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Article

Efficacy of a New Fenbendazole Treatment Protocol against *Capillaria* spp. in Northern White-Breasted Hedgehog (*Erinaceus roumanicus*)

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Abstract: Hedgehogs, *Erinaceus* spp., are commonly admitted to rescue centres in European countries. However, there are still few studies on parasitological fauna and treatment possibilities, including for *E. roumanicus*. This study aimed to evaluate parasitism in 34 hedgehogs directly after their admission to the Budapest Zoo and Botanical Garden Wildlife Rescue Centre, as well as the efficacy of fenbendazole treatment. The Mini-Flotac method was used to quantitatively evaluate and assess the efficacy of treatment with fenbendazole (100 mg/kg PO. BID for 7 days) in five selected individuals. Faecal samples were analysed at D0 and D14 after the beginning of the treatment. Globally, the prevalence of positive animals was 76%. *Capillaria* spp. were the most prevalent (68%), while coccidia showed a prevalence of 32%. Considering the range of action of this benzimidazole, the treatment provided an efficacy of 100%, eliminating all forms of *Capillaria* spp. Considering the high number of hedgehogs admitted to rescue centres and the potential effects of parasitism in morbidity and mortality during recovery, it is essential to improve our knowledge with regard to the parasitological fauna of these species and to attain effective treatment protocols.

Keywords: hedgehog; parasite; *Capillaria* spp.; deworming; anthelminthics; fenbendazole

1. Introduction

Hedgehogs belong to the family *Erinaceidae*, subfamily *Erinaceinae*, and the different species therein are grouped into five genera: *Atelerix* (the African hedgehogs—*A. albiventris*, *A. algirus*, *A. frontalis*, and *A. sclateri*), *Erinaceus* (the woodland hedgehogs—*E. europaeus*, *E. concolor*, *E. amurensis*, and *E. roumanicus*), *Hemiechinus* (the long-eared hedgehogs—*H. auritus* and H. *collaris*), *Mesechinus* (the steppe hedgehogs—*M. dauuricus* and *M. hughi*), and *Paraechinus* (the desert hedgehogs—*P. aethiopicus*, *P. hypomelas*, *P. micropus*, and *P. nudiventris*). These species have different geographical distributions across Europe, Asia, and Africa, while there are no native species on the American and Australian continents [1–7].

The growing interest of hedgehogs in wildlife population health and One Health studies can be attributed to several factors. In general, most species (especially *Erinaceus* spp.) are resilient and adaptable to environments and habitats such as urban and agricultural areas, leading to an intimate contact with humans and domestic species, thus providing an opportunity for the flow of important zoonotic agents [8,9], as well as environmental contaminants [10,11]. On the other hand, some species (especially *Atelerix albiventris*) are currently popular as exotic pets and have become frequent patients for veterinarians in exotic animal clinics.

Regarding internal parasites, hedgehogs can be hosts to a wide range of coccidia (e.g., *Isospora rastegaievae* (Yakimoff and Matikaschwili, 1933), *I. schmaltzi*, *I. erinacei*, *Eimeria perardi*, and *E. ostertagi*) [5,12,13], and *Cryptosporidium* spp. (e.g., *Cryptosporidium parvum* and *C. erinacei*) [14,15]). Hedgehogs may also have cestodes (e.g., *Hymenolepis erinacei*) [16], trematodes (e.g., *Brachylaemus erinacei*) [17], and nematodes (e.g., *Crenosoma striatum*, *Capillaria* spp. and *Physaloptera clausa*) [5,13]. Considering *Capillaria* spp., the infective larvae can be found in large quantities in earthworms, so hedgehogs can be infected by their ingestion or directly through the environment [5,13]. According to Kirilov et al. 2022 [18], considering both juveniles and adults, there are twelve agents of helminthiases in *Erinaceus* spp. with medical significance: *Dicrocoelium dendriticum* and *Alaria alata* (considering Trematoda); *Spirometra erinacei* and *Mesocestoides* sp. (considering Cestoda), *Eucoleus aerophilu*, *Thominx aerophilus*, *Trichinella spiralis*, *Trichinella native*, *Haemonchus contortus*, *Physocephalus sexalatus*, *Ascarops strongylina*, and *Spirocerca lupi* (considering Nematoda). The authors of the present article recently conducted a literature review on hedgehog parasitology [19].

