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Morphometrical analysis of *Taenia taeniaeformis* and *Taenia crassiceps* in the common vole (*Microtus arvalis*) and the water vole (*Arvicola terrestris*) in Vorarlberg, Austria

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Summary

Taenia taeniaeformis and *Taenia crassiceps* are cestodes with voles as intermediate hosts and Felidae, Canidae and Mustelidae as definitive hosts. To evaluate the influence of *T. taeniaeformis* metacestodes on voles in Vorarlberg (Western Austria), a helminthological survey was performed on 318 common voles (*Microtus arvalis*) and 93 water voles (*Arvicola terrestris*). Furthermore the metacestodes themselves were analysed by morphometric methods. Our results demonstrate that both *T. taeniaeformis* and *T. crassiceps* are endemic in Vorarlberg, and that there is a significant difference between those infected with larvae of *T. taeniaeformis* and uninfected voles regarding body weight, but not sex or body length.

Keywords: *Taenia taeniaeformis*; *Taenia crassiceps*; *Microtus arvalis*; *Arvicola terrestris*; morphometrics

Introduction

Taenia taeniaeformis, a cyclophyllidean cestode, is an almost worldwide distributed parasite with Felidae as the common definitive hosts. In Europe this cestode has been documented in wild cats, domestic cats and European lynx (e.g. Loos-Frank & Zeyhle, 1982). Furthermore this parasite can be hosted by Canidae, e.g. red foxes and dogs (e.g. Pfeiffer *et al*., 1997), and Mustelidae. In the definitive host the adult tapeworms are located in the intestine, and can cause diarrhoea and emaciation. The gravid proglottides of this tapeworm can be found in the final host's faeces. The most important intermediate hosts are Murinae and Arvicolinae, but metacestodes were also found in Soricidae and pheasants (*Phasanius colchicus*) (e.g. Schmidt, 2001; Milazzo *et al*., 2010). Metacestodes are described as being a few millimetres in size with yellowish-white cysts (*Cysticercus fasciolaris*) which are located in the intermediate host's liver tissue (Schaerer, 1987). These cysts inhabit the larvae with a 5 – 200 mm long and 1 – 7 mm broad strobilocercus attaching to a bladder. The scolex is armed with 26 – 38 hooks, of which the large hooks reach lengths of up to 400 µm and the small hooks a size of 250 µm.
For human medicine *T. taeniaeformis* is of low importance, although three confirmed cases are known. In Sri Lanka and Argentina metacestodes were found in a child (Ekanayake *et al*. 1999) and Sterba *et al*. (1977) reported a case in a woman in the CSSR. Furthermore Garin *et al*. (2005) diagnosed a cyclophyllidean metacestode in the brain of a 38-year old Cambodian patient.

Taenia crassiceps is a parasite of carnivores: Canidae, Felidae and Mustelidae. In Europe the most common definitive hosts are red foxes, stone martens, lynx and dogs (Loos-Frank & Zeyhle, 1981). Murinae and Arvicolinae are the most important intermediate hosts of this parasite. However, metacestodes have also been found in dogs, domestic cats and marmosets (e.g. Wünschmann *et al*., 2003). Metacestodes (*Cysticercus longicollis*) have a length of 1 – 6 mm and an armed scolex with 28 – 39 hooks. Large hooks have been described with lengths between 146 and 211 µm and small hooks between 114 and 170 µm. Metacestodes of *T. crassiceps* are located in the musculature, in the subcutis, in peritoneal and pleural cavities (Petavy *et al*., 2003) and rarely in the eye (Delvalle, 1989) or in the brain (Wünschmann *et al*. 2003). There they form sac- or bladder-like cavities where they multiply asexually with proliferation. Human infections are rare. Most infestations were reported in immunosuppressed patients (e.g. AIDS patients), where the metacestodes are located in the musculature or connective tissue. Occasionally lesions occur, which may reappear after treatment because of the proliferation ability of these metacestodes (Chermette *et al*., 1995). This cestode is rarely reported with other opportunistic pathogens, e.g. *Pneumocystis jirovecii* or *Toxoplasma gondii* (Klinker *et al*., 1992). Infections in immunocompetent persons are even more scarce.

Arocker-Mettinger *et al*. (1992) found metacestodes of *T. crassiceps* in the right eye of a 15-year-old girl, where the parasite caused iridocyclitis.

