Prevalence of Gastrointestinal Parasites in Horses in the Eastern Province of Saudi Arabia

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ABSTRACT

Gastrointestinal parasites have great health impact on horses and economic challenge to horse owners. Little information is available on the prevalence of these parasites in horses in Saudi Arabia. The goal of this study was to document the prevalence of gastrointestinal parasites among horses in the Eastern Province of Saudi Arabia. Three hundred and two horses were tested. Fecal samples were examined using direct smear, flotation, and sedimentation techniques. Overall rate of infection was (30.46%). *Tritrichomonas equi, Parscaris equorum, Dictyocaulus arnfieldi, Strongyle-type* ova*, Oxyuris equi* and *Eimeria leukarti* were detected. Geographic distribution of these parasites indicated higher prevalence in horses in Al-Ahsa area and seasonal prevalence. In the meantime, parasites were detected throughout the year but with higher percentage values in the warm season than those in the cold season.

Key Words: Gastrointestinal parasites, Horse, Prevalence, Saudi Arabia.

INTRODUCTION

Gastrointestinal parasites cause clinically and economically serious health problems in horses. They mainly affect the digestive system however respiratory system and other organs may be affected as well. Economical impact of these parasites is evident on many fronts including direct impact on horse health, poor performance, cost of treatment, cost of prevention and labor work (Bowman *et al.,* 2003).

Many types of gastrointestinal parasites are capable of infecting horses; however, few parasites are known to cause significant health problems. The primary class of gastrointestinal parasites that causes health problems in horses are nematodes including large and small strongyles, ascarid *Parascaris equorum* (in foals), pinworm *Oxyuris equi* and lungworm *Dictyocaulus arnfieldi* as well as cestodes which include *Anoplocephala perfoliata* (Love *et al.,* 1999). Many of the protozoan parasitic infections may affect horses. *Tritrichomonas equi* is considered one of the most common parasitic infections which is found in the large intestine of the horses and can be a potential cause of diarrhea in foals (Mair *et al.*, 2002, and Lun *et al.,* 2005). On the other hand *Eimeria leukarti* is commonly seen in foals. The goal of this study was to determine the prevalence of gastrointestinal parasites of horses in the Eastern Province of Saudi Arabia. Since understanding the parasitological problems will guide to the most appropriate prevention methods.

MATERIALS AND METHODS

Study Area

The current study was carried out in the Eastern Province of Saudi Arabia. Covered areas included horse farms that were located in Gabal Arba, Gewatha and Eastern villages in Al-Ahsa. Also Abohedrea Road in Al-Dammam and Safwa, Aljadoria, Alawjam and Alkadeeh in Al-Qatief were included. Additionally, samples were also collected from horses referred to the Veterinary Teaching Hospital at the College of Veterinary Medicine and Animal Resources, King Faisal University, Al-Ahsa. The study lasted one year starting in May, 2007 until April, 2008.

Collection, Transportation and Preparation of Samples

Purposive sampling was performed to conduct an epidemiological study on gastrointestinal parasites in horses at 90% level of confidence and 5% desired absolute precision and 50% expected prevalence as previously described (Thrusfield, 1995). Samples were examined using three techniques after macroscopic examination of samples for consistency, color, and presence of blood or mucus and also worms. These included direct smear, flotation and sedimentation techniques. McMaster slide was used for counting parasites ova. Micrometry using a Graticule was performed for measuring ova and larvae.

Direct Smear Technique

Direct smear was performed to identify parasitic protozoa using the described method (Sloss and Kemp 1978). Small amount of fecal sample was placed on a microscope slide to which few drops of saline solution were added and mixed to obtain an emulsion. Large bits of debris were removed by forceps. To enhance the internal structure of protozoan cysts a drop of lugol´s iodine was added to each smear. The slide was covered with a cover slip and examined using the 10X objective, and then the 40X magnification.

Flotation Technique

Flotation technique was carried out as described earlier (Sloss *et al.,* 1994). Two to three grams of fecal material were mixed with 15 ml of saturated salt solution 33% (Zinc sulfate salt solution SG 1.18). The mixture was filtered through double layers of tea strainer to remove the large debris. The mixture was poured in a 15 ml centrifuge tube and centrifuged for 5 minutes at 650 G. The supernatant was discarded and a salt solution was added. The centrifugation step was repeated. The surface of fluid was touched by a square-cut glass rod to remove the eggs that float on the surface then transferred to a slide and covered by a slip. The slide was scanned using 10X magnification lens of the microscope for detection of ova.

