



Short Communication

FLOTAC for diagnosis of endo-parasites in pet squirrels in southern Italy



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ABSTRACT

The present study investigated the occurrence of endoparasites in pet squirrels in southern Italy. Fresh fecal samples were collected from 50 asymptomatic pet squirrels belonging to five different species (*Callosciurus finlaysonii*, $n = 6$; *C. prevosti*, $n = 6$; *Tamias striatus*, $n = 26$; *T. sibiricus*, $n = 10$; *Sciurus carolinensis*, $n = 2$) housed both in pet shops and/or in private residences. All fecal samples were processed using the FLOTAC pellet technique to identify and count helminth eggs/larvae and protozoan cysts/oocysts. In addition, to detect *Cryptosporidium* spp. and *Giardia* spp. the samples were analyzed by the Remel Xpect[®] immunoassay. Helminth eggs were detected in 9 out of 50 squirrels. Specifically, eggs of *Dicrocoelium dendriticum* were found in 5 squirrels (*C. finlaysonii*, $n = 2$; *C. prevosti*, $n = 2$; *T. striatus*, $n = 1$); eggs of the pinworm *Syphacia* spp. in 3 squirrels (*C. prevosti*, $n = 2$; *T. striatus*, $n = 1$); and eggs of gastrointestinal nematoda (*Nippostrongylus*-like) were found in 1 subject (*C. prevosti*). Finally, two squirrels (*C. prevosti*) had multiple parasitic infections with *D. dendriticum* and *Capillaria hepatica*, and with *D. dendriticum* and *Strongyloides* spp., respectively. None of the samples were positive for *Cryptosporidium* spp. or *Giardia* spp. or any other protozoa (e.g. *Eimeria*). To the authors' knowledge, this is the first report of a *D. dendriticum* natural infection in pet rodents.

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1. Introduction

Exotic small mammals (e.g. rabbits, rodents, ferrets) are kept as pets worldwide. Pet rodents (e.g. rats, guinea pigs, hamsters) are especially popular (Lennox and Bauck, 2012) and squirrels (Family: Sciuridae) – comprising 51 genera and 278 species native to the Americas, Africa, Europe and Asia – are also commonly kept as pets (Wilson and Reeder, 2011). While knowledge of the husbandry and veterinary care of squirrels is increasing, still little information is available on the presence and prevalence of endoparasites in captive squirrels (Carrasco et al., 2006; Dubey et al., 2006;

Staebler et al., 2007). FLOTAC is a multivalent copromicroscopic technique based on centrifugal flotation of the fecal sample and subsequent translation of the top layer of the floating suspension (Cringoli et al., 2010). Due to its high accuracy and sensitivity, FLOTAC is a validated method to detect parasitic infections in livestock, companion and also exotic animals (Rinaldi et al., 2012). The aim of this study was to investigate for the first time the occurrence of endoparasites in pet squirrels in southern Italy, using FLOTAC for copromicroscopic diagnosis.

2. Materials and methods

From May to August 2013, fresh fecal samples were collected from 50 asymptomatic captive squirrels belonging to

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five different species (*Callosciurus finlaysonii*, $n = 6$; *C. prevosti*, $n = 6$; *Tamias striatus*, $n = 26$; *T. sibiricus*, $n = 10$; *Sciurus carolinensis*, $n = 2$). Twenty-two of these animals were kept in pet shops whereas 28 were owned by individuals as pets in the province of Naples (Campania region, southern Italy). For each animal, the following information was recorded: sex, age, general husbandry and feeding history, source (pet shop versus private owners), presence of cohabitant pets and use of antiparasitic treatments.

Samples were taken from the apical region of feces deposited on the ground. In order to avoid cross-contamination, only animals housed in single cages were included in the present study. The samples collected were kept at room temperature until transport to the laboratory where they were refrigerated at +4 °C and analyzed within three days.

All samples collected were processed using the FLOTAC pellet technique (Cringoli et al., 2010) for detection of helminth eggs/larvae and protozoan cysts/oocysts. Two different flotation solutions were used: FS2 (Sodium Chloride Solution) (specific gravity (s.g.) = 1200) and FS7 (Zinc Sulfate Solution) (s.g. = 1350). Parasitic elements were counted, photographed and measured using a light microscope at 20× and 40× magnifications (Leica DFC 490) and identified in accordance with the guidelines reported in textbooks (e.g. Baker, 2007). For detection of *Giardia* spp. and *Cryptosporidium* spp., the specimens were also analyzed using the Xpect® *Giardia/Cryptosporidium* snap test (Remel Thermo Scientific, Santa Fe Drive, Lenexa, KS, USA).

