Veterinary Parasitology 186 (2012) 532–537

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Anthelmintic efficacy in captive wild impala antelope (*Aepyceros melampus*) in Lusaka, Zambia

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ARTICLE INFO

Article history:
Received 29 June 2011
Received in revised form 26 October 2011
Accepted 3 November 2011

Keywords:
Anthelmintic efficacy
Captive-wild impala (*Aepyceros melampus*)
Zambia

ABSTRACT

There has been an increase in the number of wild ungulates kept in captivity for ecotourism and conservation in Zambia and these animals are susceptible to a number of diseases including gastrointestinal helminth infections. Surveys to determine anthelmintic efficacy to gastrointestinal nematodes in captive-wildlife are not common and there have been no reports of anthelmintic resistance in captive-wildlife in Zambia. This study was carried out to determine the efficacy of the benzimidazole anthelmintic fenbendazole in captive wild impala (*Aepyceros melampus*) in Zambia. During the month of April 2011, at the end of the rainy season, the faecal egg count reduction test was performed at a private game facility for assessing anthelmintic efficacy of oral fenbendazole and the anthelmintic treatment showed an efficacy of 90%. *Haemonchus* spp. and *Trichostrongylus* spp. were the predominant genera present before treatment, but *Haemonchus* spp. larvae were the only genus recovered from the faecal cultures after anthelmintic treatment. This represents the first documentation of anthelmintic treatment failure in captive wild-antelopes in Zambia. It also demonstrated the ineffectiveness of the common traditional practice of deworming captive-wild antelopes at the end of the rainy season due to the rapid re-infection of impala that occurs due to high pasture infectivity. Suggestions on changes to current anthelmintic use/practices that will make them more efficacious and reduce the possibility of development of anthelmintic resistance in captive wild game in Zambia are also made.

1. Introduction

Anthelmintic resistance is a growing problem in domestic ruminant production (Cernanska et al., 2006) and has been reported in all drug classes (Kaplan, 2004). It has arisen due to a protracted and frequent use of these drugs to control helminth parasites in livestock production systems. In Zambia, it has been shown to exist in sheep to benzimidazoles and ivermectin (Gabrei et al., 2001). In wildlife production, it is not a commonly reported problem but there are some reports in literature (Isaza et al., 1995a). However, gastrointestinal nematodes have been shown to be a cause of morbidity and mortality in captive wild ungulates (Goossens et al., 2006) and as wildlife production practices become more intensive more anthelmintics will be utilized and it will also inevitably result in a more widespread development of resistance globally. Benzimidazoles are the most commonly used anthelmintics in captive wild ungulates due to their wide safety margin (Isaza et al., 1995b) and the fact that they can be administered orally using different carriers such as feed and mineral licks. The limitation of route of anthelmintic administration to feed and mineral supplements may also encourage development of resistance since accurate doses may not be ingested by all animals and some may get sub-therapeutic doses that may not eliminate entire worm burdens in the host (Goossens et al., 2006). Idiosyncrasies have also been shown on the pharmacokinetics of anthelmintics in

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doi:10.1016/j.vetpar.2011.11.020

different wild animals that may explain the lack of efficacy in certain occasions (Ortiz et al., 2001). Reduced efficacy of anthelmintics can be confounded by incorrect administration of the anthelmintics (El Abdellati et al., 2010), individual species' pharmacokinetics variability, and lack of dose rates for captive-wild animal species, all leading to incorrect faecal egg count reduction (FECR) test results.

The impala (*Aepyceros melampus*) is a sexually segregated ungulate of the antelope family and closely related to domestic bovidae. Impala are the most abundant antelopes kept in captivity in Zambia as they are known to be quite hardy and prolific. The medium-sized antelope normally grazes when pasture is available but may browse as well. Gastrointestinal parasites reported in antelopes include coccidian species and helminths (Ezenwa, 2004a).

