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The successful elimination of mucosal dwelling cyathostome larvae with fenbendazole at a dose rate of 7.5 mg/kg bodyweight daily for 5 days

Elisabeth. M. Abbott[1], *J. L. Duncan*[2] and *K. Bairden*[3]

[1]Clinical Development Antiparasitics, Hoechst Veterinär GmbH, Wiesbaden, Germany
[2]Dept. of Veterinary Medicine, University of Glasgow, Veterinary School, Glasgow, UK
[3]Dept. of Veterinary Parasitology, University of Glasgow, Veterinary School, Glasgow, UK

Summary

Very few equine anthelmintics have been shown to be effective against mucosal dwelling cyathostome larvae. Since, increasingly, larval cyathostomosis is being recognised in the field, the efficacy of 5 daily treatments with fenbendazole, at a dose rate of 7.5 mg/kg bodyweight/day, was evaluated in a controlled necropsy study in twenty, 12 to 24-month old ponies with naturally acquired cyathostome infections. Following a 6-week pretreatment housing period, the ponies were allocated on a basis of liveweight, to treatment and control groups. Six weeks later both groups of ponies were humanely destroyed and their cyathostome worm burdens, which included luminal adults and encysted mucosal larvae, were assessed. Efficacy of fenbendazole against adult cyathostomes was 90.7% (P<0.01). Numbers of mucosal larvae, recovered following digestion, were reduced by 95.3% (P<0.0005). Within the mucosal larval population, developing 4th stage larvae were reduced by 96.4% (P<0.0001), 4th stage larvae by 99.9% (P<0.0001) and inhibited 3rd stage larvae by 91.5% (P<0.005). The strategic use of the 5-day fenbendazole treatment regime in the autumn and winter months could be beneficial in the prevention of the disease syndromes collectively known as larval cyathostomosis.

Keywords: efficacy, 5-day fenbendazole treatment, mucosal dwelling cyathostome larvae, naturally infected ponies

Die erfolgreiche Elimination von in der Mukosa befindlichen Cyathostoma-Larven mit Fenbendazol in einer Dosierung von 7,5 mg/kg Körpergewicht täglich über 5 Tage

Nur sehr wenige Pferdeentwurmungsmittel haben sich als wirksam gegen die in der Darm-Mukosa befindlichen Cyathostoma-Larven erwiesen. In steigendem Maße wird von der Infektion mit Cyathostoma-Larven Notiz genommen. Deshalb wurde die Wirksamkeit einer 5-Tage-Behandlung mit Fenbendazol in einer Dosierung von 7,5 mg/kg Körpergewicht pro Tag bei 20, 12 bis 24 Monate alten Ponies mit natürlich erworbener Cyathostoma-Infektion, untersucht. Nach einer 6wöchigen Aufstallungsperiode vor der Behandlung, wurden die Ponies auf der Grundlage ihres Lebendgewichtes in eine Behandlungsgruppe und in eine Kontrollgruppe unterteilt. Nach der 5tägigen Behandlung mit Fenbendazol wurden 6 Wochen später alle Ponies getötet und ihre Cyathostoma-Wurmbürde, sowohl die im Darmlumen befindlichen adulten Formen als auch die in der Darmmukosa eingekapselten Mukosa-Larven, bestimmt. Fenbendazol reduzierte die adulten Cyathostoma-Formen um 90,7% (P<0,01). Die Zahl der Mukosa-Larven, gewonnen mittels Aufschluß der Darmwand mit Pepsin und Salzsäure, wurde zu 95,3% (P<0,0005) verringert. Die Zahl der innerhalb der Mukosa-Larven Population sich entwickelnden 4. Stadienlarven wurden zu 96,4% (P<0,0001), 4.Stadienlarven zu 99,9% (P<0,0001), und gehemmte 3. Larvenstadien zu 91,5 (P<0,005) reduziert.
Die planmäßige Anwendung einer 5-tägigen Fenbendazol-Behandlungsstrategie in den Herbst- und Wintermonaten könnte nützlich für die Vorbeugung des Krankheitssyndroms Larven-Cyathostomiasis sein.

