, may be of public health concern.

It seems that a common perception among the public is that eating fresh berries from the forest constitutes the highest risk of *E. multilocularis* infection. Although fecal collections in this thesis were focused mainly in field habitats (Paper III), it is still of note that few feces overall and no *E. multilocularis* infected feces were found in forest habitat. Furthermore, no *E. multilocularis*

infected rodents were found in forested habitat (Paper I, II). In particular, no bank voles, which include berries as a major component of their diet compared to field voles or mice (Hansson, 1971), were found infected. While bank voles appear somewhat resistant to *E. multilocularis* infection (Woolsey *et al.*, 2016) in areas of high fox density and egg contamination, prevalence in this species can increase (Reperant *et al.*, 2009). Therefore, the results of this thesis suggest that forested areas in Sweden have low potential for egg exposure. Further investigation into, for instance, fox feces collected systematically throughout the year and in both habitats would further clarify these findings.

5.2.2 Yearly and Seasonal Effects

Although other studies have noted temporal influences on *E. multilocularis* positive findings in both feces and rodents (Hegglin *et al.*, 2007, Stieger *et al.*, 2002, Burlet *et al.*, 2011), no significant seasonal or yearly effects were noted for *E. multilocularis* positive feces in this project (Paper III). In the multivariable model for parasitized rodents performed in Paper II, season and reproduction were found to be highly associated. Breeding rodents, which were nearly 2.5 times more likely to be parasitized, were most often captured in the spring. Reproduction (e.g. stress and hormones) could decrease immunity and increase opportunity for infection (Klein, 2004). In addition, spring densities of rodents are low and made up of older individuals which have had a longer time for parasite exposure (Haukisalmi *et al.*, 1988; Myllymäki, 1977a). Therefore the association between breeding and season seemed not to be a true seasonal effect. In all Papers, the number of positive samples was too low to analyze temporal effects, if present, with any confidence.

5.2.3 Occurrence and Distribution of Other Taeniid Cestodes

In addition to *E. mutilocularis*, the other larval cestodes present in rodents included *V. mustelae*, *H. taeniaeformis*, *T. polyacantha*, and *Mesocestoides* spp. (Paper II). With few exceptions, the distribution of these parasite species seemed to be driven by an interaction between rodent species habitat preferences and rodent species susceptibility. *Versteria mustelae* and *H. taeniaeformis* were the most commonly found with parasites. Similar to *E. multilocularis* (Paper I), these parasites were most commonly found in rodents (water voles, field voles) caught in field habitat. In contrast, *T. polyacantha* and *Mesocestoides* spp., although rarely found, seemed to be restricted to rodents in forest/mix habitats (bank vole, mice). As in Paper III, several hotspots of infection, where both rodents and feces were found positive for a parasite, were identified. However, even in these cases, parasite distribution in the rodents followed the habitat and species limitations described above.

5.2.4 Field as a Risk Factor for Transmission

All nine *E. multilocularis* positive rodents were found in field habitat (Paper I, II) and the odds of parasitization (with taeniid larval cestodes in general) was ten times higher in the field than in forest habitat (Paper II). In addition, most feces (96%) were found in fields or on field/forest borders (Paper III). This percentage is biased by the fact that sampling effort was more concentrated in fields. However, the decision to concentrate in fields was due not only to the observed difficulty of finding feces in forest within this study (Paper III), but also to previous literature supporting the importance of field habitat for *E. multilocularis* transmission (Giraudoux et al., 2003). Still, as infected definitive hosts were present in both habitats, the results of these Papers I-III support the idea that infection pressure/parasite contamination is highest in field habitat.

As discussed in Paper II, the results of the multivariable model should be interpreted carefully as it is an attempt to explain a complex interaction between rodent ecology and susceptibility. In addition, it is unclear how the absence of "rodent species" affects the final results. To attempt to understand the effect of rodent species, Table 2 was created. When divided by rodent species, it is evident that the data is skewed and, in some cases, few or no infected/non-infected individuals are observed. This is likely due in part to the strong preference for each species to live in certain habitats. There also appears to be a pattern suggesting an increasing parasite prevalence along the gradient from forest, mix, to field. However, except for *M. glareolus*, there is not a significant difference ($p>0.05$, Fisher's) in parasite prevalence between the three habitats for each species. This pattern (i.e. strong effect of field), however, becomes significant when all factors are combined in the model (Paper II).

Table 2. Proportion of parasite infected rodents by rodent species (n) and habitat

	Field	**Mix**	**Forest**
Arvicola amphibius (439)	107/439 (24.4%)	--	--
Microtus agrestis (187)	36/136 (26.5%)	5/33 (15.2%)	1/18 (5.6%)
Myodes glareolus (655)	0/1 (0%)	31/167 (18.6%)	30/487 (6.2%)
Apodemus flavicollis (79)	0/1 (0%)	2/19 (10.5%)	3/59 (5.1%)
Apodemus sylvaticus (206)	0/8 (0%)	1/58 (1.7%)	1/140 (0.7%)

5.3 Monitoring Considerations

5.3.1 Sampling Methodology

In contrast to the other high endemic countries in EMIRO (e.g. Lithuania and Switzerland), Sweden had a very low estimated *E. multilocularis* national prevalence in foxes (0.1%) (Wahlström *et al.*, 2012). Furthermore rodent intermediate hosts were not identified at project start in 2013. To increase the likelihood of finding any *E. multilocularis* positive samples for comparison, sampling had to be focused in areas where the parasite was most likely to be found. In accordance to the descriptions provided in (Stärk *et al.*, 2006) and (Cameron, 2012), the study design used in this thesis could be considered as an example of a risk-based sampling approach. However, as discussed in Paper III, the sampling in this thesis was not designed to specifically test risk factors or to evaluate the efficiency of risk-based sampling in relation to other sampling strategies. Therefore, the term targeted sampling, which is a term with a broader definition and which includes concepts on which risk-based sampling is based, was adopted (Stärk *et al.*, 2006).

As discussed in Paper III, the sampling strategy used should be interpreted as a first attempt at using these concepts (risk-based and/or targeted sampling) in the Swedish environment and used as a baseline for future work. Still, the importance of the methodology used became apparent when comparing final results herein to the outcome of previous monitoring activities (Table 1). Overall, significantly more positive fox feces (41/714, 5.7% 95% CI 4.2-7.7%) were identified through the work of this thesis than in the second national screening (3/2779, 0.1%, 95% CI 0-0.3%) ($p<0.001$) (Paper III). Most importantly, through the collections of both rodents and fox feces for this thesis, two new areas (Gnesta/Nyöping, Vetlanda/Växjö) positive for *E. multilocularis* were identified in Sweden (Paper I, Paper III). Although more work is needed to improve this design for wider use (e.g. clearly defining risk factors), these results exemplify the potential efficiency of using a risk-based approach for *E. multilocularis* detection in the future.

5.3.2 Sampling Design Implications

High risk areas (i.e. areas where *E. multilocularis* was considered most likely to be found) targeted for sampling were based on presence of suitable rodent intermediate hosts (Paper III). This decision was based both on the EMIRO hypothesis that the rodent could be a limiting factor for *E. multilocularis* presence and on previous knowledge of *E. multilocularis* transmission ecology (see Section 2.1.3). The importance of considering host ecology when planning disease surveillance in wildlife has been highlighted before (Nusser *et al.*, 2008; Mörner *et al.*, 2002). In particular, Nusser *et al.* (2008) investigated different models for designing sampling for chronic wasting disease (CWD) in white-tailed deer (*Odocoileus virginianus*). By choosing a sampling regime which incorporated knowledge of deer spatial grouping, the accuracy and reliability of the results was increased as compared to convenience or random sampling usually done by wildlife authorities (Nusser *et al*., 2008). These concepts should be particularly considered for any area with an assumed low prevalence of *E. multilocularis*, and, in particular, countries designing monitoring to declare freedom from *E. multilocularis*.

