A field study on the anthelmintic resistance of *Parascaris* spp. in Arab foals in the Riyadh region, Saudi Arabia

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**ABSTRACT**

Background: In the last decade, *Parascaris* spp. resistance to anthelmintics has been recorded in many countries. In Saudi Arabia, there are limited data available on *Parascaris* spp. resistance to anthelmintics.

Objective: To determine the current status of ivermectin, abamectin and praziquantel combined, and fenbendazole resistance to *Parascaris* spp. in horses in Saudi Arabia.

Methods: Three hundred and forty-one foals from eleven different farms were examined by faecal egg count (FEC). The foals were all Arab horses aged 17.2 ± 4.5 (SD) months. Ivermectin (n = 46 foals), abamectin and praziquantel combined (n = 46), and fenbendazole (n = 46) were administered on day 0 and faeces were collected on day 14. The study comprised 41 untreated foals as controls. Animals that have FEC of ≥100 eggs per gram (EPG) were used to measure anthelmintic efficacy. *Parascaris* spp. populations were considered susceptible when faecal egg count reduction (FECR) was >95% associated with a lower 95% confidence limit (LCL) >90%, suspected resistant when FECR <90% or LCL <90% and resistant when FECR <90% and LCL <90%.

Results: Prevalence of *Parascaris* spp. infection was 53% (179/341 horses). Anthelmintic resistance to *Parascaris* spp. were highest following fenbendazole (55% of farms and 65% of foals) and to a lower extent following ivermectin or the combination of abamectin and praziquantel which comprised 27% of farms (and 46% of foals) and 18% of farms (and 10% of foals), respectively.

Conclusion: These data indicate that anthelmintics-resistant *Parascaris* spp. populations are present on horse farms in Saudi Arabia.

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Equine; foal; *Parascaris* spp.; anthelmintic resistance; ivermectin; Saudi Arabia

1. Introduction

Resistance of horse intestinal nematodes to anthelmintics is well documented worldwide. This problem is thought to be caused by intensive usage of antiparasitic drugs (Kaplan 2002). In the last decade, *Parascaris* spp. resistant to different groups of anthelmintics has been reported in horses in many countries such as the Netherlands (Boersema et al. 2002), USA (Craig et al. 2007), UK (Stoneham & Coles 2006), Canada (Hearn & Peregrine 2003; Slocombe et al. 2007), Germany (von Samson-Himmelstjerna et al. 2007), Sweden (Lindgren et al. 2008), Italy (Veronesi et al. 2010), France (Geurden et al. 2013) and Australia (Armstrong et al. 2014). Since chemical treatment is still the mainstay of parasite control measures, the detection of anthelmintic resistance in strongyles has become a critical necessity (Pook et al. 2002; Wolstenholme et al. 2004). Faecal egg count reduction test (FECRT) is the most widely used technique to detect anthelmintic resistance in this field (Coles et al. 2006). This is applicable for all types of anthelmintics for its ease of use besides it does not require expensive equipment (Taylor et al. 2002; Coles et al. 2006), but it does require at least two visits to the farm. In Saudi Arabia, the number of horses, according to the estimates of Saudi Ministry of Agriculture in 2014, was more than 27,114 horses and around 500 horses were imported every year from some European and Arab countries (Ministry of Agriculture 2014). In addition, anthelmintic drugs such as ivermectin (IVM), the combination of abamectin and praziquantel (ABA/PRZ) and fenbendazole (FBZ) are the most commonly used in Saudi veterinary practice. With the increasing number of foals in Saudi Arabia, there are extensive uses of anthelmintics and poor information on the intestinal parasites infecting horses in Saudi Arabia (Alanazi & Alyousif 2011).

The present study aims to determine the current status of *Parascaris* spp. resistance to IVM, ABA/PRZ, and FBZ on horse farms in Saudi Arabia.
2. Materials and methods

2.1. Study design

The study was carried out in the period from March to September 2016, using a total of 341 foals from different locations around the Riyadh region, Saudi Arabia. The foals were all Arab horses aged 17.2 ± 4.5 (SD) months. Farms and foals were selected according to the following criteria: foals were older than 4 months and less than 18 months and had not been dewormed in the previous 8 weeks. In addition, selected farms held at least 12 foals except for 2 farms that had only 9 foals with no control groups. Farm managers or owners were asked to formally agree to participate in the experiment and they were informed with the results of their animals.

