The role of *Apodemus* spp. in the life cycle of taeniid parasites including *Echinococcus multilocularis* in Sweden

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Abstract

Introduction: Taeniid tapeworms, which include *Echinococcus* and *Taenia* spp. are of great importance in both human and veterinary medicine. For example, alveolar echinococcosis is a zoonotic parasitic disease caused by *Echinococcus multilocularis*. After the first detection of *E. multilocularis* in Sweden in 2011, a research program was set up to identify which rodent species function as an intermediate host for this parasite. Other taeniid cestodes that have a similar life cycle can help to give further indication of the interaction between foxes and mice and a consequent possible risk of the spread of *E. mutlilocularis*. To our knowledge, nothing has been published about taeniid parasites in *Apodemus* spp. in Sweden and, because of that, this study looks specifically at the role of two *Apodemus* species native in Sweden, *A flavicollis* and *A sylvaticus*.

Materials and methods: For this study, 284 *Apodemus* mice were examined for both *E. multilocularis* and other taeniid cestodes. The investigated mice belong to the species *Apodemus flavicollis* and *A. sylvaticus* and were captured in four different areas in Sweden in the spring and fall of 2014. After storage at -20˚C, the 284 *Apodemus* mice were dissected with special focus on parasitic cysts in the liver, which were identified with a PCR specific for *E. multilocularis* and *Taenia* species and then further confirmed by sequencing.

Results: A total of 13 *Taenia* cestodes were identified from the examined *Apodemus* mice, giving an overall *Taenia* prevalence of 4.6% (13/284). The identified taeniid species were *Hydatigera taeniaeformis* (2.1%, 6/284) and *Taenia polyacantha* (0.4%, 1/284). In seven animals, the specific species could not be determined due to low identity matches. *E. multilocularis* has not been identified. The prevalence of *Taenia* infections was significantly higher in breeding than in non-breeding animals, and significantly more animals were infected in Gnesta/Nyköping than in Katerineholm. No significant difference was found in parasite prevalence between species, season and sex.

Conclusion: The results show that both *A. flavicollis* and *A. sylvaticus* function as intermediate hosts for at least two *Taenia* species in Sweden. Although it is not clear if they play a big role in the transmission of these cestodes to foxes, this study has demonstrated that there is a transmission of these parasites between foxes and rodent intermediate hosts in general. In contrast, *E. multilocularis* was not found in the *Apodemus* mice. Although the prevalence of *E. multilocularis* is still on a very low (0.1%), it has been detected in other rodent species (*Microtus agrestis* and *Arvicola amphibius*) in Sweden. This may indicate that *Apodemus* spp. are unsuitable as an intermediate host for this parasite in Sweden. A suggestion for future work is to investigate the community of *Taenia* spp. in the local rodent population. If the prevalence of *Taenia* spp. in other rodent populations is higher in areas with a higher *E. multilocularis* prevalence as well, *Taenia* spp. could maybe serve as an indicator for relative prevalence and increased risk of *E. multilocularis*.

Introduction

Taeniid tapeworms are of importance in both human and veterinary medicine. Some species within the genera *Echinococcus* and *Taenia* are zoonotic and can cause serious illness in humans. For example, alveolar echinococcosis is a zoonotic disease caused by larval stages of the parasite *E. multilocularis.* It is a rare disease, but it is considered to be one of the most serious parasitic diseases in humans in Europe.[1] *E. multilocularis* has recently been discovered in Sweden[2], however not much is known about the epidemiology of the parasite in this country yet. Because of this, a research project was set up in both foxes and rodents, to learn more about the parasite in this country. In order to find the most important intermediate hosts for *E. multilocularis*, several rodent species are examined for the parasite as well as for other taeniid cestodes. These other taeniid cestodes have a similar life cycle and may help to give an indication of the interaction between foxes and the mice, and a consequent possible risk of the spread of *E. mutlilocularis* in areas of low *E. multilocularis* prevalence. During the beginning of the project it was unknown which rodent species were functioning as intermediate hosts in Sweden. Little is known about taeniid parasites in *Apodemus* mice in Sweden and if these mice could play a role in the life cycle of taeniid parasites in general. Because of that, this study looks specifically at the role of two *Apodemus* species native in Sweden, *A flavicollis* and *A sylvaticus*, as intermediate hosts for *E. mutlilocularis* and other taeniid cestodes.

E. multilocularis in Europe and Sweden

E. multilocularis is endemic in mountainous areas in Europe, but has been increasingly reported in regions near Sweden. The highest endemic areas in central Europe occur in the northern pre-alpine regions, the high Tatra mountains between Poland and Slovakia, the Jura mountains in France, Switzerland and Germany and in the mountain areas from southern Belgium to central Germany.[3] Although the reason for these high endemic areas is not yet clear, it appears to be linked to climatic factors, land use and landscape patterns, that favour the most important mid European intermediate hosts *Microtis arvalis* (common vole) and *Arvicola terrestris* (*A.(terrestris) scherman* and *A. (terrestris) amphibius*) (water vole).[3] The drastic increase in fox population densities after the demise of rabies prevalence rates also seem to have had a positive effect on the parasite density in south-west Germany.[3]

For a long time, Sweden was considered free from *E. multilocularis*. After the detection of *E. multilocularis* in Denmark in 2000[4], a surveillance program was begun to analyse about 300 red foxes every year in southern Sweden.[5] The first detection of *E. multilocularis* in a red fox (*Vulpes vulpes*) in Sweden was after the expansion of the investigated area in February 2011.[2] Following this finding surveillance was increased, and three more cases were identified in foxes a few months later.[2] Due to these findings surveillance was continued.[5] Since Sweden is not directly connected to mainland Europe, it is not clear if the epidemiology of the disease can be compared to the other European countries. For example, the most important intermediate host in mainland Europe, *Microtus arvalis* does not occur in Sweden and only one of the two *Arvicola* species (*A. amphibius*) is present in Sweden.[2] In order to study the rodent's role in the parasite's life cycle and to determine the most important intermediate host species in Sweden, an additional research project was initiated. The overall aim of this EMIRO project, Echinococcus Multilocularis In ROdents, is to improve the understanding about the role of the different wild rodent communities for the life cycle of *E. multilocularis* and thereby providing fundamental knowledge to predict the risk for *E. multilocularis* infection and spread and to identify key factors limiting the parasite transmission

(http://www.emiro.org). Through these activities, *E. multilocularis* has now been found in fox faeces from four different investigated areas in Sweden (Västra Götaland, Södermanland, Dalarna and Småland) and in one area in Södermanland also in rodents.[6]

Taeniidae

Taxonomy

The family of Taeniidae incorporates four genera that are closely related, *Taenia*, *Echinococcus*, *Hydatigera* (e.g. *H. taeniaeformis*) and *Versteria* (e.g. *V. mustulae*) (table 1). To date, four different *Echinococcus* spp. and nearly 50 different *Taenia* spp. are recognized, with varying intermediate and definitive hosts.[1,7]

Table 1. Taxonomy of Taeniidae [8,9]

Phylum	Platyhelminths
Class	Cestoda
Order	Cyclophyllidea
Family	Taeniidae
Genus	*Taenia*
	Echinococcus
	Hydatigera
	Versteria

Life cycle

The life cycle of taeniid cestodes is indirect and involves two mammalian hosts and requires a predator-prey relationship to be completed.[10]