Material and methods

In 2004 (September to December), 318 common voles (*Microtus arvalis*) and 93 water voles (*Arvicola terrestris*) were captured within a pest control program in the areas of Lustenau, Hohenems and Dornbirn, three towns in Vorarlberg, the westernmost province in Austria, known as an endemic area of *Echinococcus multilocularis*. In a previous study at the Medical University of Vienna these voles were first examined to evaluate the presence of metacestodes of the fox tapeworm in *M. arvalis* and *A. terrestris*; metacestodes of *T. taeniaeformis* and *T. crassiceps* were also collected (Führer *et al*., 2010). These metacestodes were analysed extensively within this study.

Examination of the voles
Weight and lengths of all captured voles were measured. To exclude mix-ups with *Microtus agrestris* species, classification of *M. arvalis* was performed during necroscopy with an inspection of the inner side of the second molars of the upper jaw. Sex determination was performed during necroscopy.

Examination of Taenia taeniaeformis
Within this study we examined 63 metacestodes of *T. taeniaeformis*, of which 23 were found in common voles and 40 detected in water voles. Cysts of *T. taeniaeformis* metacestodes were measured. Afterwards the cysts were opened with forceps, the metacestode was pulled out and the width and length of the strobila was analysed. The hooks on each scolex were counted using a reflected light microscope. The scolex was then cut off and bleached with a Berlese mixture (Reichenow, 1969) on a slide for 24 hours. Afterwards the hooks were measured microscopically at 100 – 400x magnification.
Furthermore molecular biological methods (e.g. PCR, sequencing) were used to confirm species classification within the cytochrome oxidase 1 (CO1) and the 12S ribosomal RNA genes as reported previously (Kocher *et al*., 1989; Rodriguez-Hidalgo *et al*., 2002).

Examination of Taenia crassiceps
Metacestodes of *T. crassiceps* were collected in two water voles. These metacestodes were counted, measured and stained with a Berlese mixture for the counting of the hooks.

Statistical analysis
For calculating statistically significant differences regarding total body weight, total body length and sex between *T. taeniaeformis* infected and uninfected groups of each species, after calculating Levene's Test for Equality of Variances, a t-test for independent samples was calculated assuming statistical significance below a p-value of 0.05.

Results

Taenia taeniaeformis
Metacestodes of *T. taeniaeformis* were detected in 22 out of 318 screened common voles and in 30 out of 93 water voles. In total 68 intact cysts were isolated and examined. All metacestode cysts were of yellowish-white colour. Three metacestodes of the common vole and two of the water vole presented everted scoleces.
In water voles metacestodes of *T. taeniaeformis* presented cysts with a diameter of 5 – 13 mm and a weight of 0.13 – 0.52 g. Larvae presented strobilocerci with a length of 15 – 139 mm and widths between 2.5 and 5 mm (Table 1). Morphometric analysis of the scolex revealed the presence of 26 – 36 hooks per scolex. Large hooks measured between 395 and 485 µm and small hooks between 230 and 290 µm.
In common voles cysts of 4 – 8 mm and a weight of 0.04 – 0.2 g were observed. Strobilocerci were of 7 – 98 mm length and 1.5 – 4 mm width. Each scolex presented between 26 and 34 hooks, the sizes of which varied between 350 and 435 µm in the case of large hooks and between 230 and 270 µm in the case of small hooks.

Table 1. Morphometrical analysis of *Taenia taeniaeformis* metacestodes in common and water voles

	Microtus arvalis	*Arvicola terrestris*	Overall
Prevalence [a]	22/318 (6.9%)	31/93 (33.3%)	
Cysts overall	26	48	74
Intact cysts	24	44	68
Non-intact cysts	2	4	6
Intensity [b]	1 – 2 (1.18 ± 0.39)	1 – 12 (1.5 ± 2.05)	1 – 12 (1.37 ± 1.56)
Cyst length (mm) [b]	4 – 9 (7.04 ± 1.33)	5 – 13 (8.26 ± 1.79)	4 – 13 (7.8 ± 1.73)
Cyst width (mm) [b]	3 – 9 (5.91 ± 1.59)	4 – 9 (7.43 ± 1.21)	3 – 9 (6.87 ± 1.54)
Strobila length (mm) [b]	7 – 98 (30.5 ± 20.17)	15 – 139 (48.5 ± 28.37)	7 – 139 (35.5 ± 27.09)
Strobila width (mm) [b]	1.5 – 4 (2.4 ± 0.58)	2.5 – 5 (3.5 ± 0.58)	1.5 – 5 (3.13 ± 0.77)
Hooks [b]	26 – 34 (30.4 ± 2.33)	26 – 36 (29.9 ± 2.43)	26 – 36 (30.04 ± 2.39)
Large hooks (µM) [b]	315 – 435 (406.1 ± 35.61)	340 – 485 (443.8 ± 26.5)	315 – 485 (430.2 ± 34.9)
Small hooks (µM) [b]	205 – 270 (252.1 ± 17.66)	220 – 290 (261.6 ± 13.9)	205 – 290 (258.2 ± 15.9)