To estimate the horse weight in the field, a girth tape (Equi Max®, Thirsk, United Kingdom) was used around the girth of the foal running behind the front legs and the withers.

Only foals infected with Parascaris spp. egg counts ≥100 per gram faeces were included in this trial. In each farm, foals were randomly divided into up to four groups with a minimum of three foals per group. Foals in each group were given one of the three commercial oral paste preparations at the recommended dose rates or left untreated as controls. Compounds used were 200 μg/kg BW IVM (Eqvalan®, Merial Saude Animal Ltda, São Paulo, Brazil), a combination of 3.7 mg/g ABA and 46.2 mg/g PRZ (Promectin Plus, Jurox Pty Ltd., Rutherford, Australia) administered orally at a dosage of 54 mg/kg BW, or 750 mg FBZ (Fenacure 750, Ashish Life Science PVT Limited, Mumbai, India) equivalent to 7.5 mg/kg BW. To avoid drug administration errors, all treated animals were administered by the same person under veterinary supervision. Faecal egg counts (FECs) were performed on the day of treatment and at day 14 post-treatment.

2.2. Faecal sample collection and analysis

Faecal samples were taken directly from the foal’s rectum although some samples were collected from fresh deposits in the bedding but were always positively associated with a particular horse. Samples of at least 20 g were put separately into pre-labelled bags with the air excluded. In general, samples were processed on the same day, or stored at 4 °C until been processed the next day. Microscopic examination and counting were performed after faecal flotation in saturated salt solution for isolation of parasite eggs. Parasite egg counts were carried out using McMaster technique (Whitlock et al. 1980) on 3 g of faeces with a minimum detection level of 20 eggs per gram (EPG). Sturt University (2008), was used for the calculation of the % reduction and 95% confidence limits (CLs). For all tested compounds, resistance was categorised as follows: (1) resistance present if FECR < 90% and the lower confidence limit (LCL) < 90%, (2) resistance suspected if FECR < 90% or LCL < 90% and (3) no resistance if FECR ≥ 95% and LCL > 90% (Pook et al. 2002).

Data were analysed using three different methods of calculations as follows. In these calculations, AM = arithmetic mean, GM = geometric mean, CL = confidence limit.

Calculation method 1.

This method is recommended by the World Association of the Advancement of Parasitology (WAAVP) (Coles et al. 1992).

\[
\text{Efficacy} = \frac{\left(\frac{\text{mean FEC day of treatment group}}{\text{mean FEC 14 days post-treatment group}}\right) \times 100}{\text{FEC day of treatment}}
\]

Calculation method 2.

FECR proportions of individual horses ((pre-treatment EPG – post-treatment EPG)/pre-treatment EPG) were calculated with the data back-transformed. The FECR of the treatment group was calculated by back-transformation of the mean of ECRs: FECR = \([1 - e^{\frac{1}{n} \sum \log_e (\text{FECR})}] \times 100\%\), where the constant e is the base of natural logarithms. The group means were calculated as transformed mean = AM (arcsin, square root of individual FECR proportions). CL at the level of 95% was calculated using the standard deviation of the mean of the data generated in Microsoft Excel prior to retransformation (FECR% = 100 × (sin (transformed group mean))^2).

Calculation method 3.

Method 3 corrects egg count reductions by considering changes in the control group egg counts between collections:

\[
\text{Reductions-based AM values (but no CL)} = \frac{\text{Mean post – treatment EPG} \times \text{Mean pre – treatment control EPG}}{\text{Mean pre – treatment EPG} \times \text{Mean pre – treatment control EPG}}
\]

Reductions-based AM values (but no CL) were calculated by the method of Dash et al. (1998). Calculation of GM values was as follows: \(\text{GM} = e^{\frac{1}{n} \sum \log_e (X_i + 1/n)} - 1\) (where \(n\) counts \(X_i\), and \(e\) is exponential function). The 95% CL = 100[1 – \(P\times\exp\{1+(t \times s/[(n+c+1/nt)]\}]) (where \(t\) is based on \(P = 0.05\) and degrees of freedom \((n - 1)\), \(nc\) = n for control group; \(nt\) = treatment group size, \(s\) = residual standard deviation from analysis of variance of differences in log counts pre-and post-treatment).

