

***Rodentolepis straminea* in an urban population of
Apodemus sylvaticus in the UK.**

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Abstract

The presence of the cyclophyllidean cestode *Rodentolepis straminea* (Cestoda, Hymenolepididae), was confirmed by molecular DNA analysis from a wood mouse (*Apodemus sylvaticus*) population inhabiting urban woodland in Salford, Greater Manchester (UK) with a prevalence of 27.8%. It would appear that the only previous published record of this species in *A. sylvaticus* in the British Isles is that from southwest Ireland, where 24% of the wood mice examined were infected with *R. straminea*. This species has been recorded in studies on *A. sylvaticus* in continental Europe. The current report represents a new record for *R. straminea* on mainland Britain and a first study of helminth parasites in an urban wood mouse population.

Introduction

The family Hymenolepididae is the richest in number of recorded species of all families within Eucestoda with about 850 species, 230 in mammals, the rest in birds (Czaplinski & Vaucher, 1994). Czaplinski & Vaucher (1994) listed 34 valid genera of mammalian hymenolepidids with eight of them primarily or exclusively from rodents. Of these, *Hymenolepis* (sensu stricto) and *Rodentolepis* Spasskii, 1954 are the most species rich genera found in rodents.

There has been much controversy and confusion with regards to *Rodentolepis straminea* since it was first (inadequately) described by Goeze in 1782 and named as *Taenia straminea* (summarised by Baer & Tenora, 1970). Currently there is still some disagreement over whether this species should be assigned to *Hymenolepis* or *Rodentolepis*. It was originally assigned to the genus *Rodentolepis* by Spasskii, 1954 which was erected for hymenolepidids from mammals with numerous hooks and *R. straminea* was designated a type species of the group (Czaplinski & Vaucher, 1994). However, Cunningham & Olsen (2010) argued that the more common usage of *Hymenolepis* should be adopted but stated that more defined molecular analyses would be required to fully interpret the systematics. Subsequently, a study by Haukisalmi *et al*

(2010) on the systematic relationships of hymenolepidid cestodes of rodents and shrews using partial sequences of 28S ribosomal RNA placed *R. straminea* in the 'Rodentolepis' clade. Recent papers refer to *R. straminea* and in the Fauna Europaea (www.faunaeur.org) this name is listed as the accepted name (updated in 2013). The genus *Rodentolepis* includes armed cestodes with a single row of numerous hooks, testes in a row or elongated triangle separated into two groups by the female gonads. Whereas, the genus *Hymenolepis* includes only species with a rudimentary rostellar apparatus and no rostellar hooks such as *Hymenolepis diminuta*. Therefore, in this paper *Rodentolepis straminea* will be used.

R. straminea was also originally thought to be synonymous with *Rodentolepis microstoma*, however, morphological examination and protein electrophoresis have now shown these to be separate species (Casanova *et al*, 2001).

Morphologically *R. straminea* and *R. microstoma* are very similar and difficult to differentiate. The main method of distinguishing between the two species is based on identification of polar filaments in the eggs of *R. microstoma* and the absence of polar filaments in the eggs of *R. straminea* (Casanova *et al.*, 2001).

R. microstoma is commonly known as the "bile duct tapeworm" of rodents due to its predilection for the bile duct where it attaches by the scolex with the strobila extending into the duodenum. In contrast, *R. straminea* is usually found in the ileum.

R. straminea is a generalist cestode for which murid rodents and cricetids are the definitive host. The intermediate hosts of *R. straminea* are thought to be beetles, and *Tribolium confusum* has been used in experimental infections (Walkey *et al.*, 1980). In addition, cystercercoids of *R. straminea* were found occurring naturally in the oribatid mite *Archipteria coleopterata* (Prokopic,

1962). This is noteworthy as mites are usually associated with the intermediate stages of anoplocephalid cestodes rather than hymenolepidids (Prokopic, 1962).

In an experimental study on the specificity of *R. straminea*, it was shown that the preferred hosts were *A. flavicollis*, *A. sylvaticus* and *Mesocricetus auratus* (Walkey *et al.*, 1980). Only individuals of *Mus musculus* with reduced immunological competence could be experimentally infected (Walkey *et al.*, 1980). The preferred hosts of *R. microstoma* were *M. musculus* along with the golden hamster (*M. auratus*) (Dvorak *et al.*, 1961). The morphological similarity between these two hymenolepidid species could have led to erroneous identification particularly in earlier reports before the advent of molecular techniques.

