Prevalence of gastrointestinal nematodes, parasite control practices and anthelmintic resistance patterns in a working horse population in Egypt

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Summary

Background: Anthelmintic resistance is commonly reported in horse populations in developed countries, but evidence in some working horse populations is either lacking or inconclusive.

Objectives: To estimate prevalence of GI nematode infections in working horses in Egypt and to evaluate strongyle resistance to ivermectin, doramectin and fenbendazole.

Study Design: Cross-sectional study

Methods: Faecal egg count was performed on 644 working horses from 2 provinces in Egypt. A short questionnaire about horse signalment and worming history was completed for each horse. Horses identified with ≥50 strongyle type egg/g (n = 146) underwent faecal egg count reduction testing (FECRT) following treatment with ivermectin (n = 33), doramectin (n = 33) or fenbendazole (n = 30). Risk factors for strongyle (≥200 egg/g) and *Parascaris equorum* (>0 egg/g) infection were investigated using multivariable logistic regression analyses.

Results: The prevalence of low (0–199 epg), medium (200–500 epg) and high (>500 epg) strongyle infection was 88.4%, 5.9% and 5.8%, respectively. *P. equorum* eggs were detected in 5.1% (n = 33) of horses. Strongyle FECR was 100%, 99.97% and 100% following treatment with ivermectin, doramectin and fenbendazole, respectively. Anthelmintic treatment in the 12 months preceding examination was associated with reduced likelihood of strongyle infection (odds ratio [OR] = 0.26, 95% confidence interval [CI] = 0.14, 0.47, P <0.001). The likelihood of *P. equorum* infection was significantly associated with horses' age (OR = 0.78, 95% CI = 0.69, 0.90; P <0.001). Male horses were more likely to have *P. equorum* infection (OR = 2.86, 95% CI = 1.37, 5.93, P = 0.005).

Main Limitations: Nonrandomised selection of study areas and larval cultures were unsuccessful for some samples

Conclusions: There were low prevalence of strongyle and *P. equorum* infection and no evidence of macrocyclic lactones or benzimidazole resistance in strongyles in the studied working horse population.

Introduction

Several species of gastrointestinal (GI) nematodes infect equids, many of which have been associated with colic, ill thrift, reduced growth rates and performance [1-3]. Furthermore, larval cyathostominosis, that is characterized by diarrhoea, hypoproteinaemia and rapid weight loss is a significant disease in young grazing horses [4]. Frequent use of anthelmintics to control GI nematodes has resulted in development of anthelmintic resistance (AR) and reduced efficacy of available anthelmintic classes [5; 6]. Benzimidazole and tetrahydropyrimidines strongyle resistance has been widely reported in studies originated from Americas and Europe [7]. Shortened egg reappearance period (ERP) following treatment with macrocyclic lactones (ML) have also been reported [8; 9] and considered an early indicator of emerging strongyle resistance to these drugs. *Parascaris equorum* resistance to ML is also common and has been reported in several countries [7]. Such reports highlighted the need for continued monitoring of AR in all equine populations along with encouragement of practices to reduce anthelmintic usage [10].

Treatment of horses on the basis of diagnostic faecal egg count (FEC) testing has been implemented to reduce anthelmintic usage and selective pressure on parasitic populations to reduce AR [11]. However, the practice of FEC testing could be neither widely available nor affordable in many working equid populations and hence preventive measures that are based upon best available evidence about prevalence of infection and AR could be more feasible. A study in Nicaragua reported an infection prevalence of 94% with absence of AR to ivermectin and fenbendazole in a study population of 105 working horses [12]. Absence of resistance to these drugs was also reported in working horses in Morocco [13]. Resistance to fenbendazole was reported in working horses in Egypt, but the authors ascribed this to underdosing [14]. Overall, evidence regarding AR in some working horse populations is limited and further studies are required. We previously reported that working horses in Egypt are managed separately with no access to pasture grazing [15] and therefore it was hypothesized that the prevalence of strongyle infection in this working horse population would be low based on reports from other countries [16; 17]. Furthermore, whilst the extra-label use of doramectin is commonplace in this population of horses [15], information about the efficacy of doramectin in horses is limited [18; 19]. Therefore, the objectives of the current study were to quantify the GI nematode burden in working horses in Egypt, to identify factors associated with presence/severity of GI nematode infection and to evaluate strongyle resistance to ivermectin, doramectin and fenbendazole through faecal egg count reduction testing (FECRT).

