Efficacy of a Topical Formulation Containing Emodepside and Praziquantel (Profender®, Bayer) Against Nematodes in Captive Tortoises

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ABSTRACT
Gastrointestinal parasites are commonly diagnosed in captive tortoises. In response, fenbendazole has traditionally been used as an anthelmintic, either in single or repeated doses. However, fenbendazole requires oral administration and the process can be very challenging in some individuals. A topical preparation containing emodepside and praziquantel (Profender®, Bayer, Leverkusen, Germany) is promoted as effective against a broad range of nematodes, trematodes and cestodes. While this product is currently only licensed for administration to cats, previous studies have shown positive results with terrestrial tortoises. The aim of this study was to determine the efficacy of Profender® against oxyurid and ascarid parasites in captive tortoises. This was achieved by quantifying nematode eggs per gram (EPG) in feces using a modified McMaster technique before (Day 0) and after (Days 14 and 33) topical application of Profender® at a dose rate of 21.5mg emodepside and 85.5mg praziquantel per kg. Twenty nine tortoises, representing four different species, were enrolled in this study of which 14 (48%; including *Testudo hermanni* and *Testudo graeca*) were positive for intestinal nematodes. Following treatment the oxyurid EPG was slightly increased on Day 14 but declined significantly by Day 33 (59.7% reduction; p=0.01), indicating a slow onset of effect and moderate efficacy 33 days post-treatment, although no conclusions regarding efficacy against ascarids can be drawn in this study. Topical application of emodepside and praziquantel was well-tolerated in our tortoise population and could therefore be considered as a useful alternative anthelmintic treatment protocol for captive tortoises.

KEY WORDS: Chelonians, parasitology, nematodes, anthelmintics, emodepside, praziquantel.

ABBREVIATIONS
CRERB: Clinical Research Ethical Review Board; RVC: Royal Veterinary College; Ultraviolet B (UVB); EPG: Eggs per gram of feces; FEC: Fecal egg count; FECR: Fecal egg count reduction; IQ: Interquartile.

INTRODUCTION
Endoparasites are commonly diagnosed in chelonians and gastrointestinal helminthiasis has previously been reported to be the most commonly presented condition in tortoises (Holt *et al.*, 1979). While many endoparasites are minimally pathogenic, some may require therapeutic intervention and stressful or suboptimal environments are likely to exacerbate the outcome of

infection in captive terrestrial species (Wilson and Carpenter, 1996; Pasmans *et al.*, 2008). Intestinal nematodes such as oxyurids and ascarids are frequently detected during routine fecal screening in captive tortoises in the UK, although the significance of health concerns associated with nematodiasis in tortoises remain uncertain (Hedley *et al.*, 2013).

Oxyurids are the most frequently identified nematodes in tortoises, especially in Hermann's tortoises (*Testudo hermanni*) (Buńkowska *et al.*, 2011; Rataj *et al.*, 2011). There are a wide variety of oxyurid worms that can affect tortoises, including species within the genera *Tachygonetria*, *Spauligodon*, *Aleuris*, *Thelandros*, *Mehdiella*, *Ortleppnema* and *Thaparia* (Greiner and Mader, 2006) and co-infection is common (Matsuo *et al.*, 1999; Greiner and Mader, 2006). In the absence of reported variation in pathogenicity or anthelmintic susceptibility between individual parasite species, the oxyurids will not be discussed individually in this article. Oxyurids have a direct life cycle and most appear to be host-specific (Telford, 1971). Infected tortoises are usually asymptomatic, and a largely host-parasite relationship has been established in the gut of many tortoises (McArthur *et al.*, 2004). Oxyurid parasites commonly inhabit the lower intestinal tract of tortoises, where it has been suggested that they may help break down fecal content and reduce the occurrence of constipation (Frank, 1981). However, high levels of parasitaemia have been associated with fatal post-hibernation anorexia in tortoises (Wilson and Carpenter, 1996), potentially a consequence of nutrient deprivation to the host. Intestinal impaction has also been documented in severe infestations (Frank, 1981). A rise in oxyurid eggs detected in feces has been reported after hibernation, demonstrating their ability to survive the hibernation period, especially in young tortoises (Traversa *et al.*, 2005).

Angusticaecum holopterum is the most common ascarid identified in herbivorous tortoises (Holt *et al.*, 1979; Frank, 1981; Traversa *et al.*, 2005). Ascarids are relatively large parasites; adult worms can reach up to 10cm in length (Frank, 1981). They are located in the gastrointestinal tract and may be found attached to the intestinal mucosa of their host. *Angusticaecum holopterum* tend to have direct life cycles and infection of the host follows ingestion of an embryonated egg (McArthur *ei al.*, 2004; Hedley *et al.*, 2013). Gastrointestinal lesions may occur due to the migration of larvae through the viscera or embedding of adults within the mucosa (McArthur *et al.*, 2004). Low numbers of ascarids are unlikely to cause disease, but intestinal obstruction or perforation has been reported in tortoises with heavy burdens of adult ascarids (Keymer, 1978; Sprent, 1984; Wilson and Carpenter, 1996), in addition to anorexia and vomiting of worms (Holt *et al.*, 1979). This might be more likely to occur following anthelmintic treatment due to the accumulation of dead worms in the large intestine (Sprent, 1984). Other pathologies that have been associated with ascaridiasis include intussusception, gastrointestinal ulceration, coelomitis, thromboembolism and avascular necrosis (Frye, 1991).

Fenbendazole has been traditionally used as an anthelmintic in tortoises for decades, either in single or repeated doses (Benson, 1999; Carpenter, 2005; Giannetto *et al.*, 2007). It is widely regarded as a safe anthelmintic drug with a broad margin of safety and is reported to be well tolerated by tortoises (Fitzgerald and Newquist, 2008). However, there has been a recent report indicating an association between clinicopathologic alternations (including heteropenia, transient hypoglycaemia, hyperuricaemia and hyperphosphataemia) and the administration of fenbendazole in parasitized tortoises (Neiffer *et al.*, 2005). There is also recent anecdotal evidence of reduced fenbendazole efficacy in chelonians (Pellett *et al.*, 2014). Fenbendazole is

usually administered orally. This can however be a stressful experience and carries a risk of trauma, so may be impractical in some tortoises. Russian tortoises (*Testudo horsfieldii*) and Leopard tortoises (*Stigmochelys pardalis*) are particularly uncooperative and challenging for oral administration of drugs (Brames, 2008). Consequently, there is a necessity to assess alternative anthelmintic options which could be administered by a more practical route.

