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THE CARRIAGE OF LARVAL *ECHINOCOCCUS MULTILOCCULARIS* AND OTHER CESTODES BY THE MUSK RAT (*ONDATRA ZIBETHICUS*) ALONG THE OURTHE RIVER AND ITS TRIBUTARIES (BELGIUM)

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ABSTRACT: In Belgium, the carriage of *Echinococcus multilocularis* by the red fox (*Vulpes vulpes*) can be very high in some areas. This study was designed to evaluate the carriage of the larval form of *E. multilocularis* and other cestodes in a musk rat (*Ondatra zibethicus*) population trapped along the Ourthe River (southeastern Belgium). Six hundred fifty-seven musk rats were necropsied, and the larval cestodes of the abdominal and pleural cavities were identified. For *E. multilocularis*, the fertility of the cysts was verified in 58 liver samples. The following species were found: *Taenia taeniaeformis* (65.8%), *Taenia martis* (22.2%), *E. multilocularis* (22.1%), *Taenia polyacantha* (2.6%), and *Taenia crassiceps* (0.9%). Results were analyzed according to the site of capture (upper, middle, and lower Ourthe). There was a highly significant relationship between the carriage of *E. multilocularis* and the site of capture (the prevalence being higher in the upper part of the river). This difference could be due to different geoclimatic conditions. All but one hepatic lesion were found to contain protoscoleces of *E. multilocularis* (98.8%). The musk rat is probably infected through the consumption of plant material contaminated by the fox's feces. The red fox can occasionally prey on musk rats, but the musk rat cadavers that are left on the river banks by the trappers are probably also consumed. This could favor the maintenance of *E. multilocularis* life cycle. In conclusion, the musk rat seems to be highly susceptible to *E. multilocularis* and in Belgium could play the role of reservoir; when present this species could represent an inexpensive and sensitive bioindicator for the study and monitoring of the zoonosis.

Key words: Alveolar echinococcosis, Belgium, *Echinococcus multilocularis*, metacestodes, musk rat, *Ondatra zibethicus*, *Taenia* spp.

INTRODUCTION

The musk rat (*Ondatra zibethicus*) is a rodent belonging to the family Cricetidae, subfamily Arvicolinae (Newell, 2000). This North American species was introduced in Europe and is now widely distributed over the European continent and most parts of Russia (Wilson and Reeder, 1993). In Belgium, the musk rat was introduced in 1928 (Lambot, 1993). Like elsewhere in Europe, this species is responsible for marked damage to the river banks (Lambot, 1993). In the spring (March–April) and in the fall (October) during the migration periods for this species, trapping campaigns are organized by the Ministry of Agriculture and Environment of the Walloon Region (southern Belgium). The presence of larval forms of *Taenia taeniaeformis*, *Taenia martis*, *Echi-*

nococcus multilocularis, *Taenia polyacantha*, and *Taenia crassiceps* in the musk rat was reported either in the USA or in Europe (Samuel et al., 2001). The metacestode *E. multilocularis* was observed in the musk rat in France (Boussinesq et al., 1986), Germany (Baumeister et al., 1997), and more recently in the Netherlands (Borgsteede et al., 2003) and Belgium (Hanosset et al., 2004). In humans, the development of the larval form of *E. multilocularis* is responsible for a rare but potentially fatal condition called alveolar echinococcosis (Acha and Szyfres, 2005). In Belgium, 11 cases of alveolar echinococcosis were diagnosed since 1999 (Carlier, pers. comm.), and of those, four cases were recently described (Detry et al., 2005). In Europe, the adult stage of this cestode is found mainly in the red fox (*Vulpes vulpes*; Hanosset et al., 2004).

In some areas of Belgium, prevalence of *E. multilocularis* in its final host can be very high (up to 33%; Losson et al., 2003), but the potential intermediate hosts are not well known (Hanosset et al., 2008). The present study was designed to evaluate the prevalence of the larval form of *E. multilocularis* in a musk rat population trapped along the Ourthe River and its tributaries (eastern Belgium). The prevalences of the larval forms of other cestodes present in the abdominal and pleural cavities were also recorded.