Materials and Methods

Study population and recruitment

This is a cross sectional study in which working horses were recruited from 37 villages/areas within 2 provinces (Al Dakahliya and Al Sharquiya) in Egypt (Figure 1). The study was communicated to local veterinarians via telephone calls and social media groups. Those who showed willingness to assist in recruitment of horse-owners were then asked to explain the study objectives to their clients/local villagers and assign a date for a visit. On arrival to a village, announcements were made using a microphone. Horse owners were asked to bring their horses to the mobile clinic and if available, to bring a freshly voided faecal sample (3-4 faecal balls). In case of a low response rate or when it was deemed impractical for the horse owners to bring their horses to the mobile clinic, the horse owners were visited at their residences to collect the faecal samples. Reporting of the parasitological examination results back to the horse owners and free anthelmintic treatment of infected horses were offered as incentives for participation in the study.

Sample size calculations to identify a prevalence of srongyle infection ($\geq$ 200 strongyle type eggs per gram) of 20% with a precision level around the prevalence estimate of 5% and 95%

confidence level indicated that 246 horses were required to be recruited onto the study. The calculated sample size was adjusted for the clustering nature of the study population (horses were clustered within villages) using the equation $N = n\ (1 + \rho\ (m - 1)$ [20]; where N is the final sample size, n is the original sample size estimate, ρ (Rho) is the intra-cluster correlation and m is the average number of horses per village. Using an m value of 30 and ρ of 0.05, the final sample size estimate was 603 horses.

Sample and data collection

Freshly voided faecal samples (minimum 3 faecal balls) were collected in sealable plastic bags, with as much air as possible being expelled before sealing to reduce larva development. In some horses, samples were collected directly from the rectum if no fresh faeces were available at the time of the visit. All samples were stored at 4°C until being processed. The median time between sample collection and processing was 4 days (interquartile range [IQR] 3, 5). A short questionnaire was completed with the horse owners which included questions about the horse' signalment, and parasitological control strategies used by the owner (Supplementary Item 1). Questions about general management practices were not asked as this information has been previously reported for horses in the study area [15] and investigating the relationship between the level of strongyle infection and management practices was not an objective in the current study. Horses' age was estimated using dentition together with information provided by the owners. Body condition score (BCS) was recorded on a scale of 1 to 9, where 1 indicated a poor BCS and 9 indicated an extremely fat BCS [21]. Sampling was completed between December 2019 and February 2020 when clover was the main forage source for livestock in Egypt.

Parasitological examination

Faecal egg count

Faecal samples were analysed in duplicates using a modified McMaster technique that had a detection limit of 10 eggs per gram (EPG). Following thorough mixing of the sample, the technique involved mixing 4.5 g faeces with 40.5 ml tap water. The mixture was then passed through a 250 µm-aperture sieve to remove debris. The filtrate was used to fill two 15 ml centrifuge tubes that were subjected to centrifugation for 5 minutes at 1800 × g. The supernatant was discarded and each of the pellets were vortexed briefly before each being re-suspended in 15 ml saturated salt solution (specific gravity = 1.2). The top of the tube was occluded, and the tube was inverted 2-3 times to ensure even distribution of eggs. A Pasteur pipette was used to transfer 1 ml of the suspension to fill the 2 chambers of the McMaster slide. The second tube was also mixed by inverting and 1 ml of the suspension was transferred to another McMaster slide. The eggs were counted in the entire two chambers of the slide (i.e., 1 ml) and the mean count from the two slides was used to calculate the EPG count in the sample. Horses were classified as low (0-199 EPG), moderate (200-500 EPG), or high (>500 EPG) shedders according to the American Association of Equine Practitioners guidelines [22].