Emodepside belongs to a relatively new class of anthelmintics (cyclooctadepsipeptides) which achieve their anthelmintic activity by a novel mode of action (Harder, 2003). Emodepside acts at neuromuscular junctions to trigger release of an inhibitory neurotransmitter by stimulating the presynaptic receptors, inducing a flaccid paralysis in nematodes (Willson *et al.*, 2003; Harder *et al.*, 2005). Previous studies in a wide range of companion and livestock animals including chickens, mice, rats, dogs, cats, sheep, cattle and horses, have shown that emodepside displays effective anthelmintic activity against a number of gastrointestinal nematodes (Fukashe *et al.*, 1990; Sasaki *et al.*, 1992; von Samson-Himmelstjerna *et al.*, 2000). Praziquantel is a broadly effective anthelmintic drug with activity against trematodes and cestodes (Andrews, 1986). Praziquantel works by increasing the permeability of Ca^{2+} channels, causing an excessive influx of Ca^{2+} across muscle and/or tegumental membranes of parasites (Martin, 1997). This rapidly causes tegumental damage and spastic paralysis of the parasites, and increases exposure of parasite antigens at the worm surface which makes them more vulnerable to host antibody-dependent immunity (Harnett and Kusel, 1986). A commercially available topical preparation containing emodepside and praziquantel (Profender®, Bayer, Leverkusen, Germany) has been licensed for application to cats in UK since 2005 ("Profender", 2008). Profender® has been tested for drug absorption and efficacy after topical application in a wide range of reptile species (chelonians, lizards and snakes) with the studies demonstrating promising results in all specimens and no treatment-related adverse effects observed, even at dosages 15, 30 and 50 times higher than the recommended dose for cats (Mehlhorn *et al*., 2005; Brames, 2008; Schilliger *et al*., 2009).

The objective of this study was to evaluate the efficacy of Profender® spot-on, a combination product containing emodepside and praziquantel, against oxyurids and ascarids in captive tortoises in the UK. Based on evidence from previous studies (Brames, 2008; Schilliger *et al.*, 2009), we hypothesized that there would be a gradual decline in the number of nematode ova excreted in feces after the topical application of Profender®.

MATERIALS AND METHODS

This study was ethically reviewed by the Clinical Research Ethical Review Board (CRERB) at the Royal Veterinary College (RVC) and ethical approval was granted under the reference number *URN 2016 1518*. Informed consent was obtained in advance from the owners of all tortoises before enrolment.

Study animals

A panel of 29 clinically healthy tortoises, including four different species, were located in a tortoise rehoming center in Norfolk, UK and enrolled in this clinical trial between September and October 2016. These included 22 Spur-thighed tortoises (*Testudo graeca*), four Hermann's tortoises, two Russian tortoises, and one Leopard tortoise. Of those, 28 had not received anthelmintic treatment in the preceding four years. Our tortoise population were maintained in

different groups but under identical husbandry conditions; all tortoises lived in outdoor greenhouses, with supplementary heat and ultraviolet B (UVB) light provided when necessary, and they were fed a naturally wide diet of weeds and flowers, including dandelion (*Taraxacum* spp.), sow thistle (*Sonchus* spp.), mallow (*Malva* spp.), clover (*Trifolium* spp.) and nipplewort (*Lapsana* spp.). Fresh fecal samples were collected from the ground or water for microscopic fecal analysis.

Fecal examination

All samples were processed using a modified McMaster egg counting technique to establish a quantitative assessment of nematode egg counts. Briefly, where available 3 g fecal material were measured into a plastic container. Where the size of the fecal sample was limiting a minimum quantity of 1 g was used instead. For flotation, salt/sugar solution (400 g sodium chloride, 500 g sugar, dissolved in 1 L distilled water) with a specific gravity of 1.28 was added at a 14:1 (v/w) ratio and mixed well with the feces. The mixture was stirred and pipetted to fill up both chambers of a McMaster counting chamber. Both grids were visualized under the microscope (Olympus CX41) at 100x total magnification. Images were captured using a digital camera (Olympus DP20). Identification of oxyurid and ascarid eggs was based on the distinct morphological descriptions of ova (McArthur *et al.*, 2004; Greiner and Mader, 2006; Jocobson, 2007) and the number of worm eggs present was recorded. Results were presented as 'eggs per gram' (EPG) of feces (EPG = total number of eggs in both grids x 50).

Determination of anthelmintic efficacy

Tortoises with EPG in excess of 400 helminth eggs were selected for treatment. These tortoises were treated topically by the veterinary surgeon with a single dose of Profender® applied to the pre-femoral fossa at 1ml/kg (Fig. 1), corresponding to 21.5mg emodepside and 85.5mg praziquantel per kg (off-label). The dosage used in this study was seven times higher than the licensed dose for cats (Brames, 2008), due in part to the thick *stratum corneum* of the tortoise epidermis but also limiting the potential wastage of solution.

Tortoises were returned to their usual enclosures with no alterations to their environment and husbandry throughout the study period. Their owner was requested to report any adverse reactions as soon as possible to the veterinary surgeon. On day 14 post-treatment, feces collected from all enrolled tortoises were examined microscopically. Fecal egg counts (FEC) were repeated from the treated tortoises 33 days after treatment. Subsequently, the EPG determined pre- (Day 0) and post-worming (Days 14 and 33) were assessed and the percentage fecal egg count reduction (FECR) of oxyurid and ascarid eggs was calculated. For individual nematodes, the arithmetic means of EPG were calculated (Dobson *et al.*, 2009) and the percent efficacy of Profender® against individual nematode type was calculated using the formula below (Coles *et al.*, 1992).

$$\text{FECR}\ (\%) = \frac{\text{pretreatment EPG} - \text{posttreatment EPG}}{\text{pretreatment EPG}}\ \text{x}\ 100\%$$

Sufficient anthelmintic efficacy with no evidence of genetic resistance was defined as FECR ≥95% (Coles *et al.*, 2006).