MATERIALS AND METHODS

Between October 2005 and June 2006, 657 musk rats were trapped in the basin of the Ourthe by professional agents. The Ourthe River is one of the tributaries of Meuse River. It is located in southeast Belgium (50°–50°30'N, 5°30'–6°E). The altitude up the river is approximately 500 m, whereas it is only 150 m downstream. The climate is mild and humid; precipitation is approximately 800 mm/yr. For each individual, body weight was recorded. The age was evaluated according to the molar index recorded on the first upper molar (Pankakoski, 1980). After opening of the abdominal and pleural cavities, the sex of the animal was recorded, and the larval cestodes were observed and identified. The larval stage of *E. multilocularis* in the liver occurs as translucent pearly vesicles separated by fibrous areas (Petavy and Deblock, 1983). To verify the fertility of the cysts, 58 samples were fixed in neutral formalin, embedded in paraffin wax, cut in 5- μ m sections, and stained with Mayer's hematoxylin and eosin (Kruse and Pritchard, 1982). Sections were observed under the microscope (40 \times magnification) for the presence of protoscoleces (Bonnin et al., 1986). The larval form of *T. taeniaeformis* occurs as yellowish vesicles containing an invaginated scolex at the end of a long, folded, immature strobile (Schuster, 1982). Three other metacestodes were found both in the abdominal and pleural cavities. The cysticerci of *T. crassiceps* resemble rice grains ("small pearls"; Borgsteede, pers. comm.) and can be present in great numbers and are able to reproduce asexually (Miyaji et al., 1990). Metacestodes of *T. martis* and *T. polyacantha* cannot be distinguished visually; their identification is based on the number of hooks on the scolex: the scolex of *T. martis* bears 24 to 30 hooks, whereas the scolex of *T. polyacantha*

has 44 to 62 hooks (Verster, 1969). To count the hooks, the metacestodes were stained with Semichon's acetocarmine (Kruse and Pritchard, 1982). Data concerning the comparison between musk rat weight and infestation by metacestodes were analyzed by multiple regression (F of Snedecor and associated P value), whereas the data concerning the comparison between the place of capture of the musk rats, their sex or their age and infestation by metacestodes were analyzed by logistical regression (Chi-carré and its P value; Albert, 2005).

RESULTS

The most prevalent species was *T. taeniaeformis* (65.8%) followed by *T. martis* (22.2%) and *E. multilocularis* (22.1%). *Taenia polyacantha* (2.6%) and *T. crassiceps* (0.9%) were rare. The mean number of cysticerci of *T. taeniaeformis* in the musk rats trapped along the River Ourthe and its tributaries was 4.73, whereas the maximum number observed in one animal was 112. The larval stage of *T. martis* and *T. polyacantha* was observed in 22 and 2.6% of the musk rats, respectively. The mean and maximal numbers of worms were 2.57 and 14 per animal (*T. martis*) and 2.41 and 5 per animal (*T. polyacantha*).

Three hundred forty-nine (53.1%) and 308 (46.9%) musk rats were males and females, respectively. Table 1 presents the prevalences of the infections with the different metacestodes in males and females. The analysis of the results by logistical regression indicated that *T. martis* is significantly more frequently found in females than in males. This was not the case for the other species.

Results were also analyzed according to the place of capture. The basin of the Ourthe was divided arbitrarily into three different but equal parts (upper, middle, and lower Ourthe). There was a highly significant relationship between the carriage of *E. multilocularis* and the place of capture. Indeed, the prevalence for this species was found to be higher in the upper part of the river compared with the lower part ($P=0.00013$) (Table 2).

TABLE 1. Prevalences of the metacestodes of *Echinococcus multilocularis*, *Taenia taeniaeformis*, *Taenia martis*, *Taenia polyacantha*, and *Taenia crassiceps* in male and female musk rats trapped in the basin of the Ourthe River (Belgium) in 2005–2006.