Ungulates in the wild generally have manageable gastrointestinal nematode burdens with minimal effects on their overall health, but in limited spaces, such as pertains in captivity, this may be inhibited and may increase their burdens of internal and external parasites (Ezenwa, 2004b) due to natural selection processes being disturbed leading to imbalances in the ecosystem.

The FECR test is the most widely performed test to assess anthelmintic resistance (Cabaret and Berrag, 2004) and has been used in captive-wild animals (Goossens et al., 2005; Young et al., 2000). Other tests to determine anthelmintic efficacy include the egg hatch assay (EHA) and larval development assay (LDA), but these *in vitro* tests are seldom done for wild-captive ungulates (Young et al., 2000). Guidelines for evaluating efficacy of anthelmintics in domestic animals exist (Coles et al., 2006; Wood et al., 1995); but comprehensive guidelines for captive wild herbivores are non-existent.

There are no published data on the efficacy of anthelmintics in captive-wild ungulates in Zambia. The present study was thus, undertaken to evaluate the efficacy of fenbendazole administration in captive-wild free-range impala antelope as it is routinely practiced on many game facilities in Zambia.

2. Materials and methods

2.1. Study area and animals

The study was carried out on a privately owned game facility (15°32.87′S, 28°16.42′E altitude 1216 m), covering an area of 43 acres and located south of Lusaka, the capital city of Zambia. The game facility had 117 free-range captive wild impala (*A. melampus melampus*) with a male:female:juvenile ratio of 12:50:55. The country has a distinct warm, wet rainy season between November and April the following year, followed by a cool dry season (May to July) and a hot dry season that precedes the rainy season. The untreated control were captive wild impala from another facility within Lusaka that had statistically comparable faecal egg counts (FEC) as represented by eggs per gram (EPG) faeces (ANOVA: $F_{1,95} = 0.03$; $p = 0.96$, control mean $\text{Log}_{10}(1 + \text{EPG}) \pm \text{SD}$ 2.87 ± 0.28, treated mean $\text{Log}_{10}(1 + \text{EPG}) \pm \text{SD}$ 2.89 ± 0.36).

2.2. Sampling and laboratory analysis for FECRT

The anthelmintic treatment was carried out two weeks after the end of the natural rainy season in April 2011 as part of the routine twice-a-year deworming strategy. The anthelmintics were administered in a non-commercial salt lick to encourage all animals to take the anthelmintic. The amount of the fenbendazole (Fenbenat 4%, Opharma) mixed with the salt lick was estimated to provide from 5 to 8.5 mg/kg per impala daily. The amount of fenbendazole was calculated based on estimated total herd body mass multiplied by 7.5 mg/kg and uniformly mixed into 75% of the normal daily quantity of salt lick (with the assumption that some animals would consume less and others more of the salt lick). The treatment lasted for 3 days and adequate anthelmintic–salt lick intake by the majority of the impala was estimated by observation by the game keeper.

The FEC were monitored at the treatment site and the control site a day prior to anthelmintic treatment and for a further five weeks on days 4, 8, 14, 24 and 31 after the last day of anthelmintic treatment. At each sampling, groups of impala were followed around the game facility from 9 am until noon. Upon observing an animal defaecating, fresh faecal samples were collected avoiding collecting faecal pellets that were contaminated with soil.

2.3. Faecal samples were classified as small, medium, large pellets or unpelleted, diarrhoeic

The modified McMaster egg counting technique (MAFF, 1980) with a sensitivity of 50 eggs per gram (EPG) was used to quantify nematode egg burdens in the faecal samples and identification of helminth eggs was done according to Soulsby (1968). Four grams of faeces were diluted in 56 ml of saturated sodium chloride solution (specific gravity 1.2) as a floatation agent and the FEC were reported as eggs per gram of faeces. Further, the faecal samples were pooled in a pestle, mixed with vermiculite to make them crumbly, placed in large plastic containers and cultured at 27 °C for 10–14 days while stirring contents every day to avoid fungal overgrowth. Larvae were recovered using a standard Baermann technique and the third stage larvae were identified as described by Hansen and Perry (1990).