Statistics
Statistical analyses were conducted using SPSS Statistics version 22 (IBM, United States). The Shapiro-Wilk test was used to test for normality ($p<0.05$). None of the parameters were found to be normally distributed, so non-parametric tests were used for data analysis. For individual nematodes, the Wilcoxon signed-rank test was used to compare mean EPG between the three measurement days (pre-treatment, 14 and 33 days post-treatment), and the criterion for statistical significance was a p-value below 0.05 ($p<0.05$). Statistical testing was not performed for individual tortoise species due to the small sample sizes.

RESULTS

Pre-trial screening

During pre-trial screening 15 (Day 0; 52%) of the sample tortoise population had no worm eggs detected by fecal examination. In total 14 (48%) of the sample tortoise population were positive, all with EPG ≥400, including 11 *T. graeca* and 3 *T. hermanni*. Of these, 11 (79%; 8/22 *T. graeca* and 3/4 *T. hermanni*) included oxyurid-type eggs (Fig. 2) and three (21%; 3/22 *T. graeca*) included ascarid eggs putatively identified as *A. holopterum* (Fig. 3). No tortoises were found to be co-infected with both nematode types (Fig. 4).

Post-treatment screening

Fecal screening 14 days post-treatment revealed a slight, albeit not significant, increase in oxyurid EPG of 6% across all infected tortoises ($p=0.93$; Table 1 and Fig. 5). By Day 33 oxyurid EPG was significantly decreased (59.7%; $p=0.01$).

The percentage reduction in ascarid EPG was 66.9% by Day 14 ($p=0.109$; Table 1). As one of just three Day 33 post-treatment fecal samples was unavailable, the Day 33 median ascarid EPG, efficacy percentage and p-value were not calculated, although the individual FECR figures were 100% and 18% respectively.

Of the 15 tortoises found to be uninfected during pre-trial screening, fecal samples were available from nine on Day 14 and all remained negative.

DISCUSSION

Susceptibility to disease caused by parasitism can be related to a number of factors including environmental temperatures and cleanliness, stress, concurrent disease, parasite burden, nutritional status and age of the host (Wilson and Carpenter, 1996). In some tortoises, therapeutic intervention may be required in response to parasite infection. Fenbendazole has been commonly used as an anthelmintic in many reptiles, including tortoises (Carpenter, 2005; Giannetto *et al.*, 2007). To date, there is no alternative product available on the market in the UK, while the safety and efficacy of other anthelmintics is yet to be demonstrated in a wide array of tortoise species.

One possible alternative is Profender®, although previous studies have yielded conflicting results. One clinical study with 103 Russian tortoises (*T. horsfieldii*) showed a slight increase of oxyurid EPG 15 days after application of Profender® at 1ml/kg and an overall oxyurid EPG reduction of 69% by 29 days (Brames, 2008). However, another study reported a complete cessation of oxyurid and ascarid egg excretion 14 days after Profender® treatment at 1.12ml/kg (Schilliger *et al.*, 2009) while one other reported the absence of nematode eggs 24 hours after

topical Profender® application at a lower dose of 0.56ml/kg (Mehlhorn *et al.*, 2005). It is challenging to explain the disagreement between these results, although the inconsistency may be attributable to host or parasite species variation, husbandry factors and/or the different fecal egg detection and counting techniques used in these studies. As a result, an efficacy study for Profender® in a real life clinical scenario is warranted. To the authors' knowledge, this study is the first to report the efficacy of Profender® against oxyurids in Spur-thighed and Hermann's tortoises, including quantitative data using the modified McMaster egg counting technique.

In the study reported here the dosage of 1ml Profender®/ kg bodyweight applied topically to the pre-femoral fossa demonstrated delayed, moderate anthelmintic activity against oxyurids in terrestrial tortoises. No statistically significant change in mean oxyurid EPG was detected 14 days after the administration of Profender®, followed by a significant reduction of 59.7% by Day 33. Our findings concur with those from a previously published report which also showed an initial slight increase in oxyurid EPG in a large group of Russian tortoises 15 days after Profender® administration (Brames, 2008). This initial discouraging result could be explained by a slow onset of effect for Profender®, potentially associated with the relatively thick integument and less permeable skin in terrestrial tortoises. Absorption of both ingredients in Profender® has been proven to be successful in terrestrial tortoises in a previous pharmacokinetic study, although a comparatively longer period of time was required to achieve therapeutic levels in the blood (Schilliger *et al.*, 2009). It is possible that oxyurid EPG would have continued to decrease after our study period of 33 days, but such investigation was not possible within the framework of the study. Explanations for the incomplete efficacy by Day 33 may include a possible requirement for a longer time frame for emodepside to exert a complete effect against oxyurids in tortoises, or the occurrence of anthelmintic resistance in oxyurids to emodepside in tortoises. However, complete elimination of oxyurids is often not possible (Mitchell, 2007). The efficacy of Profender® against oxyurids in our tortoises did not reach 95% and was less than reported in some other published studies (Mehlhorn *et al.*, 2005; Brames, 2008; Schilliger *et al.*, 2009), although it should be noted that a substantial decline in EPG was detected which would reduce the extent of environmental contamination. Direct comparison with fenbendazole from the published literature is difficult, although a study using two fenbendazole doses at 100mg/kg 14 days apart in 77 tortoises prompted a 85.5% reduction in oxyurid EPG seven days after the second treatment (Pellett *et al.*, 2014), suggesting that this fenbendazole protocol was more effective against oxyurids in tortoises than Profender®. Nonetheless, in situations where oral administration is challenging, Profender® spot-on can provide closely comparable efficacy for deworming since no safe and effective injectable anthelmintic is currently available for the use in tortoises. Ivermectin is an injectable anthelmintic that works against nematodes; however, chelonians are generally highly susceptible to ivermectin toxicosis and this can result in paresis, flaccid paralysis, hepatic lipidosis, nephrotoxicity and death (Teare and Busch, 1983; Diaz-Figueroa and Mitchell, 2006). The use of ivermectin should therefore be avoided in tortoises. No treatment-related adverse effects were reported in any animals treated with Profender® throughout the study.