Larval culture

Faecal samples containing ≥50 strongyle type EPG (n = 146) were subjected to larval culture (LC). For each LC, 10 g of faeces from each of 10 individual samples were pooled to give a total weight of 100g. Samples from the same village or close villages were grouped together depending on the number of positive samples per village. The faecal material was mixed and moistened with water if necessary, to develop a crumble consistency and placed in sealable plastic bags. Holes were made in the top half of the bags then the bags were incubated at room temperature for 14–21 days. The cultures were checked regularly every 3 days for desiccation and water was added if necessary. The larvae were harvested using the Baermann funnel method. Harvested larvae were placed in petri dish, immobilised with a few drops of Lugol's iodine and examined under a dissecting microscope. Larva identification was performed according to Cernea *et al.* [23].

Faecal egg count reduction test (FECRT)

Horses that had FEC ≥50 strongyle type EPG [24], had been under their current owners' care for at least 8 weeks and for which the minimum ERP of the previously administered anthelmintic had passed were subjected to FECRT. The standard minimum ERP used were 6 weeks for fenbendazole (FBZ) [25] and 8 weeks for ivermectin (IVM) [26] and doramectin (DRM) [27]. Horses were treated with FBZ (Panacur suspension, 7.5 mg/kg p.o.)[a], IVM (Equiveen paste for equine; 0.2 mg/kg P.O.)[b], or DMR (Dectomax injectable solution; 0.2 mg/kg s.c.)[c] at a dose appropriate for 110% of horse body weight [24], as estimated by a weigh tape (Easy-Measure)[d] to avoid underdosing. Treatments were administered by the primary author and the type of the anthelmintic was changed approximately every 10 horses to produce balanced groups. The same batch of each anthelmintic was used throughout the study with all being purchased directly from the companies' representatives. The median time between diagnosis of strongyle infection and treatment was 7 days (IQR 3, 11). Faecal samples were collected 14 days post treatment and examined using a modified McMaster method as described above.

Data analysis

Descriptive statistics were created for all variables in the data. Inaccuracies in the results of descriptive statistics were revised against the completed questionnaires. The prevalence of strongyle infection together with 95% confidence intervals were calculated. The association between the level of infection (low, moderate or high) and BCS was evaluated using the Jonckheere-Terpstra test. EPG values on day 0 were compared between horses treated with either of investigated anthelmintics using the Kruskal–Wallis test. To evaluate the association between explanatory variables (age, sex, number of horses under the current owner's care, whether the horse was dewormed in the preceding 12 months, and whether a routine treatment program was implemented) and strongyle infection (EPG ≥200), a two-level random intercept logistic regression analysis was performed and odds ratios (OR) and their 95% confidence intervals (CI) reported. Village/recruitment area was evaluated as a random effect in all analyses (level 2). The models were fitted using the glmer::lme4 function [28] in R. Initially, a null model and a model that only included the random effect were compared using the likelihood ratio test (LRT) to investigate whether to retain the random effect in subsequent analyses. The variable age was found non-linearly related to the outcome measure based on the results of generalised additive models [29] and therefore it was categorised into < and ≥ 5 years. A multivariable two-level model was built using a manual backward selection procedure. A predictor was considered significant if the associated Wald P value was <0.05. Excluded variables were placed back into the model to assess for confounding. A change in regression coefficients of other variables in the model by ≥20% was used as a sign for confounding [30]. The amount of variability in the log odds of strongyle infection that was attributed to the recruitment area was calculated using the latent-variable approach for binary data. The method assumes the binary outcome arises from an underlying continuous distribution and that the level one (individual horses) variance on the logit scale is $\pi^2/3$. Similarly, the explanatory variables were investigated for association with patent *P. equorum* infection (>0 ascarid type EPG) using logistic regression analyses. The random effect of recruitment area was not found significant (LRT P value = 0.66) and therefore it was excluded from the analysis. The variable age was found linearly associated with the outcome variable and therefore it was included as a linear fit. The model was built using a manual backward elimination approach as described above and the final model was assessed for goodness of fit using the Hosmer-Lemeshow goodness of fit test.