6 Conclusions

The results of this thesis have provided the first identification of *E. multilocularis* in rodents in Sweden and give insight into the importance of these species as intermediate hosts in the country. These results also provide a representation of the localized distribution and local levels of environmental contamination of *E. multilocularis* within the southern half of Sweden. From these findings, a sampling strategy was proposed for monitoring *E. multilocularis* in Sweden for the future. Finally, to the author's knowledge, these results provide the first description of other taeniid larval cestodes in rodents from the south-central part of the country. Specific conclusions are discussed below.

- All rodent species investigated in this thesis are infected by taeniid larval cestodes. However, the host range of each parasite species was affected both by ecological factors and species specific host susceptibility.

- The field vole (*M. agrestis*) and the water vole (*A. amphibius*) are probably the most important rodents for the transmission of *E. multilocularis* in Sweden. These rodents were also infected by other larval taeniid cestodes in Sweden, mainly *V. mustelae* and *H. taeniaeformis*.

- Although *E. multilocularis* metacestodes were observed in both the field vole and the water vole, findings were rare. In addition, the majority of water voles (3/8) contained metacestodes which were likely not infectious to the definitive host. This, in addition to similar reports in literature, suggests a limited susceptibility of this species. The lack of highly susceptible hosts may limit *E. multilocularis* presence in Sweden.

- No *E. multilocularis* metacestodes were identified in the bank vole (*M. glareolus*) or mice (*Apodemus* spp.) despite a large samples size (n=655 and n=285, respectively). Thus, these species do not appear to play a significant role in *E. multilocularis* transmission in Sweden. These species do, however, have a role as intermediate hosts for other larval cestodes, such as *T. polyacantha* and *Mesocestoides* spp.

- Transmission patterns for other taeniid cestodes were used as a model for understanding the potential for *E. multilocularis* transmission in Sweden. From this, field habitat emerged as an important risk factor for taeniid parasite, including *E. multilocularis*, infection in rodents in Sweden.

- *Echinococcus multilocularis* has a heterogeneous distribution in Sweden. Regional differences in prevalence were recognized, and, on the local level, micro-foci were identified. Prevalence of *E. multilocularis* in foxes in these areas is probably higher than the national estimate (0.1%).

- Targeted sampling as defined in this thesis, identified more *E. multilocularis* positive samples than systematic sampling techniques employed by the national authorities. Although the sampling design outlined here needs to be further developed, these findings demonstrate that the transmission ecology of *E. multilocularis* needs to be considered when designing future monitoring/surveillance for detection of this parasite in low endemic areas or in countries where the parasite is thought to be absent.

7 Future Perspectives

Although the work of this thesis has expanded the knowledge of *E. multilocularis* lifecycle and distribution in Sweden, there are still many gaps in our knowledge. Due to the zoonotic potential of this parasite, there will be a continued need for research. Some potential areas for further investigations are discussed below.

- Little is known about water vole ecology in Sweden. Observations from fieldwork indicated that water voles seem to prefer different types of fields and different parts of chosen fields. Even within these microhabitats, the voles seem to have different tunneling patterns. These preferences and behaviors could have important implications for the success of parasite transmission and should be investigated further.

- There is very little information in the literature regarding the importance of the water vole for the red fox diet in Sweden. Although indirect evidence from bones observed in feces and signs in the field showed that foxes were eating these voles, a formal diet study is needed to examine the relative prey importance of these rodents throughout the year and what it may mean for parasite transmission.

- Knowledge of host densities is a key factor in understanding *E. multilocularis* transmission potential. However, better methods of measuring relative fox and rodent host densities need to be developed. Current methods of estimating fox density, such as collecting feces along transects, are time-consuming, expensive, and need to be performed over large areas and times to increase accuracy (e.g. Webbon *et al.*, 2004). In addition, techniques based on feces can be biased by environmental factors (Cavallini, 1994). Although techniques have been developed to estimate water vole density (Quéré *et al.*, 2000; Giraudoux *et al.*, 1995), they should be validated for use in Sweden.

- Experimental studies investigating *E. multilocularis* infection dynamics in *Arvicola* spp. are needed to clarify any differences in the susceptibility between these species.

- Interspecies interactions both outside and inside the host may affect *E. multilocularis* transmission. For instance, field studies of interactions between rodent species, particularly field voles and water voles, may reveal behaviors that limit parasite exposure. In addition, experimental studies investigating parasite communities within individual rodents may reveal interactions which increase or decrease *E. multilocularis* establishment and development.

- More research is needed to clarify human risk factors. In particular, the question of the risk of acquiring the parasite from berries remains. Although results from this thesis indicate a potentially lower risk of exposure in the forest, further investigation is needed to clarify these results.

- Risk-based sampling is a promising method of detection for *E. multilocularis*. However, risk factors relating to the presence of the parasite in the environment (particularly for the formation of micro-foci) need to be identified and, if possible, quantified before this type of sampling can be performed more widely.

- With better defined risk factors, models and/or risk maps could be developed. These could be used to estimate levels of contamination and therefore also risk for human exposure in different regions and habitats within Sweden.

- The results of this thesis have indicated a wider distribution of the parasite than previously thought found in national monitoring. Still, only four restricted areas of southern Sweden were investigated. To better understand the distribution nationally, the methods used in this thesis should be applied to more regions throughout the country where the parasite has not yet been identified. Studying differences in prevalence between the north and south of Sweden may provide further insight into factors which limit parasite transmission. For instance, the heavily forested habitat of the north may reduce opportunities for micro-foci to form. In addition, repeat monitoring in the study areas herein may be useful to understand any temporal variation in prevalence.

- Since the first finding of *E. multilocularis* in Sweden, an underlying question has been if the parasite was introduced or if it has always been in the country. Although this thesis was not designed to answer this question, parasite findings in all four regions suggest that it was not a single recent introduction. However, this does not exclude the possibility of multiple recent introductions or that it has always been here but at a very low prevalence. Although this is a question that may never be fully answered, genetic studies may provide some insight. Repeated national monitoring to evaluate any change in prevalence may also give additional insight into this question.

8 Populärvetenskaplig Sammanfattning

Rävens dvärgbandmask, *Echinococcus multilocularis*, är en parasit som överförs mellan olika hunddjur och gnagare. I Europa är rödräven parasitens viktigaste slutvärd, medan olika sorkar fungerar som mellanvärd. Som vuxen lever bandmasken i rävens tunntarm och ägg kommer ut i miljön med avföringen. När en sork äter äggen utvecklas parasiten i larvcystor i levern. Sorken måste därefter ätas upp av en räv för att bandmaskens livscykel skall fullföljas. Även människor kan infekteras genom att precis som gnagare få i sig äggen. Detta kan ske vid förtäring av förorenad mat eller genom interaktion med infekterade värddjur (t.ex. räv eller hund). I de fall en människa blir infekterad, tillväxer cystorna långsamt likt en cancer i levern. Sjukdomen echinococcos hos människa är dödlig men det finns bra behandlingsalternativ.