The FECR was calculated using the formula by Young et al. (2000) in which there is no untreated control: {([pre-treatment mean EPG] – [post-treatment mean EPG])/(pre-treatment mean EPG)} × 100 = % efficacy.

For comparison, the FECR was also calculated as recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Coles et al., 2006), with a slight modification of using an untreated impala herd on another facility with comparable FEC ($p > 0.01$) as a control. The FECR was calculated using the formula $\text{FECR}\% = 100 \times (1 - X_t/X_c)$, where X_t is the arithmetic mean EPG of the treated group and X_c is the arithmetic mean EPG of the control group. Lack of anthelmintic efficacy for both formulae is a test result of efficacy less than 95% (Coles et al., 2006; Young et al., 2000).

2.4. Statistical analysis

Minitab® version 14 was used for data analysis and graphing. The EPGs data were transformed using the formula $Log_{10}(1 + EPG)$ in order to normalise them prior to statistical analysis using analysis of variance (ANOVA) to determine differences between means. Proportion positives of pelleted faeces and helminth positive samples were subjected to the chi-square test to determine differences between day's post-anthelmintic treatment results.

3. Results

The impala found the salt lick/anthelmintic mixture palatable and each day, all the mixture was consumed. The results from FECR test show a low efficacy of the anthelmintic fenbendazole with an overall percentage efficacy of 90.6% (using the formula of Young et al., 2000) and 89.4% (using the formula of Coles et al., 2006) on day 8 post anthelmintic administration (Fig. 1). Day 4 post treatment gave better efficacies of 97.4 and 96.5% respectively. The figure also shows that re-infection occurs rapidly and by day 24 the arithmetic mean EPGs in the treated impala were statistically similar to the untreated controls ($p < 0.05$).

There was a significant reduction in the proportion of unpelleted faeces from pre-treatment 17% (9/53), to 0% day 4 (0/38), 0% day 8 (0/52) and 0% day 14 (0/49) ($\chi^2 = 24.765$, $df = 3$, $p < 0.01$), but no significant difference from day 24 11.7% (6/51) and day 31 9.8% (5/51) ($\chi^2 = 1.279$, $df = 2$, $p = 0.528$) (Figs. 2 and 3). The chi-square results comparing pre-treatment levels at days 4, 8 and 14, however, should be taken with caution since on these days there were no impala with unpelleted faeces resulting in counts that were less than five.

There was a significant effect of anthelmintic treatment on proportion of faeces positive for strongyle eggs on different days post treatment ($\chi^2 = 130.2$, $df = 5$, $p < 0.01$). The proportion positive changed from 100% (53/53) pre-treatment to 36.8% (14/38) day 4, 26.9% (14/52) day 8, 40.8% (20/49) day 14, 88.2% (45/51) day 24 and 100% (51/51) on day 31 (Fig. 2). There was no significant difference in proportion faecal samples that were nematode egg positive between pre-treatment samples and day 31 post-treatment. There was however, a significant difference between pre-treatment samples and day 4 post-treatment ($\chi^2 = 45.46$, $df = 1$, $p < 0.01$), day 8 ($\chi^2 = 60.69$, $df = 1$, $p < 0.01$), and day 14 ($\chi^2 = 43.83$, $df = 1$, $p < 0.01$) post-treatment. Although day 8 post-anthelmintic treatment had a lower percentage positive than day 4, it had a higher mean EPG (Figs. 1 and 2).

