

## Prevalence of helminths and evaluation of anthelmintic resistance in working horses and donkeys in seven regions of Egypt

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### **Abstract**

**Aims and objectives** The aim of this study was to investigate resistance to the two proprietary anthelmintic products used by Brooke Hospital for Animals, Egypt (BHA). The objective of the present study was to (i) evaluate the efficacy of the drugs against internal parasites in equines from seven Egyptian regions where Brooke hospital for animals (BHA) is operational (ii) give a current status of the evidence regarding the development of resistance in equine parasites to Fenbendazole and Ivermectin (iii) quantify the internal parasite burden of working equines and (iv) investigate associations between intensity of parasitic infection and age, sex, species and dewormer type.

**Materials and methods** In each of the seven regions covered by BHA, with owner consent, ten equids (five donkeys and five horses) were randomly selected for sampling. For each animal, signalment data on species, sex and age were recorded. On day 0, a freshly voided faecal sample was collected from the ground for each animal. Samples were stored and transported via a temperature controlled container to a regional university veterinary laboratory for a standardised evaluation via sedimentation and flotation methods. At the time of sample collection, a weight-appropriate oral dose of either Fenbendazole or Ivermectin, product standardised by region, was administered to each animal. All animals used in this study were handled according to Brooke Minimum Standards for Working Equines by trained Brooke staff. Fourteen days later, the same sample collection, transportation and evaluation protocol was followed for the same 70 animals. The efficacy of each anthelmintic product was calculated as the percentage of Faecal Egg Count (FEC) reduction (FERC) post treatment, using the arithmetic mean of day 0 FEC as the control reference. All data collected were entered into pre-prepared Excel spreadsheets prior to descriptive and inferential analysis using GenStat statistical software, including evaluation of the normality of distribution of data and the selection of appropriate statistical tests. FECs were classified into 'low', 'medium' and 'high' categories according to levels defined by Soulsby (1982). **Results** Prior to treatment, helminths were identified in 41.4% (95% CI 30.6-53.1) of animals sampled. Five species of helminths were identified; *Strongylus*, *Parascaris*, *Strongyloides*, *Fasciola* and *Anoplocephala*. Overall, median FEC was 0 eggs per gram (epg), interquartile range 0-300 epg, varying from 0 epg (0-0) to 500 epg (300-1200) according to region. Donkey FEC was significantly higher ( $p=0.02$ ) before treatment than horse FEC. There was no significant association between age and FEC. Overall, calculated FERC was 99.5% for Fenbendazole and 98.5% for Ivermectin. **Discussion and conclusions** Many working equids in the regions covered by BHA Egypt have either no or low parasite burden. There is no current evidence of resistance to the two anthelmintics studied. Further studies are required to estimate levels of infestation in local equids not subject to routine deworming to inform an evidence based deworming strategy.

## **Introduction**

There are an estimated 110 million equines (horses, donkeys, mules) in the developing world (FAOSTAT, 2008) where they provide an essential, widely used resource for traction and transport due to economic and/or topographical constraints on motorised alternatives (Ramaswamy, 1994; Gebreab, 1998; Pritchard et al. 2005).

Internal parasites are one of the most common threats to equine health and well-being, especially at young ages (Brady and Nichols, 2009).

Effective parasite control is an essential element in achieving a successful equine operation and maintaining healthy, productive horses (Brady and Nichols, 2009).

The issue of anthelmintic resistance has become increasingly important in the veterinary field during recent years and this is particularly true for equine medicine (Kaplan, 2004; Wolstenholme et al., 2004). Over the past couple of decades, parasitologists have recommended changing parasite control regimes from the intensive interval –dose treatment regime to a more sustainable approach, which has a secondary goal of also preserving the effective lifespan of the drugs (Duncan and Love, 1991; Gomez and Georgi, 1991; Lloyd et al., 2000; Kaplan et al. 2004). Despite this, most equine owners continue to rely on continuous prophylactic usage of anthelmintic compounds at frequent intervals (NAHMS, 1998; Biggin et al., 1999; Pascoe et al., 1999; Lloyd et al., 2000; Earle et al., 2002; Matthee et al., 2002).