One other potential explanation for the slow onset of effect of Profender® in our study is the relatively low ambient temperature available throughout. Tortoises are ectothermic herbivores and their metabolism is dependent on the external temperature (Pough, 1980; Lagarde *et al.*, 2003; McArthur *et al.*, 2004). Appropriate ambient temperatures for Hermann's and Spur-

thighed tortoises have been suggested to be between 26–30ºC (78.8-86ºF) (McArthur *et al.*, 2004). Since our population of tortoises were housed in outdoor enclosures with no external heat source and the temperature of the study location was between 8–18ºC (46.4-64.4ºF) during the first 14 days of our study period, it is possible that the suboptimal condition may have inhibited drug absorption through the skin, systemic circulation, distribution and/or metabolism in the tortoises. This theory can be supported by a previous study which showed that physiological performance and metabolism in reptiles are highly temperature dependent (Huey, 1982); one other study also demonstrated that a decrease in temperature had a negative impact on cardiac performance in ectothermic vertebrates (Driedzic and Gesser, 1994). Consequently, systemic circulation and distribution of emodepside and praziquantel may have been affected in our tortoise population kept at suboptimal temperatures. However, the housing condition used in our study reflects the situation for many captive tortoises in the UK as a recent retrospective survey demonstrated that 59.6% of captive tortoises were kept outside, either in an outdoor enclosure or free-ranging in the garden (Hedley *et al.*, 2013). Further study measuring the serum concentration of emodepside of tortoises kept at different temperatures would provide valuable information on the possible impact of environmental conditions on the pharmacokinetics of Profender® in tortoises. This may also help to determine the half-life and potential residual activity of emodepside in tortoises. Oxyurids in tortoises may also be less sensitive to emodepside under a suboptimal body temperature but further research needs to be undertaken to justify this speculation. However, it is of note that fenbendazole has previously been shown to take 31 days to achieve the full effect in Hermann's tortoises in a therapeutic trial study (Giannetto *et al.*, 2007). Future study comparing the efficacy of Profender® and fenbendazole is also warranted.

One of the most striking results to emerge from our present study is that 52% of our sample tortoise population showed no evidence of intestinal parasitism, despite the fact that 96.6% of them had not been dewormed in the preceding four years. This illustrates the limited requirement for routine anthelmintic treatment and confirms that the decision to deworm should depend upon fecal examination and the clinical status of a tortoise.

Limitations of the present study included the small sample size. It is also worth noting that the sampled population of tortoises could not be moved into a completely clean environment. Thus, reinfection could not be ruled out. The impact of reinfection on post-treatment FEC depends on the time of sampling and the prepatent period of the parasite species encountered. While prepatent period is highly variable between oxyurid species (Galvin, 1968; Taffs, 1976; Parsons *et al.*, 1987; Baker, 2007; Reinemeyer *et al.*, 2010) and current knowledge is limited for parasites which infect tortoises, the sampling interval employed here is likely to have precluded completion of the prepatent period. All tortoises that were nematode positive during the pre-screening were treated with Profender® on ethical grounds, preventing inclusion of an untreated control group. Further study using a larger sample population and 3-day pooled samples is recommended. This can enhance the sensitivity of ova detection and overcome the potential random error of variation in quantities of ova being shed by the nematodes in tortoises.

One disadvantage of using Profender® as an anthelmintic in tortoises is that it contains a fixed combination of emodepside, a broad spectrum nematocide, and praziquantel which targets trematodes and cestodes. Although trematodes and cestodes have been infrequently detected in

tortoises, they are suggested to be non-pathogenic (Rataj *et al.*, 2011). They also have indirect life-cycles, with intermediate hosts required for replication, so they are rarely encountered in captive tortoises (Greiner and Mader, 2006; Rataj *et al.*, 2011; Hedley *et al.*, 2013). As a result, there is generally no therapeutic indication for the use of praziquantel in most captive tortoises, even though it has been shown to be well tolerated.

Despite the delayed, moderate efficacy demonstrated in this study, caution should be exercised in applying this medication. Further research is necessary to fully elucidate the safety and optimum dosage of Profender® in a wide range of tortoise species, especially in clinically unwell tortoises. It is possible that a higher or repeated dose of Profender® may be able to provide sufficient anthelmintic activity (FECR ≥95%) against oxyurid and ascarid parasites in tortoises. Due to the relatively slow onset of effect of Profender® shown in our study, it is advisable not to hibernate the tortoises within 30 days after the application of Profender® in order to allow sufficient time for the killing process and ensure all dead nematodes are passed in feces.

CONCLUSION

Our findings showed moderate efficacy of Profender® against oxyurid parasites in two species of terrestrial tortoises. Although sufficient anthelmintic efficacy of Profender® cannot be concluded from this study since FECR ≥95% was not achieved, one application of Profender ® to the pre-femoral fossa at 1ml/kg was effective in reducing the burden of the most common gastrointestinal nematode encountered in captive tortoises. Efficacy of Profender® against ascarids cannot be concluded in this present study.

From our investigations, we can conclude that the topical application of Profender® was well-tolerated and no treatment-related adverse effects were observed on Spur-thighed and Hermann's tortoises. The method of administration of Profender® is advantageous; it provides a more practical option and a less stressful deworming experience for tortoises, especially in situations where oral administration is problematic. This topical preparation, containing a combination of emodepside and praziquantel, can be considered as an alternative treatment protocol to fenbendazole in tortoises, although independent clinical judgement is required when selecting the optimum anthelmintic for tortoises.

Conflict of interest

The author of this paper has no financial or personal relationship with any people or organizations that could inappropriately influence or bias this paper.

Authors' contributions

PKT participated in the design of the study, performed fecal analysis and data analysis, and drafted the manuscript. JH participated in the design of the study and reviewed the manuscript. SP participated in the design of the study and procurement of samples. DB provided support with coproscopical examination and reviewed the manuscript.

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REFERENCES

Andrews P. 1985. Praziquantel: mechanisms of anti-schistosomal activity. Pharmacology & Therapeutics, 29(1): 129-156.

Baker DG. Flynn's Parasites of Laboratory Animals. Blackwell Publishing, Hoboken.

Benson KG. 1999. Reptilian gastrointestinal diseases. Semin. Avian Exot. Pet Med, 8(2):90–97.

Brames H. 2008. Efficacy and tolerability of Profender in reptiles: spot-on treatment against nematodes. Exot. DVM, 10:29–34.