FECR was calculated for each anthelmintic according to the equation, $FECR(\%) = (mean\ EPG\ at\ day\ 0 - mean\ EPG\ at\ day\ 14)\ \times 100/(mean\ EPG\ \ at\ day\ 0)$, where the arithmetic group mean FEC for day 0 and day 14 were used to estimate the group FECR [24]. The thresholds used to determine if the investigated anthelmintics achieved the appropriate efficacy were FECR of >95% for IVM and DRM and >90% for FBZ [22]. The 95% confidence limits of % mean FECR were calculated using Bayesian hierarchical models [31] implemented using the eggCounts statistical package [32] in R. Anthelmintic resistance was concluded if the % mean FECR and the lower confidence limit fell below the designated threshold. All analyses were performed in R version 3.6.3 [33]. Critical probability was set at P = 0.05. The data analysed in the current study are available in https://figshare.com/s/a2d3c67a5f0b29cbf529.

Results

Study population

The study included 644 working horses that were recruited from 37 villages/areas within two Egyptian provinces. The horses were owned by 535 different owners and had a median age of 6 years (IQR 2.5, 10) and included 508 females (78.9%) and 136 (21.1%) entire males. Horses had been under the present owner care for a median of 3 years (IQR 1, 5) which may either indicate a high turnover rate of these horses or there were many new people who started to breed horses for working purposes in the study area. The body condition score was recorded for 511 horses and it had a median score of 4 (IQR 4, 5).

Parasitic control measures

A routine anthelmintic program was implemented in 31.6% (n = 192) of horses of which owners reported 3 were dewormed every 4 weeks or less, 2 were dewormed every 5-6 weeks, 2 were dewormed every 7-8 weeks, 95 were dewormed every 2-6 months, 14 were dewormed every >6-9 months, 45 were dewormed every >9-12 months and 6 were dewormed less frequently. The control program was not specified for 25 horses. Anthelmintic treatment in the preceding 12 months was reported for 47.6% (n = 302) of the horses. This was administered a median of 4 months (IQR 2, 7) before the questionnaire administration. The most recently administered anthelmintic products were DRM (n = 86), IVM injectable preparations (n = 56), IVM oral paste (n = 50), Albendazole (n = 33), piperazine citrate (n = 20), pyrantel tartarate (n = 11), FBZ (n = 1) and herbal preparations (fenugreek seeds and wormwood) (n = 11). Owners could not specify the products used in 34 horses. Reasons for the last anthelmintic administration were; as a part of routine management (n = 211), due to weight loss (n = 42), worms were seen in faeces (n = 22), colic (n = 9), pruritus (n = 5), anal pruritus (n = 6) and presence of external parasites on the horses' skin (n = 7). Treatments were administered according to body weight as estimated by eye and only two owners reported that their horses had a FEC test performed before treatment.

Prevalence of strongyle burden

The prevalence of low, medium and high strongyle infection were 88.4% (n = 569, 95% confidence interval [CI] = 85.6, 90.6), 5.9% (n = 39, 95% CI = 4.3, 8.0) and 5.75% (n = 37, 95% CI = 4.2, 7.8), respectively. Around two thirds (60.9%, 95% CI = 57.1, 64.6) of the horses were identified with a parasitic burden that was below the detection limit of the FEC method used. The level of infection was not significantly associated with the BCS of the horses (P = 0.5).

Factors associated with strongyle burden

Table 1 presents the results of logistic regression analyses of variables investigated for association with strongyle infection (≥200 EPG). The variable anthelmintic treatment in the preceding 12 months was the only variable retained in a final multivariable logistic regression model (OR = 0.26, 95% CI of OR = 0.14, 0.47). The model showed that the odds of strongyle infection was 74% lower in horses that had deworming treatment in the 12 months preceding the time of sample collection. Area of recruitment was significantly related to the odds of strongyle infection (LRT P <0.001). The random effect of recruitment area was responsible for 19% of variation in the data. Two villages had residuals greater than the mean (zero) of the random effect, whereas the rest of the residuals were not different from the mean because the zero value lies within the CIs (Figure 2).