Parasitic species	Total sample	Males	Females
	n=657 (100%)	n=349 (53.1%)	n=308 (46.9%)
<i>E. multilocularis</i>	145 (22.07)	78 (22.35) <i>P</i> =0.85	67 (21.75)
<i>T. taeniaeformis</i>	432 (65.75)	239 (68.48) <i>P</i> =0.14	193 (62.66)
<i>T. martis</i>	146 (22.22)	64 (18.34) <i>P</i> =0.011	82 (26.62)
<i>T. polyacantha</i>	17 (2.59)	11 (3.15) <i>P</i> =0.33	6 (1.95)
<i>T. crassiceps</i>	6 (0.91)	3 (0.86) <i>P</i> =0.88	3 (0.97)

Prevalences of the infections with the different metacestodes in subadult and adult musk rats are given in Table 3. Logistical regression results indicated that *T. taeniaeformis* was more frequently found in adults than in subadults. The prevalences with the other metacestodes (*T. martis*, *T. polyacantha*, and *T. crassiceps*) were not significantly related to body weight ($P>0.05$). There was also a significant positive relationship between body weight of the musk rats and infection with the cysticerci of *T. taeniaeformis* ($F=15.89$; $P=0.000075$; Fig. 1).

The *P* values obtained by comparing the effect of a given infection on another infection are shown in Table 4. With the exception of *T. crassiceps*, significant associations were observed between the other species of cestodes. All but one of the sampled liver lesions were found to contain protoscoleces of *E. multilocularis*,

which indicates a high percentage of fertility in this species (99%).

DISCUSSION

In the present study, the most frequently observed metacestode was that of *T. taeniaeformis*. High prevalences were also reported in the neighboring regions: 45% in the Netherlands (Borgsteede et al., 2003), 42% in Lower Saxony (Baumeister et al., 1997), and 64% in East Germany (Schuster, 1987). In Belgium, a survey conducted in 1982 on musk rats trapped in different areas of the country revealed a global incidence of 47% (Cotteleer et al., 1982). Borgsteede et al. (2003) in the Netherlands reported a mean of 3.3 and a maximum of 28 cysticerci per animal. In Germany, maximal parasitic burdens reached 125 and 74 in Lower Saxony and East Germany, respectively (Schuster,

TABLE 2. Prevalences of the metacestodes of *Echinococcus multilocularis* in musk rats trapped in 3 parts (upper, middle, and lower) of the Ourthe River (Belgium) in 2005–2006.

Parts of Ourthe River	Upper Ourthe	Middle Ourthe	Lower Ourthe
	n=235 ^a	n=108 ^a	n=314
Percentages of musk rats infected (no. of musk rats infected by this helminth)	32.3 (76) <i>P</i> =0.00013	25 (27)	13.4 (42)

^a n, number of musk rats by parts of Ourthe River.

TABLE 3. Prevalences of the metacestodes of *Echinococcus multilocularis*, *Taenia taeniaeformis*, *Taenia martis*, *Taenia polyacantha*, and *Taenia crassiceps* in adult and subadult musk rats trapped in the basin of the Ourthe River (Belgium) in 2005–2006.

Parasitic species	Total sample	Adults	Subadults
	<i>n</i> =657 (100%)	<i>n</i> =271 (41.25%)	<i>n</i> =386 (58.75%)
<i>E. multilocularis</i>	145 (22.07)	67 (24.72) <i>P</i> =0.17	78 (20.21)
<i>T. taeniaeformis</i>	432 (65.75)	193 (71.22) <i>P</i> =0.013	239 (61.92)
<i>T. martis</i>	146 (22.22)	63 (23.25) <i>P</i> =0.60	83 (21.50)
<i>T. polyacantha</i>	17 (2.59)	6 (2.21) <i>P</i> =0.61	11 (2.85)
<i>T. crassiceps</i>	6 (0.91)	2 (0.74) <i>P</i> =0.69	4 (1.04)