Buńkowska K, Okulewicz A, Perec-matysiak A, Hildebrand J. 2011. Preliminary coproscopic examination of tortoises in the City Zoological Garden in Wroclaw , Poland. Wiadomoœci Parazytol, 57(4):249–251.

Carpenter J. 2005. Exotic animal formulary. Elsevier Saunders, St. Louis, Mo.

Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA, Waller PJ. 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol, 44(1-2):35–44.

Coles GC, Jackson F, Pomroy WE, Prichard RK, Von Samson-Himmelstjerna G, Silvestre A, Taylor MA, Vercruysse J. 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol, 136(3-4):167–85.

Diaz-Figueroa O, Mitchell MA. 2006. Gastrointestinal Anatomy and Physiology. In: Reptile Medicine and Surgery. Elsevier Inc., Amsterdam, Netherlands:145–162.

Dobson RJ, Sangster NC, Besier RB, Woodgate RG. 2009. Geometric means provide a biased efficacy result when conducting a faecal egg count reduction test (FECRT). Vet. Parasitol, 161(1-2):162–167.

Driedzic WR, Gesser H. 1994. Energy metabolism and contractility in ectothermic vertebrate hearts: hypoxia, acidosis, and low temperature. Physiol. Rev, 74(1):221–59.

Fitzgerald KT, Newquist KL. 2008. Poisonings in Reptiles. Vet. Clin. North Am.: Exot. Anim. Pract, 11(2):327–357.

Frank W. 1981. Endoparasites. Dis. Reptil, 1:291–358.

Frye F. 1991. Biomedical and Surgical Aspects of Captive Reptile Husbandry. Krieger, Malabar, Florida.

Fukashe T, Koike T, Chinone S, Akihama S, Iagake H, Takagi M, Shimizu T, Yaguchi T, Sasaki

T and Okada T. 1990. Anthelmintic effects of PF 1022, a new cyclic depsipeptide, on the intestinal parasitic nematodes in dogs and cats. Proc Jpn Soc Vet Sci, 110:122.

Cook GC. 1994. Tropical infection of the gastrointestinal tract and liver series. Gut online, 35:1159–1162.

Galvin TJ. 1968. Development of Human and Pig Ascaris in the Pig and Rabbit. J. Parasitol. 54(6):1085–1091.

Giannetto S, Brianti E, Poglayen G, Sorgi C, Capelli G, Pennisi MG, Coci G. 2007. Efficacy of oxfendazole and fenbendazole against tortoise (Testudo hermanni) oxyurids. Parasitol. Res., 100(5):1069–1073.

Greiner EC, Mader DR. 2006. Parasitology. *In* Mader, DR. (ed): Reptile Medicine and Surgery. Saunders Elsevier, St. Louis, Mo:343–364.

Harder A, Schmitt-Wrede HP, Krücken J, Marinovski P, Wunderlich F, Willson J, Amliwala K, Holden-Dye L, Walker R. 2003. Cyclooctadepsipeptides—an anthelmintically active class of compounds exhibiting a novel mode of action. Int. J. Antimicrob. Agents, 22(3):318–331.

Harder A, Holden-Dye L, Walker R, Wunderlich F. 2005. Mechanisms of action of emodepside. Parasitol. Res., 97(1):S1-S10.

Harnett W, Kusel JR. 1986. Increased exposure of parasite antigens at the surface of adult male Schistosoma mansoni exposed to praziquantel in vitro. Parasitology, 93(2):401–405.

Hedley J, Eatwell K, Shaw DJ. 2013. Gastrointestinal parasitic burdens in UK tortoises: a survey of tortoise owners and potential risk factors. Vet. Rec., 173(2013):525.

Holt PE, Cooper JE, Needham JR. 1979. Diseases of tortoises: a review of seventy cases. J. Small Anim. Pr., 20(5):269–286.

Huey RB. 1982. Temperature, physiology, and the ecology of reptiles. Biol. Reptil., 12:25–91.

Jocobson E. 2007. Parasites and Parasitic Diseases of Reptiles. *In* Jocobson E (ed), Infectious Diseases and Pathology of Reptiles: Color Atlas and Text. CRC Press, Boca Raton, Florida:571–665.

Keymer IF. 1978. Diseases of chelonians: (1) Necropsy survey of tortoises. Vet. Rec., 103(25):548–552.

Lagarde F, Bonnet X, Corbin J, Henen B, Nagy K, Mardonov B, Naulleau G. 2003. Foraging behaviour and diet of an ectothermic herbivore: Testudo horsfieldi. Ecography, 26(2):236–242.

Martin RJ. 1997. Modes of action of anthelmintic dugs. Vet. J., 154(1):11–34.

Matsuo K, Ganzorig S, Oku Y, Kamiya M. 1999. Nematodes of the Indian star tortoise, Geochelone elegans (Testudinidae) with description of a new species Alaeuris geochelone sp. n. (Oxyurida: Pharyngodonidae). J. Helminthol. Soc. Washingt., 66(1):28–32.

McArthur S, Wilkinson R, Meyer J. 2004. Medicine and surgery of tortoises and turtles. Blackwell Pub, Oxford.

Mehlhorn H, Schmahl G, Frese M, Mevissen I, Harder A, Krieger K. 2005. Effects of a combination of emodepside and praziquantel on parasites of reptiles and rodents. Parasitol. Res., 97:65–69.

Mitchell MA. 2007. Parasites of Reptiles. *In* Baker DG (ed): Flynn's Parasites of Laboratory Animals. Blackwell Publishing, Hoboken, New Jersey:177–216.

Neiffer DL, Lydick D, Burks K, Doherty D. 2005. Hematologic and plasma biochemical changes associated with fenbendazole administration in Hermann's tortoises (testudo hermanni). J. Zoo Wildl. Med., 36(4):661–72.

Nielsen MK, Vidyashankar AN, Andersen UV, DeLisi K, Pilegaard K, Kaplan RM. 2010. Effects of fecal collection and storage factors on strongylid egg counts in horses. Vet. Parasitol., 167(1):55–61.

Parsons JC. 1987. Ascarid Infections of Cats and Dogs. Vet. Clin. North Am. Small Anim. Pract., 17(6):1307–1339.

Pasmans F, Blahak S, Martel A, Pantchev N. 2008. Introducing reptiles into a captive collection: The role of the veterinarian. Vet. J., 175(1):53-68.