Ascarid burden and associated risk factors

P. equorum eggs were identified in 5.1% (n = 33) of horses. These horses had a median age of 2 years (IQR 1, 3). A large proportion of horses with *P. equorum* infection (58%) had been treated within the last 12 months (median = 3, IQR = 1.1, 4). Results of univariable analysis of variables investigated for the association with *P. equorum* infection is presented in Table 2. Table 3 presents a final multivariable logistic regression model of the risk factors associated with *P. equorum* infection. Male horses (OR = 2.86, 95% CI = 1.37, 5.93) were more likely to be diagnosed with *P. equorum* infection. The likelihood of infection was 22% lower for each year increase in the horses' age (OR = 0.78 95% CI = 0.69, 0.90). The Hosmer-Lemeshow goodness of fit test indicated that there was not significant lack of fit (P = 0.1).

Larval culture

Some of the larval cultures were not successful because of dissection or excessive moisture content of the cultures. Cultures identified with excessive moisture contained intact eggs, whereas those that were found desiccated were identified with dead larvae. Retrieved larvae were morphologically identified as *Oesophagodontus robustus, Strongylus edentatus* and *Triodontophorus spp*; all were members of subfamily *Strongylinae* (large strongyles). Larvae that belong to subfamily Cyathostominae (small strongyles) were not detected in any of the cultures.

Faecal egg count reduction testing

The % mean FECR was 64.94% (95% CI = 59.8, 69.8) for the first 11 horses treated with DRM. This has raised concerns that the anthelmintic used could have been adulterated. The batch was discarded, and another batch of the drug was purchased from the company representative. Out of these 11 horses, seven were re-treated with FBZ and included in the FBZ group. The other 4 horses were not treated as they did not reach the treatment threshold of ≥50 EPG. There were 146 (22.7%) horses that had ≥50 strongyle type EPG and therefore they were eligible to be included in the FECRT. Of these only 96 horses were included in the FECRT. The owner of 25 horses were uncontactable, 18 owners refused to participate in the FECRT, 2 horses were sold, and a horse had anthelmintic treatment 4 weeks before testing. In addition, 4 horses were excluded after initial treatment with DRM. The number of horses treated with IVM, DRM and FBZ were 33 (34.4%), 33 (34.4%) and 30 (31.3%), respectively. The median FEC on day 0 did not differ significantly between treatment groups (Kruskal-Wallis P = 0.2). The % mean FECR was 100% (95% CI = 99.7, 100), 99.7% (95% CI = 99.3, 99.9) and 100% (95% CI = 99.6, 100) for IVM, DRM and FBZ, respectively. Of the horses included in the FECRT, 4 had both strongyle and *P. equorum* infection and all were treated with DRM. The % mean ascarid FECR in these horses was 64.16% (95% CI = 31.9, 79.8).

Discussion

This is the first study to deliver evidence-based information about prevalence of GI nematodes in working horses in Egypt and to investigate the efficacy of three anthelmintic drugs in treatment of strongyle infection in this horse population. This information is important to optimise parasitic control measures, especially as FEC testing is seldom utilised in this working equid population [15].

Most horses in the current study (88.35%) had low (0 -199 EPG) strongyle infection intensity with around two thirds (60.35%) of the study population identified with a parasitic burden that was below the detection limit of the FEC method used. This low prevalence was identified despite 52.37% of horses had not received anthelmintic treatment in the previous 12 months and 68.37% of horse owners reported lack of routine anthelmintic treatment. The prevalence estimate was also lower than previously reported in working horses in Lesotho (88.2%) [34], Nicaragua (94%) [12] and Ethiopia (69.4%) [35]. The low prevalence observed in the current study could be due to differences in management practices between the current horse population and those previously studied. Strongyle infection is common in grazing horses where daily access to pasture for 30 days prior to parasitological examination was associated with greater risk of egg shedding [17]. Conversely, horses that are stall managed with limited access to pasture have been reported to be at reduced risk of developing strongyle infection [16]. Management practices of working horses in the study area have been previously reported and showed no access to pasture grazing and limited contact between horses [15], which could explain why most horses were identified with low infection intensity in the current study. Previous studies have reported seasonal variation in worm egg shedding in working equids with dry conditions and short rainy seasons being associated with lower prevalence compared with a long rainy season in Ethiopia [36; 37]. Additionally, studies on grazing horses reported that most egg shedding occurred in summer and autumn in the USA [17] or in summer and spring in the UK [38]. There might be some element of seasonal effect in the current study, but this cannot be fully elucidated unless a longitudinal study is performed.