Other hosts of *R. straminea* include *Cricetus cricetus* in Hungary (Tenora & Murai, 1970); *Apodemus agrarius* in Serbia and Slovakia (Ondrikova *et al.*, 2010, Debenedetti, 2014;): *Apodemus flavicollis* in Slovakia (Ondrikova *et al.*, 2010); *Apodemus sylvaticus* in Spain (Fuentes *et al.*, 2004); *Rattus norvegicus* and *Rattus rattus* in the islands of the Southern Indian Ocean (Pisanu *et al.*, 2001) and in *Rhabdomys pumilia* (four striped grass mouse) and *Mastomys natalensis* (African soft-furred rat) in South Africa (Collins, 1972). *R. straminea* has also been recorded from *Mus spretus*, *Rattus rattus* and *A. sylvaticus* in the Iberian peninsula (Feliu *et al.*, 1997).

Rodentolepis straminea has apparently been recorded from *Mus musculus* and *Rattus norvegicus* in the UK (Corbett & Harris, 1991). In a study carried out in Essex (UK), *R. straminea* was recorded from *Clethrionomys glareolus* and *Microtus agrestis* (Nasher, 1976). It would appear that the only published record of *R. straminea* in *A. sylvaticus* in the British Isles is that from Ross

Island, Kilarney, southwest Ireland, where *R. straminea* was found infecting 24% of the wood mice examined (O'Sullivan *et al.*, 1984). The current study represents the first molecular confirmation of *R. straminea* in the British Isles and the first report of *R. straminea* infection of *A. sylvaticus* on the mainland of Great Britain.

Materials and Methods

Collection and examination of helminths

Wood mice (*A. sylvaticus*) were trapped using Longworth and Ugglan traps over the period from October to December 2012, June and July 2013, October to December 2013 and January to March 2014, at Castle Irwell in Salford, Greater Manchester. Castle Irwell is a large area comprising student accommodation for the University of Salford, university sports pitches and an area of woodland within a dense urban area. It is bounded by the river Irwell on three sides and by a 2 meter brick wall, with a road and housing estate beyond on the other. Trapped wood mice were taken back to the laboratory, euthanized with isoflurane followed by cervical dislocation. Each mouse was sexed, weighed, and its dimensions measured then either dissected immediately following a standard dissection procedure or frozen at -20°C for later examination. The intestinal tract and the liver were examined under a dissecting microscope for the presence of helminth parasites. Retrieved tapeworms were relaxed in water before being counted and fixed in 70% ethanol.

Wood mice were grouped on the basis of their weight into rough age groups, adults (≥ 20 g), young adults (13-19g) and juveniles (< 13 g) (Behnke *et al.*, 1999). Seasonality was assessed by grouping the sampling times into seasons: spring (March-May); summer (June-August); autumn (September-November); and winter (December-February).

Morphological identification of *Rodentolepis* and *Hymenolepis* species is difficult because they are very similar, they also have a wide geographical distribution and often low host specificity. Furthermore, after freezing or fixation *R. straminea* would appear to readily lose some or all of its scolex hooks, especially if its rostellum is everted, making identification more difficult (O'Sullivan *et al.*, 1984; R. Rushworth, personal observation).

Morphological identification of cestodes was determined using keys (Khalil *et al.*, 1994), and drawings from Haukisalmi *et al.*, (2010). Nematodes were identified using keys (Anderson, 1974). The only trematode found was identified by the position of the testes. Tapeworms were stained with Mayer's paracarmine using a protocol from the Natural History Museum, London (E. Harris, personal communication). The hooks on the scolex were cleared with a drop of lactic acid.