Pellett S, Smith K, Mainwaring S, Cope I. 2014. Lack of Efficacy to Fenbendazole - Is It a Growing Problem in Tortoises? *In* British Small Animal Veterinary Congress. Unpublished, Birmingham, United Kingdom.

Pough FH. 1980. Advantages of ectothermy for tetrapods. Am. Nat., 115(1):92–112.

European Medicines Agency, 2008. *Profender: EPAR - Product Information*. [pdf] European Medicines Agency. Available at: <http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/veterinary/000097/WC500063851.pdf > [Accessed 7 February 2017]

Rataj AV, Lindtner-Knific R, Vlahović K, Mavri U, Dovč A. 2011. Parasites in pet reptiles. Acta Vet. Scand., 53,(1):33.

Reinemeyer CR, Prado JC, Nichols EC, Marchiondo AA. 2010. Efficacy of pyrantel pamoate and ivermectin paste formulations against naturally acquired Oxyuris equi infections in horses. Vet. Parasitol., 171(1):106–110.

Sasaki T, Takagi M, Yaguchi T, Miyadoh S, Okada T, Koyama M. 1992. A new anthelmintic cyclodepsipeptide, PF1022A. J. Antibiot., 45(5):692–697.

Schilliger L, Betremieux O, Rochet J, Krebber R, Schaper R. 2009. Absorption and efficacy of a spot-on combination containing emodepside plus praziquantel in reptiles. Rev. De Méd. Vét., 160(12):557–561.

Sprent JFA. 1984. Ascaridoid Nematodes. *In* Hoff GL, Frye FL, Jacobson ER. (ed): Diseases of Amphibians and Reptiles. Springer US, New York:219–245.

Taffs LF. 1976. Pinworm infections in laboratory rodents: a review. Lab. Anim., 10(1):1–13.

Teare JA, Busch M. 1983. Toxicity and efficacy of ivermectin in chelonians. J. Am. Vet. Med. Assn., 183(11):1195–1197.

Telford SR. 1971. Parasitic Diseases of Reptiles. Am. Vet. Med. Assoc., 159(11):1644–1652.

Traversa D, Capelli G, Iorio R, Bouamer S, Cameli A, Giangaspero A. 2005. Epidemiology and biology of nematodofauna affecting Testudo hermanni, Testudo graeca and Testudo marginata in Italy. Parasitol. Res., 98(1):14.

von Samson-Himmelstjerna G, Harder A, Schnieder T, Kalbe J, Mencke N. 2000. In vivo activities of the new anthelmintic depsipeptide PF 1022A. Parasitol. Res., 86(3):194–199.

Willson J, Amliwala K, Harder A, Holden-Dye L, Walker RJ. 2003. The effect of the anthelmintic emodepside at the neuromuscular junction of the parasitic nematode Ascaris suum. Parasitology, 126(1):79–86.

Wilson SC, Carpenter JW. 1996. Endoparasitic diseases of reptiles. Semin. Avian Exot. Pet Med., 5(2):64–74.

Figure 1. Profender® was applied to the prefemoral fossa at 1 mL/kg using a disposable syringe.

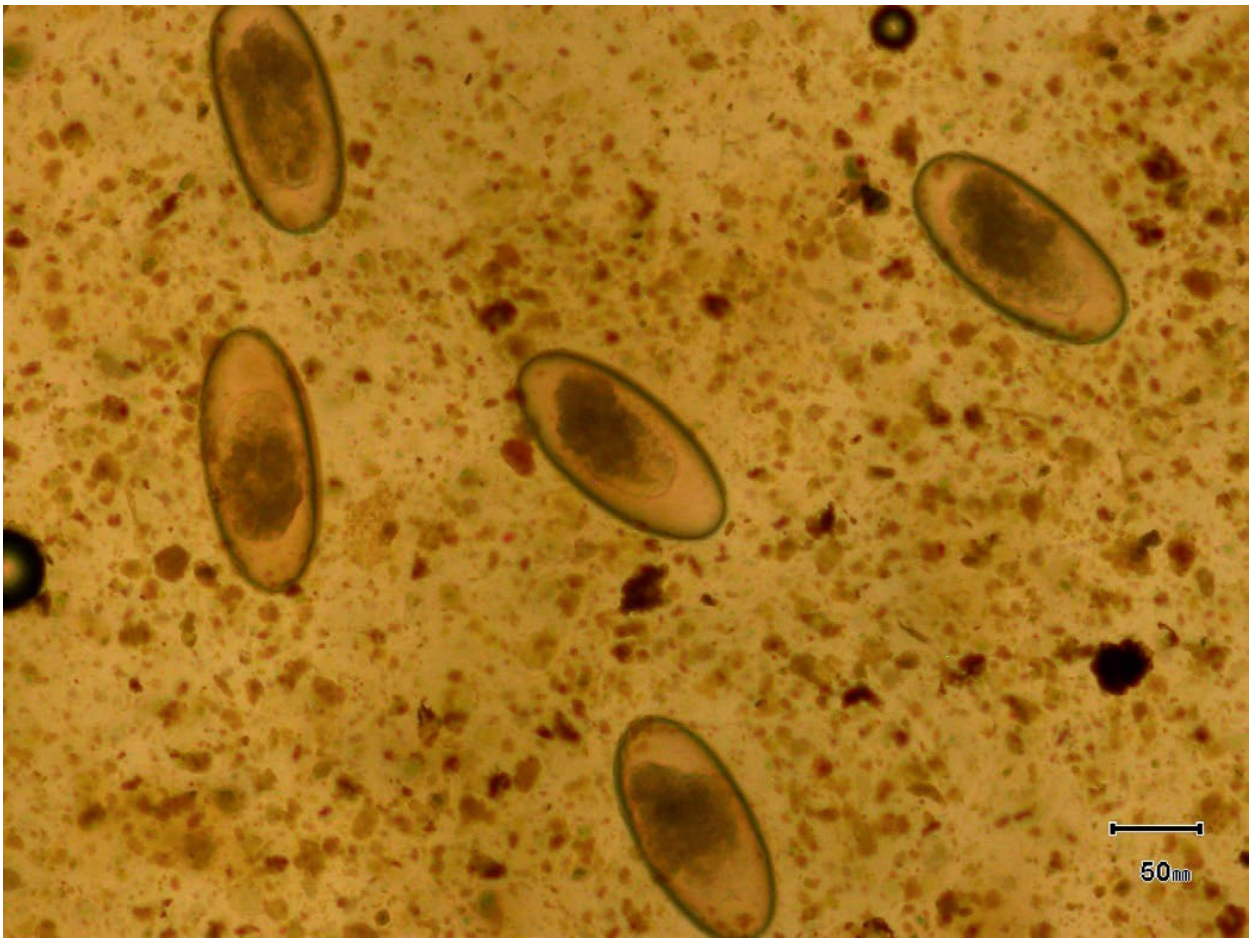

Figure 2. Oxyurid ova of Hermann's tortoise (Testudo hermanni). Photomicrograph magnification, 100×.