BCS was not associated with the level of strongyle infection. A previous study that investigated the same horse population reported that poor BCS was associated with the presence of severe dental disease [39]. Perceived weight loss was a reason for previous anthelmintic treatment in 42 horses in our study, but most treatments (n = 211) were given as a part of routine management. Lack of association between BCS and the intensity of strongyle infection [40; 41] or between anthelmintic treatment and weight gain [13] have been previously reported in other working equid populations. Other studies, however, reported high parasitic burdens were associated with poor BCS [35]. It would therefore appear that BCS of working equids depends on a combination of factors such as workload, the amount of available feed and the presence of underlying disease conditions.

Anthelmintic treatment in the preceding 12 months was associated with around 70% decrease in the odds of strongyle infection in the current study. This finding is self-intuitive and might indicate that treatments used were effective in reducing parasitic burdens in the study population. This agrees with previous studies that reported either lack of anthelmintic treatment or longer time since last worming were positively associated with GI parasitic burdens [17; 42]. It is also of note that many horses that did not receive anthelmintic treatment in the preceding 12 months, or even for longer periods based on discussions with horse owners over the telephone when reporting the FEC results back to them, were not diagnosed with clinical strongyle infection. Individual horse variation in egg shedding has been frequently reported in grazing horses where it was found that 80% of eggs were shed by 20% of horses with some horses were consistently high shedders and others shed low numbers of eggs [43]. Low levels of exposure to infection could be another reason why many horses in the current study did not develop infection despite lack of anthelmintic treatment [16]. The prevalence of strongyle infection varied significantly between recruitment areas and this was responsible for 19% of variation in the likelihood of strongyle infection. This might have been caused by differences in availability of veterinary services, knowledge of horse owners about the importance of preventive veterinary care, type of anthelmintics commonly used (e.g. proprietary vs herbal treatment) or other unknown factors between study areas.

Older horses were less likely to be diagnosed with *P. equorum* infection in the current study. It is widely acknowledged that horses develop age-related immunity to *P. equorum* infection making infection being a problem only for younger horses [44; 45], with maximum ascarid egg shedding being reported in foals [46]. Several studies in working equids, however, reported lack of a relationship between age and *P. equorum* infection in working horses [34] and donkeys [36; 47]. Several older horses in the current study were identified with *P. equorum* infection that should be considered when designing parasitic control programs for this horse population. Differences in infection patterns between horses in developed and developing countries could be due to compromised immunity as a result of underlying diseases or malnutrition in working equids [34].

A large proportion (58.1%) of horses identified with *P. equorum* infection had a history of recent anthelmintic treatment. This raises concerns about reduced efficacy of anthelmintics used. Half of these horses were treated with DRM (n = 9), 3 with IVM oral paste, 2 with IVM injection preparations, and 3 with piperazine citrate. Four horses in the current study underwent ascarid FECRT following treatment with DRM and they had % mean FECR of 64.16%, which may have indicated reduced efficacy of DRM against *P. equorum* in the study population. Resistance of *P. equorum* to ML has been reported worldwide [7], and our findings highlighted the need for further studies to fully investigate ML resistance in *P. equorum* in working horses in Egypt