Rävens dvärgbandmask är sedan årtionden vanligt förekommande hos rödräv i Centraleuropa (t ex i delar av Tyskland, Schweiz och Frankrike). I Sverige upptäcktes den för första gången 2011. På grund av att människor kan infekteras finns behov av att förstå var och hur parasiten kan uppträda i Sverige. När detta projekt inleddes 2013 saknades exempelvis kunskap om vilka gnagararter som är parasitens mellanvärdar i Sverige. Syftet med avhandlingen var att undersöka hur överföringen av rävens dvärgbandmask går till, med särskilt fokus på svenska gnagares roll.

Genom arbetet upptäcktes rävens dvärgbandmask för första gången hos vattensork och åkersork i Sverige. Däremot påträffades den varken hos skogssork eller skogsmöss. Även undersökningen av närbesläktade bandmaskar hos dessa smågnagare visade att arter som fångades i ängsmiljöer (dvs. vattensork och åkersork) var parasiterade i högre grad än de som fångades i beskogade områden (dvs. skogsmöss och skogssork). Detta tyder på att det i Sverige är vattensork och åkersork som är viktigast för överföringen av olika bandmasklarver inklusive rävens bandmask.

De två sorkar som är viktigast som mellanvärdar för dvärgbandmasken i övriga Europa (fältsork och en typ av vattensork) saknas i Sverige. Fältsork är i Sverige ersatt av åkersork. Dessutom skiljer sig den vattensork vi har i Sverige, både vad gäller livsstil och livsmiljö, från den i centrala Europa. Det visade sig att endast en av närmare 200 åkersork var infekterad. Av åtta infekterade vattensorkar var larverna infektionsdugliga bara hos tre individer. Då det i Sverige verkar saknas optimala mellanvärdar är det inte så konstigt att förekomsten av rävens dvärgbandmask generellt är låg.

För att ta reda på de svenska sorkarnas exponering för dvärgbandmaskens ägg, samlades rävspillning in på de olika fångstplatserna. Spillning med parasitägg påvisades därigenom i fyra regioner i Sverige. Rävens dvärgbandmask var dock inte jämnt fördelad i den svenska miljön. På några fångstplatser var andelen parasitinfekterad spillning mycket högre än på andra platser. Det är naturligtvis där som sorkarna är mest exponerade för bandmasken. Det var på en sådan plats som flertalet av de infekterade sorkarna hittades. Man kan inte utesluta att även människor exponeras där.

Den provtagningsmetodik som har använts i detta avhandlingsarbete visade sig vara effektivare för att hitta spillning från infekterade rävar jämfört med den som använts i tidigare svenska studier. Genom att rikta insamlingsinsatserna till de platser där det är mest troligt att hitta dvärgbandmasken (det vill säga där vattensork och/eller åkersork lever), påvisades dvärgbandmasken i fyra undersökta regioner. Noteras kan att i två av dessa var dvärgbandmasken inte känd sedan tidigare. Dessa resultat har utgjort underlag till något som kallas riktad provtagning och baseras på kunskap om olika faktorer som är av betydelse för smittspridning.

Efter ytterligare utveckling skulle riktad provtagning kunna användas för att på ett kostnadseffektivt sätt ta reda på om dvärgbandmasken finns etablerad på fler platser där den ännu inte påvisats, exempelvis i tätortsnära områden och i Norrland. Metodiken skulle även kunna tillämpas i andra länder och särskilt i länder som betraktas som fria från parasiten och där det finns behov av att dokumentera smittfrihet.

References

Al-Sabi, M.N.S., Chriél, M., Jensen, T.H. & Enemark, H.L. (2013a). Endoparasites of the raccoon dog (*Nyctereutes procyonoides*) and the red fox (*Vulpes vulpes*) in Denmark 2009–2012–A comparative study. *International Journal for Parasitology: Parasites and Wildlife,* 2, pp. 144-151.

Al-Sabi, M.N.S., Jensen, P.M., Christensen, M.U. & Kapel, C.M.O. (2013b). Morphological and molecular analyses of larval taeniid species in small mammals from contrasting habitats in Denmark. *Journal of Helminthology,* 89(1), pp. 112-117.

Ammann, R. & Eckert, J. (1995). Clinical Diagnosis and Treatment of Echinococcosis in Humans. In: Thompson, R.C.A. & Lymbery, A.J. (Eds) *Echinococcus and hydatid disease*: Wallingford, UK: Cab International, pp. 411-464.

Bang, P., Dahlstrøm, P. & Walters, M. (2001). *Animal tracks and signs*. (Pocket Guides, Natural History (Oxford, England). New York, USA: Oxford University Press.

Barabási, S.S., Marosfői, L., Barabási, Z.S. & Cozma, V. (2011). Natural alveolar echinococcosis with *Echinococcus multilocularis* in wild rodents. *Scientica Parasitologica,,* 12(1), pp. 11-21.

Beery, T.H. (2013). Nordic in nature: friluftsliv and environmental connectedness. *Environmental Education Research,* 19(1), pp. 94-117.

Behnke, J.M., Bajer, A., Harris, P.D., Newington, L., Pidgeon, E., Rowlands, G., Sheriff, C., Kuliś-Malkowska, K., Siński, E., Gilbert, F.S. & Barnard, C.J. (2008). Temporal and between-site variation in helminth communities of bank voles (*Myodes glareolus*) from N.E. Poland. 1. Regional fauna and component community levels. *Parasitology,* 135(8), pp. 985-997.

Beiromvand, M., Akhlaghi, L., Massom, S.H.F., Meamar, A.R., Darvish, J. & Razmjou, E. (2013). Molecular identification of *Echinococcus multilocularis* infection in small mammals from Northeast, Iran. *PLoS Neglected Tropical Diseases,* 7(7), p. e2313.

Bergstedt, B. (1965). Distribution, reproduction, growth and dynamics of the rodent species *Clethrionomys glareolus* (Schreber), *Apodemus flavicollis* (Melchior) and *Apodemus sylvaticus* (Linné) in southern Sweden. *Oikos,* 16(1/2), pp. 132-160.

Brunetti, E., Kern, P., Vuitton, D.A & Writing Panel for the WHO-IWGE. (2010). Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Tropica,* 114(1), pp. 1-16.

Burlet, P., Deplazes, P. & Hegglin, D. (2011). Age, season and spatio-temporal factors affecting the prevalence of *Echinococcus multilocularis* and *Taenia taeniaeformis* in *Arvicola terrestris*. *Parasites & Vectors,* 4, p. e6.

Cameron, A.R. (2012). The consequences of risk-based surveillance: Developing output-based standards for surveillance to demonstrate freedom from disease. *Preventative Veterinary Medicine,* 105 (4), pp. 280-286.

Cavallini, P. (1994). Faeces count as an index of fox abundance. *Acta Theriologica,* 39(4), pp. 417-424.

Chechulin, A.I., Zav'yalov, E.L., Gerlinskaya, L.A., Krivopalov, A.V., Makarikov, A.A. & Muzyka, V.Y. (2010). Helminthes of the water vole *Arvicola terrestris* Linnaeus of forest-bog

biocenoses in the north of the Baraba Lowland. *Contemporary Problems of Ecology*, 3(1), pp. 4-9.

Christiansen, E. (1983). Fluctuations in some small rodent populations in Norway 1971–1979. *Holarctic Ecology*, 6(1), pp. 24-31.