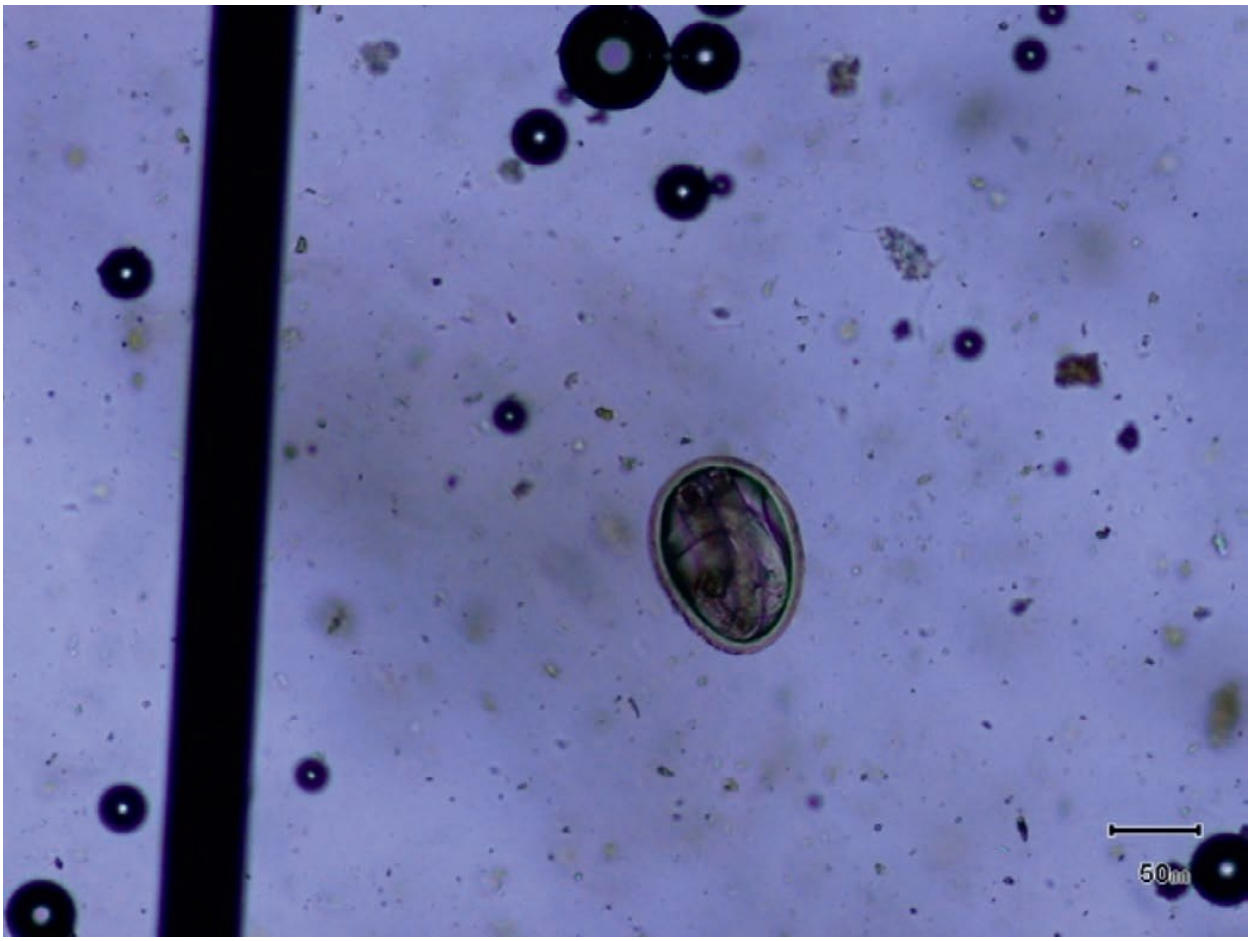

Figure 3. Ascarid ovum of spur-thighed tortoise (Testudo graeca). Photomicrograph magnification, 100×.