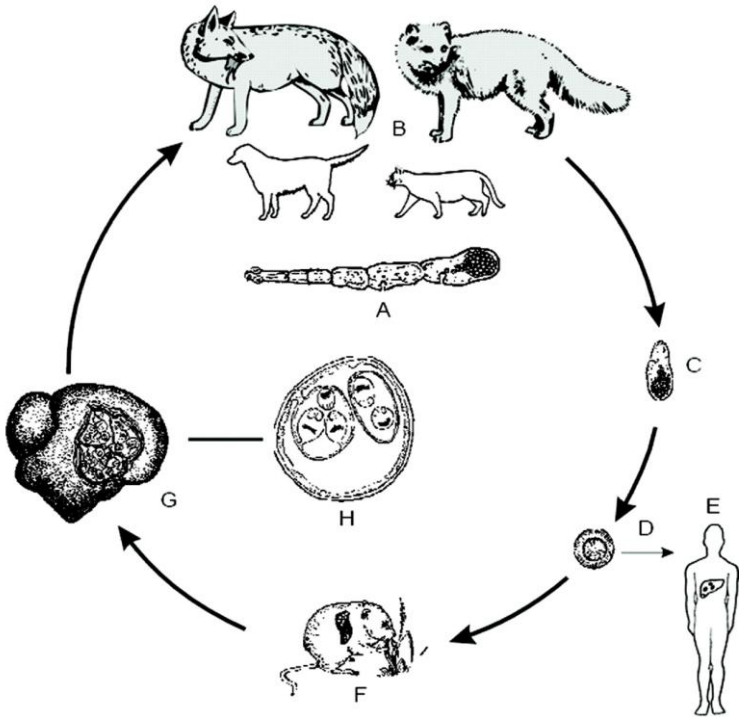

Fig. 1: Life cycle of *E. multilocularis*. A) Adult cestode, B) Carnivorous host (red fox, artic fox, dog and cat), C) Proglottid with eggs, D) Eggs with oncosphere, E) Human as an aberrant host, F) Infected rodent, G) Rodent liver with metacestodes, H) Metacestode with protoscoleces.

Source: Eckert J, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin Microbiol Rev. 2004;17(1):107-135.

Adult stage

The adult cestodes inhabit the small intestine of a carnivore definitive host. The infection is usually harmless and in general causes mild local inflammation in the small intestine.[10] The adult worms vary in length, from less than two millimetres (eg. *Echinococcus*) up to more than ten meters long.[11] In general, the structure of adult taeniids is similar, all containing a scolex with four suckers and sometimes a cone-like rostellum with a crown of chitinized hooks, a neck region and proglottids. The scolex makes it possible for the worm to anchor to the intestinal mucosa. Proglottisation takes place from the neck region and new proglottids are continuously produced. Eggs are produced by sexual reproduction within the proglottids until the proglottids at the posterior terminus of the worm are filled with eggs and will be shed from the worm, passing within the faeces of the carnivorous host into the environment.[10]

Larval (metacestode) stage

After oral uptake of eggs by an intermediate host, for example with contaminated plant material or water, they hatch within the small intestine and release an oncosphere, a hexacanth (six-hooked) embryo, that penetrates the intestine wall.[11] Via the circulatory or lymphatic system the oncosphere is brought to the site of predilection where it develops into a larval cystic stage (metacestode), which is infective to canids. Taeniid metacestodes can parasitize various organs and tissues or develop free in the body cavity, depending on species.[10] Taeniids in rodents mainly have a predilection for the liver or the peritoneal cavity. The cycle completes when an intermediate host containing mature metacestodes is eaten by a suitable definitive host. [11,12]

Types of metacestodes

A metacestode is generally a bladder-like cestode larva with an invaginated scolex. Five types of metacestodes are recognized; cysticercus, coenurus, strobilocercus, fimbriocercus and echinococcus (fig. 2.) The types we can find in rodents are cysticerci, strobilocerci and echinococcus.

Asexual proliferation has been observed in all types of taeniid metacestodes but is generally rare. Within the *E. multilocularis* metacestode, asexual reproduction is more common. Numerous protoscoleces are produced, which have the potential to develop into an adult cestode in a suitable definitive host.[10]

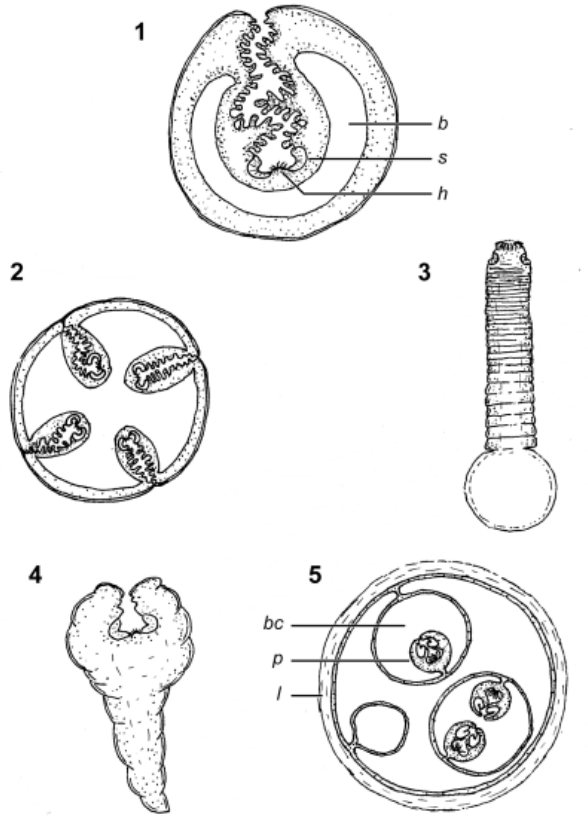

Fig. 2. Taeniid metacestodes. 1) Cysticercus, b = bladder, h = rostellar hooks, s = sucker; 2) Coenurus, a polycephalic cysticercus, 3) Strobilocercus, with segmentation and a terminal bladder, 4) Fimbriocercus, a solid-bodied larva, 5) Echinococcus, a cystic or multicystic structure with, bc = brood capsule, l = laminated layer, p = protoscolex.
Source: Lavikainen A.; A taxonomic revision of the taeniidae ludwig, 1886 based on molecular phylogenies. 2014.

Transmission

E. multilocularis is usually transmitted between foxes of the genera *Vulpes* and *Alopex* and certain rodents.[13] In mainland Europe the most important intermediate hosts are *M. arvalis* and *A. terrestris*.[3] Humans can become infected after accidental uptake of eggs. Dogs are also susceptible definitive hosts and can play a role in the transmission to humans due to close contact.[3] In humans, a slowly progressive larval mass with an alveolar structure will develop in the liver or other internal organs after a long incubation period of 5-15 years, which can lead to severe disease and even death if untreated.[1]

Cestodes that belong to the genus *Taenia* have a great range of different definitive and intermediate hosts, varying from cattle, pigs, humans and domestic and wild carnivores to lagomorphs and rodents. *Taenia* cestodes that are known to be transmitted between rodents and carnivores in Sweden are *T. crassiceps, T. martis martis, V. mustelae, T. polyacantha* and *H. taeniaeformis.*[7,9]Among these only *T. crassiceps, T. polyacantha* and *H. taeniaeformis* are only? transmitted between foxes and rodents.[7]

Table 2. Most important definitive and intermediate hosts , zoonotic risk and metacestode stage of *E. multilocularis* and *Taenia* spp. that can be found in rodents[7,14]

Cestode	Definitive host	Intermediate host	Zoonotic	Metacestode (predeliction place, type and appearance)
E. multilocularis	Foxes, *dogs*	Rodents	Yes	Echinococcus: Liver, cystic or multicystic structure with protoscoleces
T. crassiceps	Foxes, *cats*	Rodents	Yes	Cysticercus: Subcutaneously or in body cavities, unencysted, transparent and up to 3mm long
T. martis martis	Martens	Rodents	No	Cysticercus: In very early stage in lungs, where it can undergo asexual reproduction. Later in pleural and peritoneal cavity, free floating and up to 50mm long
V. mustulae	Weasels	Rodents	No	Cysticercus: Liver, usually on the surface. The cysts are 1 – 3mm in diameter and contain a 0.8 – 2mm oval larva
T. polyacantha	Foxes	Rodents	No	Cysticercus: Starts as a vesicle in the liver that undergoes asexual reproduction and passes to the peritoneal cavity where it is a free floating, unencysted, 4 – 12mm long larva
H. taeniaeformis	Cats, *foxes*	Rodents	Yes	Strobilocercus: Yellowish cyst up to 12mm in length that contains a larva that can be up to 350mm long. It does not undergo asexual reproduction

Apodemus spp. as a possible intermediate host for *E. multilocularis*

During the beginning of the EMIRO project, it was unknown which rodent species were functioning as intermediate hosts for *E. multilocularis* in Sweden.[15] The most important intermediate host in mainland Europe, *Microtus arvalis* does not occur in Sweden and only one of the two *Arvicola* species (*A. amphibius*) is present in Sweden.[2] Four types of different rodent species are frequently captured in the areas in Sweden where *E. multilocularis* has been identified in foxes,[16] *A. amphibius* (water vole), *M. agrestis* (common vole), *Myodes glareolus* (bank vole) and two *Apodemus* species, *Apodemus flavicollis* (yellow-necked mouse) and *A. sylvaticus* (wood mouse). In order to find out which species function as an intermediate host in Sweden, all captured animals are examined for *E. multilocularis* and other taeniid parasites. Examination is still ongoing, but *E. multilocularis* has been detected in two species already, *A. amphibius* and *M. agrestis.*[17] Nothing is known about the role of *Apodemus* spp. in the life cycle of *E. multilocularis* and other taeniid cestodes in Sweden yet and, for this reason,I focus on the *Apodemus* spp. captured as part of the EMIRO project for this study.