3. Results

3.1. Faecal egg counts (FEC)

The prevalence of Parascaris spp. eggs was 53% (179/341 horses). Only 179 foals were involved in this study.
Eggs of *Strongyloides westeri*, *Strongyles* spp. and *Habronema* spp. were found in limited number of the faecal samples on the day of treatment and few of these eggs still existed two weeks post-treatment. All horses tolerated the treatments and no adverse effects were observed.

### 3.2. Faecal egg count reduction test (FECRT)

Tables 1–3 show the summary of the results of FECRT using different methods of calculations. *Parascaris* spp. resistance to IVM was found in 27% (3/11) farms of the farms examined using the three calculation methods. However, by using geometric means, the resistance is estimated to be in 18% of the farms (2 out of 11). On the other hand, the first calculation method detected resistance to ABA/PRZ in only one farm compared to two farms using the second and third methods of calculation. Finally, *Parascaris* spp. had the highest resistance to FBZ in five farms (45%) using the first method and in six farms (55%) using both the second and third calculation methods (Tables 2 and 3). In all tested farms, the control groups mostly had higher EPG at day 14 compared to day zero.

#### Table 1. Pre-treatment and post-treatment mean egg per gram (EPG) counts by faecal egg count reduction percentage (FECR%) with 95% lower confidence limits (LCL) calculated for the three methods for ivermectin (IVM).

| Farm | N  | PrT AM of EPG (min–max) | PoT AM of EPG (min–max) | FECR% LCL | FECR% LCL | FECR% LCL |
|------|----|-------------------------|-------------------------|-----------|-----------|-----------|
| 1    | 12 | 303 (280–340)           | 0                       | 100       | 100       | 100       |
| 2    | 24 | 300 (120–640)           | 46.7 (10–120)           | 89.1      | 73.1      | 86.5      |
| 3    | 16 | 290 (160–440)           | 60 (40–80)              | 90.8      | 83.3      | 78.9      |
| 4    | 9  | 173 (120–280)           | 26.7 (0–80)             | Np        | Np        | 96.5      |
| 5    | 9  | 167 (100–280)           | 0                       | Np        | 100       | 100       |
| 6    | 20 | 200 (120–320)           | 8 (0–40)                | 97.3      | 77.7      | 99.4      |
| 7    | 25 | 263 (200–400)           | 33.3 (0–80)             | 88.4      | 74.2      | 91.3      |
| 8    | 12 | 200 (180–240)           | 13.3 (0–40)             | 95.4      | 61.8      | 98.0      |
| 9    | 21 | 164 (100–240)           | 8 (0–40)                | 97.4      | 79.0      | 99.2      |
| 10   | 12 | 200 (160–240)           | 0                       | 100       | 100       | 100       |
| 11   | 19 | 216 (100–280)           | 72 (40–80)              | 70.6      | 60.6      | 65.1      |

PrT, pre-treatment; PoT, post-treatment; N, number of horses; AM, arithmetic means; GM, geometric means; Np, not performed; controls were not used in properties No. 4 and 5.

#### Table 2. Pre-treatment and post-treatment mean egg per gram (EPG) counts by faecal egg count reduction percentage (FECR%) with 95% lower confidence limits (LCL) calculated for the three methods for abamectin/praziquantel combination (ABA/PRZ).

| Farm | N  | PrT AM of EPG (min–max) | PoT AM of EPG (min–max) | FECR% LCL | FECR% LCL | FECR% LCL |
|------|----|-------------------------|-------------------------|-----------|-----------|-----------|
| 1    | 12 | 327 (180–480)           | 0                       | 100       | Np        | 100       |
| 2    | 24 | 287 (160–560)           | 20 (0–80)               | 95.3      | 79.8      | 98.5      |
| 3    | 16 | 335 (120–580)           | 30 (0–80)               | 95.2      | 81.0      | 96.6      |
| 4    | 9  | 280 (120–440)           | 53 (40–80)              | Np        | Np        | 78.5      |
| 5    | 9  | 227 (120–320)           | 0                       | Np        | Np        | 78.5      |
| 6    | 20 | 284 (140–40)            | 8 (0–40)                | 97.3      | 77.7      | 99.4      |
| 7    | 25 | 207 (120–360)           | 6.7 (0–40)              | 97.6      | 80.9      | 99.6      |
| 8    | 12 | 220 (100–360)           | 13.3 (0–40)             | 95.4      | 61.8      | 98.7      |
| 9    | 21 | 224 (120–360)           | 64 (40–120)             | 79.79     | 63.6      | 72.1      |
| 10   | 12 | 140 (100–200)           | 0                       | 100       | 100       | 100       |
| 11   | 19 | 140 (100–220)           | 8 (0–40)                | 96.7      | 73.1      | 98.1      |

PrT, pre-treatment; PoT, post-treatment; N, number of horses; AM, arithmetic means; GM, geometric means; Np, not performed; controls were not used in properties No. 4 and 5.