For the treatment and control of parasite infections in equines two major anthelmintic classes are available in Egypt: endazole (Benzimidazole group, BZs) and Ivermectin (macrocyclic lactones group, MLs). According to numerous studies, BZs resistance in cyathostomins is present on the most of the horse farms in the majority of the industrialised countries (Kaplan, 2004; Lind et al., 2007; Traversa et al. 2007; Kuzmina and Kharchenko, 2008; Traversa et al. 2009a). Finally, the ML, Ivermectin and moxidectin still show high efficacies on nearly all examined farms (Wirtherle et al., 2004; Lind et al., 2007; Traversa et al., 2007; von Samson –Himmelstjerna et al., 2007b; Lyons et al., 2008).

The objective of the present study was to evaluate the efficacy of two anthelmintic drugs against internal parasites in equines from seven Egyptian regions where Brooke hospital for animals (BHA) is operational. To give a current status of the evidence regarding the development of resistance in equine parasites to Fenbendazole and Ivermectin, to quantify the internal parasite burden of working equines and to investigate the association between intensity of parasitic infection and age, sex, species and dewormer type.

## **Materials and Methods**

### **Geographical Locations**

This project was undertaken in seven Brooke operation regions of Egypt (Cairo, Alexandria, Luxor, Aswan, Delta, Edfu and Matrouh).

### **Study Animals**

Ten study animals (5 donkeys and 5 horses) from each of the seven operational areas (70 equines in total) were selected by Brooke Hospital for Animals (BHA) Egypt for study following owner consent. Only those equines which had not been wormed within the last 14 days and which belonged to owners known to BHA were included in the study. Equines were sourced from the field via mobile clinics and from in-patients within the static clinics. Animal based information including age (classified into four groups:- less than one year, 1-5 years, 6- 15 years and 16 years plus), sex, and species was recorded for all study animals.

### Animal Handling

Animals used in this study were handled according to Brooke Minimum Standards for Working Equines by trained Brooke staff.

### Anthelmintic drug

This study was designed to assess the level of parasite resistance to two anthelmintics which are commonly used by Brooke Egypt. These are Ivermectin (Equiven paste<sup>®</sup>, ADWIA Pharmaceuticals) and Fenbendazole (Curazole 10% oral drench, Univet limited). The appropriate dose (Equiven: 200 microgram ivermectin /kg; Curazole: 7.5 mg/kg) of drug was provided to each equine on day 0 (day of treatment). The type of anthelmintic used within the seven regions prior to the start of the study and during the study (day 0) is displayed in table 1.

	Alexandria	Aswan	Cairo	Delta	Edfu	Luxor	Matrouh
Anthelmintic prescribed prior to the study	Equiven	Curazole	Curazole	Equiven	Equiven (horses) and Curazole (donkeys)	Curazole	Equiven
Anthelmintic prescribed in the study	Equiven	Curazole	Curazole	Curazole	Equiven (horses) and Curazole (donkeys)	Equiven	Equiven

Table 1. Regional anthelmintic use prior (within the previous three months) and during the study. Cells shaded in grey indicate the two regions which used a different anthelmintic treatment during the study when compared to prior to the study.

### Collection, preservation and analysis of faeces

Fresh faeces were collected from the ground on day 0 (day of treatment) and day 14 (13 days post treatment, PT). On day 0 faeces were collected either before or immediately after treatment. A total of 20-25 g of faeces was collected from each equine by a Brooke veterinarian. Each 20-25gm sample comprised a pooled sample of faeces collected from three distinct areas within each pile of faeces to ensure an 'even' collection of sample for each animal (Denwood et al. 2012). Once collected, the samples were kept cool by storage in an ice tank or refrigeration until arrival at the designated laboratory. Three laboratories in Egypt were contracted for analysis of faeces, these were Clinical Laboratory Diagnosis, Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University; Department of Parasitology, Faculty of Veterinary Medicine, Alexandria University and Parasitology Department, Faculty of Veterinary Medicine, Mansoura University,

### Faecal worm egg count

Both qualitative and quantitative parasitological techniques were used for each sample within 48 hours of collection. The total faecal worm egg count (eggs per gram: e.p.g) was determined using the

quantitative McMaster technique (MAFF, 1984) by using a flotation fluid to separate eggs from faecal material in a counting chamber (McMaster) with two compartments. Nematode, cestode eggs and coccidia oocysts were recovered using qualitative flotation and trematode eggs were recovered using sedimentation methods (MAFF, 1984).