There were significant differences in the mean EPGs between the controls and treatment groups 4 and 8 days after anthelmintic administration ($F_{3,167} = 153.7$; $p < 0.001$, control mean $Log_{10}(1 + EPG) \pm SD$ 2.82 ± 0.26 (day 4) and 2.86 ± 0.32 (day 8); post-treatment day 4 mean $Log_{10}(1 + EPG) \pm SD$ 0.62 ± 0.86; post-treatment day 8 mean $Log_{10}(1 + EPG) \pm SD$ 0.52 ± 0.92) but no differences before anthelmintic administration ($F_{1,95} = 0.03$; $p = 0.96$, control mean $Log_{10}(1 + EPG) \pm SD$ 2.87 ± 0.28, treated mean $Log_{10}(1 + EPG) \pm SD$ 2.89 ± 0.36). There were differences in mean $Log_{10}(1 + EPG)$ transformed EPGs between controls and post treatment samples on days 14 and 24 but no differences in mean Log transformed EPGs between controls and treated animals on day 31 after treatment ($F_{1,101} = 4.64$; $p = 0.03$, control means $Log_{10}(1 + EPG) \pm SD$ 2.42 ± 0.59, post-treatment day 31 mean $Log_{10}(1 + EPG) \pm SD$ 2.62 ± 0.29).

Using the Baermann method on pooled faecal samples demonstrated *Haemonchus* spp. and *Trichostrongylus* spp. as the predominant genera present prior to anthelmintic treatment (31.4% and 58.3% respectively, others unidentified larvae). After the anthelmintic treatment, only *Haemonchus* spp. larvae were recovered from the faecal cultures of pooled positive faecal samples.

4. Discussion

This study shows the presence of anthelmintic treatment failure in captive wild impala in Zambia and this is being reported for the first time. The study also shows that anthelmintic treatment immediately after the end of the rainy season is generally ineffective as within one month of such treatment, the FEC are comparable to pre-treatment levels and those of untreated control animals. This can be attributed to a high re-infective pressure from pasture since during the first few weeks after the end of the rainy season, the pasture and ground will have moisture and temperature that are still very favourable for hatching of strongyles eggs and larval development into infective larvae. Immediately after anthelmintic treatment the faecal consistency improved with all samples being pelleted at 4, 8 and 14 days post anthelmintic treatment. However, by day 24 post anthelmintic treatment, the proportion of high-EPG unpelleted faeces began to increase to almost pre-treatment levels. Unpelleted faecal samples have been shown to be those with high EPGs, and in this study this was attributed to the rapid re-infection of the impala due to high pasture larval burdens and a large proportion of parasitically naïve young.

The individual pre-treatment EPG values in this study were all higher than the recommended WAAVP minimum of 200 EPG for bovidae and high pre-treatment EPGs have been shown to result in more reliable results (Goossens et al., 2005).

There are various criteria for the diagnosis of anthelmintic resistance by FECR (McKenna, 1994) by either using a control group or not. This study demonstrated an agreement between the WAAVP recommended protocol but using an untreated control group with comparable ecological, management and FECs not resident on the facility and the method recommended by Young et al. (2000) and also used by Goossens et al. (2006) in Belgium in which there is no untreated control and the pre-treatment mean EPG is used for comparison. The use of a control group in the same ecosystem/area with statistically comparable EPGs is thus proposed as a viable means for determining anthelmintic efficacy in free-range captive wild ungulates, where it may not be possible to separate the groups to leave one as an untreated control. This proposed approach will however, need further validation.

Haemonchus spp. was the only genus present after the anthelmintic treatment. *Haemonchus* has been shown to