The transmission of taeniid cestodes depends on a large number of interacting factors, including the intermediate host density, predation of the intermediate host and the dietary specialisation of the definitive host.[18] The fox is an opportunistic species and its diet is variable in time and space. About 65% of fox diet consists of rodents and they tend to prey

mostly on grassland species, especially *Microtus* voles.[19] The habitat of both *Apodemus* species mainly contains woodland areas, but they are also often found in human proximity in parks and gardens or even in houses.[20] Research has shown that *Apodemus* spp. occurs less frequently in fox diet.[18,19,21-23] One hypothesis for this is that rodent species in woodland areas are less accessible due to their habitat and behavioural ability to avoid predation, but this hypothesis has not been tested yet.[24] If so, it would be expected that there is little interaction between foxes and *Apodemus* mice and that the transmission of taeniid cestodes would also be low. However, when the population density of grassland species declines, the fox's diet can be more diversified and it is possible that forest species will make up a greater amount of their diet.[24] Still, other studies show that forest species are not significantly preyed upon more at low densities of *Microtus* voles.[21-23]

To our knowledge nothing has been published about taeniid cestodes in *Apodemus* spp. in Sweden yet, but *Apodemus* spp. has been confirmed to be a suitable intermediate host for taeniid species in other countries.[25] In contrast, *E. multilocularis* is rarely found in *Apodemus* mice, even in endemic areas.[26,27] Although this indicates that *Apodemus* spp. are not the most important host for the maintenance of the life cycle of *E. multilocularis*, there are rare reports of *E. multilocularis* in *Apodemus* spp. In two studies from 1984, *E. multilocularis* was identified in *A. argenteus* in Japan[28], and *A. flavicollis* in Slovenia.[29] *E. multilocularis* has also been reported from *A. agrarius* in Belarus in 1963 and between 1980–1999 from *A. sylvaticus* and *A. agrarius*.[30] A more recent report comes from Iran, 2013, where an *E. multilocularis* cyst was confirmed by PCR in *A. witherbyi.*[31] However, it is not clear if these metacestodes were well developed and harboured infectious protoscoleces.

Objective/Aim of the study

The prevalence of taeniid cestodes in rodents is a critical component for understanding the intensity of interaction between the carnivorous definitive hosts and *Apodemus* spp. and to determine the risk for *Apodemus* spp in the spread of *E. multilocularis* in Sweden. The aim of this study was to evaluate the prevalence of taeniid metacestodes in two *Apodemus* species, *A. flavicollis* and *A. sylvaticus*, and in particular their role as an intermediate host for *E. multilocularis* in four low endemic areas. An investigation was initiated to answer the following two hypotheses:

> *"*Apodemus flavicollis *and* Apodemus sylvaticus *do play a significant role as an intermediate host for taeniid cestodes in Sweden."*
>
> *"*Apodemus flavicollis *and* Apodemus sylvaticus *do not play a significant role as an intermediate host for E. multilocularis in Sweden."*

Materials and methods

Study area

Fieldwork (snap trapping) was conducted during 4 to 11 day trapping periods in the spring (April 2 – May 12) and fall (September 10 – October 13) of 2014 in four different areas in Sweden: Katrineholm, Gnesta/Nyköping, Uddevalla and Växjö. The areas in Katrineholm and Uddevalla were selected by the EMIRO project on the basis of previous positive *E. multilocularis* findings in foxes.[5] Gnesta/Nyköping and Växjö were chosen due to collaboration with an ongoing surveillance and monitoring project through FoMA Zoonos (Fortlöpande MiljöAnalys/Environmental Assessment; http://www.slu.se/en/environment/) These were areas where the status of *E. multilocularis* was unknown at the beginning of the EMIRO project.

Trapping method

Study areas were ~20km^2. The snap traps were placed in small quadrats based on the method described in Myllymäki 1971.[32] In short, three snap-traps were placed within a 1m radius in each of the four corners of a 15x15m square. The traps were placed in a position in which they were most likely to catch a rodent and provided with a special bait (fig. 3). Each trap site consisted of two to four quadrats placed at least 50 meters apart. The traps were left at the same position for two nights and examined for trapped animals every day. The locations of the quadrats was recorded by GPS and marked with plastic sticks (fig. 4); therefore, the same quadrats were used for the duration of the study. Once animals were removed from traps, they were identified to species, put in a bag with a unique identification number and frozen at -20°C.

Fig. 3. Bait used in the snap traps, hemp seed sandwiched by two pieces of beeswax
Photo: Nannet Fabri

Fig. 4. Snap trap marked with a plastic stick
Photo: Nannet Fabri

Snap trap sites in Katrineholm and Uddevalla were chosen in a semi-random fashion in order to avoid inaccessible areas (i.e. lakes, roads) and to ensure adequate sampling of both forest and field habitat types. The trapping sites in Gnesta/Nyköping and Växjö were predetermined on the basis of an ongoing FoMA project. At the predetermined FoMA location, the specific criteria for setting trap sites were relative closeness to roads and the presence of forest area (coniferous, deciduous or combination). In all four areas, most of the traps were located along ecotones (i.e. habitat borders). Animals used in this study were previously trapped in 2014.

The number of *Apodemus* trapped per 100 trap-nights can be used as an index for mouse density.

Dissection and parasite collection

The collected animals were frozen at -20°C in the field and kept frozen until analysis. The mice were dissected in a fumehood in order to protect from potential aerosol virus exposure.

Species determination

The species (*A. flavicollis* or *A. sylvaticus*) were determined on the basis of biometric and morphometric data, looking at weight, total length, tail length, foot length (right hind foot, from the heel to the tip of the central toe), colour and the presence of a coloured chest band (fig. 5). The right front paw of each animal was saved in 95% ethanol for possible further research to look at the genomic differentiation between both species.

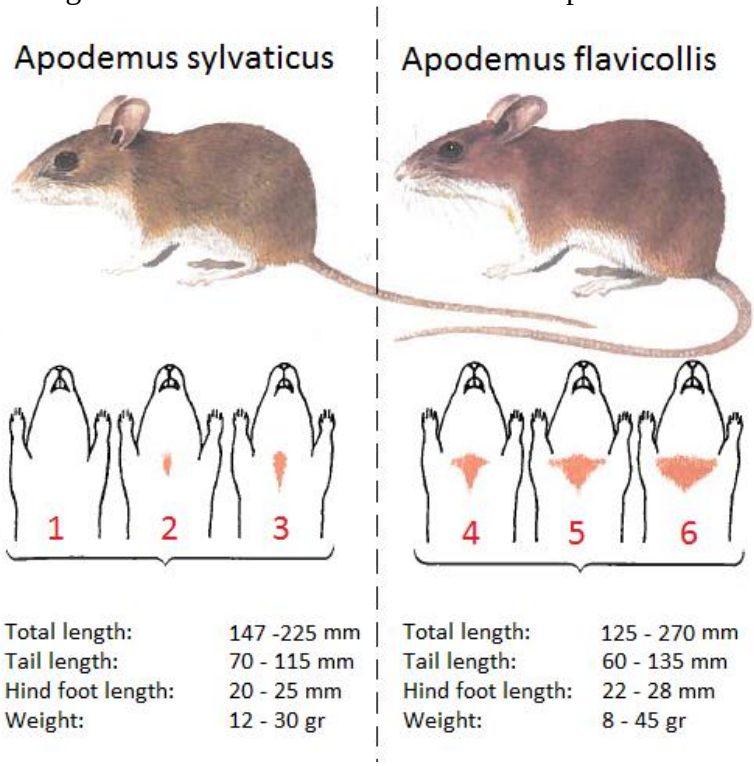

Fig. 5. Biometric and morphometric differences between *Apodemus* spp.
Drawing modified from: Lundberg P, de Jong J. Sveriges smådäggdjur. Fourth edition ed. Fältbiologerna Förlag; 1995.