DNA amplification and sequence analysis

Ten specimens of putative *R. straminea* retrieved from the ileum of *A. sylvaticus* were included in this study. Tapeworms were fixed in 70% ethanol and genomic DNA was extracted using a Bioline Isolate II genomic DNA kit according to the manufacturer's instructions (Bioline, London, UK). A fragment within the ITS 1 and 5.8S ribosomal RNA gene was amplified using the hymenolepid primers F3 5'GCGGAAGGATCATTACACGTTC 3' and R3 5'

GCTCGACTCTTCATCGATCCACG 3' (Foronda et al., 2011). PCR was carried out in a 50 µl reaction containing 10 x NH₄ buffer (Bioline), 0.5 µM of each primer, 200 µM of each deoxynucleoside triphosphate (dNTPs; Bioline), 2mM MgCl₂, 2.5U of BioTaq DNA polymerase (Bioline) and 5µl of template DNA. A Stratagene (La Jolla, CA) Robocycler was used with a cycling profile of 5 minutes at 94°C for 1 cycle, followed by 30 s at 94°C, 30 s at 56°C, 30 s at 72°C, for 35 cycles and a final extension of 5 min at 72°C for 1 cycle.

PCR products were electrophoretically resolved on a 1.5% Tris-borate-EDTA (TBE) agarose gel, stained using gel red DNA dye and visualised using UV illumination (Syngene G:Box gel documentation and analysis system, Cambridge, UK). The amplified products were commercially sequenced (Beckman Coulter, Essex, UK) using the reverse PCR primer. Nucleotide sequences were analysed using FinchTV software package (Geospiza, Seattle, WA) and compared with those deposited on the NCBI database using BLAST (Basic Local Alignment Search Tool) (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Data Analysis

Data analysis was conducted using R statistical software version 3.1.1 (R core team, 2014). The sampled mouse population was briefly described and the prevalence, mean intensity (average worm burden amongst infected individuals), mean abundance (average worm burden throughout the whole population) and burden distribution of *R. straminea* were described (Bush *et al.*, 1997). Basic univariable assessments of differences in prevalence were conducted, using hypothesis testing as appropriate (Fisher's exact test for categorical variables; Cochran-Armitage trend test for ordered categorical variables; Mann-Whitney U test for continuous variables). A logistic regression model was developed in order to identify possible risk factors for infection

with *R. straminea*. Due to the lack of *R. straminea* tapeworms during the spring sampling period, the “bayesglm” procedure in the “arm” package was used to stabilise the model regression coefficients. Although this procedure uses elements of Bayesian methodology (incorporation of prior distributions of the model coefficients), the model output was interpreted in a frequentist fashion. The model was first parameterised using all variables of interest (mouse weight, sex, sampling season, sampling year), and a manual stepwise process was used to remove those variables not contributing to the model (as assessed using a Likelihood ratio test, with a p-value of 0.05 or less to suggest model contribution). Possible confounding was assessed by comparing model coefficients, with any change greater than 20% suggestive of confounding. Differences in mean intensity were largely assessed using bootstrapped t-tests and ANOVAs (Efron & Tibshirani, 1993), using tailored code written in R and 1000 iterations. For comparison of the burden and mouse weight, the Spearman rank correlation test was used (Best & Roberts, 1975).

Results

Sequencing

PCR products were successfully obtained from all 10 putative *R. straminea* tapeworms tested. Unambiguous sequence data were obtained for all PCR products. A BLAST search of these data (a 513bp ITS1 and 5.8S ribosomal RNA fragment) indicated all were indistinguishable from one another and shared 100% sequence identity to *R. straminea* (GenBank Accession number JN258054).

Morphology and distribution of adult R. straminea tapeworms

The tapeworms were relatively long and thin, ranging from 60mm to 165mm (mean length = 112 mm), with a body width of up to 2mm. The proglottids were wider than long, crapesdote, with maturation progressing down the strobila. The scolex is small with four suckers and a retractable rostellum armed with single crown of small hooks. There are 21-26 cricetoid hooks (short handle and pronounced guard) which are approximately 14 μ long (Fig. 1). Each proglottid contained three testes arranged in a row with a single lobed ovary and vitellarium in the centre (Fig. 2). The eggs were slightly oval with no polar filaments and measured approximately 62 μ m - 66 μ m x 36 – 50 μ m (Fig.3.). The oncosphere hooklets were longer than those on the scolex.

The distribution of *R.straminea* burden within wood mice showed a clear right skew (moment coefficient of skewness 3.7), suggesting an over dispersed distribution, as is commonly seen in macroparasitic infections (Crofton, 1971). The mean intensity of infection was 7.8 worms per mouse, and the mean abundance was 2.2 worms per mouse.