### Statistical analysis

Number of helminths recorded for each equine was classified according to established infection intensity categories (Soulsby, 1982) into one of four categories: none, low (up to 500 e.p.g), medium (501-1000 e.p.g), high (>1000 e.p.g).

Aggregate FEC (e.p.g) data were examined for normality using Shapiro-Wilks test and by examining the data using probability plots. Data which were not normally distributed were transformed (logarithm to base 10) prior to statistical analysis. Multiple univariable regression screening tests were used to highlight interesting relationships present in the data. General linear regression was used to further investigate relationships with p values <0.05 identified by the univariable screening test. All analyses were performed using GenStat version 15.1.0.8035 (VSN International Ltd); the level of significance was set at p<0.05.

The efficacy of treatment product (Curazole or Equiveen) was based on the arithmetic mean of the group's faecal egg count (FEC) (e.p.g) and calculated as the percentage of FEC reduction PT, using the arithmetic mean of day 0 FEC as the control reference (as no control group was used in this study).

%Efficacy faecal egg count reduction (%FERC)

$$=100 \times \frac{\text{mean}_{\text{FECday0}} - \text{mean}_{\text{FECPT}}}{\text{mean}_{\text{FECday0}}}$$

The criteria for determination of anthelmintic resistance were defined as a reduction in FEC of less than 80% (Kaplan et al., 2004).

## Results

### Equines

The data set consisted of 35 horses and 35 donkeys of which 23 were female and 47 male. There were three categories of age with 20 equines between 1-5years, 47 between 6-15 years and three 16 years plus.

### Before treatment

#### Coprological prevalence of helminth species

Laboratory analysis identified the presence of five species of helminths (*Strongylus*, *Parascaris*, *Strongyloides*, *Fasciola* and *Anoplocephala*) for which the recorded prevalence varied between laboratory techniques (Figure 1). *Strongylus* was the most common helminth identified with a prevalence of 17% CI 9.9-27.8(sedimentation) and 33% CI 23-44.5 (flotation). *Parascaris* and *Fasciola* were the next most common with prevalence of 5.7% CI 1.8-14.2 (*Parascaris*) and 4.3% CI 1-12.4 (*Fasciola*) by sedimentation and 7% CI 2.7-16 (*Parascaris*) by flotation. *Strongyloides* were only detected by flotation with a prevalence of 5.7% CI 1.8-14.2, whereas *Anoplocephala* was only detected by flotation with a prevalence of 1.4% CI 0-8.4.

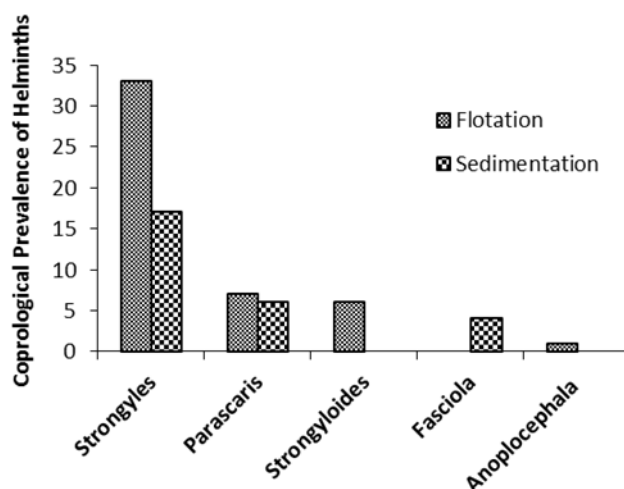


Figure 1. Coprological prevalence of helminth species by technique for working equines in Egypt.

Four species of helminths (*Strongylus*, *Parascaris*, *Strongyloides* and *Fasciola*) were identified in both horse and donkey, however the prevalence varied between laboratory techniques and species (Figure 2). *Strongylus* was the most common helminth identified in both species, however the prevalence was greater in donkeys than horses (Figure 2). The prevalence of *Parascaris*, *Strongyloides* and *Fasciola* was similar between horses and donkeys, whereas *Anoplocephala* was only detected by flotation in one horse.