Sex and age determination

Sex and reproductive status (breeding/non-breeding) of the animals were also determined. The animals were placed in three categories, breeding (adults), non-breeding (sub-adults and juveniles) and unknown. Female animals were categorized as breeding when they were lactating, had a perforated vagina and/or placental scars or embryos in the uterus. In males, the functional group was determined by the presence of seminal vesicles and testes size. *A.flavicollis* males were categorized as breeding when they had seminal vesicles present and testes size of 11mm and above. *A. sylvaticus* males were categorized as breeding when they had seminal vesicles present and testes testes size of 9mm and above. Females categorized as unknown were females that had a uterus that was thicker than in non-breeding animal or in which it was not clear if the vagina was perforated or not. *A. sylvaticus* males placed in the group unknown had seminal vesicles present but a testes size smaller than 9mm, or a testes size of 9mm and above but no indication of seminal vesicle presence written in the notes. *A.flavicollis* males placed in the group unknown had a testes size of 11mm and above but no

indication of seminal vesicle presence written in the notes. All the other animals were categorized as non-breeding.

Sample collection

The intestines were collected, weighed and frozen for possible further research. Blood was also collected from the heart using filter paper for use in another project examining these rodents for other pathogens. Although special attention was paid to the liver, all abdominal organs were macroscopically examined for suspect parasitic cysts. The livers were cut out and examined over a light source to detect cysts located within the parenchyma. Any suspected lesion or mass in the peritoneal cavity or organs was collected.

Liver lesions

If cysts or suspect lesions were found in the liver, its location and the size were determined. They were then cut out and stored at -20°C for molecular identification. If there were more than three lesions, only three (different looking) lesions from different areas of the liver were collected and identified by PCR. Free floating larvae in the peritoneal cavity were also collected for identification. Well-developed cysts of *H. taeniaeformis* were identified morphologically and were collected and saved but not further identified by PCR.

Identification of parasites

Genomic DNA was isolated from suspect parasitic cysts using a commercial DNA extraction kit (Qiagen, QIAamp DNA Mini Kit, Sollentuna, Sweden). A multiplex PCR (Qiagen, Qiagen Multiplex PCR Kit, Sollentuna, Sweden), was performed with primers targeting regions of the mitochondrial genome specific for *E. multilocularis* and *Taenia* spp. (Cest 1, Cest 2, Cest3, Cest4, and Cest5) as described in Trachsel 2007.[33] The PCRs were always run with one negative and two positive (DNA extracted from *E. multilocularis* and *T. crassiceps*.) controls. In case of positive reaction for *Taenia* spp. or *E. multilocularis*, the amplicons were sequenced for species confirmation. PCR products were cleaned with an enzyme (illustra ExoProStar 1-step, GE Healthcare Europe, Sweden Branch, Uppsala Sweden) and shipped to an outside laboratory (Macrogen, Amsterdam, The Netherlands) for sequencing. Results were analysed using the software CLC Main Workbench v5.6.1. and submitted for a nucleotide BLAST through the NCBI database. Only sequences with more than 95% quality cover and identity were accepted.

Data analysis

Data were analysed using SPSS 20 with $p \leq 0.05$ used as a cut-off for inferring statistical significance. Sample prevalence and 95% exact binominal confidence intervals (CIs) were calculated for overall *Taenia* prevalence and prevalence in species, area, season, sex, and functional group. A chi-square test was used to determine if there was a difference in sex or functional status of the trapped mice in different seasons. A Fisher's exact test was used to determine whether *Taenia* prevalence differed among species, area, season, sex, and functional group. A logistic regression was used to determine if species, study area, season, sex and functional group contribute significantly to the chance of having a taeniid infection. Sampling seasons were defined as spring (April 2 – May 12) and fall (September 10 – October 13) 2014.

Results

Captured mice

Over 14,034 trap nights, a total of 284 *Apodemus* mice were captured, 79 *A. flavicollis* and 205 *A. sylvaticus.* Trapping results for the *Apodemus* spp. are summarized in Table 3 and in the appendix. Most animals were captured in Växjö (87), followed by Katerineholm (84), Uddevalla (77) and Gnesta/Nyköping (36) (fig. 6) and more mice were captured in the fall (207) than in the spring (77) (fig. 7).

Table 3. Number of captured *Apodemus*, traps, trap nights and trapping indices (captures/100 trap nights) per area and season in 2014

Area	Season	Number of captured *Apodemus*	Number of traps	Trap nights	Trapping indices
Katrineholm	Spring	19	1104	2	0.8
	Fall	65	1056	2	3.1
Gnesta/Nyköping	Spring	7	624	2	0.5
	Fall	29	576	2	2.5
Uddevalla	Spring	40	1053	2	1.9
	Fall	37	1068	2	1.7
Växjö	Spring	11	768	2	0.7
	Fall	76	768	2	4.9