Schlüsselwörter: Wirksamkeit, Fenbendazol, Mukosa, Cyathostoma, Larven, Pferd

Introduction

Larval cyathostomosis, the clinical syndrome associated with the emergence of large numbers of 4th stage cyathostome larvae from the large intestinal mucosa, is seen in Britain, mainly between November and May. It can cause severe debility and even death (*Kelly* and *Fogarty,* 1993: *Mair,* 1994). Several disease syndromes have been described namely seasonal sudden onset diarrhoea with accompanying weight loss and ventral oedema (*Mair* et al., 1990), seasonal sudden weight loss with ventral oedema (*Kelly* and *Fogarty,* 1993: *Mair,* 1993), chronic weight loss (*Ogbourne,* 1978) and seasonal "malaise" (*Mathews* and *Morris,* 1995). Epidemiological features associated with the diagnosis of the disease are its seasonality, prevalence in younger horses and recent treatment with anthelmintics (*Reid* et al., 1995). Blood analyses usually reveals a leucocytosis due to a neutrophilia, hypoalbuminaemia and elevated β-globulins whilst faecal egg counts are generally low or negative though larvae may be seen in the faeces. The prognosis must be guarded as the underlying pathology can be severe without the horse showing marked clinical signs (*Lyons* et al., 1994). The need for prevention of the disease is emphasised by the fact that only about half of the affected horses recover following treatment (*Love* et al., 1992).

There is no doubt that the use of effective anthelmintics can keep pasture contamination at a low level by minimising egg output (*Duncan,* 1974). However, increasingly, decisions on worm control measures are taken by owners without veterinary consultation and this, together with a lack of understanding of pasture management, may be contributing to the increased prevalence of larval cyathostomosis. Whilst in the mucosa, larvae are refractory to treatment with most of the broad spectrum anthelmintics at their routine dose rates. However, five daily treatments with fenbendazole at 7.5 mg/kg b.w. has been shown to be >95% effective against 4th stage cyathostome larvae in the mucosa (*Duncan* et al., 1980). The following study was designed to evaluate the efficacy of a 5-day treatment with fenbendazole against various larval stages in the mucosa of naturally infected ponies.

Materials and methods

A treatment regimen of 7.5 mg fenbendazole/kg bodyweight, daily for 5 days was selected as this regime is already licensed in the United Kingdom and Ireland for the control of encysted mucosal stages of the Cyathostomes.
Twenty 12 to 24-month old ponies with naturally acquired cyathostome infections were communally grazed for three weeks prior to being housed at the end of January. Each pony received about 1 kg of a high energy coarse ration daily. Water and hay were freely available. After a 6-week housing period, the ponies were ranked on liveweight and replicates of two formed. Within each replicate the ponies were allocated at random to either treatment or control group.
Pre- and post treatment, the ponies were maintained in their respective groups. During the treatment period they were individually housed and those in the treatment group were given a 10% fenbendazole suspension (Panacur Equine Guard®) at a dose rate of 7.5 mg fenbendazole/kg bodyweight per day mixed in their daily concentrate ration. The untreated control ponies were handled identically but were given an equivalent volume of saline daily, also mixed in with their feed. At the end of the treatment period the ponies were group housed for a further 39 to 40 days until necropsy.
After euthanasia the contents of the caecum, ventral colon and dorsal colon were collected in separate containers. The mucosal surface of each was washed under running water and the washings added to the contents which were then made up to a final volume of 10 litres for the caecum, and 20 litres for both the ventral and dorsal colon. After thorough mixing, a 10% sample was taken from each and formalised. The entire 10% sample was screened, adult cyathostomes removed and counted. Following washing, the caecum, ventral colon and dorsal colon were weighed separately. Ten percent, by weight, was then taken by dissection of longitudinal strips from the proximal, median and distal portions of each. The mucosal layers were stripped from the muscularis and serosa and digested in a pepsin/hydrochloric acid (HCl) mixture at 37°C. At two hour intervals the supernatant was removed, formalised and the remaining mucosal material re-incubated with fresh pepsin/HCl. This process was repeated on average six times to ensure the release of the majority of larvae from the tissue. The formalised supernatants were left to sediment, bulked and the final volume reduced to 400 ml and formalised. A 1% sub-sample of each digest was examined microscopically and since the cyathostome larvae appeared as one of three distinct morphological forms they were classified as third, fourth or developing fourth larval stages.
In samples prepared and digested as described above three types of cyathostome larvae were recovered i.e. the large, coiled larvae designated in this study as developing fourth stage larvae (DL_4); smaller stages designated here as fourth stage larvae (L_4); extremely small forms, designated here as L_3, similar in size to exsheathed third stage cyathostome larvae.