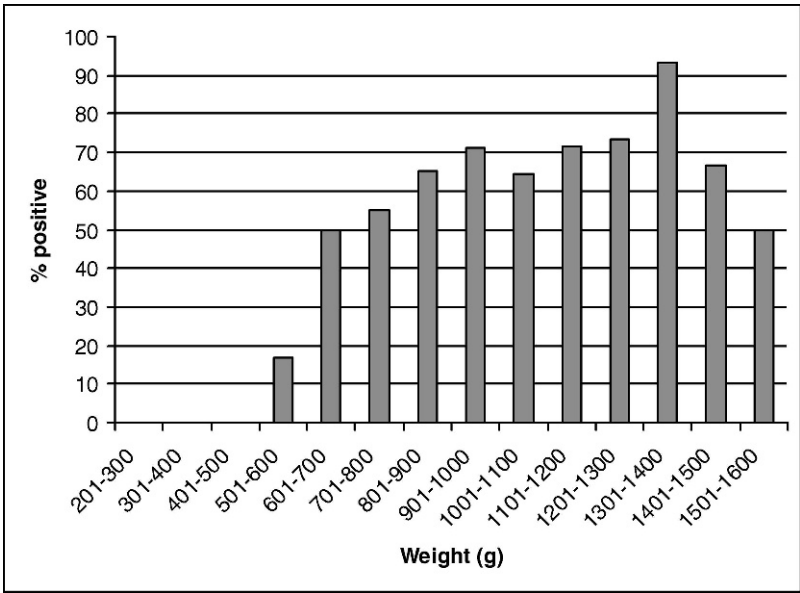


FIGURE 1. Frequency distribution of musk rats from Ourthe (Belgium) infected with larval stages of *Taenia taeniaeformis* (*n*=657) according to their weight.

TABLE 4. Co-occurrence of the different species of metacestodes (*Echinococcus multilocularis*, *Taenia taeniaeformis*, *Taenia martis*, *Taenia polyacantha*, and *Taenia crassiceps*) found in the musk rats trapped in the basin of the Ourthe River (Belgium) in 2005–2006. Data were analyzed by logistical regressions. *P*<0.05 are indicated in bold.

	<i>E. multilocularis</i>	<i>T. taeniaeformis</i>	<i>T. martis</i>	<i>T. polyacantha</i>	<i>T. crassiceps</i>
<i>E. multilocularis</i>	—	0.032	0.61	0.00013	0.74
<i>T. taeniaeformis</i>	0.032	—	0.00025	0.67	0.43
<i>T. martis</i>	0.61	0.00025	—	0.022	0.53
<i>T. polyacantha</i>	0.00013	0.67	0.022	—	0.14
<i>T. crassiceps</i>	0.74	0.43	0.53	0.14	—

1987; Baumeister et al., 1997). Adult musk rats seem to be more often infected by this cestode (Baumeister et al., 1997; Borgsteede et al., 2003). There was also the case in the observations during our study. In the Netherlands, a significant relationship was observed between the level of infection and the body weights of the animals (Borgsteede et al., 2003), and this is in agreement with the present work. From these figures, it can be concluded that this tapeworm is very prevalent in the study area where the musk rat seems to act as a highly sensitive intermediate host.

The larval stage of *T. martis* was observed in 22% of the musk rats necropsied during the present study, whereas the mean number of larval stages was 2.57 and the maximum parasitic burden was 14 per animal. This metacestode is found in the pleural and abdominal cavities. In comparison, 6, 3, and 19% of the musk rats were found to be infected in the Netherlands, Lower Saxony, and East Germany, respectively (Schuster, 1987; Baumeister et al., 1997; Borgsteede et al., 2003). The mean number of worms reported by Borgsteede et al. (2003) was 2.5. Maximal parasite burdens of 14, 13, and 10 were recorded in Lower Saxony, the Netherlands, and East Germany, respectively (Schuster, 1987; Baumeister et al., 1997; Borgsteede et al., 2003). From these figures, it can be concluded that there is no difference between the study area and the closest neighboring country (i.e., the Netherlands). Borgsteede et al. (2003) indicated that the adult animals were more heavily infected than the juveniles, whereas no difference was reported by Baumeister (1997) and in the present study. The present work indicated that the incidence of *T. martis* metacestode was higher in females and this observation deserves confirmation. In the Netherlands, there was no significant difference between musk rat sex and infestation by the metacestode (Borgsteede et al., 2003).