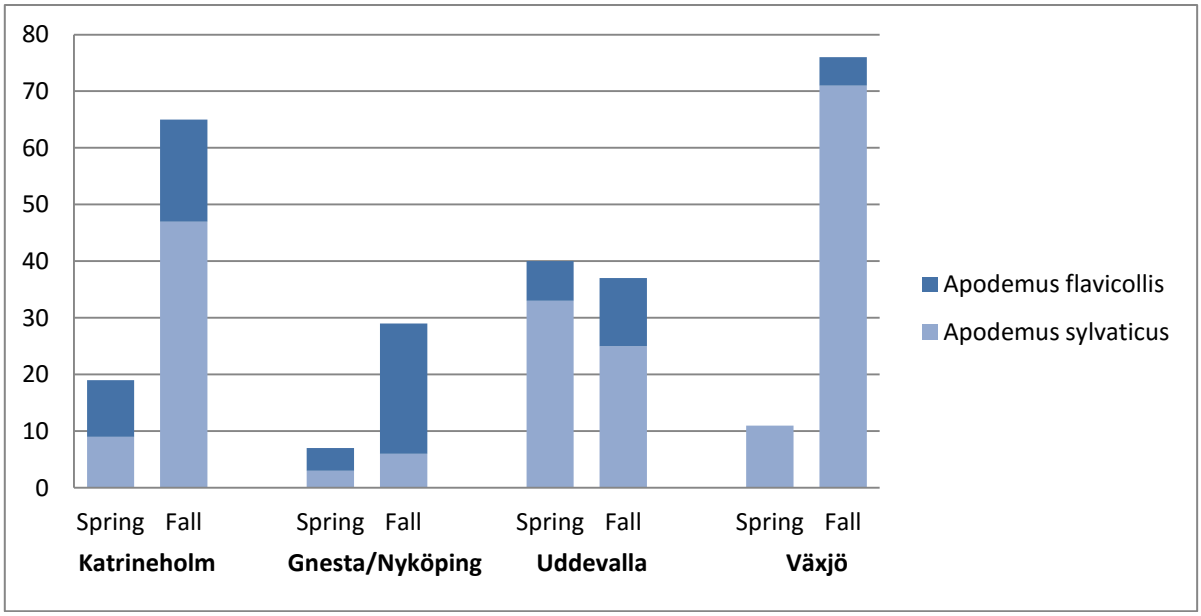

Fig. 6. Examined *Apodemus* spp. classified per location and season

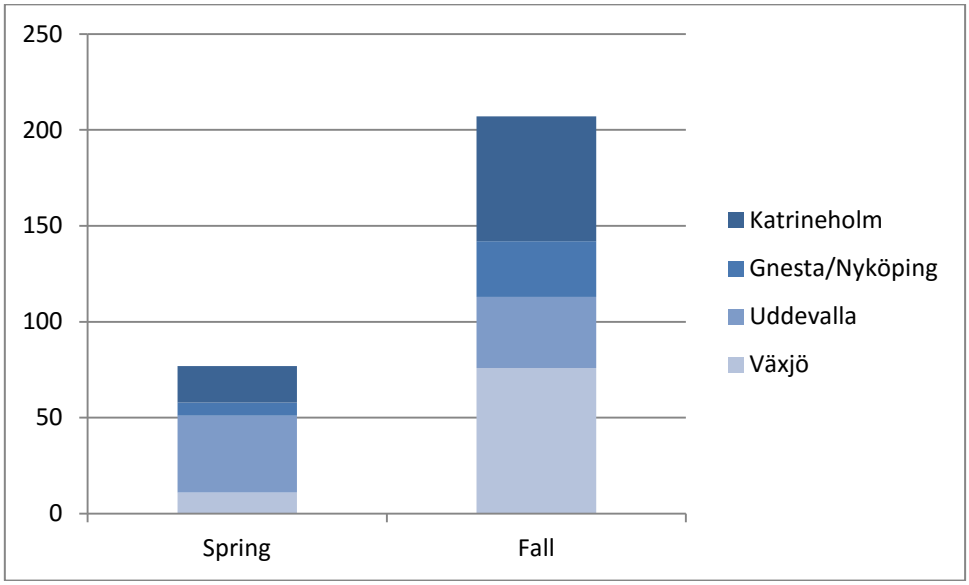

Fig. 7. Examined *Apodemus* mice classified per area and season

Of the 284 examined mice, there were 135 females and 149 males. The M:F sex ratio was 1:0.9 and there was no significant difference in sex ratio between spring and fall (Chi^2, p = 0.845).
There were more non-breeding (149) than breeding (107) animals caught. For 28 animals the reproductive status could not be determined, and they were placed in the category 'unknown'. Significantly more non-breeding animals were caught during the fall than the spring (Chi^2, p = 0.000) (fig. 8).

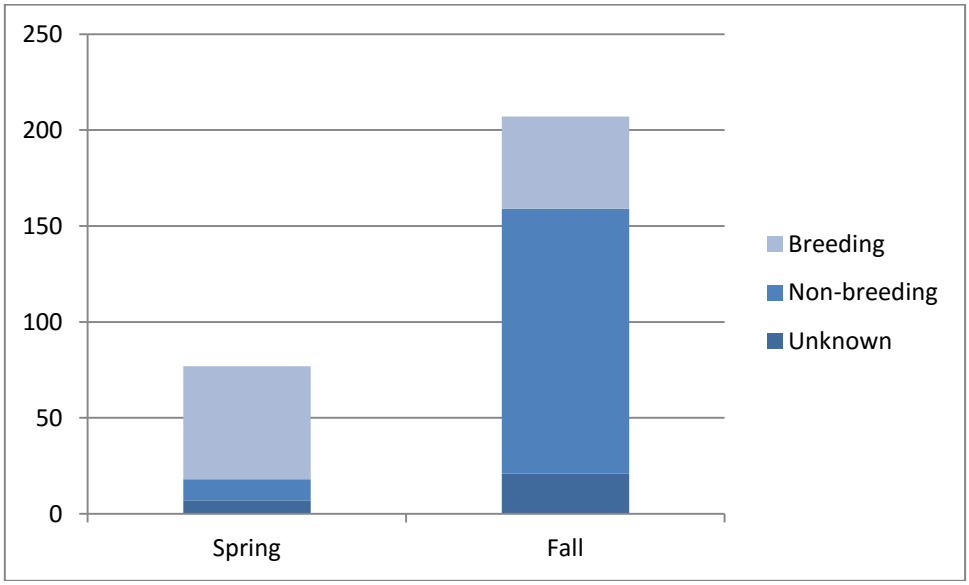

Fig. 8. Examined mice classified per reproductive status and season

Identification of taeniid species
A total of twenty-nine mice (10.2%, n=284) contained cysts, visible spots or lesions in the peritoneal cavity or liver (fig. 9). Most animals had only 1 or 2 small (0.5–2 mm diameter) cysts or lesions in the liver, but 8 animals (n=29, 27.6%) had multiple small cysts/lesions diffusely spread over all liver lobes. A well-developed *H. taeniaeformis* cyst was

morphologically identified in 3 animals (n=29, 10.3%). One animal contained free floating larvae in the peritoneal cavity (n=29, 3.4%). This animal was also co-infected with a well developed *H. taeniaeformis* cyst and multiple other smaller cysts in the liver. No other co-infections were found.

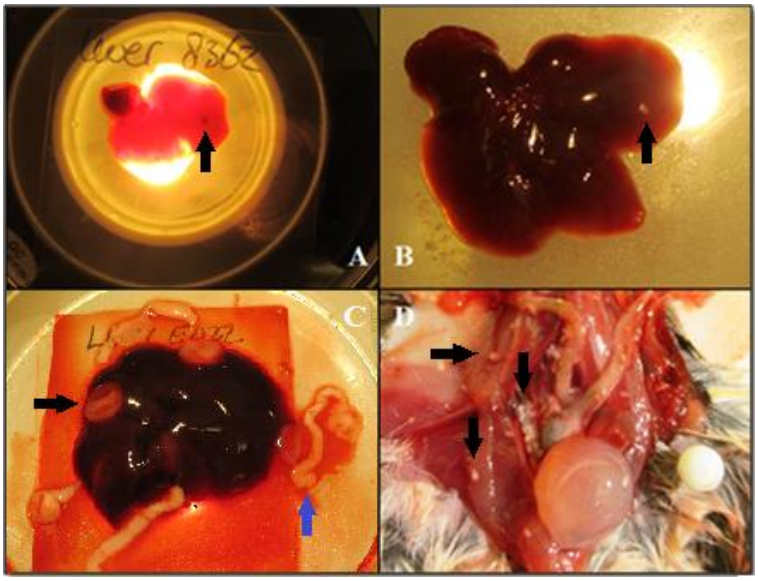

Fig. 9. Example of *Taenia* metacestodes. A,B) Small undeveloped *Taenia* metacestode in left liver lobe, C) Big metacestode (black arrow) containing *H. taeniaeformis* larva (blue arrow), D) Multiple free floating larvae in the abdomen

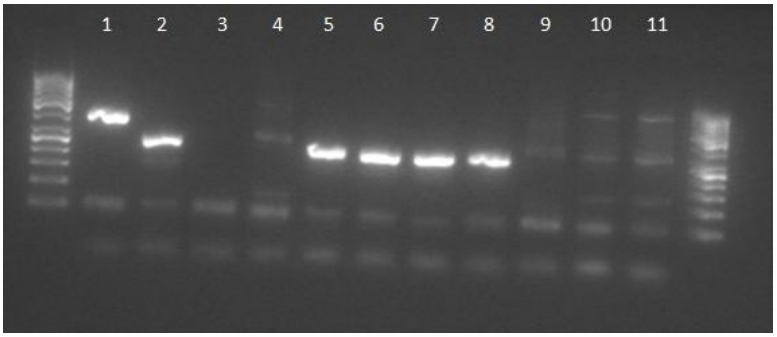

Figure 10. Example of the multiplex PCR amplification. Left and right lane 50bp DNA ladder, 1) Positive control, a standard DNA of *E. multilocularis*, 2) Positive control, a standard DNA of a *Taenia crassiceps*, 3) Negative control, 5 – 8) samples with *Taenia*-DNA, 3,4,9,10 and 11) negative samples

A total of 13 animals showed a positive *Taenia* lesion, giving an overall prevalence of *Taenia* infections of 4.6% (n=284, 95% CI: 2.5 – 7.7%). Of the 8 livers with multiple lesions, 7 (87.5%) were positive for *Taenia*. None of the animals were positive for *E. multilocularis*. The number of *Taenia* infections, prevalence and 95% exact binominal confidence intervals in different species, areas, seasons, sexes and functional groups are listed in table 4. No significant differences could be detected among species, (Fisher's exact, p= 0.360), study areas (Fisher's exact, p = 0.091), seasons (Fisher's exact, p = 0.349) or sex (Fisher's exact, p = 0.578). A significant difference was only found between functional groups, where breeding animals had significantly more *Taenia* infections than non-breeding animals (Fisher's exact, p = 0.002).