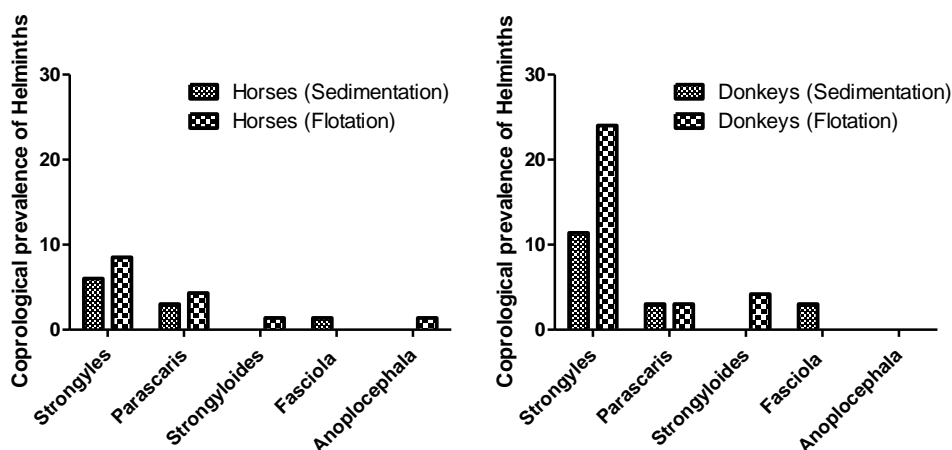


Figure 2. Coprological prevalence of helminths for working horses (a) and donkeys (b) in Egypt and by technique.

### Frequency and intensity of helminth infection

Before treatment the estimated coprological prevalence of helminth eggs was 41% CI 30.6-53.1 of equines sampled (29 out of 70 equines sampled) i.e. 59% (41 equines) of equines sampled had no detectable helminth eggs. Egg counts on day 0 ranged from 0-6750 and were not normally distributed, a median value of 0 e.p.g with interquartile range (IQR) of 0-300. The frequency distribution of four levels of intensity of infection for individual animals before treatment is shown in Figure 3. Seventeen (24%) equines had **low** ( $\leq 500$  e.p.g) FEC; 4 (6%) equines had **medium** (501-1000 e.p.g) FEC; 8 (11%) equines had **high** ( $>1000$  e.p.g) FEC.

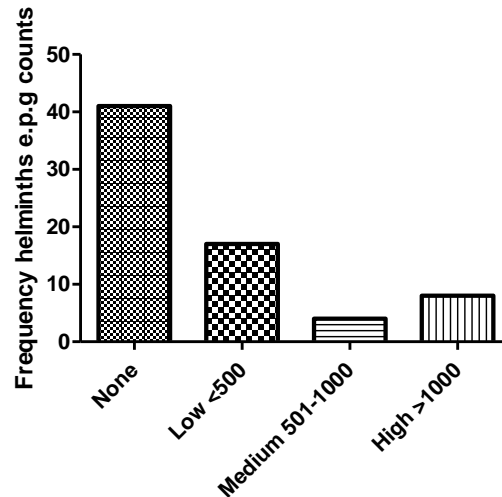


Figure 3. Frequency of intensity of helminth infection in working equines in Egypt

### Region

Number of infected equines and patterns of FEC varied between the seven regions (Table 2 and Figure 4) however this was not quite significant ( $p=0.06$ ). Cairo had the most equines (9/10) and Matrouh had the least equines (0/10) with detectable e.p.g. The (9/10) within categories Cairo had the highest number of equines with e.p.g within the **low** (<500 e.p.g) and **high** (>1000 e.p.g) intensity categories. Luxor had the highest number of equines with e.p.g within the **medium** intensity (501-1000) category.

	Region						
	Alex	Aswan	Cairo	Delta	Edfu	Luxor	Matrouh
No of infected equines	5/10	4/10	9/10	4/10	3/10	4/10	0/10
Arithmetic mean FEC (e.p.g)	85	290	700	430	1315	160	0
Standard Error of the mean	37.1	225.8	188	225.1	869	91.23	0
Median	12.50	0	500	0	0	0	0
Interquartile range	0-200	0-200	300-1200	0-600	0-100	0-100	0

Table 2. Number of infected equines and mean/median FEC per region.