Calculation of Efficacy and Statistical Analyses of Data
Adult and larval worm counts, including mucosal-dwelling larvae, were summarised for the treated and control groups using mean and geometric mean worm counts. A two-sample t-test was undertaken on the log transformed larval and adult worm counts to test for a significant treatment effect. Tests for significance

Tab. 1: Group geometric mean (G.M.) and range of cyathostome worm burdens at necropsy in naturally infected ponies treated with fenbendazole at a dose rate of 7.5 mg/kg daily for five days and the reduction (%) relative to untreated control ponies

Group	Adults	DL_4	L_4	L_3	Total mucosal larvae
Controls					
G.M.	10,398	9,399	64,809	74,602	150,666
Range	5,000–33,500	5,500–16,100	30,000–128,700	47,200–167,500	98,300–312,300
Treated					
G.M.	963	340	27	6,348	7,100
Range	0–4,500	0–5,500	0–8,100	100–126,900	100–140,500
% Reduction	90.7	96.4	99.9	91.5	95.3
Significance	P<0.01	P<0.0001	P<0.0001	P<0.005	P<0.0005

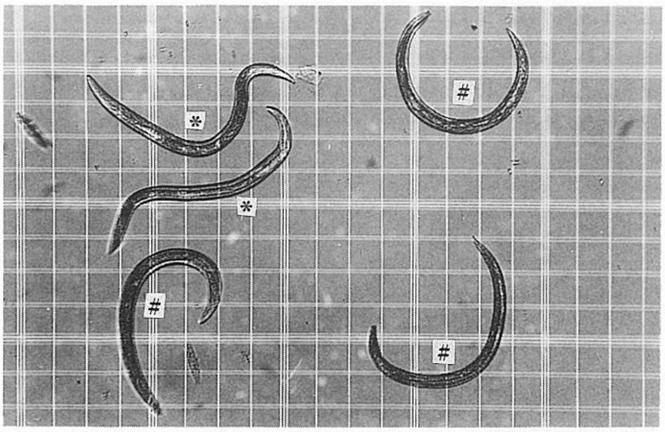

Fig. 1: Comparison of freshly exsheated cyathostome infective larvae following faecal culture and larvae recovered following pepsin-hydrochloric acid digestion of the large intestinal mucosal of naturally infected ponies necropsied 12 weeks after housing

* = freshly exsheathed cyathostome infective larvae (L_3)
# = larvae recovered following digestion of the large intestinal mucosa (inhibited L_3)
Each square is 80 μ x 80 μ

were carried out at the 5% significance level. Efficacy was calculated using the percentage reduction in geometric mean (G.M.) worm count of the treated group compared with the control group. This takes account of lack of normal distribution of worm counts.

Results

Adult and mucosal larval worm burdens in the treated and control ponies at necropsy are presented in Tab. 1. Adult worm burdens of the treated ponies were reduced by >90% ($P<0.01$) and total mucosal larval burdens by 95.3% ($P<0.0005$). The 3rd stage larval burdens were high in the control ponies and comprised 50% of the total mucosal larval burden. Third stage larval burdens of the treated ponies ranged from 100–15,000 with the exception of one pony which had 126,900 L_3 present. The group mean burden was 6,348, including this pony, which represents a 91.5% reduction ($P<0.005$) relative to the controls.

Discussion and conclusion

The results of this study clearly demonstrate that consecutive treatments with fenbendazole at 7.5 mg/kg bodyweight daily for five days is highly effective against mucosal dwelling cyathostome larvae thus confirming the findings of *Duncan* et al. (1980). In contrast to this earlier study, larvae were differentiated into 3rd, 4th and developing 4th stage larvae on size and morphology. The smallest larvae recovered were compared with freshly exsheated infective 3rd stage cyathostome larvae and were essentially similar (Fig. 1).
The interval between acquisition of infection and the appearance of strongyle eggs in the faeces (the pre-patent period), varies according to cyathostome species and ranges between 35 and 42 days (*Round* 1969). Since the ponies were housed for a 6-week period prior to treatment there is little doubt that the small 3rd stage larvae identified at necropsy were inhibited in their development at the time of treatment. At necropsy, 12 weeks after housing, 3rd stage larvae were still present thus strengthening the argument that they were inhibited in their development.
The presence of small numbers of adult worms in the treated ponies could have been due either to lack of efficacy because of the presence of benzimidazole resistant strains or the consequence of a few late 4th stage larvae surviving treatment and emerging from the mucosa to continue their development to adults in the gut lumen. The presence of relatively large numbers of inhibited 3rd stage larvae in one of the treated ponies (Pony 25) is unknown. Since inhibited 3rd stage larval burdens in excess of 2 million have been found in naturally infected yearling ponies in the winter months (*Eysker* et al., 1984), the presence of about 120,000 L_3 in pony 25 could, in fact, represent a greater than 90% reduction in inhibited larval burdens.
The adult cyathostome worm burdens comprised about 7% of the total cyathostome worm burdens, the remaining 93% of the population being mucosal dwelling larvae. Of the latter, about 6% were classified as developing L_4 whilst 44% were L_4 and 50% inhibited L_3. *Eysker* and his colleagues (1984 and 1989) have also observed the presence of large numbers of inhibited 3rd stage larvae (60 to 90% of the total worm burdens) in the mucosa of ponies in the Netherlands during the winter months. These data support *Ogbourne's* (1976) observation that in Western Europe there is an annual turnover in the adult population in the late winter / early spring when the previous year's dwindling adult population dies off to be replaced by a new population derived from emerging 4th stage larvae.
In conclusion, the present study clearly demonstrates that 5 consecutive daily treatments with fenbendazole at a dose rate of 7.5 mg/kg bodyweight were highly effective in reducing mucosal dwelling 3rd and 4th stage cyathostome larval burdens and could be beneficial in the prevention of the disease syndromes collectively known as larval cyathostomosis.