Table 4. Number of *Taenia* infections, prevalence and 95% exact binominal confidence intervals

		Total examined in group	Number of animals with *Taenia* infections	Prevalence (%)	95% CI (%)
Species					
	A. flavicollis	79	5	6.3	2.3-13.1
	A. sylvaticus	205	8	3.9	1.8-7.1
Area					
	Katrineholm	84	1	1.2	0.1-5.1
	Gnesta/Nyköping	36	4	11.1	3.6-24.0
	Uddevalla	77	3	3.9	1.0-9.8
	Växjö	87	5	5.7	2.1-11.9
Season					
	Spring	77	5	6.5	2.4-13.4
	Fall	207	8	3.9	1.8-7.1
Sex					
	Female	135	5	3.7	1.3 – 7.8
	Male	149	8	5.4	2.5 – 9.8
Functional group					
	Breeding	107	11	10.3	5.5 – 17.0
	Non-breeding	149	2	1.3	0.2 – 4.1
	Unknown	28	0	0.0	
All animals		284	13	4.6	2.5 – 7.4

Logistic regression analysis was conducted to predict *Taenia* infections in mice using season, area, species, sex and functional group as predictors. The Wald criterion demonstrated that area (Gnesta/Nyköping) (p=0.035) and functional group (breeding animals) (p = 0.009) made a significant contribution to prediction. The other variables were not significant predictors. The Exp(B) value indicates that an *Apodemus* mouse trapped in Gnesta/Nykoping is 13 times more likely to be infected than an *Apodemus* mouse trapped in Katrineholm and that breeding animals are 14.9 times more likely to have a *Taenia* infection than non-breeding animals (table 5).

Table 5. Binary logistic regression outcome. Season = Spring (0) and fall (1), area = Katrineholm (0), Gnesta/Nyköping (1), Uddevalla (2) and Växjö (3), species = *A. sylvaticus* (0) and *A. flavicollis* (1), sex = Male (0) and female (1), functional group = Breeding (0), non-breeding (1) and unknown (2). Outcome = *Taenia* infection (0) or no *Taenia* infection (1); * = Significant P-value

Variables	b-coëfficient	P-value	Odds ratio	95% CI for odds ratio
Species	-.611	.462	.543	.11 - 2.78
Area		.121		
Area (1)	-2.533	.035*	.079	.008 - .835
Area (2)	-1.004	.415	.367	.03 – 4.09
Area (3)	-2.001	.096	.135	.01 - 1.43
Season	-.282	.706	.754	.17 - 3.26
Sex	.764	.250	2.148	.58-7.90
Functional group		.009*		
Functional group (1)	2.699	.002*	14.864	2.65-83.50
Functional group (2)	19.468	.998	284886354.2	.000
Model X^2	= 23.857	p = 0.002		
n	= 284			

Taenia spp. specification

Table 6. Number of specific *Taenia* infections in both *Apodemus* spp.

	A. flavicollis	*A. sylvaticus*
H. taeniaeformis	5	1
T. polyacantha	0	1
Unknown	1	6

H. taeniaeformis

Overall, 6 mice (2.1%, n=284) were infected with *H. taeniaeformis.* Three *H. taeniaeformis* metacestodes were well-developed and were macroscopically recognizable due to the presence of a big (9-12mm diameter) cyst containing a larva (strobilicercus). The other three cysts were two small cysts of 1mm diameter and one cyst of 3 mm diameter. The three well-developed cysts were found in breeding *A. flavicollis* females, the three smaller cysts in two non-breeding *A. flavicollis* males one breeding *A. sylvaticus* male. The mice with *H. taeniaeformis* originate from three different areas, Katrineholm (n=1), Gnesta/Nyköping (n=3) and Växjö (n=2) caught in both spring (n=1) and fall (n=5). There was no significant difference in prevalence in season (Fisher's exact, p=1.000), sex (Fisher's exact, p=1.000) or functional group (Fisher's exact, p=0.240). Prevalence of *H. taeniaeformis* differed significantly between species (Fisher's exact, p=0.007) and area (Fisher's exact, p=0.039).

T. polyacantha

One *A. sylvaticus* was infected with *T. polyacantha* (0.4%, n=284). The *T. polyacantha* metacestode was a small (1x2mm) cyst in the median liver lobe of a breeding male that was caught in the spring in Växjö.

Unknown

In a total of seven *Apodemus* mice the specific *Taenia* spp. could not be identified. All sequence results had a very low identity match (84-90%). All seven animals had multiple cyst diffusely spread over the whole liver. One of the animals, an *A. flavicollis*, also had 0.5-1mm sized free floating larvae in the peritoneal cavity. This animal was also co-infected with an *H. taeniaeformis*. This was the only co-infection detected in the examined animals.

Discussion

In this study I particularly focussed on the rodent's role in the life cycle of taeniid cestodes. The life cycle of taeniid cestodes however contains two hosts. Because of this, the transmission of cestodes also depends on factors that include the definitive host. For example, the population densities of carnivorous definitive hosts can also influence the taeniid prevalence in rodents. We know little about the fox and cat densities in the four investigated areas. Because of this, I cannot exclude the influence of the fox and cat population density on my observations.

Apodemus spp. as an intermediate host for taeniid cestodes

The results of this study show that *Apodemus spp.* are equally exposed to infective taeniid eggs in all of the four investigated areas in Sweden. The results also show that taeniid metacestodes can develop to maturity in the *Apodemus* mice.. From this, we can conclude that *Apodemus* spp. are suitable intermediate hosts for *Taenia* spp. in Sweden. However, we cannot conclude that the *Apodemus* spp. play a significant role as an intermediate host in the life cycle for these parasites. Voles (e.g. *Arvicola, Microtus* and *Myodes spp.*) occur in the same areas as the examined mice and they can also harbour infective taeniid cysts of the same genera and species.[17] As stated before, these voles make up a much greater amount of the fox diet [19,21-23] and therefore possibly play a much larger role in the *Taenia* life cycles. On the other hand, from these results we can conclude that the two species of *Apodemus* studied do come in contact with *Taenia* infected faeces. We also can conclude that there is interaction between carnivorous definitive hosts and suitable rodent intermediate hosts in general, as an interaction between both host species is needed to establish the parasites' life cycle in the environment.

Taeniid cestodes can be transmitted by several carnivorous definitive hosts. The *Taenia* cestodes found in this study are mainly transmitted between cats or foxes and different rodent intermediate hosts.[7] The trapping areas are in rural inhabited areas where house and farm cats are likely to be. The most prevalent taeniid in this study (*H. taeniaeformis*) is mainly transmitted by cats. Thus, it is possible that the examined mice obtained the infection with *H. taeniaeformis* via cat faeces. However, *T. polyacantha* is almost exclusively transmitted by foxes.[7] *T. polyacantha* was identified in one *A. sylvaticus* is Växjo. The sequence retults gave a high identity match (97%) but a quite low query cover (95%). However, due to the high identity match, we can say the parasite is most likely a *T. polyacantha*. The finding of this parasite in an *Apodemus* mouse in Växjö shows that the mice come in direct contact with fox faeces in this area.