Conraths, F.J. & Deplazes, P. (2015). *Echinococcus multilocularis*: Epidemiology, surveillance and state-of-the-art diagnostics from a veterinary public health perspective. *Veterinary Parasitology*, 213(3-4), pp. 149-161.

Conraths, F.J., Staubach, C. & Tackmann, K. (2003). Statistics and sample design in epidemiological studies of *Echinococcus multilocularis* in fox populations. *Acta Tropica*, 85(2), pp. 183-189.

Craig, P.S., Giraudoux, P., Shi, D., Bartholomot, B., Barnish, G., Delattre, P., Quéré, J.P., Harraga, S., Bao, G., Wang, Y., Lu, F., Ito, A., & Vuitton, D.A. (2000). An epidemiological and ecological study of human alveolar echinococcosis transmission in south Gansu, China. *Acta Tropica*, 77(2), pp. 167-177.

Dalén, L., Kvaløy, K., Linnell, J.D.C., Elmhagen, B., Strand, O., Tannerfeldt, M., Henttonen, H., Fuglei, E., Landa, A. & Angerbjörn, A. (2006). Population structure in a critically endangered arctic fox population: Does genetics matter? *Molecular Ecology*, 15(10), pp. 2809-2819.

Danson, F.M., Craig, P.S., Man, W., Shi, D. & Giraudoux, P. (2004). Landscape dynamics and risk modeling of human alveolar echinococcosis. *Photogrammetric Engineering & Remote Sensing*, 70(3), pp. 359-366.

Davidson, R.K., Romig, T., Jenkins, E., Tryland, M. & Robertson, L.J. (2012). The impact of globalisation on the distribution of *Echinococcus multilocularis*. *Trends in Parasitology*, 28(6), pp. 239-247.

Delattre, P., Giraudoux, P., Baudry, J., Musard, P., Toussaint, M., Truchetet, D., Stahl, P., Poule, M.L., Artois, M., Damange, J.-P. & Quéré, J.-P. (1992). Land use patterns and types of common vole (*Microtus arvalis*) population kinetics. *Agriculture, Ecosystems & Environment*, 39(3-4), pp. 153-168.

Dell'Arte, G.L., Laaksonen, T., Norrdahl, K. & Korpimäki, E. (2007). Variation in the diet composition of a generalist predator, the red fox, in relation to season and density of main prey. *Acta oecologica*, 31(3), pp. 276-281.

Deplazes, P., Eckert, J., Mathis, A., Samson-Himmelstjerna, G.v. & Zahner, H. (2016). *Parasitology in Veterinary Medicine*. Wageningen, the Netherlands: Wageningen Academic Publishers.

Deplazes, P., van Knapen, F., Schweiger, A. & Overgaauw, P.A.M. (2011). Role of pet dogs and cats in the transmission of helminthic zoonoses in Europe, with a focus on echinococcosis and toxocarosis. *Veterinary Parasitology*, 182(1), pp. 41-53.

Duhamel, R., Quéré, J.-P., Delattre, P. & Giraudoux, P. (2000). Landscape effects on the population dynamics of the fossorial form of the water vole (*Arvicola terrestris* sherman). *Landscape Ecology*, 15(2), pp. 89-98.

Eckert, J. (1998). Alveolar echinococcosis (*Echinococcus multilocularis*) and other forms of echinococcosis (*Echinococcus vogeli* and *Echinococcus oligarthrus*) In: Palmer, S.R., Lord Soulsby & Simpson D.I.H (Eds) *Zoonoses: Biology, Clinical Practices, and Public Health Control*. New York, New York: Oxford University Press, pp. 689-716.

Eckert, J., Conraths, F.J. & Tackmann, K. (2000). Echinococcosis: An emerging or re-emerging zoonosis? *International Journal for Parasitology*, 30(12-13), pp. 1283-1294.

Eckert, J.P., Deplazes, P.S., Craig, M.A., Gemmell, M.A., Gottstein, B., Heath, D., Jenkins, D.J., Kamiya, M. & Lightowlers, M. (2001a). Echinococcosis in animals: Clinical aspects, diagnosis and treatment. In: Eckert, J., Gemmel, M.A., Meslin, F.X., & Pawłowski, Z.S. (Eds) *WHO-OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern*. Paris, France: WHO/OIE, pp. 72-99.

Eckert, J.P., Gottstein, B., Heath, D. & Liu, F.-J. (2001b). Prevention of echinococcosis in humans and safety precautions. In: Eckert, J., Gemmel, M.A., Meslin, F.X., & Pawłowski, Z.S. (Eds) *WHO-OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern*. Paris, France: WHO/OIE, pp. 238-248.

Eckert, J. & Deplazes, P. (2004). Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clinical Microbiology Reviews*, 17(1), pp. 107-135.

Englund, J. (1965). Studies on food ecology of the red fox (*V. vulpes*) in Sweden. *Viltrevy*, 3(4), pp. 377-485.

Englund, J. (1980). Yearly variations of recovery and dispersal rates of fox cubs tagged in Swedish coniferous forests. In: Zimen, E. (Ed.) *The Red Fox, Symposium on Behaviour and Ecology (Biogeographica, Volume 18)*. The Hague, the Netherlands: Dr. W. Junk B.V. Publishers, pp. 195-207.

Erlinge, S. (1981). Food preference, optimal diet and reproductive output in stoats *Mustela erminea* in Sweden. *Oikos*, 36(3), pp. 303-315.

Erlinge, S., Hoogenboom, I., Agrell, J., Nelson, J. & Sandell, M. (1990). Density-related home-range size and overlap in adult field voles (*Microtus agrestis*) in southern Sweden. *Journal of Mammalogy*, 71(4), pp. 597-603.

Erlinge, S., Göransson, G., Hansson, L., Högstedt, G., Liberg, O., Nilsson, I.N., Nilsson, T., von Schantz, T. & Sylvén, M. (1983). Predation as a regulating factor on small rodent populations in southern Sweden. *Oikos*, 40(1), pp. 36-52.

European Commission. (2011). Commission delegated regulation (EU) No 1152/2011 of 14 July 2011 supplementing Regulation (EC) No 998/2003 of the European Parliament and of the Council as regards preventive health measures for the control of *Echinococcus multilocularis* infection in dogs. *Official Journal of the European Union*, L 296, pp. 6-12.

European Food Safety Authority (EFSA) Panel on Animal Health and Welfare (2015). *Echinococcus multilocularis* infections in animals (Scientific Opinion). *EFSA Journal 2015*, 13(12), p. 4373.

European Parliament & Council of the European Union (2003). Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. *Official Journal of the European Union*, L325, pp. 31-40.

Fichet-Calvet, E., Giraudoux, P., Quéré, J.-P., Ashford, R.W. & Delattre, P. (2003). Is the prevalence of *Taenia taeniaeformis* in *Microtus arvalis* dependent on population density? *Journal of Parasitology*, 89(6), pp. 1147-1152.

Freeman, R.S. (1956). Life history studies on *Taenia mustelae* Gmelin, 1790 and the taxonomy of certain taenioid cestodes from *Mustelidae*. *Canadian Journal of Zoology*, 34(4), pp. 219-242.

Fuglei, E., Stien, A., Yoccoz, N.G., Ims, R.A., Eide, N.E., Prestrud, P., Deplazes, P. & Oksanen, A. (2008). Spatial distribution of *Echinococcus multilocularis*, Svalbard, Norway. *Emerging Infectious Diseases*, 14(1), p. 73-75.

