**Short Communication**

## Ovicidal efficacy of fenbendazole after treatment of horses naturally infected with cyathostomins

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**Abstract**

The ovicidal activity of benzimidazole (BZ) anthelmintics is unique and not seen in other drug classes. Such ovicidal efficacy is not widely reported for equine cyathostomins, nor has this activity been tested in the face of BZ resistance. Although the product label states that fenbendazole is for use against BZ-susceptible cyathostomins, susceptibility testing is rarely performed. In this field-based study, the ovicidal efficacy of fenbendazole in horses (n=39) harbouring BZ-resistant cyathostomins was compared when dosed at 7.5 mg/kg body weight (BW) orally, as a single dose per os (n=21) or daily for five consecutive days in feed (n=18). Suppression of egg hatch rate was observed in the single and five- day treatment groups; a significant difference between pre- and post-treatment egg hatch rates (*P*<0.05) was observed for three days after treatment with a single dose of fenbendazole (on premises with BZ-resistant cyathostomins), and for three days after treatment for five consecutive days with fenbendazole (on premises with BZ-resistant cyathostomins). Post treatment numbers of eggs and larvae remained significantly lower (*P*<0.05) than pre-treatment levels to the end of the trial. We conclude that in the face of BZ-resistant cyathostomins the ovicidal effect of fenbendazole persist for three days after both a single oral dose of 7.5 mg/kg per os and after treatment orally for five consecutive daily doses at 7.5 mg/kg in feed.

*Keywords:* Benzimidazole; cyathostomin; efficacy; egg hatch assay; ovicidal;

**Introduction**

Benzimidazoles (BZ) are the only class of anthelmintics with ovicidal efficacy (Lacey *et al.,* 1990). BZ resistance in cyathostomins in the UK continues increasing in prevalence (Lester *et al.,* 2013). The marketing authorisation for fenbendazole states that it is only suitable for use in animals with BZ-sensitive cyathostomins (NOAH Compendium), however efficacy testing is not common practice in the field (Allison *et al.,* 2011; Relf *et al.,* 2011).

To date, most of the work investigating BZ ovicidal activity has been conducted on ruminant nematodes, particularly *Haemonchus contortus*. To the best of the authors’ knowledge there are no published studies on BZ ovicidal efficacy on equine cyathostomins in the face of resistance.

The objective of this study was to investigate the ovicidal efficacy of fenbendazole in horses under field conditions when given at 7.5 mg/kg BW as a single dose, or on five consecutive days.

**Materials and Methods**

*Pilot Study and Sample size estimates*

A pilot study was carried out (n=6) to inform sample size estimates. Estimates were conducted in SiZ (Cytel v 1.0, Woburn, MA, USA), using a Wilcoxon-Mann-Whitney model. Using standard deviation data derived from this pilot study (0.141) we adopted a sample size of 40 horses (20 in each group) which gave 80% power and 95% confidence to detect a 7-fold difference in egg hatch rate.

*Ethical consent*

This study received ethics approval from the University of Liverpool Ethics Committee, RETH000363.

*Sample Population*

From 109 animals at four premises in South-West England and one in South Wales, 39 horses met the inclusion criteria of: faecal egg count (FEC) ≥150 eggs per gram faeces (epg) (Coles *et al.,* 2006), no anthelmintics within 13 weeks and in good health (Table 1). All farms harboured BZ resistant cyathostomins. Horses were sampled August – October 2012. The study population consisted of 19 females and 20 males, aged 0.5-20 years mean 6 ± 4.7 years. Breeds represented were: 20 Warmbloods, four Thoroughbreds, two Warmblood cross Thoroughbreds, seven native ponies, five cobs and one Irish Draft horse. Pre-treatment FEC for the population ranged from 150 epg to 1300 epg with an arithmetic mean of 550 epg ± 328 epg and a median of 475 epg. The remaining 70 horses that did not meet the inclusion criteria were 32 males and 38 females, ranging from 0.5-25 mean 9 ± 6.17 years, representing the breeds noted above, FEC <150 epg.

Premises one, two and three had not used BZ anthelmintics for 10 years, premises four and five used BZ infrequently (Table 1). Horse exposure to BZ prior to arrival on these premises was unknown. None of the premises quarantined new animals on arrival. Time on the current premises varied, <1 month to >5 years. Premises were deemed high risk for intestinal parasite transmission due to a high proportion of immunologically immature animals and frequent movement of horses on or off the premises (Nielsen *et al.,* 2010a; Relf *et al.,* 2011).