Sedimentation Technique

Sedimentation technique was used for diagnosing trematode ova (Urquhart *et al.*, 1987). The test was carried out as described by Zajac and Conboy (2006). One gram of fecal sample was mixed with about 10 ml of distilled water. The emulsion was poured into a 15 ml centrifuge tube and capped. Ethyl acetate was added until the tube was almost full. Centrifuge tube was capped, shaken approximately 50 times and centrifuged for 3 minutes at 500 g. The plug in the interface was rung and the supernatant was poured carefully to leave the pellet at the bottom of the tube intact. The sediment was resuspended in few drops of water. Two drops of the sediment was placed on a slide and covered with a slip. Slide was scanned using the 10X magnification lens of the microscope to detect ova.

Fecal Egg Count

Fecal egg count technique was adopted using a modified McMaster technique as described by Zajac and Conboy (2006). One gram of fecal sample was obtained from the positive sample. Each positive sample was homogenized with 14.0 ml of saturated salt solution (33% zinc sulfate solution SG 1.18). Then, it was sieved through a nylon tea strainer into another container. Additional 15.0 ml of saturated salt solution were added to wash the remaining debris. The mixture was agitated thoroughly and immediately a sample was taken and placed in the two counting chambers of a McMaster slide using a Pasteur pipette. Care was taken to ensure that no air bubbles were introduced in the slide. The slide was allowed to settle for at least five minutes before examination. The eggs were counted in both chambers under the microscope using 10X magnification using the following formula:

Eggs per gram= Total number of eggs counted × 200/ Number of chambers counted

Statistical analysis

Statistical analysis of the data was carried out using SAS statistical program (1986). Differences among means were de-

tected by chi-square and t-test. $P < 0.05$ was considered significant.

RESULTS

Prevalence of gastrointestinal parasites

Ova of some gastrointestinal parasites were detected in the feces of horses after applying either a direct smear method, where it was possible to encounter movements of some protozoan parasites and also other immotile parasites, or concentration methods for further detection of parasites. Most of the detected parasites were of nematode-types with the exception of trichomonad-type protozoa which only exhibited either cysts and/or trophozoite forms. Positive samples for parasites comprised 30.46% of all tested samples and the numbers of positive samples for each parasite was also expressed in a percentage are shown in Table 1. *T. equi* was amongst those detected parasites and possessed the highest percentage albeit, its protozoal nature. Percentage value pertaining to the detected *P. equorum* was found to be considerably the highest followed by other nematodes (Fig. 1). Again, although being protozoa, *E. leuckarti* was only detected in one sample throughout the study. In addition, it was found that nematode ova of *P. equorum* had the highest count per gram followed by *D. arnfieldi* (Fig. 2), *O. equi* (Fig. 3), Strongyles (Fig. 4),and oocysts of *E. leuckarti* respectively. Cysts of *T. equi* were determined per field.

Table 1
Gastrointestinal parasites detected in the feces.

	Positive Cases	Mean Count
Tritrichomonas equi	41 (13.58%)	6 cyct/field
Parascaris equorum	38 (12.58%)	971 ova/g
Dictyocaulus arnfield	7 (2.32%)	114 ova/g
Strongyle -type ova	4 (1.32%)	99 ova/g
Oxyuris equi	1 (0.33%)	99 ova/g
Eimeria leuckarti	1 (0.33%)	99 Oocyst/g
Total	92 (30.50%)	

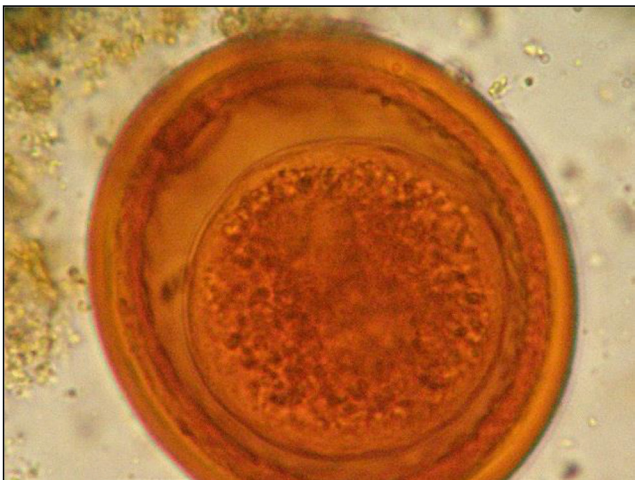

Fig. 1. *P. equorum* ovum at 100X magnification.