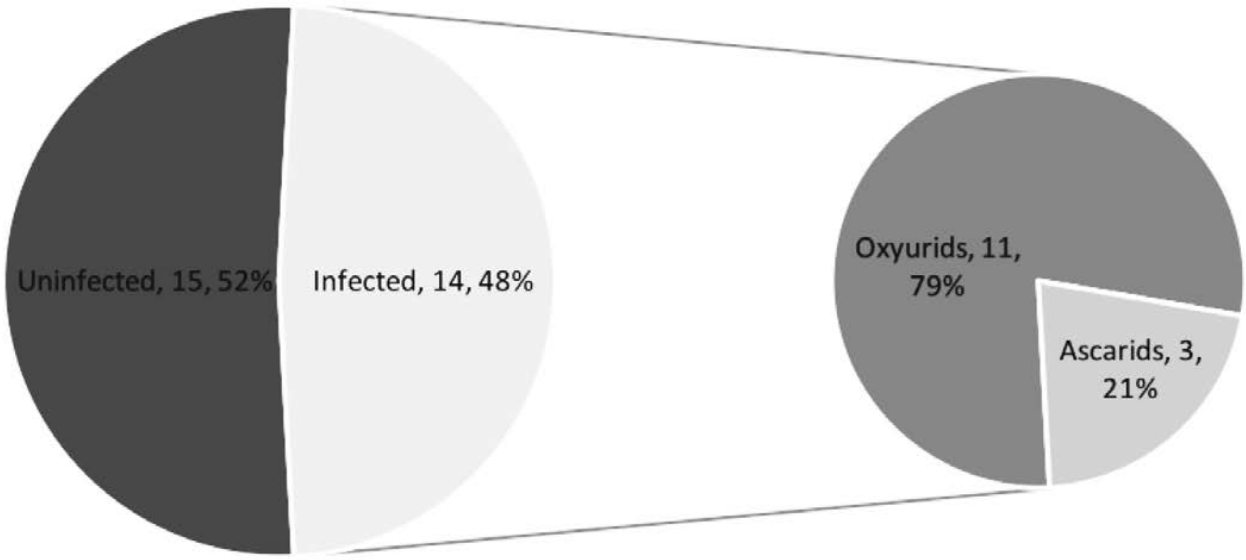

Figure 4. Pie of pie diagram to illustrate the percentages of uninfected and infected tortoises, and the proportionate occurrence of oxyurid- and ascarid-type nematodes eggs detected before anthelmintic treatment (Day 0).

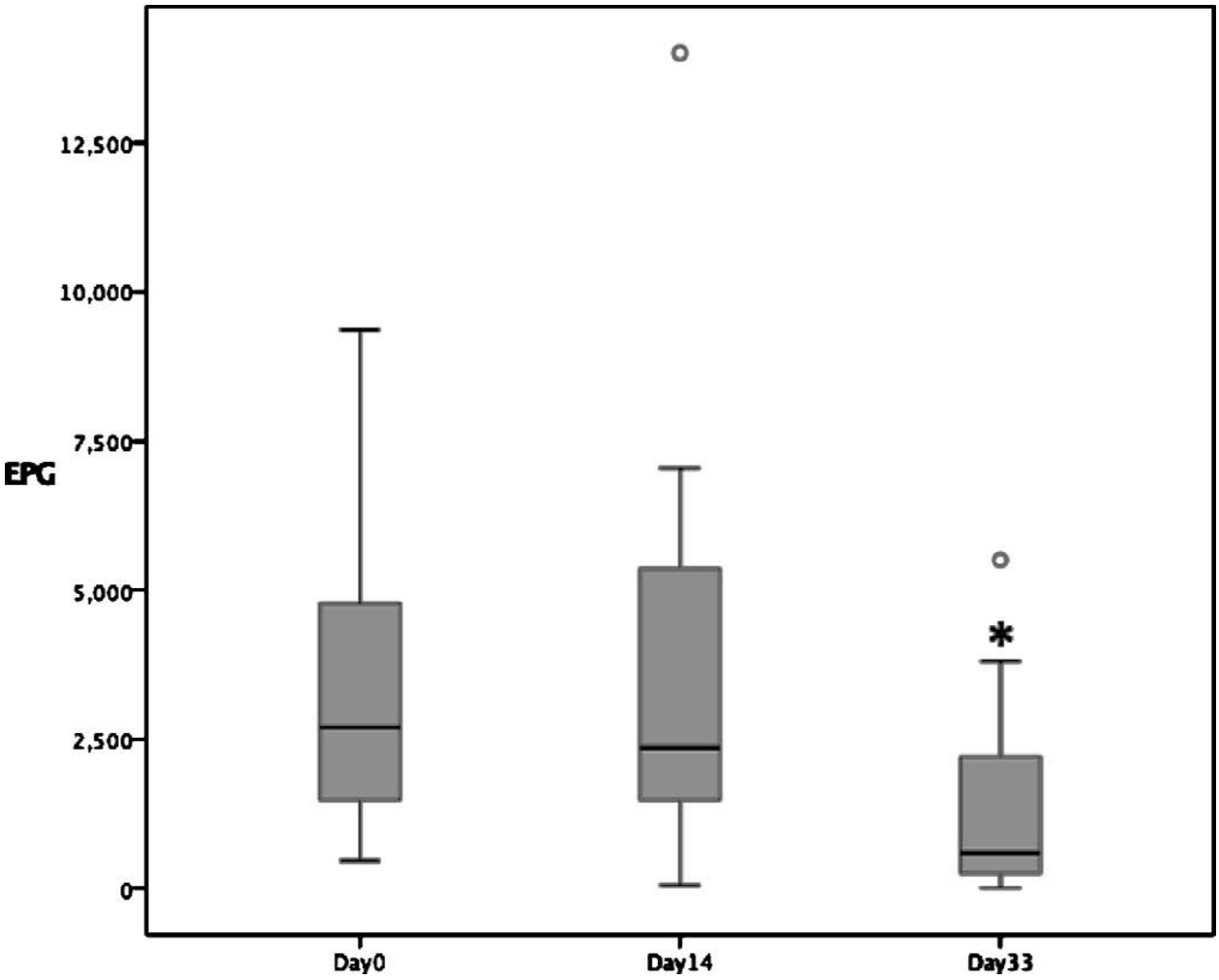

Figure 5. Box-and-whisker plots to show the oxyurid EPG (median, interquartile, and range) on trial Days 0, 14, and 33. *Wilcoxon signed-rank test comparison $P < 0.05$ between Days 0 and 33.

Table 1. Fecal nematode egg counts before (Day 0) and after (Days 14 and 33) topical treatment using Profender®, percentage efficacy, and data analysis in clinical trial.

Analysis of fecal egg counts

		Median (IQ) (EPG)[b]			FECR (%)[a]		*P*-value[d]	
Nematode	**N[a]**	**Day 0**	**Day 14**	**Day 33**	**Day 14**	**Dave 33**	**Day 14**	**Day 33**
Oxyurid	11	2,700.0 (1,475.0–4,775.0)	2,350.0 (1,475.0–5,350.0)	600.0 (250.0–2,200.0)	-6.0	59.7	0.929	0.01
Ascarid	3	1,900.0 (1,500.0–3,000.0)	950.0 (475.0-1,175.0)	-	66.9	-	0.109	-

[a] *N* = Number of tortoises analyzed.
[b] Median fecal egg count and interquartile (IQ) range, EPG = eggs per gram of feces, pretreatment (Day 0) and posttreatment (Days 14 and 33).
[c] Fecal egg count reduction (FECR), a measure of efficacy.
[d] (Two-sided) probability comparing Day 0 with Day 14/ Day 33 for the treatment using Profender within each nematode group.