Fujita, O., Oku, Y., Okamoto, M., Sato, H., Ooi, H. K., Kamiya, M. & Rausch, R. L. (1991). Early development of larval *Taenia polyacantha* in experimental intermediate hosts. *Journal of the Helminthological Society of Washington* 58(1), 100-109.

Giesecke, J. (2002). *Modern Infectious Disease Epidemiology*. Second Edition. London, Great Britain: Arnold.

Giraudoux, P., Craig, P.S., Delattre, P., Bao, G., Bartholomot, B., Harraga, S., Quéré, J.-P., Raoul, F., Wang, Y., Shi, D. & Vuitton, D.A. (2003). Interactions between landscape changes and host communities can regulate *Echinococcus multilocularis* transmission. *Parasitology*, 127(Supplement1), pp. S121-S131.

Giraudoux, P., Delattre, P., Takahashi, K., Raoul, F., Pierre Quéré, J., Craig, P. & Vuitton, D.A. (2002). Transmission ecology of *Echinococcus multilocularis* in wildlife: What can be learned from comparative studies and multiscale approaches? In: Craig, P. & Pawlowski, Z. (Eds) *Cestode Zoonoses: Echinococcosis and Cystercercosis: An emergent and global problem*. Amsterdam, the Netherlands: IOS Press, pp. 251-266.

Giraudoux, P., Pradier, B., Delattre, P., Deblay, S., Salvi, D. & Defaut, R. (1995). Estimation of water vole abundance by using surface indices. *Acta Theriologica*, 40(1), pp. 77-96.

Giraudoux, P., Raoul, F., Pleydell, D., Li, T., Han, X., Qiu, J., Xie, Y., Wang, H., Ito, A. & Craig, P.S. (2013). Drivers of *Echinococcus multilocularis* transmission in China: Small mammal diversity, landscape or climate? *PLoS Neglected Tropical Diseases*, 7(3), p. e2045.

Goszczyński J. (1990). Scent marking by red foxes in Central Poland during the winter season. *Acta Theriologica*, 35(1-2), pp. 7-16.

Gottstein, B., Saucy, F., Deplazes, P., Reichen, J., Demierre, G., Busato, A., Zuercher, C. & Pugin, P. (2001). Is high prevalence of *Echinococcus multilocularis* in wild and domestic animals associated with disease incidence in humans? *Emerging Infectious Diseases*, 7(3), p. 408-412.

Gottstein, B., Stojkovic, M., Vuitton, D.A., Millon, L., Marcinkute, A. & Deplazes, P. (2015). Threat of alveolar echinococcosis to public health–a challenge for Europe. *Trends in Parasitology*, 31(9), pp. 407-412.

Goüy de Bellocq, J., Sarà, M., Casanova, J.C., Feliu, C. & Morand, S. (2003). A comparison of the structure of helminth communities in the woodmouse, *Apodemus sylvaticus*, on islands of the western Mediterranean and continental Europe. *Parasitology Research*, 90(1), pp. 64-70.

Guislain, M.-H., Raoul, F., Giraudoux, P., Terrier, M.-E., Froment, G., Ferté, H. & Poulle, M.-L. (2008). Ecological and biological factors involved in the transmission of *Echinococcus multilocularis* in the French Ardennes. *Journal of Helminthology*, 82(2), pp. 143-151.

Guislain, M.H., Raoul, F., Poulle, M.L. & Giraudoux, P. (2007). Fox faeces and vole distribution on a local range: Ecological data in a parasitological perspective for *Echinococcus multilocularis*. *Parasitology*, 14(4), pp. 299-308.

Hanosset, R., Saegerman, C., Adant, S., Massart, L. & Losson, B. (2008). *Echinococcus multilocularis* in Belgium: Prevalence in red foxes (*Vulpes vulpes*) and in different species of potential intermediate hosts. *Veterinary Parasitology*, 151(2-4), pp. 212-217.

Hansson, L. (1967). Index line catches as a basis of population studies on small mammals. *Oikos*, 18(2), pp. 261-276.

Hansson, L. (1968). Population densities of small mammals in open field habitats in south Sweden in 1964-1967. *Oikos*, 19(1), pp. 53-60.

Hansson, L. (1969). Home range, population structure and density estimates at removal catches with edge effect. *Acta Theriologica*, 14(11), pp. 153-160.

Hansson, L. (1971). Small rodent food, feeding and population dynamics: A comparison between granivorous and herbivorous species in Scandinavia. *Oikos*, 22(2), pp. 183-198.

Hansson, L. (1972). Evaluation of the small quadrat method of censusing small mammals. *Annales Zoologici Fennici*, 9, pp. 184-190.

Hansson, L. (1977). Spatial dynamics of field voles *Microtus agrestis* in heterogeneous landscapes. *Oikos*, 29(3), pp. 539-544.

Hansson, L. (1979). Field signs as indicators of vole abundance. *Journal of Applied Ecology*, 16(2), pp. 339-347.

Hansson, L. (1989). Landscape and habitat dependence in cyclic and semi-cyclic small rodents. *Holarctic Ecology*, 12(4), pp. 345-350.

Hardorn, D.C., Rüfenacht, J., Hauser, R. & Stärk, K.D.C. (2002). Risk-based design of repeated surveys for the documentation of freedom from non-highly contagious diseases. *Preventive Veterinary Medicine*, 56(3), pp. 179-192.

Haukisalmi, V. & Henttonen, H. (1993). Population dynamics of *Taenia polyacantha* metacestodes in the bank vole *Clethrionomys glareolus*. *Annales Zoologici Fennici*, 30(1), pp. 81-84.

Haukisalmi, V., Henttonen, H. & Tenora, F. (1988). Population dynamics of common and rare helminths in cyclic vole populations. *The Journal of Animal Ecology*, 57(3), pp. 807-825.

Hegglin D, Bontadina F, Contesse P, Gloor S, Deplazes P. (2007). Plasticity of predation behaviour as a putative driving force for parasite life-cycle dynamics: The case of urban foxes and *Echinococcus multilocularis* tapeworm. *Functional Ecology*, 21(3), pp. 552-560.

Henttonen, H. (1987). The impact of spacing behavior in microtine rodents on the dynamics of least weasels *Mustela nivalis*: A Hypothesis. *Oikos*, 50(3), pp. 366-370.

Henttonen, H., Fuglei, E., Gower, C.N., Haukisalmi, V., Ims, R.A., Niemimaa, J. & Yoccoz, N.G. (2001). *Echinococcus multilocularis* on Svalbard: introduction of an intermediate host has enabled the local life-cycle. *Parasitology*, 123(6), pp. 547-552.

Hoberg, E.P., Aubry, K.B. & Brittell, J.D. (1990). Helminth parasitism in martens (*Martes americana*) and ermines (*Mustela erminea*) from Washington, with comments on the distribution of *Trichinella spiralis*. *Journal of Wildlife Diseases*, 26(4), pp. 447-452.

Hofer, S., Gloor, S., Müller, U., Mathis, A., Hegglin, D. & Deplazes, P. (2000). High prevalence of *Echinococcus multilocularis* in urban red foxes (*Vulpes vulpes*) and voles (*Arvicola terrestris*) in the city of Zürich, Switzerland. *Parasitology*, 120(2), pp. 135-142.

Hosmer, D.W., Lemeshow, S. & Sturdivant, R.X. (2013). *Applied Logistic Regression*. Third Edition. Hoboken, New Jersey: John Wiley & Sons.