Seasonality, age and sex of A. sylvaticus

A total of 79 wood mice were captured over the study period, of which 47 (59%) were female ($\chi^2(1)=2.85;p = 0.1$). Most of the mice captured (53 mice; 67%) were young adults, and 20 (25%) were adults. Although all six juvenile mice (8% of the total) were trapped during 2013, there was no evidence of any significant temporal trends in age (Fisher's exact test p -value = 0.2) or weight (Kruskal Wallis test $\chi^2(5) = 5.72; p = 0.3$) distribution over the sampling period. Only one sampling collection was conducted in the spring and summer months (2014 and 2013, respectively), and two were conducted in autumn and winter. The number of mice collected per season ranged from eight (spring 2014) to 22 (autumn 2013).

Helminth species and levels of infection

Seven species of intestinal helminths were recorded in *A. sylvaticus* from Castle Irwell, including 3 nematode, 1 trematode and 3 cestode species (Table 1).

Overall 90% of the wood mice were infected with one or more helminth species. Intestinal cestodes were identified in 47 (59.5%) of the wood mice trapped in Castle Irwell, Salford. Cestodes now identified as *R. straminea* were found in 22 wood mice (27.8%) and could be seen through the intestinal wall of the posterior part (ileum) of the intestine prior to dissection. The most commonly identified cestode was *Skjrabotaenia lobata* which was found in 32 (41%) of the wood mice. The presence of this worm often caused an enlargement of the anterior part of the intestine. Of the 22 wood mice infected with *R. straminea*, 10 (45%) were also infected with *S. lobata*, which suggested there was no interaction between infection with the two worms ($\chi^2(1) = 0.09; p = 0.8$).

The only other hymenolepidid species found in 4 wood mice (5.1% from Castle Irwell) was *Rodentolepis nana*. *R. nana* is considerably smaller than *R. straminea* measuring up to 35mm in length and 0.6 – 1mm in length, it is often firmly attached to the gut wall. It has a retractable rostellum with a single crown of approximately 23 fraternoid (Baer & Tenora, 1970) hooks (long curved handle and a short blade and guard) measuring approximately 16 μ .

There was no evidence of any difference in prevalence of *R. straminea* between male and female mice ($\chi^2(1) = 0.66; p = 0.4$). The Cochran-Armitage test for trend identified evidence of a borderline linear increase in prevalence as age increased ($\chi^2(1) = 3.51; p = 0.06$), with the prevalence in juveniles, young

adults and adults estimated as 17%, 23%, and 45%, respectively. Similarly, a Mann-Whitney U test showed evidence that mice infected with *R.straminea* had a higher median weight than those not infected (18.7g vs 15.9g; $p = 0.01$).

There was evidence of temporal variation in the prevalence of infection with *R. straminea* over the sampling period (Fisher's exact test $p = 0.01$). This effect remained apparent when the year alone was assessed (Fisher's exact test $p = 0.02$), with a higher prevalence in 2012 compared to subsequent years (Table 2). There was weak evidence of variation in prevalence according to season (Fisher's exact test $p = 0.05$), which remained when only autumn and winter (the only seasons for which two samplings were available) were compared (Fisher's exact test $p = 0.06$). This suggested that the prevalence in winter was higher than that in autumn.

In order to account for possible confounding, a multivariable logistic regression model was developed - the results of which are shown in Table 3. Due to possible collinearity between the spring season and the year 2014, the year of sampling was condensed into two categories: period 1 (autumn/winter 2012) and period 2 (summer 2013-spring 2014). As expected from univariable analysis, there was a significant effect of weight, sampling period and season. The model fit, as assessed by the Hosmer-Lemeshow goodness of fit test with 10 groups, was good ($\chi^2(8) = 6.05$; $p = 0.6$).

There was no evidence of any difference in the mean abundance of infection between mice of different sexes ($t(76) = 0.28$; bootstrapped $p = 0.4$), or of different estimated ages ($F(2) = 0.65$; bootstrapped $p = 0.4$). However, there was evidence of a positive correlation between mouse weight and burden (Spearman's rho = 0.3; $p = 0.01$). There was also evidence of variation in mean intensity of infection during different years, with a mean burden of 6 worms

per mouse in 2012, 0.8 in 2013 and 0.7 in 2014 ($F(2) = 7.58$; bootstrapped $p = 0.01$). There was no evidence of any difference in mean worm burden in different seasons ($F(3) = 0.55$; bootstrapped $p = 0.5$).