The three drugs investigated in the present study were identified with high efficacy against strongyle infection. Early indications of strongyle resistance to IVM in the form shortened ERP have been reported in multiple studies from developed countries [8; 48]. Studies in working equids reported high IVM efficacy [12; 49] with ERPs being similar to that when the drug was first prescribed [12]. Two studies on working equids in Egypt, however, reported reduced efficacy of IVM [14; 50], which contradicts the findings of the current study. Hamed *et al.* [50] evaluated an IVM injectable preparation at a dose rate of 0.2mg/kg in working donkeys and reported % mean FECR of 99.6% and 91.6% on days14 and 28 post treatment, respectively. A recent study that compared the efficacy of IVM following intramuscular and oral administration reported reduced efficacy following intramuscular administration [51], which could explain the results reported by Hamed *et al.* [50]. The study by Ali *et al.* [14] reported a % mean FECR of 70% following IVM oral paste treatment in horses. The authors claimed that horses were resenting drug administration with subsequent underdosing and reduced efficacy. DRM is an avermectin that is produced by mutational biosynthesis. It shares similarities to IVM including the mechanism of action and the broad nematode and ectoparasite spectrum of activity [52]. The drug is not registered for use in equids, but the extra-label use of DRM is common in working equid populations and several studies reported >99% efficacy [19; 53; 54], which agrees with the results of the current study. Resistance to FBZ is widely reported in nonworking horse populations [7], but studies in working horses reported contradictory findings. Lack of FBZ resistance was reported in a working horse population in Nicaragua [12], whereas resistance was reported in working horses in northwest Ethiopia [49] and India [55]. Noticeable reduction in FBZ efficacy in horses was reported in a study originating from Egypt but the authors ascribed this to underdosing making it difficult to draw a conclusion from the study [14]. Based on the findings of these multiple studies it seems that the level AR is population-specific, mostly due to the type of dominant strongyle population, and there must be continued monitoring of AR in each equid population with parasitic control programs being devised accordingly.

Some of the larval cultures in the current study were not successful limiting the ability to draw strong conclusions on the type of strongyle population in horses investigated and a further study considering the technical issues identified in the current study is required. Two post-mortem studies investigated the population of internal parasites in donkeys slaughtered in the zoological garden of Giza, Egypt with one study reporting only 6.2% of donkeys harboured cyathostomins [56] and the other study reporting 83.3% of donkeys were infested with cyathostomins [57]. However, these donkeys are not representative of the general donkey population in Egypt and parasite control practices in donkeys are likely to be different from those in horses making a direct comparison of these results difficult. Although the study reported high efficacy of IVM, DRM and FBZ, ERP was not investigated which is important to determine the optimum deworming frequency in the study population. Repeated sampling would not be tolerated by the horse owners and likewise it involves difficulty due to the highly scattered nature of the study population. Selection of study areas was not randomised due to logistical issues relating to access to equine populations, and therefore, it may not be appropriate to extrapolate the current results to other regions of Egypt. There could be some element of selection bias of horses recruited onto the current study as the owners who believed that their horses were infected might have been tempted to participate in the study. However, this source of bias was less likely to have occurred given the low prevalence of strongyle infection identified and our experience working with these horse owners indicate that offering free treatment was an enough incentive for most owners to participate regardless of the infection status of their horses.

In conclusion, the current study reported low prevalence of strongyle infection and high efficacy of three anthelmintic drugs (ivermectin, doramectin and fenbendazole) in a working horse population in Egypt. There was also an indication of *P. equorum* resistance to ML is emerging in this working horse population, but further studies are required to investigate this. Taken together, it could be recommended that a single anthelmintic treatment every 12 months could be enough to control strongyle infection in adult working horses in Egypt. However, FEC testing is advised to identify those animals that require more regular anthelmintic treatment as opposed to low shedders which can be treated less frequently.

Ethical Animal Research

Ethical approval for the study was granted by the Institutional Animal Care and Use Committee, Zagazig University, Egypt (ZU-IACUC/2/F/22/2020). Horse owners gave verbal consent for their animals’ inclusion in the study.

Competing Interests

No competing interests have been declared.