References

Duncan, J. L. (1974): Field studies on the epidemiology of mixed strongyle infection in the horse. Veterinary Record 94, 337–345.

Duncan, J. L., McBeath, D. G. and *Preston, N. K.* (1980): Studies on the efficacy of fenbendazole used in a divided dosage regime against strongyle infections in ponies. Equine Veterinary Journal 12 (2), 78–80.

Eysker, M., Jansen, J. and *Mirck, M. H.* (1984): Inhibited development of Cyathostominae in the horse in the early third stage. Research in Veterinary Science 37, 355–356.

Eysker, M., Boersema, J. H. and *Kooyman, F. N. J.* (1989): Effect of repeated oxfendazole treatments on small strongyle infections in Shetland ponies. Research in Veterinary Science 46, 409–412.

Kelly, J. C. and *Fogarty, U. M. G.* (1993): Outbreak of larval cyathostomiasis on a thoroughbred stud farm. Irish Veterinary Journal 46, 133–136.

Love, S., Mair, T. S. and *Hillyer, M. H.* (1992): Chronic diarrhoea in adult horses: a review of 51 referred cases. Veterinary Record 130, 217–219.

Lyons, E. T., Swerczek, T. W., Tolliver, S. C., Drudge, J. H., Stamper, S., Granstrom, D. E. and *Holland, R. E.* (1994): A study of natural infections of encysted small strongyles in a horse herd in Kentucky. Veterinary Medicine 89, 1146–1155.

Mair, T. S., de Westerlaken, L. V., Cripps, P. J. and *Love, S.* (1990): Diarrhoea in adult horses: A survey of clinical cases and an assessment of some prognostic indices. Veterinary Record 126, 479–481.

Mair, T. S. (1993): Recurrent diarrhoea in aged ponies associated with larval cyathostomiasis. Equine Veterinary Journal, 25 (2), 161–163.

Mair, T. S. (1994): Outbreak of larval cyathostomiasis among a group of yearling and two-year-old horses. Veterinary Record 135, 598–600.

Mathews, A. G. and *Morris, J. R.* (1995): Cyathostomiasis in horses. Veterinary Record 136, 52.

Ogbourne, C. P. (1976): The prevalence, relative abundance and site distribution of nematodes of the subfamily Cyathostominae in horses killed in Britain. Journal of Helminthology 50, 203–214.

Ogbourne, C. P. (1978): Pathogenesis of cyathostome (Trichonema) infections of the horse. Commonwealth Agricultural Bureaux, Misc. Publications, A Review 3, 1–25.

Reid, S. W. J., Mair, T. S., Hillyer, M. H. and *Love, S.* (1995): Epidemiological risk factors associated with a diagnosis of clinical Cyathostomiasis in the horse. Equine Veterinary Journal 27 (2), 127–130.

Round, M. C. (1969): The pre-patent period of some horse nematodes determined by experimental infection. Journal of Helminthology XLIII, Nos. 1/2, 185–192.

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Dr. Eisabeth M. Abbott

Clinical Development
Antiparasitics
Hoechst Veterinär GmbH
Wiesbaden, Germany

Prof. J. L. Duncan

Dept. of Veterinary Medicine
University of Glasgow
Veterinary School
Glasgow, UK

Dr. K. Bairden

Dept. of Veterinary Parasitology
University of Glasgow
Veterinary School
Glasgow, UK