[a] Number (Percent) of voles infested.
[b] Minimum-Maximum (Mean intensity ± SD)

The hooks, strobilae and cysts were smaller in common voles than in water voles.
In total 34 metacestodes of *T. taeniaeformis* were analysed with *Taenia*-specific PCRs (CO1 and/or 12 rRNA). Species classification was confirmed by sequencing of two isolates (JN882300 and JN882301).

Taenia crassiceps
Those 318 common voles and 93 water voles were further examined for the presence of *T. crassiceps*. Metacestodes were only found in two water voles (31 and 43 larvae each) captured in Hohenems. Of those, 15 metacestodes from each vole (n = 30) were examined in this study. Metacestodes of *T. crassiceps* had lengths between 1.5 and 4.5 mm. The scolex presented between 28 and 36 hooks, of which the large hooks were sized between 150 and 192 µm and the small hooks between 120 and 150 µm (Table 2).

Table 2. Morphometrical analysis of *Taenia crassiceps* metacestodes in water voles

	Arvicola terrestris
Prevalence [a]	2/93 (2.2 %)
Cysts overall	74 [c]
Intensity [b]	31 – 43 (37 ± 8.49)
Cyst length (mm) [b]	1.5 – 4.5 (3.2 ± 0.64)
Cyst width (mm) [b]	1 – 2 (1.5 ± 0.4)
Hooks [b]	28 – 36 (31.1 ± 2.47)
Large hooks (µM) [b]	150 – 192 (177 ± 11.76)
Small hooks (µM) [b]	120 – 150 (137 ± 9.5)

[a] Number (Percent) of voles infected
[b] Minimum-Maximum (Mean intensity ± SD)
[c] 30 cysts were used for analysis

Discussion

In this study the relations of sex, weight and length of 318 common voles (22 infected with *T. taeniaeformis*) and 93 water voles (30 infected with *T. taeniaeformis*) to infestations with this metacestode were analysed with biostatistical methods.
The examined water voles presented body weights between 37.3 and 139 g. Water voles infected with metacestodes of *T. taeniaeformis* had a mean body weight of 82.1 g (n =29; Standard Deviation = 16.3; Standard Error Mean = 2.5), whereas uninfected water voles had a mean body weight of 73.5 g (n = 64; Standard Deviation = 19.6; Standard Error Mean = 3). Common voles presented body weights between 6.2 and 35.8 g. Non-infected *M. arvalis* had a mean body weight of 17.9 g (n = 296; Standard Deviation = 4.5; Standard Error Mean = 0.2), whereas those infected with *T. taeniaeformis* metacestodes had a mean weight of 21.3 g (n = 22; Standard Deviation = 5.6; Standard Error Mean = 1.2). Significant differences between non-infected and infected common voles and water voles were found regarding the body mass (p value = 0.045 for *A. terrestris*, p value < 0.001 for *M. arvalis*) (Fig. 1).

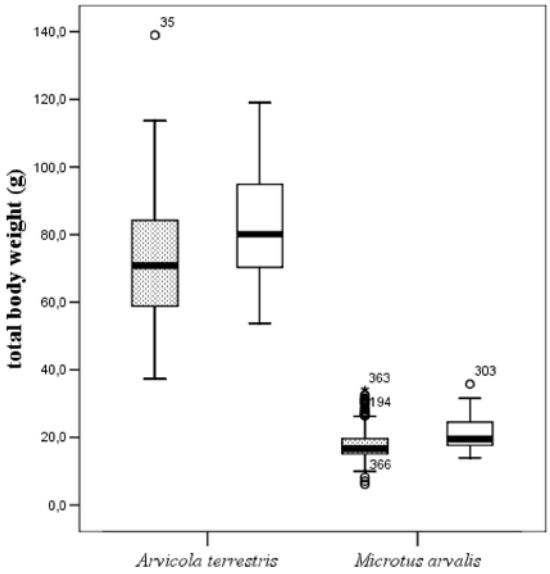

Fig. 1. Boxplots of total body weight distributions of captured *Arvicola terrestris* and *Microtus arvalis* populations. Each with *Taenia taeniaeformes* infestation (left dotted boxplot) and without infestation (right blank boxplot) respectively.