Body weight was estimated by weigh tape and rounded up by 50 kg for light/small breeds and 100 kg for heavy/large breeds to counter inaccuracy (Ellis and Hollands, 1998).

*Treatment group allocation*

At treatment BZ susceptibility was unknown. Treatment was not randomized; approximately 50% of treatments at each premises were single doses and the remaining 50% received a five day dose (Table 1). Allocation of delivery route depended upon management, e.g. feasibility of offering medicated feed once daily.

*FECR sample collection and analysis*

Prior to treatment a single, fresh, spontaneously voided, faecal sample was collected from each animal for FEC analysis. A modified McMaster method ([Coles *et al.,* 1992](#_ENREF_6)) was used, where each egg count represented 25 epg. A second FEC was conducted 14 days post-treatment to calculate FEC reduction (FECR) using 90% as the cut off for BZ resistance.

From FECR results, strongyle populations per premises were categorized *post hoc* as “BZ susceptible” or “BZ resistant”. FECR results identified that on no premises could the strongyle population (in all tested horses) be classified as BZ susceptible. Two groups are considered in the analysis of the results from this study: a single dose group (n=21) and a five day dose group (n=18).

*Anthelmintic treatment and sample collection for egg hatch tests*

Before treatment a fresh faecal sample was collected from all animals and stored under anaerobic conditions (Coles *et al.,* 1992). All 21 horses in the single dose group were administered a dose of 18.75% w/w fenbendazole at 7.5 mg/kg on day 0 orally using the oral dosing device supplied, ensuring that the entire dose was swallowed. Further faecal samples were collected, and stored, for five consecutive days from the day after dosing with fenbendazole. All 18 of the horses receiving five consecutive days’ treatment were administered fenbendazole (10% w/v) at 7.5 mg/kg BW daily for five consecutive days (day -4 to 0), orally in feed following the manufacturer’s recommendations. Feed intake was monitored to ensure the full dose was ingested. Further faecal samples were collected and stored from one day after the last dose of fenbendazole and then on days 3, 5, 7 and 9 post-treatment for the five consecutive day dosing group. All samples were stored as described by Coles *et al.* (1992) and were used for egg hatch testing.

*Laboratory analysis*

We modified the egg hatch assay described by Matthews *et al.* (2012), to an egg hatch test (EHT) that did not expose eggs to anthelmintics, simply testing the ability of excreted strongyle eggs to hatch. Twenty-four well cell culture plates were used for incubation of eggs. For each sample, duplicates of 3 ml of water containing approximately 100 eggs per ml were incubated for 48 hours at 26°C. It was difficult to estimate the exact number of eggs directly after anthelmintic dosing and by using this quantity of eggs ensured a minimum 100 eggs per well were incubated at each time point. After incubation plates were examined using an inverted microscope. For each well the number of hatched larvae and eggs were recorded for hatch rate analysis. Data were entered into a Microsoft® Excel spreadsheet (Microsoft Excel 2007, Washington, USA) and the percentage egg hatch was automatically calculated. The number of eggs and larvae per sample per time point were also calculated.

From faecal cultures no large strongyles were identified, therefore all strongyles were classified as cyathostomins.

*Statistical analysis*

Differences pre and post treatment within each group were analysed using the Friedman non-parametric ANOVA. Post *hoc* testing and Bonferroni correction applied. All tests were carried out in SPSS (IBM v 20. New York, USA).

 **Results**

All premises demonstrated BZ-resistant parasites (Table 1). On three premises 11 animals had individual FECR values of 91.6-100% after a five consecutive day dose of fenbendazole. At day 14 post-treatment five of the horses from the 39 (8%) had a FEC greater than before treatment and a further two had the same FEC as before treatment indicating a 0% FEC reduction. Mean FEC at 14 days post-treatment for all 39 horses was 250 ± 270 epg with 34 of the 39 horses recording a positive result (≥25 epg).

Median egg hatch rate after treatment is illustrated for both treatment groups in Fig. 1. In the single dose group there was a significant difference between the pre- and post-treatment egg hatch rates (*P*= 0.0001). There was a significant reduction in the number of eggs and larvae post treatment (*P*=0.001), at day five total eggs and larvae remained significantly lower than at day zero (*P*=0.003) (Fig. 2A).