Discussion

Morphological and molecular methods have confirmed the presence of the hymenolepidid tapeworm *R. straminea* in *A. sylvaticus* with a prevalence of 27.8% from an urban area in Salford, Greater Manchester (UK). As far as can be ascertained, this would appear to be the first record of this species in *A. sylvaticus* on mainland Britain. *R. straminea* has however been recorded from *Apodemus spp* in continental Europe (Gouy de Bellocq *et al.*, 2003; Fuentes *et al.*, 2004).

There would appear to be no published surveys of the helminth parasites of *A. sylvaticus* in urban areas in the UK or Europe. However, there have been many published surveys of the helminth parasites of *A. sylvaticus* in the British Isles (Elton *et al.*, 1931, Sharpe, 1964; Lewis, 1968; Lewis & Twigg, 1972; Murua, 1978; Langley & Fairley, 1982; Montgomery & Montgomery, 1990; Behnke, 1999; Abu-Madi *et al.*, 2000; Rogan *et al.*, 2007) *R. straminea* was not reported from any of these studies, the majority of which were from rural areas and only one of the areas studied (in Bristol) could be considered to be peri-urban (Murua, 1978).

The results reported in this study suggest that prevalence of *R. straminea* infection in *A. sylvaticus* increases with age and weight. This is not surprising as generally older animals have a higher level of infection (Behnke *et al.*, 2005). There was no significant difference of infection rate between male and female

mice. The mean intensity of infection was higher in 2012 than in a similar period in 2013. This could be due to the bumper seed harvest in 2013 leading to fewer arthropods being eaten.

Populations of wild vertebrates inhabiting urban environments often show several significant ecological and behavioural differences compared with populations of the same species living in their natural non-urban habitat. These include higher density, reduced territories, prolonged breeding season, reduced losses from predators, shift in diet composition, nesting in or on man-made constructions and reduced fear of man (Gliwicz, 1994). Dickman *et al.* (1987) found that *A.sylvaticus* in urban areas of Oxford had a higher density than their rural counterparts. Urban areas tend to have a slightly higher temperature than rural areas (Bradley & Altizer, 2006), and this could lead to an increase in the population of the potential arthropod intermediate hosts. The (experimental) intermediate host for *R.straminea* is *Trilobium confusum* (Walkey *et al.*, 1980), a beetle commonly found in stored grains. However, the oribatid mite *Archipteria coleopterata* was shown to be a naturally infected intermediate host in the Czech Republic by Prokopic (1962).

Commensal rodents such as *Rattus rattus*, *Rattus norvegicus* and *Mus musculus* may be relatively common in urban areas, and Walkey *et al.* (1980) showed that only immunocompromised *Mus musculus* could be infected with *R.straminea*. In contrast *R. straminea* has been recorded in *R. rattus* in continental Europe (Feliu *et al.*, 1997) and thus could be a reservoir for cross-infection to *Apodemus* in urban sites. *Apodemus sylvaticus* has also been considered a species well adapted to urban ecosystems (Dickman *et al.*, 1997).

In conclusion, this study represents the first molecular confirmation of *Rodentolepis straminea* in the British Isles and a new host record for this

species in *A. sylvaticus* on the mainland of Great Britain. Further work could include studies on the parasites found in *A. sylvaticus* in other urban sites throughout the UK and the potential role of oribatid mites in the transmission of *R. straminea*.

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Statement of interest

None.

Ethical standards

Wood mice were humanely euthanized according to UK Home Office guidelines.

Fig.1. Scolex of *R. straminea*. *R*, rostellum (retracted); *S*, sucker; *H*, rostellar hooks. Inset: individual rostellar hooks.

Fig. 2. Strobila of *R. straminea* stained with Mayer's paracarmine. *CS*, cirrus sac; *O*, ovary; *SV*, seminal vesicle; *T*, testes.

Fig. 3. Egg of *R. straminea*. Scale bar = 10µm.