Water voles presented body lengths between 177 and 255 mm. Uninfected voles had a mean length of 204.5 mm (n = 64; Standard Deviation = 21.3; Standard Error Mean = 2.7). Voles infected with *T. taeniaeformis* presented a mean body length of 199 mm (n = 29; Standard Deviation = 36.4; Standard Error Mean = 6.8). Common voles had body lengths between 79 and 190 mm. Uninfected common voles had a mean body length of 122.5 mm (n=296; Standard Deviation = 14; Standard Error Mean = 0.8), whereas those common voles infected with metacestodes presented a mean body length of 127 mm (n = 22; Standard Deviation = 15.1; Standard Error Mean = 3.2). No significant differences between non-infected and infected voles were found regarding body length (Fig. 2).
With water voles we observed a sex ratio of 35 % male to 65 % female voles. Wieland (1973) reported a gender ratio of one to one at the birth of water voles. At high population densities competition and migration leads to a decrease in male water voles. These circumstances make it obvious that the population densities in the examined areas were high. Out of 93 examined *A. terrestris* 12 (36.4 %) male and 17 (28.3 %) female voles were infected with metacestodes of *T. taeniaeformis*.
We observed a sex ratio of 52.5 % male and 47.5 % female among the common voles. Stein (1957) reported a sex ratio of 53 % female embryos under laboratory conditions. Furthermore metacestodes of *T. taeniaeformis* were found in 13 (7.8 %) male and nine (6 %) female common voles and no relation between the gender and infestations with *T. taeniaeformis* were observed. The range of samples of infected *M. arvalis* voles was too small.

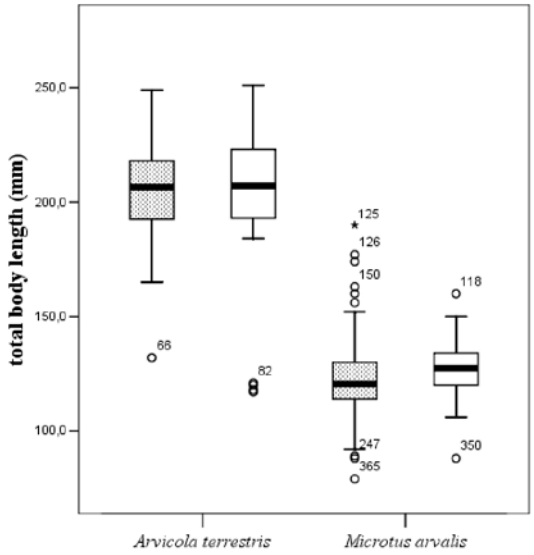

Fig. 2. Boxplots of total body length distributions of captured *Arvicola terrestris* and *Microtus arvalis* populations. Each with *Taenia taeniaeformes* infestation (left dotted boxplot) and without infestation (right blank boxplot) respectively.

No significant differences regarding sex and infections with *T. taeniaeformis* were found in murides in previous studies (e.g. Theis & Schwab, 1992). However, several studies reported significant differences between infected and non-infected rodents regarding body weight, age, total lengths and abdomen circumferences (e.g. Kowal *et al*., 2010). It is documented that the youngest mice are not infected with this metacestode (Loos-Frank, 1987). Tenora *et al*. (1979) only found *Taenia* metacestodes among bank voles (*Myodes glareolus*) older than three months. A recent study conducted in Switzerland documented that water voles older than five months were approximately two times more frequently infected than animals younger than three months (Burlet *et al*. 2011). This is caused by the fact that metacestodes need about two months after infection to become visible.

The results of this study can be summarised as follows: Both *T. taeniaeformis* and *T. crassiceps* could be found in Vorarlberg (Western Austria). Cysts, strobilae and hooks of *T. taeniaeformis* were larger in water voles than in common voles. Furthermore we documented a significant difference between uninfected individuals and those infected with *T. taeniaeformis* regarding body weight, whereas no significant differences were observed regarding body size or sex.

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Ethical standards

All voles were caught in 2004 in accordance with the Vorarlberger provincial law as published in Lg. Bl. Nr. 50/2002.

Conflict of interest

The authors declare that they have no conflict of interest.

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