Several species of Mustelidae harbor

the adult stage of *T. martis*: the Siberian polecat (*Mustela eversmannii*) in Belarus (Shimalov and Shimalov, 2002) and East Germany (Schuster et al., 1988), the otter (*Lutra lutra*) in East Germany (Schuster et al., 1988), and the stone marten (*Martes foina*) in different parts of Germany (Loos-Frank and Zeyhle, 1982; Schuster et al., 1988), in Switzerland (Zimmerli, 1982), and in Spain (Torres et al., 2004). The stone marten is an important final host of *T. martis*. In the Netherlands, a large population of stone martens is present in the southern province of Limburg, and this seems to be associated with a higher prevalence in the musk rat. Elsewhere in this country, the parasite is rare in the musk rat (Borgsteede et al., 2003). In this study area, the stone marten is very common (Libois, 2006).

The larval stage of *T. polyacantha* was observed in 2.6% of the musk rats available for the present study. The mean and maximal numbers of worms were 2.4 and five per animal. This metacestode is found also in the pleural and abdominal cavities. The prevalences reported by other authors are also low: 0.2% in the Netherlands (Borgsteede et al., 2003), 0.4% in Lower Saxony (Baumeister et al., 1997), and 1.3% in East Germany (Schuster, 1987). Despite the low prevalence in the musk rat, Baumeister et al. (1997) stated that the species was an important intermediate host for this tapeworm; they also reported that the prevalence in the red fox, the final host, was low in Lower Saxony.

Taenia crassiceps metacestode was the least abundant parasite species in the study area (0.91%). However, when present, the cysticerci were numerous and are found both in the pleural and abdominal cavities and sometimes in the mouth (Mathy, pers. obs.). A similar observation was reported by Delvalle (1989). Other reported prevalences are 0.3% in the Netherlands (Borgsteede et al., 2003), 2.7% in Lower Saxony (Baumeister et al., 1997), and 6.3% in East Germany (Schus-

ter, 1987). The Siberian polecat is considered as the principal predator of the musk rat. The otter, ermine (*Mustela erminea*), stone marten, and the red fox may also play a role in the maintenance of the life cycle (Boussinesq et al., 1986; Lambot, 1993). The red fox feeds on various prey; red foxes also will eat carrion and musk rats found dead on the river banks are consumed (Lambot, 1993).

The adult stages of *T. polyacantha* and *T. crassiceps* were found in red fox in France (Petavy and Deblock, 1980, 1983; Petavy et al., 1990), Spain (Alvarez et al., 1995), the Netherlands (Borgsteede, 1984), Germany (Loos-Frank and Zeyhle, 1982; Ballek et al., 1992; Wessbecher et al., 1994; Pfeiffer et al., 1997), Belarus (Shimalov and Shimalov, 2003), and Greece (Papadopoulos et al., 1997).

The adult stage of *T. taeniaeformis* was found in red fox in Germany (Loos-Frank and Zeyhle, 1982; Ballek et al., 1992; Wessbecher et al., 1994; Pfeiffer et al., 1997) and Belarus (Shimalov and Shimalov, 2003). However, the reported prevalences in this species are usually very low (between 0.2% and 3.2%) and cannot explain the very high levels of larval infection found in the present and other studies (Schuster, 1987; Baumeister et al., 1997; Borgsteede et al., 2003). The wild cat (*Felis silvestris*) could represent the main final host for *T. taeniaeformis* (Schuster et al., 1993). Indeed, in two different regions of Germany a few wild cats were necropsied and a high proportion of them harbored *T. taeniaeformis* (Loos-Frank and Zeyhle, 1982; Schuster et al., 1993).