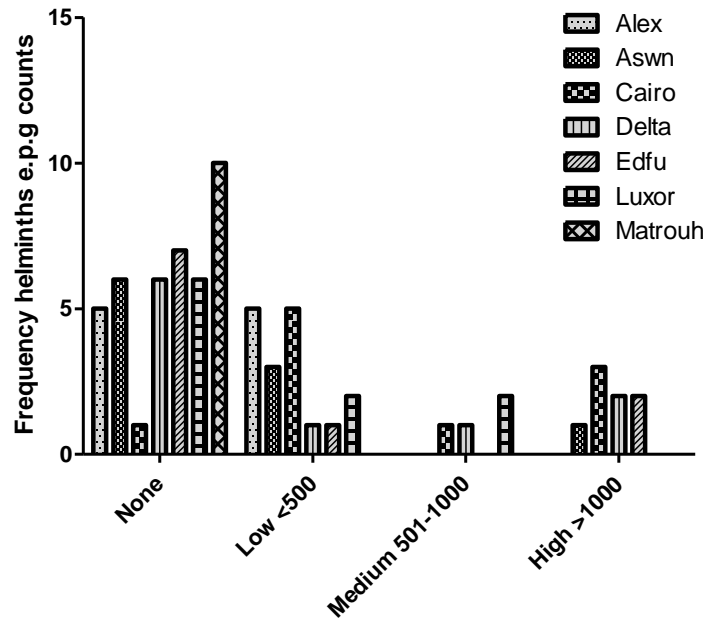


Figure 4. Frequency and intensity of helminth infection per region for working equines in Egypt

#### Sex and age

Before treatment, FEC for female equines ranged from 0-1400 with a median value of 0 e.p.g (IQR 0-100) and male equines ranged from 0-6750 with a median value of 0 e.p.g (IQR 0-500). Male equine FEC was significantly higher before treatment than female FEC ( $p=0.04$ ). There was no significant association between age and FEC.

#### Species

Before treatment FEC for horses ranged from 0-600 with a median value of 0 (IQR 0-100) and for donkeys ranged from 0-6750 with a median value of 100 (IQR 0-725). Donkey FEC was significantly higher ( $p=0.02$ ) before treatment than horse FEC.

#### Dewormer type

Before treatment FEC for equines to be treated with Curazole ranged from 0-6750 with a median value of 200 e.p.g (IQR 0-600) and equines to be treated with Equiveen ranged from 0-750 with a median value of 0 e.p.g (IQR 0-81.25). Equines to be treated with Curazole had a significantly ( $p=0.02$ ) higher FEC prior to treatment.

#### Post treatment

The mean and median FEC's pre and post treatment and efficacy of treatment expressed by the FERC are depicted in Table 3. Post treatment two equines had a FEC of 100 e.p.g. Both equines were male and 6-15 years, however of different species and from different locations (Edfu and Luxor) and treatment groups.

Based on %FERC, no evidence of resistance to Curazole and Equiveen was found with FERC's of 99.5% and 98.5% respectively. Helminth species were found in four equines post treatment and were identified as *Strongylus* ( $n=2$ ) by flotation and *Fasciola* ( $n=2$ ) by sedimentation.

Day

		0	14
Curazole (n=35)	Mean FEC	602	2.9
	SD	1197	16.9
	Median (IQR)	150 (0-600)	0 (0-0)
	%FERC		99.5
Equisen (n=35)	Mean FEC	258	2.9
	SD	1126	16.9
	Median (IQR)	0 (0-100)	0 (0-0)
	%FERC		98.5

Table 3. Mean and median FEC's pre and post treatment and efficacy of treatment expressed by the %FERC for working equines in Egypt

## Discussion

### *Pre treatment*

To the best of the authors' knowledge this is the first study to report prevalence of working equine helminth infection and intensity of infection in Egypt. This is also the first time that the efficacy of anthelmintic products has been evaluated within working equines in Egypt.

Laboratory analysis identified five species of helminths within the faeces of working equines in Egypt. Species identified are consistent with previously published reports on coprological prevalence of helminths of working equines in a range of other countries in Latin America, Africa and Europe (Burden et al. 2010, Getachew et al. 2010, Güiris et al. 2010, Upjohn et al. 2010, Haridy et al. 2007, Pereira and Vianna, 2006, Matthee et al. 2002, Wells et al. 1998, Gawor, 1995, Sotiraki, Badouvas and Himonas, 1997).