Six of the seven *Taenia* spp. that could not be identified were identified as most likely *T. hydatigena*. However, this was only with a very low identity match. (84-85%). This means that these lesions are most likely not *T. hydatigena*. Also, rodents are only very rarely described as an intermediate host for this *Taenia* species.[7,34] Since there is no >95% match with any known taeniid species, it is possible that we found a new *Taenia* species that has not been identified yet.

Apodemus spp. as an intermediate host for *E. multilocularis*

No *E. multilocularis* was detected in the *Apodemus* mice in this study despite the fact that *E. multilocularis* has been detected in fox faeces in all four areas included in this study.[16] The infection with other taeniid cestodes shows that the examined mice do come in contact with

cat faeces and in one area also with fox faeces. The role of cats in the transmission of *E. multilocularis* is questionable. [35,36] They could be a carrier of the disease, but experimental infections studies have shown that they are unlikely to develop viable eggs.[36] Foxes however do play a key role in the transmission of *E. multilocularis* and interaction between foxes and rodents can make it possible for *E. multilocularis* to establish in the population once introduced. The finding of *T. polyacantha* in an *Apodemus* mouse in Växjö shows that the mice do come in contact with fox feces in this area and thus have a possible risk of getting infected with *E. multilocularis*.

The prevalence of *E. multilocularis* in foxes in Sweden is generally very low (0.1%).[5,6] However in one area in Gnesta/Nyköping, several studies showed a high prevalence of *E. multilocularis* in fox faeces with main surveillance (0.8%, n=790)[6,16] *E. multilocularis* has also been detected in several rodents of the species *Arvicola* and *Microtus* in this area.[17] Still, no *E. multilocularis* was detected in the studied *Apodemus* mice, which further indicates that mice plays no role in the transmission.

This indicates that *Apodemus spp.* are unsuitable intermediate hosts for *E. multilocularis* and most likely do not play a significant role in its life cycle in Sweden. This agrees with the findings in previous research in Japan, which showed that *Apodemus* mice are not able to develop metacestodes after experimental infection with *E. multilocularis* eggs.[37] However, in that study a different *Apodemus spp.* (*A. speciosus*) was used than those investigated herein. In order to answer this question, experimental infections studies on *A. flavicollis* and *A. sylvaticus* are needed. Further, only a small population (n=284) was investigated by me and with the low prevalence of *E.multilocularis* in the examined areas it it cannot be excluded that the infection was missed in this study.

Mesocestoides spp.

In one animal in which we could not identify the *Taenia* spp., the sequence results had a 89-90% identity match with *Mesocestoides* spp.. The first 28-30 matches belonged to the species of *Metacestoides*. *Mesocestoides* spp. cestodes are also a member of the order Cyclophyllidea, but belong to the family of Mesocestoididae. The life cycle of these cestodes requires two intermediate hosts and a carnivorous definite host. The first intermediate hosts are small insects, generally mites or ants. The second intermediate hosts can be small mammals, birds, amphibians and reptiles.[34] In Europe, *Mesocestoides* spp. are mostly transmitted by foxes (up to 70%) and only rarely by cats and dogs.[38] The larval stage of *Mesocestoides* spp., called tetrathyridia, are mainly found in the peritoneal cavity, liver, lung and several other organs of the second intermediate host.[38-40] *Apodemus* mice predominately feed on seeds and plants, but they also eat small insects and thus have a possible risk of getting infected.[41,42] It is possible that the parasite was not a *Taenia* spp. but a *Mesocestoides* spp. since the the Trachsel 2007[33] primers designed for *Taenia* spp. can also amplify DNA from *Mesocestoides* spp. The appearance of the parasite also matches the Mesocestoides tetrahyridia, since this was the *Apodemus* with multiple cyst diffusely spread over the whole liver and 0.5-1mm sized free floating larvae in the peritoneal cavity. However, since the sequencing results did not give a >95% identity match, we cannot identify if and which *Mesocestoides* it is and it is possible that the detected parasite is also a new, not earlier identified cestode.

Taeniid parasite detection by PCR

The Trachsel 2007 multiplex PCR that was used in this study has been designed to detect taeniid eggs in the faeces of carnivores.[33] It has not been validated for the detection of taeniid lesions in liver tissue yet. Because of this, the sensitivity of this method for taeniid parasite detection in liver lesions is not known. Two lesions in this study were morphologically identified as taeniid infections but had a negative result by multiplex PCR. Both lesions looked like small *Taenia* cysts imbedded in the parenchyma. Both had a diameter of approximately 1mm. It is possible that they were morphologically misidentified. Since the sensitivity is not known, it is also possible that the metacestodes were too small to be detected by the PCR.

***Taenia* prevalence in different areas**

No significant difference was found in *Taenia* prevalence between the four investigated areas in general. The lack of statistical significance is probably due to the low frequency of positive cases and small sample population. However, the numerical prevalence of *Taenia* infections was much higher in this area than in the other three areas (11.4% in Gnesta/Nyköping compared to 5.7%, 3.9% and 1.2% in the other areas). The logistic regression also showed that *Apodemus* mice trapped in Gnesta/Nyköping were significant more likely to be infected than *Apodemus* mice trapped in Katrineholm. This may indicate a greater interaction between foxes and rodents in this area. This is interesting because a high prevalence of *E. multilocularis* in fox faeces is found in this area (0.8% n=790)[6,16]. *E. multilocularis* has also been detected in several rodents of the species *Arvicola* and *Microtus* in this area.[17] It is unknown how the establishment of *E. multilocularis* in this area is caused and why the prevalence is higher in this particular area. A higher intensity of interaction between foxes and rodents may contribute to the establishment and continuation of the *E. multilocularis* life cycle in this area. However, to be able to say anything about this, other factors should also be taken in account, like the local fox population density and the numbers of alternative intermediate hosts.

A suggestion for future work is to investigate the community of *Taenia* spp. in the local rodent population. If the prevalence of *Taenia* spp. in other rodent populations is higher in areas with a higher *E. multilocularis* prevalence, *Taenia* spp. could maybe serve as an indicator for relative prevalence and increased risk of *E. multilocularis*.

***Taenia* prevalence in the two *Apodemus* spp.**

Determination of the specific species of the mice was done by using biometric and morphometric differences. Although the species can be morphologically distinguished in Northern Europe[43], it can be quite challenging for an inexperienced observer to distinguish some of the animals as the weight, total length, tail length and foot length partially overlap. In those animals, the colour and presence of a coloured chest band gave the only reliable indication. Particularly for the younger animals, in which the colour difference is less distinct, it is possible that some misidentifications were made. In order to avoid misidentifications, other more reliable methods can be used for the distinguishing between both species. These methods include skull and/or dental mophometrics, C-band karyotyping, protein electrophoresis, sequencing of mitochondrial cytochrome B region and microsatellite analysis.[44,45] Although not performed for this study, the right front foot of all the examined *Apodemus* mice were collected and stored in 95% alcohol to facilitate species determination in the future.

Overall, more *A. sylvaticus* (205) than *A. flavicollis* (78) were caught. A possible explanation for this is the difference in habitat utilisation preference of these species. The traps in all four investigated areas were often placed in ecotones. Ecotones are transition areas between two ecological communities, e.g. forest and grassland, and are considered as habitat borders for most rodent species. *A. flavicollis* occurs for example almost exclusively in woodland. However *A. sylvaticus* occurs in both woodlands and grassland areas and reaches its maximal abundance in ecotones including forest margins, bushes, fields and parks.[46,47] Another study showed that *A. flavicollis* and *A. sylvaticus* are likely to compete with each other, since the habitats of both species partially overlap.[41] It is possible that the *A. sylvaticus* outcompeted the *A. flavicollis* at the time of trapping. This could also be a possible explanation for the higher *A. sylvaticus* abundance. However, another behavioural study showed that this interaction is comparatively weak and one species could not exclude the other.[48]

Infections with *Taenia* parasites are found in both *Apodemus* species. Although a higher prevalence of *Taenia* infections has been found in *A. flavicollis* than in *A. sylvaticus*, this difference was not significant. This means that both species are exposed to taeniid eggs and are both susceptible for the parasites. The lack of significance could be due to the small sample size, but no literature was found about one species being more likely infected than the other.