The metacestode of *E. multilocularis* was observed in 22% of the musk rats available in the present study, whereas Borgsteede et al. (2003) found it only in 0.1% of their musk rat population. The parasite larval form is found usually in the liver, but different metastases are observed in different locations (e.g., spleen, genital tract, and diaphragm). In Lower Saxony, Baumeister et al. (1997) reported

a prevalence of 4.1% in the musk rat, and all the sampled lesions ($n=41$) were found to be fertile. In the present work, only one of 58 tissue samples was unfertile. This lesion was probably too young (from 1.5 mo to 3 mo are necessary for the appearance of protoscoleces; Marchiondo and Andersen, 1984; Veit et al., 1995). This would suggest that *O. zibethicus* is a very effective intermediate host for *E. multilocularis*. The carriage of *E. multilocularis* by the musk rat varied along the Ourthe River, the upper part of the basin having a significantly higher prevalence than the lower part. The spring of the Ourthe River is located in the Ardenne area, which together with the Belgian Lorraine, is a highly endemic region for *E. multilocularis* (Losson et al., 2003; Hanosset et al., 2004). The geoclimatic conditions prevailing in these two areas are suitable for the maintenance of the *E. multilocularis* life cycle (Hanosset et al., 2008). One of the key factors is probably the altitude: *E. multilocularis* is a parasite that originates from cold regions (Hanosset et al., 2004). Indeed, the eggs of *E. multilocularis* have a survival time higher in cold temperatures (Veit et al., 1995). Delattre et al. (1991) showed that alveolar echinococcosis is more prevalent in regions with cold temperatures and a relative humidity. The altitude has an effect on climatic conditions especially on temperature. This could explain the marked difference observed in the different sections of the river and the very low prevalence recorded in the Netherlands (Borgsteede et al., 2003).

It must be pointed out that Schuster in 1987 in East Germany did not record the presence of *E. multilocularis* in the musk rat. This would suggest that at that time the parasite was very rare or absent from this part of Germany. Similarly, it must be pointed out that *E. multilocularis* was also absent in the musk rat population examined in 1982 in Belgium by Cotteleer et al. It is known that the geographic distribution of *E. multilocularis* in Europe is

expanding. Indeed, in the late 1980s only four European countries were considered as endemic versus 14 in 2004 (Hanosset et al., 2004).

In the province of Luxemburg (southern Belgium), only two other species of rodents were found to harbor the metacestode of *E. multilocularis*: the bank vole (*Clethrionomys glareolus*; 4.34% in 2004) and the common vole (*Microtus arvalis*; 0.11% in 2004) (Hanosset et al., 2008). In France, the main intermediate hosts are the bank vole, the common vole, and the water vole (*Arvicola terrestris*) (Petavy and Deblock, 1980, 1983; Delattre et al., 1985; Bonnin et al., 1986). The latter species is often found infected in France (Houin et al., 1982), but its ability to develop fertile lesions is contested (Petavy et al., 1980; Petavy and Deblock, 1983). Furthermore, in *A. terrestris* coinfections with *E. multilocularis* and the metacestodes of other tapeworms were rare (Petavy et al., 2003). This was not the case in the present study, which indicates that a single animal may harbor several species. In the red fox, *E. multilocularis* was present in 33.3% of the animals killed in the basin of the Ourthe River (Hanosset, unpubl. data). This figure is very close to the results of a previous survey (Losson et al., 2003), which indicated a global prevalence of 20.2% in Wallonia (southern Belgium) and 33.1% in the Ardenne area. Taking into account that the red fox is very common in Belgium and elsewhere in Europe and that other carnivores such as the dog and the cat play a minor role in Europe in the maintenance of the life cycle (Delattre et al., 1985), one can postulate that the musk rat is probably infected through the consumption of plant material contaminated by the fox' feces. The red fox itself can occasionally prey on living musk rats. However, it is also tempting to postulate that musk rat cadavers left on the river banks by the trappers are also consumed. If so, a human practice would have a marked impact on the epidemiology of alveolar

echinococcosis in the regions where the musk rat is present. This hypothesis requires additional study. In conclusion, the musk rat seems to be highly susceptible to *E. multilocularis* and could play the role of reservoir in Belgium. In the regions where it is present, trapping campaigns are often organised by local or national services and consequently this species could represent a cheap and sensitive bioindicator for the study and monitoring of alveolar echinococcosis.

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