*Strongylus* was the most commonly found helminth which is in line with previous reports in working equines (Burden et al. 2010, Getachew et al. 2010, Güiris et al. 2010, Upjohn et al. 2010, Pereira and Vianna 2006, Matthee et al. 2002, Wells et al. 1998, Gawor, 1995, Sotiraki, Badouvas and Himonas, 1997). However the prevalence reported in this study varied from 17% to 33% depending on type of laboratory analysis. This prevalence is considerably lower than the majority of similar studies which report prevalences in excess of 70% (Burden et al. 2010, Getachew et al. 2010, Güiris et al. 2010, Upjohn et al. 2010, Pereira and Vianna, 2006, Matthee et al. 2002, Wells et al. 1998, Gawor, 1995, Sotiraki, Badouvas and Himonas, 1997). This could be related to the frequent deworming regime of the equines in this study consisting of treatment every three months compared to other studies which do not report a regular worming strategy (Burden et al. 2010, Getachew et al. 2010, Upjohn et al. 2010, Pereira and Vianna 2006, Matthee et al. 2002, Gawor, 1995, Sotiraki, Badouvas and Himonas, 1997) or where it is infrequent (Wells et al. 1998) or greater than 4 years previous (Güiris et al. 2010).

Prevalence of *Strongylus* infection was higher in donkeys when compared to horses, which is consistent with findings from Sotiraki, Badouvas and Himonas (1997) who reported prevalences for helminths of 59% for working horses and 73% for donkeys. Wells et al. (1998) reported prevalence of strongyle eggs as high as 97.1% and Matthee et al. (2002) 95% in working donkeys. In the context of Egypt, higher prevalence of strongyles in donkeys could be related to a number of factors such as i) access and frequency of treatment (donkeys generally being located further from Brooke treatment

clinics than horses), ii) donkeys could be subjected to more unfavourable husbandry practices than horses (communal grazing on contaminated pasture, not dewormed).

The prevalence of *Parascaris*, *Fasciola* and *Anoplocephala* reported in this study are generally lower than other reports (Getachew et al. 2010, Güiris et al. 2010, Khan et al. 2010, Upjohn et al. 2010, Haridy et al. 2007, Pereira and Vianna, 2006, Gawor, 1995 and Sotiraki, Badouvas and Himonas, 1997). As above the lower prevalence of the helminths observed in this study could be related to the regular deworming strategy employed in Egypt. In order to confirm this, it would have been necessary to document the deworming history of the equines used in this study, however this was beyond its scope.

The prevalence of *Strongyloides* is consistent with previously published studies from Africa and Europe (Getachew et al. 2010, Matthee et al. 2002, Gawor, 1995 and Sotiraki, Badouvas and Himonas, 1997).

Prior to treatment there was a low frequency (41%) of equines with measureable FEC, of which the majority were in the low intensity category (<500 e.p.g). This is in contrast to other studies carried out on working equines (Getachew et al. 2010, Upjohn et al. 2010, Güiris et al. 2010, Wells et al. 1998, Matthee et al. 2002) which frequently report equines with high intensity (>1000 e.p.g) FEC. Based on this pattern of infestation this study suggests that equines in Egypt are being dewormed when it is not necessary to do so.

Interestingly, it has been noted in the literature that a large proportion of adult equines are able to maintain low worm burdens from natural infection without any treatment intervention which is an observation which is worth investigating in Egypt (Nielsen, 2012). If this was also found to occur in naturally infected equines it would lend weight to designing a deworming strategy whereby only those with higher intensity FEC would need to be dewormed.

Previous studies have reported seasonal variation with FEC higher in summer months than winter (Güiris et al. 2010 and Matthee et al. 2002). This current study was carried out in the autumn (October to December) and therefore according to other studies it could be hypothesised that FEC would be lower, however average temperatures in the seven regions at the time of this study are comparable with those reported for the summer months in the regions of Mexico (Güiris et al. 2010) and South Africa (Matthee et al. 2002). Although it was beyond the scope of this study differences may be explained by variances in rainfall seen in Egypt which would be expected to be lower when compared to Mexico and South Africa.