Taenia **prevalence in different season and functional groups**

The functional group of both species was determined by the development of the reproductive organs. The categorisation of the rodents into these different functional groups was subjective and was sometimes very difficult. Most animals could be categorized as breeding or non-breeding by the use of this method, but for some of the animals it was quite challenging.

Female reproduction status was determined by the presence of active mamma tissue, a perforated vagina and/or scars/embryo's in the uterus. However, some of the female mice had a thicker uterus than non-breeding animals, but none of the other signs. These animals were placed in the category unknown. It is possible that the animal had just been bred or will soon be ready for breeding. In males, the reproduction status was determined by the presence of seminal vesicles and testicle size. Some animals had no seminal vesicles present but very large testicles and vice versa. There is no literature published about the exact testes size of reproductive males of both *Apodemus* species. Because of this, I looked at mean testes size of the animals with and without presence of the seminal vesicles and took the mean of these two values as a cut-off point. Animals with seminal vesicles present but testicle size below the cut-off point, or testicle size above the cut-off point but no seminal vesicles were also placed in the category unknown. It is possible that these animals were already breeding or probably would have been breeding soon.

More *Apodemus* mice were caught in the fall. As the number of adult animals did not differ much between spring and fall, this increase in catch was mainly due to the the increase of non-breeding animals. The non-breeding animals could be either juvenile animals or sub-adults. Sub-adults are animals that are old enough to be breeding adults, but are not breeding yet. The fact that most non-breeding animals were trapped in the fall corresponds to the life cycle of the mice. The reproduction cycle of *Apodemus* mice usually occurs from the beginning of May until the second half of October.[41] Reproductive organs will be fully developed in both male and female mice at the beginning of the reproduction cycle. The first

young will be born around the first week of May.[41] Only spring litters attain sexual maturity in the same season. Young born in the latter part of the summer grow more slowly due to less favourable circumstances and reach sexual maturity the next season.[41] Research has shown that juvenile animals are hormonally suppressed by the sexually active animals when the population density is high.[49,50] Because of this, juvenile animals tend to disperse from their place of birth whenever possible. When the breeding season progresses, the surrounding habitats will become saturated and the juveniles have to remain in their birth habitat.[51] The population density of juveniles will increase and the animals are reproductively suppressed and reach sexual maturity later.

This research has shown that breeding animals have significantly more *Taenia* infections and are 15 times more likely to have an infection than non-breeding animals. There are several explanations for this. The first explanation could be time, as the degree of exposure should increase with age. Another explanation is the relatively smaller home range of immature animals of non-breeding animals,[52] since the animals have more chance of getting infected when they have a larger home range.

Research has also shown that neonatal mice are protected against infection with *H. taeniaeformis* by a prenatal transfer of antibodies. Neonatal mice born of immune mothers but suckled on normal mothers were significantly protected against infection with *H. taeniaeformis* at 21 days of age. This suggests that transplacentally transferred IgA or IgG may contribute to the immunity of neonatal mice.[53] Juveniles are also protected against infection when administered colostral IgA from adult mice previously orally infected with the parasite. [54] This maternal immunity could protect the juvenile specimen against infections and this could also explain the lower prevalence of *Taenia* infections in juvenile animals, although the protection is limited.

Lastly, hormones can influence the immune status of rodents. Sex and stress hormones have an effect on the immunological system. Male sex hormones (androgens) decrease both humoral and cell mediated immunity, while female sex hormones (oestrogens) are shown to have a positive effect on immunoregulation.[55,56] Elevated levels of corticosteroids also impair the immunity of mature animals. [55,56] Pregnancy and lactation decrease resistance to helminth infection in females, probably also due to hormonal effects.[55,56] The suppressed sub-adults and juvenile animals are not influenced by sex hormones yet and are thus less susceptible to infection.

Although there were more juvenile mice caught in the fall, no significant difference was found between the two seasons. However, the parasite prevalence was numerically higher in the spring, probably because the amount of breeding animals is relatively higher in the spring. But if we only look at the breeding animals, the parasite prevalence in breeding animals was higher in the fall (12.5% compared to 8.5%). This could be explained by the fact that the mice show their greatest activity during the reproductive period, in order to find a mate.[52] During this activity they have a greater risk of exposure and more animals are infected at the end of the reproductive period than at the beginning.

Taenia prevalence in different sexes

The overall *Taenia* prevalence was somewhat higher in males than in females, although not significant. This would suit the fact that males have more activity than females in order to find

a mate and thus are more likely to be exposed and get infected.[52] During the non-reproduction periods the activity does is not significantly different between both species.[41] Previous research has also shown that male mice are more susceptible to an infection with *H. taeniaeformis*.[57] However, in this study there was no difference in *H. taeniaeformis* prevalence between sexes.

Conclusion

The results show that both *A. flavicollis* and *A. sylvaticus* function as intermediate hosts for several *Taenia* species in Sweden. Although some of these infections (eg. *H. taeniaeformis*) develop into maturity in the mice, it is unclear what role *Apodemus* spp. play in the transmission of these cestodes to foxes. Still this study has demonstrated that there is a transmission of these parasites between foxes and rodent intermediate hosts in general and that *Apodemus* spp. contribute to this. In contrast, *E. multilocularis* has not been found in the *Apodemus* mice in this study, which indicates that they are not a good intermediate host for this parasite and do not play a role as such in Sweden. The prevalence of *E. multilocularis* is generally very low in Sweden (0.1%), but has been identified in other rodent species in one of the investigated areas.[17] Even though an infection of *E. multilocularis* could have been missed in this study due to the small sample size, the prevalence would still be too low for *Apodemus* spp. to play a significant role in maintaining the *E. multilocularis* life cycle. It is also possible that *A. flavicollis* and *A. sylvaticus* are not able to develop *E. multilocularis* metacestodes, but, in order to answer this question, an experimental infection study on *A. flavicollis* and *A. sylvaticus* is needed. In addition, the results showed a higher prevalence of *Taenia* spp. in an area with higher prevalence of *E. multilocularis* in both foxes (0.8%) and rodents. A suggestion for future work is to investigate the general community of *Taenia* spp. in the local rodent population. If the prevalence of *Taenia* spp. in other rodent populations is higher in areas with a higher *E. multilocularis* prevalence, *Taenia* spp. could serve as an indicator for relative prevalence and increased risk of *E.multilocularis*.

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Appendix

Numbers of examined *A. flavicollis* and *A. sylvaticus* by area, season, sex and age. K = Katrineholm, G/N = Gnesta/Nyköping, U = Uddevalla, V = Växjö. Spring = April and May, Fall = September and October. B = Breeding, N = Non-breeding, U = Unknown

		K			G/N			U			V				
		B	*N*	*U*	*B*	*N*	*U*	*B*	*N*	*U*	*B*	*N*	*U*	*Total by sex*	*Total by season*
								Apodemus flavicollis							
Spring	*Female*	4	2	0	0	0	2	3	0	1	0	0	0	12	
	Male	4	0	0	1	0	1	2	1	0	0	0	0	9	
	Total	8	2	0	1	0	3	5	1	1	0	0	0		21
Fall	*Female*	7	3	2	4	6	0	1	6	1	2	0	0	32	
	Male	0	6	0	2	10	1	0	4	0	0	3	0	26	
	Total	7	9	2	6	16	1	1	10	1	2	3	0		58
								Apodemus sylvaticus							
Spring	*Female*	3	1	0	2	0	0	5	6	1	5	1	0	24	
	Male	3	0	2	1	0	0	21	0	0	5	0	0	32	
	Total	6	1	2	3	0	0	26	6	1	10	1	0		56
Fall	*Female*	7	16	1	0	1	0	3	7	0	9	22	1	67	
	Male	3	16	4	0	4	1	4	7	4	6	27	6	82	
	Total	10	32	5	0	5	1	7	14	4	15	48	7		149
Total by age		31	44	9	10	21	5	39	31	7	27	52	7		284