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None

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Authorship

S. E. Salem, A. M. Abdelaal, S. P. Daniels and R. Ras contributed to study design. S. E. Salem, A. M. Abd El-Ghany, M. H. Hamad, H. A. Elsheikh, A. A. Hamid, M. A. Saud and R. Ras contributed to study execution. S. E. Salem contributed to data analysis and interpretation. S. E. Salem, A. M. Abd El-Ghany, M. H. Hamad, A. M. Abdelaal, H. A. Elsheikh, A. A. Hamid, M. A. Saud, S. P. Daniels and R. Ras contributed to the preparation of the manuscript. All authors gave their final approval of the manuscript.

Manufacturers' addresses

a. MSD Animal Health, Cairo, Egypt
b. Adwia Pharmaceuticals Co., 10th of Ramadan, Egypt
c. Zoeitis, Cairo, Egypt
d. Chandelles Saddlery, St Ouen, Jersey

Figure legends

Figure 1. A map of locations from which horses were recruited onto the study

Figure 2. A caterpillar plot of the village/area random effect. Y-axis is the estimated residuals for area, x-axis is the rank of location residuals. Vertical lines represent the 95% CI for the estimated residuals.

Supplementary information

Supplementary item 1. The study questionnaire

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Table 1: Descripts statistics and results of logistic regression analyses of variables investigated for association with strongyle infection (≥200 EPG). Anthelmintic treatment in the preceding 12 months was the only variable retained in a final multivariable model. β = coefficient, SE = standard error, OR = odds ratio, CI = confidence interval, Ref. = reference category.

Variable	**Level**	**n**	**No. of infected horses (75)**	**% of infected horses (11.65)**	**β**	**SE**	**OR**	**95% CI of OR**	**Wald P value**
Sex	Female	508	63	12.40	Ref.				
	Male	136	12	8.82	–0.17	0.37	0.85	0.41, 1.75	0.6
Number of horses owned	one	397	43	10.83	Ref.				
	two or more	247	32	12.96	0.35	0.28	1.42	0.83, 2.44	0.2
Anthelmintic treatment in the last 12 months	No	332	57	17.17	Ref.				
	Yes	302	16	5.30	–1.36	0.31	0.26	0.14, 0.47	<0.001
Routine deworming	No	415	59	14.22	Ref.				
	Yes	192	12	6.25	–0.75	0.35	0.47	0.24, 0.94	0.03
Age (year)	<5	283	28	9.89	Ref.				
	≥5	361	47	13.01	–0.07	0.28	0.93	0.54,1.62	0.8

Table 2: Descriptive statistics and results of univariable logistic regression analyses of variables investigated for association with *Parascaris equorum*. β = coefficient, SE = standard error, OR = odds ratio, CI = confidence interval, Ref. = reference category. Descriptive statistics for continuous variables are presented as median (IQR).

Variable	**Level**	**n**	**No. of infected horses (33)**	**% of infected horses (5.12)**	**β**	**SE**	**OR**	**95% CI of OR**	**Wald P value**
Sex	Female	508	17	3.35	Ref.				
	Male	136	16	11.76	1.35	0.36	3.85	1.89, 7.84	>0.001
Number of horses owned	one	397	13	3.27	Ref.				
	two or more	247	20	8.10	0.97	0.37	2.60	1.27, 5.33	0.01
Anthelmintic treatment in the last 12 months	No	332	13	3.92	Ref.				
	Yes	302	18	5.96	0.44	0.37	1.58	0.75, 3.23	0.2
Routine worming	No	415	15	3.61	Ref.				
	Yes	192	14	7.29	0.74	0.38	2.09	0.99, 4.44	0.05
Continuous variables	**Infected**		**Not infected**						
Age (year)	2 (1,3)		6 (3, 10)		–0.27	0.07	0.76	0.67, 0.88	<0.001

Table 3: A final multivariable analysis model of variables associated with *Parascaris equorum* infection. β = coefficient, SE = standard error, OR = odds ratio, CI = confidence interval

Variable	Level	β	SE	OR	95% CI of OR	Wald P value
Sex	Female	Ref.				
	Male	1.05	0.37	2.86	1.37, 5.93	0.005
Age (year)	-	–0.24	0.07	0.78	0.69, 0.90	<0.001