3. Results and discussion

The results of this survey showed endoparasites in 18% (9/50; 95% CI = 9.0–31.9%) of pet squirrels in southern Italy. All the animals included in this survey were asymptomatic and had not received any antiparasitic treatment. Squirrels were fed a pelleted-formulated feed, plus fresh vegetables and fruits daily. Four of the positive animals were males, and five females; six of them were from pet shops and three were kept as pets at private home. All species but *Tamias* spp. (farm-raised species) were caught in the wild and then imported to Italy. The median age of the infected animals was 2.0 years (range: 0.8–2.0 years). Two squirrels lived with cohabitant dogs, but the owners reported no contact between squirrels and dogs. The following helminth eggs (prevalence and 95% Confidence Interval – CI) were found: *Dicrocoelium dendriticum* (10.0%; 95% CI = 3.7–22.6%), pinworms (*Syphacia* spp.; 6.0%; 95% CI = 1.6–27 17.5%), *Capillaria hepatica* (2.0%; 95% CI = 0.1–12.0%), *Strongyloides* spp. (2.0%; 95% CI = 0.1–12.0%) and gastrointestinal nematoda (*Nippostrongylus*-like eggs; 2.0%; 95% CI = 0.1–12.0%). Table 1 shows the parasitological results for the different squirrel species (no. of positives and mean eggs per gram of feces). Microscopic photos of the helminth eggs found along with their key features are reported in Fig. 1a–e.

The present survey gave preliminary data on the parasitofauna of pet squirrels in Italy obtained by using FLOTAC; the major limitation of the study was the impossibility in identifying at species level all the eggs retrieved at the coprological examination. Conversely, Pisanu et al.

Table 1

Squirrel species infected by parasites in southern Italy and parasites detected (eggs per gram of feces – EPG).

Squirrel ID	Squirrel species	Parasite
1	<i>Callosciurus finlaysonii</i>	<i>Dicrocoelium dendriticum</i> (12 EPG)
6	<i>Callosciurus prevosti</i>	<i>D. dendriticum</i> (15 EPG)
16		<i>D. dendriticum</i> (10 EPG) and <i>Capillaria hepatica</i> (24 EPG)
17		<i>D. dendriticum</i> (12 EPG) and <i>Strongyloides</i> spp. (24 EPG)
21		Pinworms (<i>Syphacia</i> spp.) (20 EPG)
25		Pinworms (<i>Syphacia</i> spp.) (30 EPG)
32		Gastro-intestinal nematoda (<i>Nippostrongylus</i> -like) (44 EPG)
21	<i>Tamias striatus</i>	Pinworms (<i>Syphacia</i> spp.) (20 EPG)
43		<i>D. dendriticum</i> (12 EPG)

(2007) and Romeo et al. (2013) have reported the incidence of helminth fauna in wild squirrels (*Tamias sibiricus* and *Sciurus vulgaris*) by necropsy and identification of adult parasites. Nematode species, *Brevistriata skrjabini* and *Aonchotheca annulosa*, were present in 87% and 47%, respectively, of the Siberian chipmunks (*Tamias sibiricus*) studied (Pisanu et al., 2007). Romeo et al. (2013) reported a monoinfection by the pinworm *Trypanoxyuris* (*Rodentoxyuris*) *sciuri* in 87% of the Eurasian red squirrels (*Sciurus vulgaris*) investigated. In agreement with both of the previous studies, nematode species dominated the helminth fauna in our survey. In addition, to the authors' knowledge, none of the nematode taxa found in this survey, with exception of *C. hepatica* (Carrasco et al., 2006), have been described in captive squirrels so far (Rausch and Tiner, 1948).

Pinworms (e.g. *Syphacia* spp.) are frequently reported from pet rodents (Brown and Donnelly, 2012) and are considered ubiquitous and non-pathogenic, although diarrhea, rectal prolapse and poor condition may be seen in young animals with high worm burdens (Ravindran and Pillai, 1999; Richardson, 2003). While both gastrointestinal nematodes (e.g. *Nippostrongylus* spp.) and whipworms (e.g. *C. hepatica*) have been detected in wild rodents (Easterbrook et al., 2008), the authors were unable to find data on their prevalence in captive squirrels. In particular, no report of *Nippostrongylus* spp. infection has been documented in Sciurids so far. Therefore, as the authors could not isolate adult parasites in live or dissected animals for further confirmation, it is also possible that a pseudoinfection has been occurred in the single case detected in the present study.

The most common endoparasite found in the present survey was the small liver fluke *D. dendriticum*. Dicrocoeliosis is widespread in domestic ruminants, wild ungulates and rabbits. Human infection with *D. dendriticum* has also been reported (Gualdieri et al., 2011; Jeandron et al., 2011). Infection of definitive hosts occurs through the ingestion of ants infected with metacercariae; nevertheless, pseudoinfections (presence of *D. dendriticum* eggs in stool in the absence of adult worms) may occur. However,

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