Hrčkova, G., Miterpáková, M., O'Connor, A., Šnábel, V. & Olson, P.D. (2011). Molecular and morphological circumscription of *Mesocestoides* tapeworms from red foxes (*Vulpes vulpes*) in central Europe. *Parasitology*, 138(5), pp. 638-647.

Ihama, Y., Sato, H., Makino, Y. & Kamiya, H. (2000). Two *Taenia* species found in Japan, with new distribution record of *Taenia polyacantha* Leuckart, 1856 (Cestoda: Taeniidae). *Parasitology International*, 48(4), pp. 303-306.

Isaksson, M., Hagström, Å., Armua-Fernandez, M.T., Wahlström, H., Ågren, E.O., Miller, A., Holmberg, A., Lukacs, M., Casulli, A., Deplazes, P. & Juremalm, M. (2014). A semi-automated magnetic capture probe based DNA extraction and real-time PCR method applied in the Swedish surveillance of *Echinococcus multilocularis* in red fox (*Vulpes vulpes*) faecal samples. *Parasites & Vectors*, 7, pp. e583.

Iwaki, T., Abe, N., Shibahara, T., Oku, Y. & Kamiya, M. (1995). New distribution record of *Taenia mustelae* Gmelin, 1790 (Cestoda) from the least weasel *Mustela nivalis* in Hokkaido, Japan. *The Journal of Parasitology*, 81(5). p. 796.

Iwaki, T., Abe, N., Shibahara, T., Oku, Y. & Kamiya, M. (1996). Developmental study of *Taenia mustelae* in the intermediate and definitive hosts, with a note on the life cycle of *T. mustelae* in Hokkaido, Japan. *The Journal of Parasitology*, 82(5), pp. 840-842.

Jeppsson, B. (1990). Effects of density and resources on the social system of water voles. In: Tamarin, R.H., Ostfeld, R.S., Pugh, S.R. & Bujalska, G. (Eds) *Social Systems and Population Cycles in Voles*. Basel, Switzerland: Birkhäuser Basel, pp. 213-226.

Kapel, C.M.O., Torgerson, P.R., Thompson, R.C.A. & Deplazes, P. (2006). Reproductive potential of *Echinococcus multilocularis* in experimentally infected foxes, dogs, raccoon dogs and cats. *International Journal for Parasitology*, 36(1), pp. 79-86.

Kern, P., Ammon, A., Kron, M., Sinn, G., Sander, S., Petersen, L.R., Gaus, W. & Kern, P. (2004). Risk factors for alveolar echinococcosis in humans. *Emerging Infectious Diseases*, 10(12), pp. 2088-2093.

Kjellander, P. & Nordström, J. (2003). Cyclic voles, prey switching in red fox, and roe deer dynamics–A test of the alternative prey hypothesis. *Oikos*, 101(2), pp. 338-344.

Klein, S.L. (2004). Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunology*, 26(6-7), pp. 247-264.

Knapp, J., Nakao, M., Yanagida, T., Okamoto, M., Saarma, U., Lavikainen, A. & Ito, A. (2011). Phylogenetic relationships within *Echinococcus* and *Taenia* tapeworms (Cestoda: Taeniidae): An inference from nuclear protein-coding genes. *Molecular Phylogenetics and Evolution*, 61(3), pp. 628-638.

Knapp J, Umhang G, Poulle M-L, Millon L. Development of a real-time PCR for a sensitive one-step coprodiagnosis allowing both the identification of carnivore feces and the detection of *Toxocara* spp. and *Echinococcus multilocularis*. *Applied and Environmental Microbiology*, 82(10), pp. 2950-2958.

Lavikainen, A., Iwaki, T., Haukisalmi, V., Konyaev, S. V., Casiraghi, M., Dokuchaev, N. E., Galimberti, A., Halajian, A., Henttonen, H., Ichikawa-Seki, M., Itagaki, T., Krivopalov, A. V., Meri, S., Morand, S., Näreaho, A., Olsson, G. E., Ribas, A., Terefe, Y. & Nakao, M. (2016). Reappraisal of *Hydatigera taeniaeformis* (Batsch, 1786) (Cestoda: Taeniidae) sensu lato with

description of *Hydatigera kamiyai* n. sp. *International Journal for Parasitology* 46(5-6), pp: 361-374.

Le Pesteur, M.H., Giraudoux, P., Delattre, P., Damange, J.P. & Quéré, J.P. (1992). Spatiotemporal distribution of four species of cestodes in a landscape of mid-altitude mountains (Jura, France). *Annales de Parasitologie Humaine et Comparée*, 67(5), pp. 155-160.

Liberg, O. (1984). Food habits and prey impact by feral and house-based domestic cats in a rural area in southern Sweden. *Journal of Mammalogy*, 65(3), pp. 424-432.

Lidicker WZ. (1999). Responses of mammals to habitat edges: an overview. *Landscape Ecology*. 14(4), pp. 333-343.

Lindström, E. (1982). *Population ecology of the red fox (Vulpes vulpes L.) in relation to food supply*. PhD Thesis. Stockholm, Sweden: Department of Zoology, University of Stockholm Sweden. pp 1-154.

Lindström, E. (1989). Food limitation and social regulation in a red fox population. *Holarctic Ecology*, 12(1), pp. 70-79.

Loos-Frank, B. (1980). The common vole, *Microtus arvalis* Pall. as intermediate host of *Mesocestoides* (Cestoda) in Germany. *Zeitschrift für Parasitenkunde*, 63(2), pp. 129-136.

Loos-Frank, B. (1991). One or two intermediate hosts in the life cycle of *Mesocestoides* (Cyclophyllidea, Mesocestoididae)? *Parasitology Research*, 77(8), pp. 726-728.

Lucchini, V., Fabbri, E., Marucco, F., Ricci, S., Boitani, L. & Randi, E. (2002). Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the western Italian Alps. *Molecular Ecology*, 11(5), pp. 857-868.

Mathis, A., Deplazes, P. & Eckert, J. (1996). Improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. *Journal of Helminthology*, 70(3), pp. 219-222.

McDonald, J.H. (2014). *Handbook of Biological Statistics*. 3rd. ed. Baltimore, Maryland: Sparky House Publishing.

Meijer, T., Mattsson, R., Angerbjörn, A., Osterman-Lind, E., Fernández-Aguilar, X. & Gavier-Widén, D. (2011). Endoparasites in the endangered Fennoscandian population of arctic foxes (*Vulpes lagopus*). *European Journal of Wildlife Research*, 57(4), pp. 923-927.

Mironov, A.D. (1990). Spatial and temporal organization of populations of the bank vole, *Clethrionomys glareolus*. In: Tamarin, R.H., Ostfeld, R.S., Pugh, S.R. & Bujalska, G. (Eds) *Social Systems and Population Cycles in Voles*. Basel, Switzerland: Birkhäuser Basel, pp. 181-192.

Monterroso, P., Castro, D., Silva, T.L., Ferreras, P., Godinho, R. & Alves, P.C. (2012). Factors affecting the (in)accuracy of mammalian mesocarnivore scat identification in South-western Europe. *Journal of Zoology*, 289(4), pp. 243-250.

Montgomery, S.S.J. & Montgomery, W.I. (1988). Cyclic and non-cyclic dynamics in populations of the helminth parasites of wood mice, *Apodemus sylvaticus*. *Journal of Helminthology*, 62(1), pp. 78-90.

Myllymäki, A. (1977a). Demographic mechanisms in the fluctuating populations of the field vole *Microtus agrestis*. *Oikos*, 29(3), pp. 468-493.