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Table 1. Prevalence, mean intensity and mean abundance of intestinal helminths infecting 79 *Apodemus sylvaticus* sampled from Castle Irwell, Salford, UK.

Parasite species	Prevalence %	Mean intensity (± S.E.M.)	Range of intensity	Mean abundance (± S.E.M.)
<i>Heligmosomoides polygyrus</i>	61	7.2 (±1.74)	1-55	4.34 (±1.12)
<i>Syphacia obvelata</i>	43	196.0 (±46.99)	1-1130	84.34 (±22.86)
<i>Aonchotheca murissylvatici</i>	5	21.0 (± 17.1)	1-72	1.06 (±0.92)
<i>Brachylaimus recurva</i>	20	27.3 (± 7.46)	1-98	5.52 (±1.92)
<i>Skjrabotaenia lobata</i>	40.5	5.9 (± 1.57)	1-47	2.39 (±0.71)
<i>Rodentolepis straminea</i>	28	7.8 (1.84)	1-36	2.15 (±0.64)
<i>Rodentolepis nana</i>	5	21.5 (5.78)	11-32	1.09 (±0.59)

Table 2. Prevalence, mean intensity and mean abundance of *Rodentolepis straminea* amongst 79 *Apodemus sylvaticus* sampled from Castle Irwell, Salford, UK.

Season	Number of wood mice	Number of <i>R. straminea</i>	Prevalence %	Mean Intensity (range)	Mean abundance
Autumn 2012	9	76	44	19.0 (4-36)	8.4
Winter 2012	12	50	58	7.1(1-17)	4.2
Summer 2013	10	18	30	6 (2-9)	1.8
Autumn 2013	22	13	0.9	6.5 (1-12)	0.6
Winter 2013	18	14	0.33	2.3 (1-8)	0.8
Spring 2014	8	0	0	0	0
Overall Total	79	171	28	7.8 (1-36)	2.2

Table 3. Odds ratio estimates and confidence intervals for variables retained in the final logistic regression model. Dates and seasons relate to timing of sampling.

Variable	Odds ratio	95% confidence interval
Increase in weight by 1g	1.3	1.1 – 1.6
2012	1.0	-
2013-2014	0.2	0.1 – 0.6
Autumn	1.0	-
Winter	3.6	1.0 – 12.5
Spring	0.1	0.01 – 2.1
Summer	1.8	0.3 – 12.8



Fig. 1

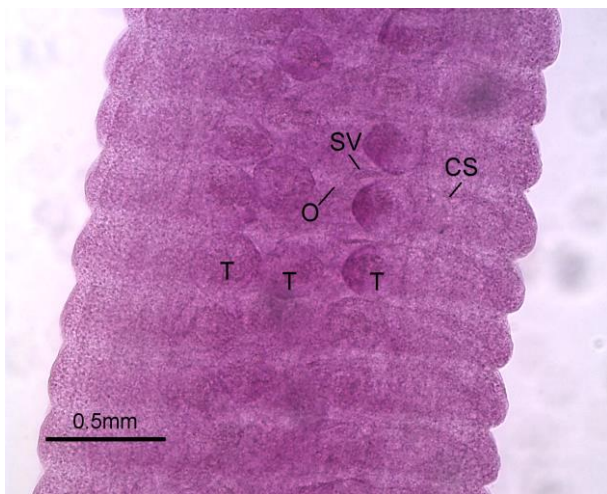


Fig. 2

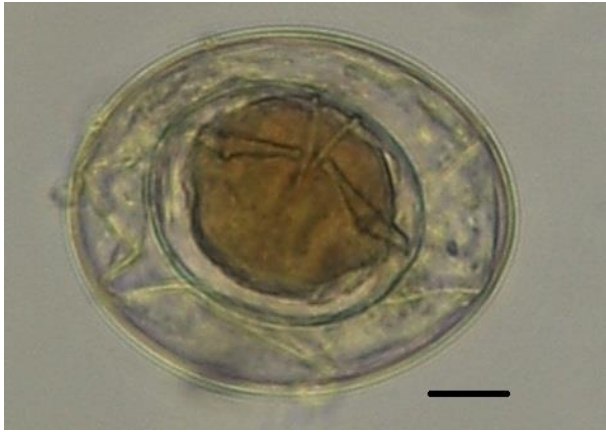


Fig. 3