Five consecutive daily doses of fenbendazole demonstrated significant differences between pre- and post-treatment egg hatch rate (*P*=0.001). Significant differences between sampling points are indicated in Fig. 1B. There was a significant reduction in eggs and larvae immediately post dosing (*P*=0.0001) and levels of eggs and larvae remained significantly lower than pre-treatment at day nine (*P*=0.004) (Fig. 2B).

**Discussion**

We report significant reduction in egg hatch rate for three days following a single dose or five consecutive day dose of fenbendazole in horses with resistant cyathostomins. While all premises were classified as resistant based on FECR tests, there was nonetheless a significant reduction in the number of eggs and larvae present in samples post treatment.

There is limited published data on the ovicidal effect of BZ on susceptible cyathostomins, making it difficult to compare our findings to previous work. Miller and Morrison (1992) reported fenbendazole treatment resulted in little or no development of strongyle eggs in cattle faeces from 12 to 72 hours after treatment. In comparison to the findings of Miller and Morrison (1992) the number of egg present post treatment in our study remained low at 72 hours, however the hatching of those eggs significantly increased returning to baseline hatch rate. This could be due to species variation or due to the influence of the resistant proportion of cyathostomins within our population. It should be assumed that most BZ-susceptible cyathostomins were removed by the fenbendazole treatment, thus skewing the population to the resistant proportion of cyathostomins which continued to hatch post treatment. It is important to consider that horses commonly harbour multiple species of cyathostomins. Previous work has identified that the efficacy of benzimidazoles and susceptibility to anthelmintic resistance can differ between cyathostomin species (Traversa *et al*., 2009).

Hotson *et al.* (1970) reported a 70% FECR in small ruminants demonstrating BZ resistance, however the BZ treatment showed little effect on the adult parasite burden in a post slaughter worm count. Compared to untreated controls the female worms had fewer eggs present. Hotson *et al.* (2009) suggested that fenbendazole treatment temporarily supressed egg laying but allowed the parasites to survive (Hotson *et al.,* 1970). This suggests anthelmintics have the ability to temporarily damage adult parasites and parasite eggs in resistant nematodes. To the authors’ knowledge there have been no such reports from equine cyathostomins of the viability of the eggs shed post-treatment with BZ (Chandler *et al.,* 2000; [Chandler and Love, 2002](#_ENREF_5); Rossano *et al.,* 2010). When considering the numbers of eggs and larvae post treatment from our findings it is important to remember that there is no direct linear correlation between the numbers of nematode eggs excreted and the number of intestinal worms present within horses (Nielsen *et al.,*2010b). However our results do indicate the presence of egg laying cyathostomins within these horses post treatment.

Our study population of horses comprised animals with a range of ages. The pharmacokinetics of anthelmintics can differ in younger animals in comparison to older animals (Gonzalez Canga *et al.,* 2009). Older animals often have lower FEC results due to immunological defence from previous cyathostomin exposure (Klei and Chapman, 1999), yet some animals never develop a sufficient immune response. The grazing environment is also known to influence parasite burden (Osterman Lind *et al*., 1999). Interestingly the age range and mean age of the animals that did not meet our inclusion criteria were similar to our study population. As these animals also resided on the same five premises, this would suggest that age alone did not influence FEC.

Both the single dose and five day doses of fenbendazole were administered according to the manufacturer’s instructions. There is evidence in other species, dogs and small ruminants, that fenbendazole administration with food significantly increases bioavailability compared to an oral bolus (McKellar *et al.,* 2008). Interestingly McKellar *et al*. (2002) suggested that in horses administering fenbendazole by oral bolus directly followed by food appeared to reduce bioavailability, however these conclusions were drawn from a very small sample. Differences in the administration routes should be considered when directly comparing these groups.

Use of any anthelmintic product should be based on local, regional and farm-level, epidemiological information about susceptibility of nematodes (NOAH Compendium). Treatment histories for premises in this study (Table 1) highlighted reliance on anthelmintics without diagnostic testing. Similar parasite control strategies were reported within commercial high risk premises by Relf *et al.* (2011). Greater education is required in the sector encouraging diagnostic testing and targeting of anthelminthic treatment (Nielsen *et al.,* 2010c).