Myllymäki, A. (1977b). Interactions between the field vole *Microtus agrestis* and its microtine competitors in Central-Scandinavian populations. *Oikos*, 29(3), pp. 570-580.

Myllymäki, A., Paasikallio, A., Pankakoski, E. & Kanervo, V. (1971). Removal experiments on small quadrats as a means of rapid assessment of the abundance of small mammals. *Annales Zoologici Fennici* 8, pp. 177-185.

Mörner, T., Obendorf, D.L., Artois, M. & Woodford, M.H. (2002). Surveillance and monitoring of wildlife diseases. *Revue Scientifique et Technique-Office International des Epizooties*, 21(1), pp. 67-76.

National Veterinary Institute (SVA). (2016). The red fox tapeworm as a zoonosis (in Swedish). http://www.sva.se/djurhalsa/zoonoser/ravens-dvargbandmask Accessed 12 June 2016.

Nakao, M., Lavikainen, A., Iwaki, T., Haukisalmi, V., Konyaev, S., Oku, Y., Okamoto, M. & Ito, A. (2013). Molecular phylogeny of the genus *Taenia* (Cestoda: Taeniidae): Proposals for the resurrection of *Hydatigera* Lamarck, 1816 and the creation of a new genus *Versteria*. *International Journal for Parasitology*, 43(6), pp. 427-437.

Nusser, S.M., Clark, W.R., Otis, D.L. & Huang, L. (2008). Sampling considerations for disease surveillance in wildlife populations. *The Journal of Wildlife Management*, 72(1), pp. 52-60.

Oksanen, L. & Oksanen, T. (1992). Long-term microtine dynamics in north Fennoscandian tundra: The vole cycle and the lemming chaos. *Ecography*, 15(2), pp. 226-236.

Osterman Lind, E., Juremalm, M., Christensson, D., Widgren, S., Hallgren, G., Ågren, E.O., Uhlhorn, H., Lindberg, A., Cedersmyg, M. & Wahlström H. (2011). First detection of *Echinococcus multilocularis* in Sweden, February to March 2011. *Euro Surveillance*, 16(14), p. e19836.

Paisley, L.G. (2001). Economic aspects of disease monitoring with special reference to bovine paratuberculosis. *Acta Veterinaria Scandinavica*, 42(Suppl 1), p. 17-25.

Pearson, D.E. & Ruggiero, L.F. (2003). Transect versus grid trapping arrangements for sampling small-mammal communities. *Wildlife Society Bulletin*, 31(2), pp. 454-459.

Pétavy, A.-F., Tenora, F. & Deblock, S. (2003). Co-occurrence of metacestodes of *Echinococcus multilocularis* and *Taenia taeniaeformis* (Cestoda) in *Arvicola terrestris* (Rodentia) in France. *Folia Parasitologica*, 50, pp. 157-158.

Piras, P., Marcolini, F., Claude, J., Ventura, J., Kotsakis, T. & Cubo, J. (2012). Ecological and functional correlates of molar shape variation in European populations of *Arvicola* (Arvicolinae, Rodentia). *Zoologischer Anzeiger-A Journal of Comparative Zoology*, 251(4), pp. 335-343.

Pyke, G.H. (1984). Optimal foraging theory: A critical review. *Annual Review of Ecology and Systematics*, 15, pp. 523-575.

Quéré, J.P., Raoul, F., Giraudoux, P. & Delattre, P. (2000). An index method of estimating relative population densities of the common vole (*Microtus arvalis*) at landscape scale. *Revue d'Ecologie: La Terre et la Vie*, 55, pp. 25-32.

Raoul, F., Deplazes, P., Nonaka, N., Piarroux, R., Vuitton, D.A. & Giraudoux, P. (2001). Assessment of the epidemiological status of *Echinococcus multilocularis* in foxes in France using ELISA coprotests on fox faeces collected in the field. *International Journal for Parasitology*, 31(14), pp. 1579-1588.

Raoul, F., Deplazes, P., Rieffel, D., Lambert, J.-C. & Giraudoux, P. (2010). Predator dietary response to prey density variation and consequences for cestode transmission. *Oecologia*, 164(1), pp. 129-139.

Raoul, F., Hegglin, D. & Giraudoux, P. (2015). Trophic ecology, behaviour and host population dynamics in *Echinococcus multilocularis* transmission. *Veterinary Parasitology,* 213(3-4), pp. 162-171.

Rausch, R.L. & Fay, F.H. (1988a). Postoncospheral development and cycle of *Taenia polyacantha* Leuckart, 1856 (Cestoda: Taeniidae). First part. *Annales de Parasitologie Humaine et Comparée,* 63(4), pp. 263-277.

Rausch, R.L. & Fay, F.H. (1988b). Postoncospheral development and cycle of *Taenia polyacantha* Leuckart, 1856 (Cestoda: Taeniidae). Second part. *Annales de Parasitologie Humaine et Comparée,* 63(5), pp. 334-348.

Rausch, R.L., Wilson, J.F. & Schantz, P.M. (1990). A programme to reduce the risk of infection by *Echinococcus multilocularis*: The use of praziquantel to control the cestode in a village in the hyperendemic region of Alaska. *Annals of Tropical Medicine and Parasitology,* 84(3), pp. 239-250.

Reperant, L.A., Hegglin, D., Tanner, I., Fischer, C. & Deplazes, P. (2009). Rodents as shared indicators for zoonotic parasites of carnivores in urban environments. *Parasitology,* 136(3), pp. 329-337.

Robardet, E., Giraudoux, P., Caillot, C., Augot, D., Boue, F. & Barrat, J. (2011). Fox defecation behaviour in relation to spatial distribution of voles in an urbanised area: An increasing risk of transmission of *Echinococcus multilocularis*? *International Journal for Parasitology,* 41(2), pp. 145-154.

Romig, T., Dinkel, A. & Mackenstedt, U. (2006). The present situation of echinococcosis in Europe. *Parasitology International,* 55, pp. S187-S191.

Saeed, I., Maddox-Hyttel, C., Monrad, J. & Kapel, C.M.O. (2006). Helminths of red foxes (*Vulpes vulpes*) in Denmark. *Veterinary Parasitology,* 139(1-3), pp. 168-179.

Said-Ali, Z., Grenouillet, F., Knapp, J., Bresson-Hadni, S., Vuitton, D.A., Raoul, F., Richou, C., Millon, L. & Giraudoux, P. (2013). Detecting nested clusters of human alveolar echinococcosis. *Parasitology,* 140(13), pp. 1693-1700.

Saitoh, T. & Takahashi, K. (1998). The role of vole populations in prevalence of the parasite (*Echinococcus multilocularis*) in foxes. *Researches on Population Ecology,* 40(1), pp. 97-105.

Schantz, P.M., Wang, H., Qiu, J., Liu, F.J., Saito, E., Emshoff, A., Ito, A., Roberts, J.M. & Delker, C. (2003). Echinococcosis on the Tibetan Plateau: Prevalence and risk factors for cystic and alveolar echinococcosis in Tibetan populations in Qinghai Province, China. *Parasitology,* 127(Supplement 1), pp. S109-S120.

Schweiger, A., Ammann, R.W., Candinas, D., Clavien, P.-A., Eckert, J., Gottstein, B., Halkic, N., Muellhaupt, B., Prinz, B.M., Reichen, J., Tarr, P.E., Torgerson, P.R. & Deplazes, P. (2007). Human alveolar echinococcosis after fox population increase, Switzerland. *Emerging Infectious Diseases,* 13(6), p. 878-882.