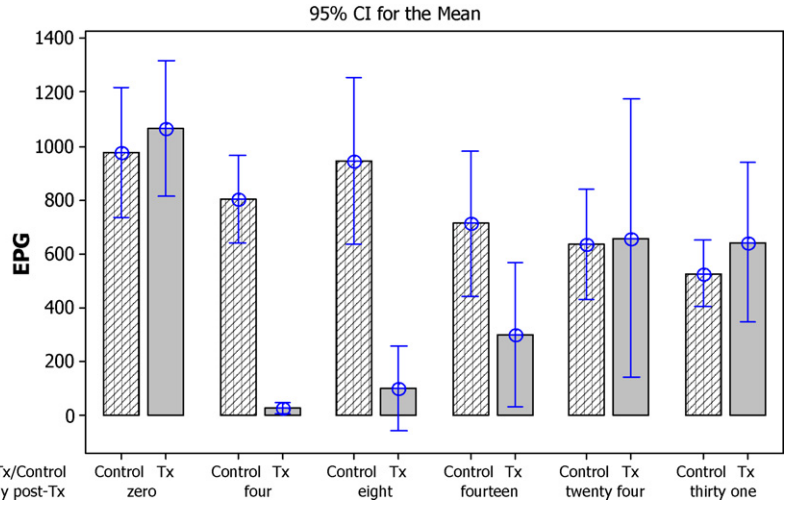

Fig. 1. Mean overall EPGs of pre-treatment and 4, 8, 14, 24 and 31 days post-treatment. *Abbreviations*: Tx: treatment – treated with anthelmintic; Control: untreated herd.

be one of the most pathogenic species of GI nematodes in small ruminant production (Cernanska et al., 2006) and its infection of captive wild impala would pose a similar or possibly more severe risk.

The results could have been compounded by incorrect administration of anthelmintics as discussed by El Abdellati et al. (2010). Nevertheless, this low dosing may encourage the development of resistance by helminths that are exposed to low-dose anthelmintic. Due to the close relationship of these captive wild antelope with domestic ruminants – the increase in proportion of anthelmintic resistant helminths in the captive wild ungulates may introduce these resistant strains into the domestic livestock production systems with very serious consequences. Further exploration of the efficacy of anthelmintics in captive wild impala with tests for resistance such as EHA and LDA would need to be done in Zambia.

Young impala, especially those that are still suckling, may not take in as much of the anthelmintic impregnated salt lick as the older ones but will be parasitized thus their treatment will appear to be less efficacious. A similar phenomenon has been shown by other researchers in Soay sheep in Belgium (Goossens et al., 2006). Further, since the young animals are parasitically naïve and more prone to helminth infection they will have increased EPGs until they attain 'self-cure' – unfortunately, these would be the same animals that would not have taken in enough anthelmintic resulting in them having high EPGs and continued contamination of pasture.

It is further proposed that prophylactic anthelmintic treatment in captive wild ungulates with low to medium stocking densities should only be limited to those herds with clinical disease or at risk of clinical disease, as determined by extremely high EPGs accompanied with early clinical signs of helminthiasis. The indiscriminate use of 'routine' anthelmintics with a high frequency of administration in captive wild ungulates runs the risk of encouraging the development of anthelmintic resistance

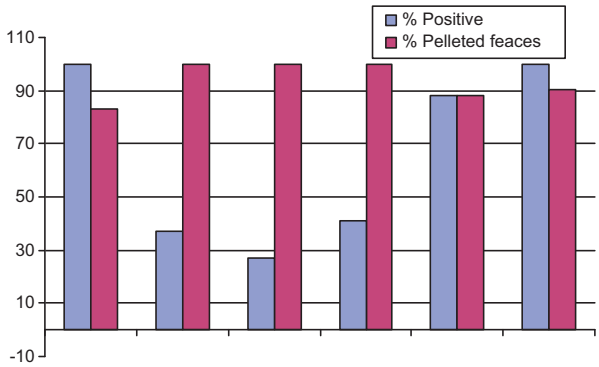

Fig. 2. Proportion of impala faeces that were positive for strongyle nematode eggs and those that were pelleted in consistency from pre-treatment samples to day 31 post treatment.