In most cases, hedgehogs do not present significant clinical signs due to parasitism. Frequently, clinical disease does not occur even in cases of very high parasite load and it is often seen as an incidental finding on necropsy when the cause of death is not associated with parasitism [20]. Nevertheless, in instances where there is a high parasite load, severe and complicated infections may occur and influence the health condition. Some authors have suggested that parasitism may make it difficult for clinically ill animals to recover in a rehabilitation centre, preventing reintroduction and, consequently, contributing to a low genetic diversity [21] and the current decline of *Erinaceus* spp. in several European countries [7]. Considering the status of *Erinaceus* spp. and the impact of parasitism in general health, parasitology and parasitology treatment studies on wildlife species contribute to their conservation. Nevertheless, according to other authors, hedgehogs are generally resistant to parasitism, since only in severe cases of lungworm infection (usually associated with pneumonia) does parasitism hinder reintroduction, and even then, only until the end of the treatment regime [20].

In this project, the aim was to perform a faecal parasitological examination in a group of Northern white-breasted hedgehogs (*E. roumanicus*) at the Wildlife Rescue Center and the Budapest Zoo and Botanical Garden rescue centre and evaluate the efficacy of a new fenbendazole treatment protocol (100 mg/kg orally, once a day for seven days) in this species.

2. Results

2.1. Parasite Evaluation

Considering all the analysed samples (N = 34), endoparasites were identified in a total of 26 Northern white-breasted hedgehogs (76%), while 8 individuals had no parasite eggs in their faeces (24%). The intensity of parasitism, considering the 34 Northern white-breasted hedgehogs, was 1350 EPG (CI 95%: 383–2317). Figure 1 presents the individual egg counts of each hedgehog included in the study.

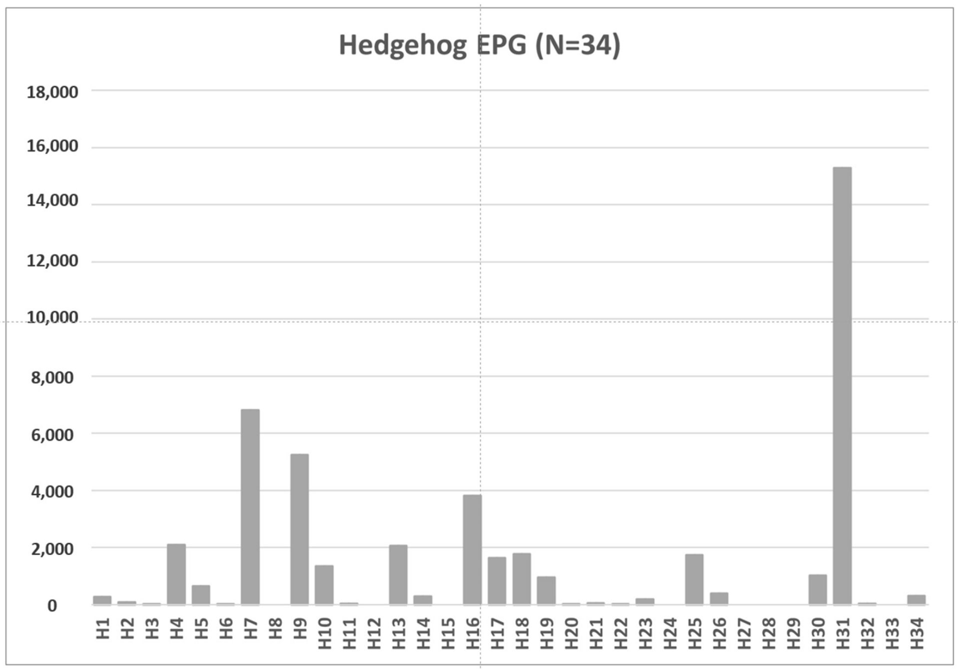

Figure 1. Quantitative evaluation of parasitism for each Northern white-breasted hedgehog (H1–H34, x-axis) in eggs per gram (EPG, y-axis).

Capillaria spp. eggs and coccidia oocysts were identified visually (Figure 2), and owing to this, it was possible to divide the 34 hedgehogs into three groups according to their parasitological fauna. More than half of the positive animals harboured exclusively *Capillaria* spp. (58%; 15/26). In three cases, only coccidia oocysts were visualised (12%; 3/26), and eight had a mixed infection (30%; 8/26), where it was possible to find both *Capillaria* spp. eggs and coccidia oocysts. Overall, the total prevalence of *Capillaria* spp. was 68%, and it was 32% for coccidia.