#### Table 3. Pre-treatment and post-treatment mean egg counts (EPG) and FECR% with 95% lower confidence limits (LCL) calculated for the three methods for fenbendazole (FBZ).

| Farm | N  | PrT AM of EPG (min–max) | PoT AM of EPG (min–max) | FECR% LCL | FECR% LCL | FECR% LCL |
|------|----|-------------------------|-------------------------|-----------|-----------|-----------|
| 1    | 12 | 360 (280–460)           | 13.3 (0–40)             | 96.3      | 69.4      | 98.9      |
| 2    | 24 | 252 (120–560)           | 28.6 (0–120)            | 87.6      | 74.3      | 86.6      |
| 3    | 16 | 310 (240–400)           | 70 (40–80)              | 88.8      | 82.5      | 77.6      |
| 4    | 9  | 200 (120–320)           | 66.7 (40–120)           | Np        | Np        | 68.1      |
| 5    | 9  | 240 (120–360)           | 13.3 (0–40)             | Np        | Np        | 98.7      |
| 6    | 20 | 260 (120–320)           | 48 (0–80)               | 84.0      | 67.3      | 79.2      |
| 7    | 25 | 217 (120–420)           | 6.7 (0–40)              | 97.6      | 80.9      | 99.7      |
| 8    | 12 | 160 (120–240)           | 13.3 (0–40)             | 95.4      | 61.8      | 98.0      |
| 9    | 21 | 172 (100–240)           | 56 (40–80)              | 82.3      | 72.2      | 65.8      |
| 10   | 12 | 173 (120–260)           | 13.3 (0–40)             | 94.4      | 52.8      | 98.1      |
| 11   | 19 | 160 (100–240)           | 64 (40–80)              | 73.8      | 62.3      | 59.1      |

PrT, pre-treatment; PoT, post-treatment; N, number of horses; AM, arithmetic means; GM, geometric means; Np, not performed; controls were not used in properties No. 4 and 5.
4. Discussion

The current study documented, for the first time, the prevalence of *Parascaris* spp. infection in foals in Saudi Arabia (53%). The recorded prevalence is within the range (31%–61%) reported in previous studies from North America (Austin et al. 1990; Lyons et al. 2008), Europe (Lind & Cristensson 2009; Laugier et al. 2012) and Australia (Armstrong et al. 2014) which confirm the common incidence of *Parascaris* spp. in foals.

Our study showed that IVM and ABA/PRZ were ineffective against *Parascaris* spp. in three and two farms, respectively. This is the first report of *Parascaris* spp. resistance to macrocyclic lactones (MLs) (e.g. IVM and ABA/PRZ) in Saudi Arabia. However, reduced IVM efficacy against *Parascaris* spp. has been reported from different parts of the world including North America (Lyons et al. 2006; Slocombe et al. 2007), Europe (Boersema et al. 2002; Lind & Christensson 2009; Veronesi et al. 2009; Näreaho et al. 2011; Laugier et al. 2012), Brazil (Molento et al. 2008), Australia and New Zealand (Armstrong et al. 2014; Bishop et al. 2014; Beasley et al. 2015). Intensive use of MLs such as IVM appears to be associated with development of resistance in *Parascaris* spp. populations (Fritzen et al. 2010). Selection for resistance probably follows the same course as for other parasites. MLs are persistent anthelmintics in horses as drug plasma levels may persist for several days or weeks (for ivermectin) after a single treatment (Pérez et al. 2003). Drug concentrations inevitably decline over time and parasites that are newly acquired during this phase may be exposed to subtherapeutic concentrations (Sangster 1999). In this case, *Parascaris* spp. larvae may develop resistance to IVM during the 10 weeks of prepatency (Boersema et al. 2002) and may carry resistance alleles to next generations. In addition, foals have poor immunity against *Parascaris* spp. and there are no other means for removing parasites other than with anthelmintics (Craig et al. 2007).