Soveri, T., Henttonen, H., Rudbäck, E., Schildt, R., Tanskanen, R., Husu-Kallio, J., Haukisalmi, V., Sukura, A. & Laakkonen, J. (2000). Disease patterns in field and bank vole populations during a cyclic decline in central Finland. *Comparative Immunology, Microbiology and Infectious Diseases,* 23(2), pp. 73-89.

Stieger, C., Hegglin, D., Schwarzenbach, G., Mathis, A. & Deplazes, P. (2002). Spatial and temporal aspects of urban transmission of *Echinococcus multilocularis*. *Parasitology*, 124(6), pp. 631-640.

Stärk, K.D.C., Regula, G., Hernandez, J., Knopf, L., Fuchs, K., Morris, R.S. & Davies, P. (2006). Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health: Review of current approaches. *BMC Health Services Research*, 6, p. e20.

Tenora, F., Wiger, R. & Baruš, V. (1979). Seasonal and annual variations in the prevalence of helminths in a cyclic population of *Clethrionomys glareolus*. *Holarctic Ecology*, 2(3), pp. 176-181.

Thompson, R.C.A. & McManus D.P. (2001). Aetiology: Parasites and life-cycles. In: Eckert, J., Gemmel, M.A., Meslin, F.X., & Pawłowski, Z.S. (Eds) *WHO-OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern*. Paris, France: WHO/OIE, pp. 1-19.

Thompson, R.C.A. (1995). Biology and systematics of *Echinococcus*. In: Thompson, R.C.A. & Lymbery. A.J. (Eds) *Echinococcus and Hydatid Disease*, Wallingford, UK: Cab International, pp. 1-50.

Torgerson, P.R., Schweiger, A., Deplazes, P., Pohar, M., Reichen, J., Ammann, R.W., Tarr, P.E., Halkik, N. & Müllhaupt, B. (2008). Alveolar echinococcosis: From a deadly disease to a well-controlled infection. Relative survival and economic analysis in Switzerland over the last 35 years. *Journal of Hepatology*, 49(1), pp. 72-77.

Trachsel, D., Deplazes, P. & Mathis, A. (2007). Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. *Parasitology*, 134(6), pp. 911-920.

Wabakken, P., Sand, H., Liberg, O. & Bjärvall, A. (2001). The recovery, distribution, and population dynamics of wolves on the Scandinavian peninsula, 1978-1998. *Canadian Journal of Zoology*, 79(4), pp. 710-725.

Wahlström, H., Enemark, H.L., Davidson, R.K. & Oksanen, A. (2015). Present status, actions taken and future considerations due to the findings of *E. multilocularis* in two Scandinavian countries. *Veterinary Parasitology*, 213(3-4), pp. 172-181.

Wahlström, H., Isomursu, M., Hallgren, G., Christensson, D., Cedersmyg, M., Wallensten, A., Hjertqvist, M., Davidson, R.K., Ulhorn, H. & Hopp, P. (2011). Combining information from surveys of several species to estimate probability of freedom from *Echinococcus multilocularis* in Sweden, Finland and mainland Norway. *Acta Veterinaria Scandinavica*, 53, p. e9.

Wahlström, H., Lindberg, A., Lindh, J., Wallensten, A., Lindqvist, R., Plym-Forshell, L., Osterman Lind, E., Agren, E.O., Widgren, S., Carlsson, U., Christensson, D., Cedersmyg, M., Lindström, E., Olsson, G.E., Hörnfeldt, B., Barragan, A., Davelid, C., Hjerteqvist, M. & Elvander, M. (2012). Investigations and actions taken during 2011 due to the first finding of *Echinococcus multilocularis* in Sweden. *Euro Surveillance.*, 17(28), p. e20215.

Webbon, C.C., Baker, P.J. & Harris, S. (2004). Faecal density counts for monitoring changes in red fox numbers in rural Britain. *Journal of Applied Ecology*, 41(4), pp. 768-779.

Wiger, R., Lien, L. & Tenora, F. (1974). Studies on the helminth fauna of Norway XXXIII: *Tetratirotaenia polyacantha* (Leuckart, 1856) Abdulaze, 1964, a parasite of *Clethrionomys glareolus* in Norway. *Norwegian Journal of Zoology*, 22, pp. 61-64.

Wilson, D.E. & Reeder, D.M. (Eds) (2005). *Mammal Species of the World: A Taxonomic and Geographic Reference, Third Ed.* Baltimore, Maryland: Johns Hopkins University Press. Online database from Smithsonian National Museum of Natural History. http://vertebrates.si.edu/msw/mswCFApp/msw/index.cfm. Accessed Jan. 26, 2016.

Woolsey, I.D., Jensen, P.M., Deplazes, P. and Kapel, C.M.O. (2016). Peroral *Echinococcus multilocularis* inoculation in *Myodes glareolus*, *Mesocricetus auratus* and *Mus musculus* (CD-1 IGS and C57BL/6j). *International Journal for Parasitology: Parasites and Wildlife*, 5(2), 158-163.

Woolsey, I.D., Bune, N.E.T., Jensen, P.M., Deplazes, P. & Kapel, C.M.O. (2015a). *Echinococcus multilocularis* infection in the field vole (*Microtus agrestis*): An ecological model for studies on transmission dynamics. *Parasitology Research*, 114(5), pp. 1703-1709.

Woolsey, I.D., Jensen, P.M., Deplazes, P. & Kapel, C.M.O. (2015b). Establishment and development of *Echinococcus multilocularis* metacestodes in the common vole (*Microtus arvalis*) after oral inoculation with parasite eggs. *Parasitology International*, 64(6), pp. 571-575.

Veit, P., Bilger, B., Schad, V., Schäfer, J., Frank, W. & Lucius, R. (1995). Influence of environmental factors on the infectivity of *Echinococcus multilocularis* eggs. *Parasitology*, 110(1), pp. 79-86.

Viel, J.F., Giraudoux, P., Abrial, V. & Bresson-Hadni, S. (1999). Water vole (*Arvicola terrestris scherman*) density as risk factor for human alveolar echinococcosis. *The American Journal of Tropical Medicine and Hygiene*, 61(4), pp. 559-565.

von Schantz, T. (1981). Female cooperation, male competition, and dispersal in the red fox *Vulpes vulpes*. *Oikos*, 37(1), pp. 63-68.

Vuitton, D.A., Demonmerot, F., Knapp, J., Richou, C., Grenouillet, F., Chauchet, A., Vuitton, L., Bresson-Hadni, S. & Millon, L. (2015). Clinical epidemiology of human AE in Europe. *Veterinary Parasitology*, 213(3-4), pp. 110-120.

Vuitton, D.A. & Gottstein, B. (2010). *Echinococcus multilocularis* and its intermediate host: a model of parasite-host interplay. *Journal of Biomedicine and Biotechnology*, 2010, pp. e923193.

Vågsholm, I. An assessment of the risk that EM is introduced with dogs entering Sweden from other EU countries without and with antihelmintic treatments. Uppsala, Sweden: National Veterinary Institute. http://www.sva.se/upload/pdf/ask/qra_emdogsaug06.pdf.

Yamamoto, N., Kishi, R., Katakura, Y. & Miyake, H. (2001). Risk factors for human alveolar echinococcosis: A case-control study in Hokkaido, Japan. *Annals of Tropical Medicine and Parasitology*, 95(7), pp. 689-696.

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