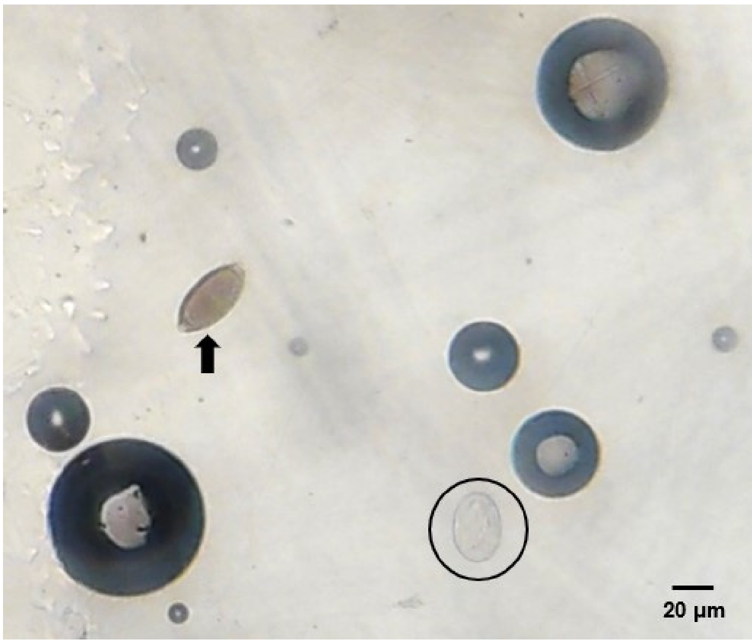

Figure 2. Morphological difference in *Capillaria* spp. egg (arrow) and coccidia oocyst (circle). A *Capillaria* sp. egg has a length of 45 μm, and a coccidia oocyst has a diameter of 28 μm, as seen in a mixed infection in a Northern white-breasted hedgehog (10×; 10×).

2.2. Fenbendazole Trial

The results of the fenbendazole treatment regime are presented in Table 1. Regarding coccidia, there were two animals (H25 and H30) presenting only coccidia oocysts on D14. On the other hand, in one of these five animals (H25) there was an increase in the number of coccidia oocysts of 64% post-treatment, compared to the values recorded pre-treatment.

Table 1. Efficacy of treatment measured with FECRT for *Capillaria* spp. Level of parasitism (in EPG) of each treated animal (fenbendazole 100 mg/kg, PO, SID 7 days) at day 0 (D0) and day 14 (D14).

Hedgehog ID	EPG on D0	EPG on D14	Efficacy for *Capillaria* spp.
H25	280	0	100%
H26	260	0	100%
H30	760	0	100%
H31	610	0	100%
H34	350	0	100%
Arithmetic Mean	-	-	100%

3. Discussion

This study revealed a general prevalence of endoparasite infection of 76% in the Northern white-breasted hedgehogs included. The most prevalent parasites belonged to the genus *Capillaria*, with a prevalence of 68%, followed by coccidia, with an average parasite load of 32%. Of the hedgehogs yielding a positive result, some animals were infected exclusively with *Capillaria* spp. (58%), others exclusively with coccidia (12%), and the other group elicited mixed infections (30%). Regarding the parasite load, this group of Northern white-breasted hedgehogs revealed an average endoparasite load of 1350 EPG. Considering the therapeutic protocol with fenbendazole (100 mg/kg body weight, PO, SID, for 7 days), the efficacy was 100% for *Capillaria* spp. and 89% in cases of coinfection.

Several factors can influence the parasite load in hedgehogs, including their genetics [22] and geographical provenance [23–25], which may explain the differences found between individuals in the present study. These Northern white-breasted hedgehogs arrived to the Wildlife Rescue Center at the Budapest Zoo and Botanical Garden from the urban area of Budapest and the surrounding natural and rural areas. Some of these areas may present more suitable conditions to the development and proliferation of parasites. On the other hand, the level of urbanisation, car traffic, and human activity may also expose hedgehogs to higher stress levels and, consequently, immunosuppression, which could lead to higher EPG values [26,27].