The findings of this study indicate that *Parascaris* spp. populations are resistant to multiple anthelmintic drugs, which is the first report outside of North America and Australia. In previous studies, FBZ was found to be highly effective against *Parascaris* spp., with FECRs of 98%–100% (Slocombe et al. 2007; Lind & Christensson 2009), while in the current study, *Parascaris* spp. resistant to FBZ was present in six of the eleven farms where the drug was tested. Currently, *Parascaris* spp. resistant to FBZ has been reported in horses from Australia (Armstrong et al. 2014). FBZ resistance was possibly selected by intensive use in some horse farms for prevention of gastrointestinal parasites infections. In fact, the development of FBZ resistance in *Parascaris* spp. populations in horses in Saudi Arabia was not surprising, as it has been used widely on Saudi horse farms. However, these results support the recommendation that the efficacy of available drug classes against *Parascaris* spp. and other equine parasites should be monitored regularly by FECR testing (Veronesi et al. 2009; Reinemeyer 2012). In addition, although the animals were selected for being infected by *Parascaris* spp., the appearance of other parasites pre- and post-treatment might indicate some resistance which needs further investigations.

FECRT is the most widely used technique for detecting anthelmintic resistance under field conditions (Coles et al. 2006) and it is considered as a useful method in diagnosing and monitoring the anthelmintic resistance (Denwood et al. 2010). The advantage of this technique is that it can be performed with equipment that is typically available in veterinary laboratories. However, it has several limitations including costs in terms of labour, animal usage and time (Boersema 1983). In addition, FECs can be affected by several factors such as differing immunity depending on the horse age (Uhlinger 1993; Klei & Chapman 1999) and differences in grazing management (Dopfer et al. 2004). However, FECRT has been used as an in vitro test for the diagnosis of resistance in many countries (White et al. 1980; Ihler & Bjorn 1996; Pook et al. 2002; Taylor et al. 2002; Kaplan et al. 2004; Kuzmina & Kharchenko 2008; Millillo et al. 2009).

The current method for FECRT is advocated by WAAVP and it involves calculating the mean and variance of egg counts before and after treatment (eg. comparing untreated controls and treated animals post-treatment), calculating the mean reduction and estimates of the 95% confidence intervals to correct for the variation of the data (Coles et al. 1992). Studies done on horses present additional challenges such as only small numbers of horses being available on a farm, especially for controls, and the variable histories and experience of infection. These issues were partially resolved by using animals as their own control (Pook et al. 2002). However, these methods do not take into account the difference between uncertainty regarding the true mean of a sample, introduced by the Poisson variability of the sample and counting process, and variability in the true mean of different samples (Denwood et al. 2010). A non-parametric bootstrapping approach has recently been suggested as an appropriate method to generate CLs from equine FECRT data (Vidyashankar et al. 2007). The technique involves resampling and summarising the observed data and makes no assumptions about the underlying distribution or processes generating the data (Mooney & Duval 1993). The bootstrapping approach is extremely useful when the underlying distribution of data is unknown, but is more complex and time-consuming than the currently advocated WAAVP method (Denwood et al. 2010). Other options for analysis of FECRT data include probability profile tools proposed by Torgerson et al. (2005).
In the current study, FECR% was determined by three different methods of calculations used previously by other researchers (Dash et al. 1988; Coles et al. 1992; Craven et al. 1998; Pook et al. 2002; Milillo et al. 2009). Calculation of FECR% by method 2 in field trials was deemed more suitable as it requires no control group and generally has lower variances (Pook et al. 2002). The cut-off% for efficacy remains debatable as the current choices of 90% or 95% are not based on any formal analysis and may require re-evaluation with new approaches. Developing cut-off values requires knowledge of the field efficacy patterns, the variability inherent in the data and simulations of resistant populations.

5. Conclusion

In conclusion, the current study detected, for the first time, the presence of anthelmintic resistance in *Parascaris* spp. populations in Saudi Arabia horse farms. The patterns of drug efficacy on individual farms varied considerably. FBZ resistance was most common, and IVM and ABA/PR2 resistance was apparent. Therefore, anthelmintic efficacy against *Parascaris* spp. and other parasites should be monitored regularly for optimisation of local parasite control programmes.

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Disclosure statement

The authors declare that they have no competing interests.

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