In our study the mean FEC at 14 days post-treatment was 250 ± 270 epg with 34 of the 39 horses recording a positive result (≥25 epg). Lester *et al.* (2013) reported FECR for BZ at day 14 where the FEC was higher than at day 0 (pre-treatment). This was also a finding in our study; at day 14 post-treatment in 39 horses with BZ-resistant strongyles five (8%) had a FEC greater than before treatment and a further two had the same FEC as before treatment indicating a 0% reduction. Resistance in equine cyathostomins appears highly prevalent within the UK horse population (Relf *et al.,* 2011), indicating the need to understand how anthelmintics perform in the face of resistance.

We acknowledge a number of limitations to this study which limit the extrapolation of our findings. Our sample size estimates indicated the need for a study of 20 horses per treatment group in order to detect a 7-fold difference in egg hatch rate with 95% confidence. Our study comprised only 39 horses, allocated to two treatment groups, and was therefore underpowered to detect differences of this magnitude or smaller.

Our study population may not be representative of the UK horse population as a whole, but represents a category of high risk horses within this population where sustainable anthelmintic treatment should be targeted. A study design issue that we struggled with was the low prevalence of premises with cyathostomin populations that were demonstrably BZ-susceptible making comparisons difficult. Notwithstanding these limitations, we believe that this study adds valuable information to our understanding of the ovicidal effect of fenbendazole in UK horses under field conditions.

**Conclusion**

Our results suggest that the ovicidal effect of fenbendazole appears to be present but short lived in benzimidazole-resistant cyathostomins when dosed as a single oral dose per os, or for five consecutive days administered in feed. Numbers of eggs and larvae present immediately post treatment were significantly lower than before treatment. Hatch viability in post treatment eggs appeared to return to pre-treatment values within three days of a single dose or five consecutive day’s treatment at 7.5 mg/kg BW.

**Conflict of interest statement**

MSD Animal Health funded this work and supplied the anthelmintics used in this study. MSD Animal Health was involved in the study design but not involved in the collection, analysis and interpretation of data. MSD Animal Health was involved in reviewing the manuscript submitted for publication. Neither of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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**Table 1**

Population data including parasite control strategies employed and premises FECR.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Premises** | **Type** | **Total Animals on Premises** | **Number Sampled (%)** | **Number of animals given a single dose of FBZ** | **Median Age years (range)** | **Treatment History** | **FECs in use** | **FECR % Mean of treated animals** | **FECR % Median of treated animals** |
| 1 | Stud | 10 | 6 (60) | 3 | 6.5 (0.5-15) | Blanket chemical – No BZ | No | -27 | 32 |
| 2 | Dealer | 21 | 8 (38) | 4 | 7 (5-20) | Blanket chemical – No BZ | Randomly | 61 | 86 |
| 3 | Stud | 4 | 4 (100) | 2 | 8 (4-15) | Blanket chemical – No BZ | No | 54 | 64 |
| 4 | Stud | 26 | 4 (15) | 2 | 1 (0.5-7) | Blanket chemical - BZ | No | 35 | 27 |
| 5 | Stud | 48 | 17 (35) | 10 | 5 (1-14) | Targeted from FEC - BZ | Yes | 38 | 83 |
| All |  | 109 | 39\*(36) |  |  |  |  |  |  |

\* Only 39 out of the 109 horses had FEC ≥150 epg

**Figure Legends**

Fig. 1. Box and whisker plot illustrating the distribution of egg hatch rates, following (A) single dose fenbendazole in animals harbouring resistant cyathostomins; and following (B) treatment for 5 consecutive days in horses with BZ resistant cyathostomins. Population values for egg hatch rate that are significantly different from the previous sampling point are labelled “#”. For both the single dose and five day dose groups’ egg hatch was significantly reduced for three days after treatment, before returning to baseline levels.

Fig. 2. Box and whisker plot illustrating distribution of eggs and larvae pre and post treatment with a (A) single dose of fenbendazole and (B) five consecutive daily doses of fenbendazole. Significant differences compared to the previous sampling point are labelled “#”. Numbers of eggs and larvae at day five and nine days post-treatment remain significantly lower than at day zero following single dose and five consecutive days dosing respectively.

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