In Italy, a prevalence of infection of 55% was obtained after the *post-mortem* analysis of 40 western-European hedgehogs (*Erinaceus europaeus*), and *Crenosoma striatum* and *Capillaria erinacei* were the most frequently found species with prevalences of 45% and 42.5%, respectively [28]. Therefore, the prevalence of infection in the present study (76%) was comparatively higher than the results obtained in Italy. However, it should be mentioned that the studies were conducted in different periods of the year (January to August in the case of the Italian study and September to December in the case of the current study), which can be related to the different activity levels during these periods. The prevalence of *Capillaria* spp. was higher in the present study (68%), which may contribute to an increased mortality and morbidity during this critical time of the year, when hibernation usually begins and when food availability may be low. Thus, parasitology assessments in rescue centres are especially critical during these months to improve recovery rates in this species. Moreover, in 2017, Raue et al. analysed more than 200 hedgehog faecal samples, and *Capillaria* spp. was found in 39% [29]. Similarly, Majeed et al. (1989) [30] found a prevalence of 60% of *Capillaria* spp. In a recovery centre in Greece, after the parasitological analysis of 19 hedgehogs, the presence of *Capillaria* spp. was found in 7 animals (37%) [31]. Therefore, the results of the current study are in line with the information available in the literature, and it can be stated that *Capillaria* spp. can be considered to be amongst the predominant nematode of these species of European hedgehogs.

Regarding coccidia, some authors have detected their presence in hedgehogs as well. In China, a study recorded the presence of *Cystoisospora* sp. in hedgehogs of the species *E. amurensis*, with a prevalence of 62.5% [32]. In 2016, in Poland, the presence of *Isospora rastegaievae* was detected in *E. roumanicus*, which was the first report at a national level [33].

Considering the therapeutic trial with fenbendazole, 100% efficacy was achieved under the spectrum of action of the drug. As an anthelmintic, it is expected to have an effect on *Capillaria* spp. but be ineffective against protozoans, such as coccidia. Some protocols for fenbendazole in hedgehogs have already described in the literature, such as 25 mg/kg every 24 h or 10–30 mg/kg every 24 h for 5 days, both administered orally [34]. However, these therapeutic formulations had already been used at this recovery centre, with no significant results in reducing the parasite load, and that was an incentive for the use of a higher dose of 100 mg/kg fenbendazole for 7 days. Compared to previous therapies, this new dose had noticeably more positive effects and allowed for a 100% reduction in nematode elimination. Therefore, it is possible to admit that conventionally used fenbendazole treatment dosages worked only at subtherapeutic doses previously. However, other factors may influence the efficacy of a treatment as well. In general, the effectiveness of an antiparasitic drug always depends on the parasites being subjected to an adequate concentration of a drug for a sufficient period to cause irreversible damage to the parasite specimens [35]. Most antiparasitic medications are absorbed and transported to the parasites via the bloodstream. However, in the specific case of benzimidazoles (as fenbendazole), when administered orally, a low gastric pH is necessary for them to become soluble and be absorbed [35,36]. Some gastric diseases as well as some parasites can increase stomach pH and therefore affect the absorption of drugs such as fenbendazole [35]. In addition, the metabolism of each individual or at a specific time of the year (especially in hibernating species) may be variable. This has been proven to be a determining factor in the doses required for each animal since individuals with a faster metabolism will metabolise and eliminate antiparasitic drugs more rapidly [35,36]. As mentioned, H25 recorded a 64% increase in coccidia oocysts compared to the pre-treatment values. Possibly, due to the elimination of *Capillaria* spp., the coccidia no longer has to compete with the former for the resources present in the host, which may explain the post-treatment increase in the number of oocysts [37]. The choice of antiparasitic should consider not only the most abundant parasite species, but also those existing in smaller quantities in the host. However, the incremental increase in coccidian oocysts may not be concerning. Coccidia are ubiquitous parasites, frequent in wild animals, and often harmless in natural and wild conditions. Nevertheless, they may cause signs of disease in densely housed animals [38].

Other pilot protocols to treat *Capillaria* spp. infections have also been evaluated in hedgehog species, even though not in *E. roumanicus*. Van de Weyer et al. [39] reported that those containing only moxidectin had a significantly lower reduction rate of *Capillaria* spp. ($\geq$28.1%) compared to those with ivermectin or levamisole ($\geq$86.6%), even though protocols with levamisole had better reduction rates than those that had only ivermectin ($\geq$69.3%).

Unfortunately, there are no consistent guidelines regarding the need to deworm rescued wild animals. The decision is mainly based on the clinical presentation (such as diarrhoea, vomiting, or weight loss). Animals with these clinical signs and/or detectable parasites in qualitative evaluations should be submitted to a quantitative exam (using McMaster or Mini-FLOTAC® methods) to establish the level where treatment is recommended. The choice of an antiparasitic drug should always be based on evidence-based veterinary medicine. The ineffectiveness of fenbendazole against coccidia should alert our colleagues at rescue centres. The use of this deworming treatment alone in hedgehogs in cases of mixed infections with helminths and protozoa is, therefore, not recommended. If only fenbendazole is used, the recovery of animals after deworming may not always be what we would expect, as already seen in small ruminants [40].