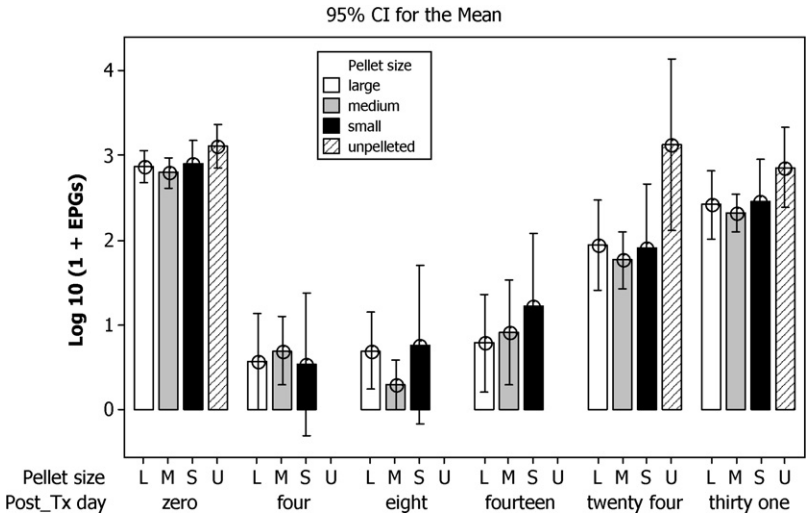

Fig. 3. 95% CI of the mean of pre-treatment and 4, 8, 14, 24 and 31 days post-treatment $Log_{10}(1 + EPG)$ according to size of pellets. *Abbreviations*: Tx: treatment with anthelmintic; S: small; L: large; M: medium; U: unpelleted.

as has been shown in other livestock (Isaza et al., 1995b).

In the immediate interim, it is proposed that anthelmintics should be administered to these captive-wild impala for a longer period of more than 3 days using various delivery methods including feed and mineral licks. Since benzimidazoles have a high therapeutic index, no untoward effect is expected but this will ensure that all members of the herd ingest sufficient quantities of anthelmintic impregnated feed or lick so that a higher proportion of the helminths are killed precluding the development of resistance via residual 'resistant' populations that are spared by the low dose anthelmintic.

It is further proposed that the anthelmintic treatment be carried out when maximum benefit is expected such as a few weeks (e.g. >6 weeks) after the end of the rainy season in order to reduce the re-infection of the impala from pasture that inevitably results immediately after the end of the rainy season due to conducive moisture and temperature conditions on pasture that will guarantee high larval survival on pasture and therefore high uptake by the impala as shown by this study. A mid dry season deworming, when the environmental conditions are less conducive for larval development, would reduce faecal shedding during the rest of the dry season and at the beginning of the rainy season and result in lowered numbers of larvae emerging from hypbiosis at this time. Hypobiosis in impala in Uganda has been reported (Ocaido et al., 1999). This will also ensure that there is low periparturient egg shedding at the time that the impala have their young soon after the beginning of the rainy season and thus the parasitically naïve young will have lower strongyle infective pressure.

Alternating of the anthelmintic class annually between a benzimidazole (e.g. fenbendazole) and ivermectins should also be employed. In order to reduce the possibility of anthelmintic resistance developing in captive-wild ungulates, keepers should also consider the use of alternative parasite control strategies that have been adopted in livestock production such as pasture hygiene for small establishments, reducing stocking densities (Isaza et al., 1995b), and dietary modulation for increased immunity to GI nematodes (McClure, 2008).

A suitable and efficacious anthelmintic program for captive-wild free-range ungulates needs to be formulated for Zambia. Such a program however, can only be formulated after taking into account uniqueness of species', seasonal factors, and other epizootiological factors to determine the best time of the year and frequency of anthelmintic use.

Conflict of interest

The authors of this paper declare that none of them have financial or personal relationships with individuals or organizations that would unacceptably bias the content of this paper.

Acknowledgements

The authors are grateful for the excellent technical assistance of Mr. Maxwell Masuku. The authors are also thankful to Zambia Wildlife Authority (ZAWA), the Management of Lafarge and staff at Chilanga golf course for facilitating this study.

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