There was some evidence to suggest regional variation in FEC. In particular, all faecal samples from equines from Matrouh were negative for helminth eggs. Whereas, 9/10 equines in Cairo were positive for helminth eggs. This may be due to differences in geography, density and husbandry of equines. For example, Matrouh is desert with widely dispersed populations of equines, whereas Cairo is urban with a high density of equines. This suggestion is consistent with the findings of Wells et al. (1998) who demonstrated that working donkeys which had opportunity to graze and interact with other donkeys had a higher natural infection of helminths. Edfu had only 3/10 equines infected, however two of these equines had the highest FEC (>1000) recorded in this study. Both equines were male donkeys which may be contributing factors, however in the absence of any other animal specific information it is impossible to say for sure why these donkeys had such high FEC.

Consistent with donkeys having higher prevalence of *Strongylus*, total FEC was also statistically higher in donkeys when compared to horses. This finding is consistent with reports from Getachew et al. (2010) who indicate in their study that more than 55% of donkeys sampled had FEC >1000

e.p.g. with counts as high as 30,650. In the context of Egypt higher FEC for donkeys is most likely linked to equine owners taking greater care of horses including frequent anthelmintic treatment in comparison to donkeys.

Before treatment FEC in equines allocated to the Equiveen treatment group had significantly lower FEC than those allocated to the Curazole treatment group. This could relate to the worming practices at a regional level from where these equines were sourced. For example, in the three months leading to the start of this study (July, August, September) the equines in Alexandria, Delta and Matrouh had received Equiveen, the equines in Aswan, Cairo and Luxor had received Curazole, whereas the equines in Edfu had received Curazole (July and August) and Equiveen (September). The majority of those equines grouped to receive Equiveen had been sourced from a region using Equiveen for the previous three months and the majority of equines grouped to receive Curazole had been sourced from a region using Curazole for the previous three months. Based on published knowledge that the egg reappearance period for Fenbendazole (Curazole) is shorter than for Ivermectin (Equiveen) (Mercier et al. 2001) the fact higher FEC was found in those equines which had been source from a region previously using Curazole agrees with published studies.

#### *Post treatment*

The efficacy of treatment is expressed as the %FERC. Fourteen days post treatment Curazole and Equiveen were both evaluated to be fully effective with mean FERCs of 99.5% and 98.5% respectively. The efficacy over time (egg reappearance period) was not evaluated in this study. This result is in line with previously reported studies on efficacy of Fenbendazole and Ivermectin in working equines in Nicaragua (Kyvsgaard et al. 2011) and adds further weight to existing reports that resistance to these anthelmintic products is not yet a problem in non-westernised countries.

#### **Conclusion and recommendations**

Working equines in Egypt have a lower estimated prevalence of helminths and total faecal FEC (e.p.g) when compared to reported values from working equines in other countries. According to FEC intensity categories defined by Soulsby (1982), the majority of equines in this study had mild to no helminth infection prior to administration of anthelmintic which indicates that worming was unnecessary.

Recognising that at least low levels of parasitism is and should be a normal and natural state for horses, further studies are required to investigate seasonal variation of helminths in naturally infected equines. These should be undertaken in non-Brooke operational areas where anthelmintics have not been used, to understand to what extent equines can naturally maintain FEC within acceptable levels (<500 e.p.g) and how seasonality affects this. Understanding this will enable BHA Egypt to be better informed on when equines need deworming according to seasonality and to be able to approximate how many equines fall into the high intensity helminth category. Knowing the answer to these questions would enable decisions to be made regarding the plausibility of testing equines prior to treating so that only those which need intervention receive it. This approach would also enable the identification of those animals which are persistently subject to higher FECs; in this way it would potentially also be a means of identifying whether other risk factors for high FEC can be identified (e.g. common husbandry, environmental factors) which could form the basis of developing other (non-drug based) interventions to reduce FEC.

Once the problematic seasons and regions have been identified in naturally infected equines the efficacy of anthelmintic at different dosing frequencies should be carried out to determine which regime maintains parasitic levels within the acceptable range. Evidence gathered from this type of study could then be used to inform and improve current deworming strategies used by Brooke Hospital for Animals in Egypt to ensure they are evidence based for the specific local environment

This study found no evidence to suggest development of resistance to Ivermectin or Fenbendazole within helminth populations in Egypt.

### **Acknowledgements**

The authors are very grateful to support staff at the clinics, area managers and the senior team of Brooke hospital for animals –Egypt for their encouragement and support during writing of this article, Also we acknowledge the cooperation of the Ethics committee, Research and welfare team of Brooke IDUK for their support in reviewing the manuscript.

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