Regarding a limitation of this study, the present work could certainly have benefited from a slightly larger sample size. Moreover, this study would also have benefited with the use of a control group to statistically compare with these results and evaluate their significancy. However, for practical reasons, this was not possible for the present study, and this possibility should be considered for future research. Notwithstanding, relevant results were obtained and may work as a starting point for future assessments regarding the parasitological fauna of *E. roumanicus*, which has not been studied as much as *E. europaeus*, and in terms of the efficacy of therapy with fenbendazole at a higher a dose than the one described in the literature.

4. Materials and Methods

4.1. Sampling Procedure

During the study period (1 September to 31 December 2022), a total of 34 *E. roumanicus* from the Wildlife Rescue Center at the Budapest Zoo and Botanical Garden were included in the present survey. Upon arrival, fresh faeces were collected using identified plastic bags and stored under 4–5 °C until analysis. All included hedgehogs were housed and rehabilitated in individual boxes to avoid cross-contamination and cross-infection.

4.2. Parasite Egg Counting and Identification

All samples were processed and analysed less than 24 h after sampling in the laboratory of the Budapest Zoo veterinary clinic. Hedgehogs' faecal samples were analysed using the Mini-FLOTAC® technique. Briefly, 2 g of faeces were diluted in 38 mL of saturated solution (specific gravity, 1.2) using the Fill-FLOTAC device (1:20 dilution). Then, the faecal suspension was transferred to the previously assembled reading chamber, which has a total volume of 2 mL, and after a flotation of 10 min, the chamber's upper disc was turned 90 degrees clockwise to the reading position. All coccidia oocysts and helminth eggs were identified based on their morphology and counted, using an analytic sensitivity of 10 oocysts or eggs per gram of faeces (OPG or EPG, respectively) [41]. The morphology of the eggs was also examined under a microscope in order to identify the taxonomic group of all parasites present [40,42]. There was only one person responsible for the morphological identification and quantification of the parasites of the whole project.

4.3. Fenbendazole Trial

A therapeutic trial was conducted with five animals selected from the group. Inclusion criteria included the need for an anti-helminthic treatment, according to the internal rescue centre protocols, the presence of a mixed infection (*Capillaria* spp. and coccidia), and not having gone into hibernation. Ethical review and approval were waived for the present trial, due to the absence of interference with the normal animal management of the rescue centre designed by the veterinary team at the institution. This study followed the daily activity and preventive protocols of the rescue centre, and none of the animals received any treatment strictly for experimental purposes.

The treatment protocol consisted of administering 100 mg/kg of body weight of fenbendazole PO SID administered over 7 consecutive days [43]. Fenbendazole was added to a small portion of food and only after this portion had been wholly ingested was the rest of the meal given to the hedgehogs to ensure that the medication was consumed. This procedure was carried out to ensure that the total amount of ingested fenbendazole could be carefully controlled. Fresh faecal samples were collected and analysed (using the same methodology) from the five individuals on day 0 (the day treatment was initiated before the first dose) and on day 14 (fourteen days after the beginning of treatment) [44,45]. The calculations performed were based on the formulas used in faecal egg count reduction tests (FECRT): FECRT % = $100 \times (1 - [T2/T1])$, where T1 and T2 represent the pre- and post-treatment EPG values, respectively [45,46]. Considering that there are no FECRTs designed specifically for hedgehogs or even wildlife, we revised the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines to adapt and create a pilot method with the use of mini-FLOTAC® with this purpose [47,48].

5. Conclusions

The rescued Northern white-breasted hedgehogs included in this study showed a prevalence of parasite infection of 76%. *Capillaria* spp. were the most frequently observed, followed by coccidia. The therapeutic efficacy of this protocol (100 mg/ PO SID 7 days) against *Capillaria* spp. was 100%. Further studies regarding the parasitological fauna of *E. roumanicus* and the evaluation of treatment protocols in wildlife species (including hedgehogs) are necessary to improve recovery rates at rescue centres and, ultimately, for the conservation of wildlife species.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

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Conflicts of Interest: The authors declare no conflicts of interest.

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