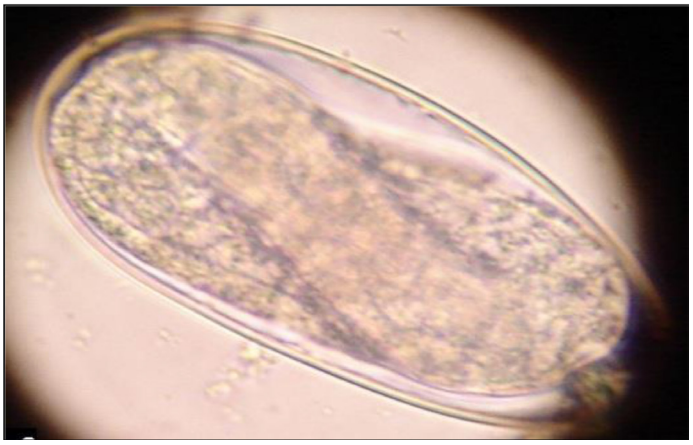

Fig. 2. *D. arnfieldi* ova at 100X magnification.

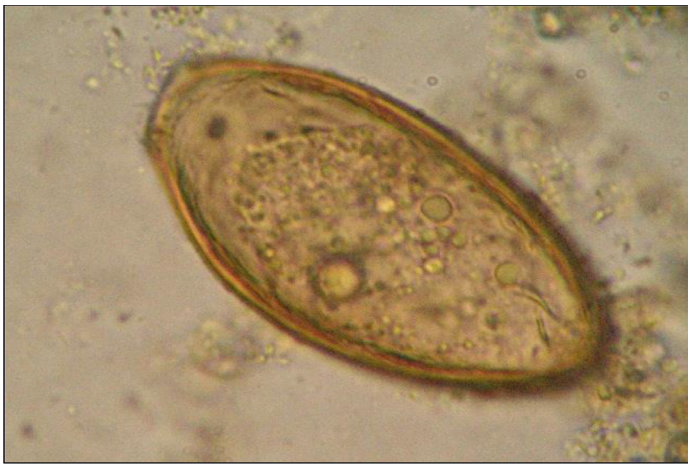

Fig. 3. *O. equi* ova at 100X magnification.

Fig. 4. Strongyles – type ovum at 100X magnification.

Seasonal prevalence

Collected data showed that the detected *P. equorum*, *D. arnfieldi* and *T. equi* were found in the feces of horses throughout the year while, strongyles, *O. equi* and *E. leuckarti* were only found in the cold season (Table 2). A general observation was that parasites detected throughout the year, exhibited higher percentage values in the warm season than those in the cold season.

Table 2
Number of gastrointestinal parasites according to the season and its % of total number of animals.

Season	Positive Cases						
	Strongyle	*P. equorum*	*D. arnfield*	*O. equi*	*T. equi*	*E.leuckarti*	Total
Warm	0.00	26 (15.20%)	5 (2.92%)	0.00	31 (18.13%)	0.00	171
Cold	4 (3.05%)	12 (0.16%)	2 (1.53%)	1 (0.76%)	10 (7.63%)	1 (0.76%)	131

Prevalence according to geographic source

Detailed values of the numbers and percentages of positive samples for each detected parasite in relation to places where groups of horses were raised in different locations are shown in Table 3. The majority of the values with high prevalence were noticed in Al-Ahsa region while the majority of low values for prevalence in Al-Dammam region.

Table 3
Number of gastrointestinal parasites from study regions in the Eastern Province of Saudi Arabia and its % of total number of animal.

Region	Positive Cases						
	Strongyle	*P. equorum*	*D. arnfield*	*O. equi*	*T. equi*	*E. leuckarti*	Total
Al-Ahsa	2 (0.99%)	29 (14.36%)	7(3.47%)	0.00	30(14.85%)	1(0.50%)	202
Al-Dammam	2 (2.00%)	9 (9.00%)	0.00	1(1.00%)	11(11.00%)	0.00	100
Total	4 (1.32%)	38 (12.58%)	7(2.30%)	1(0.33%)	41(13.57%)	1(0.33%)	302

Clinical signs associated with helminths infections

Although no clinical signs were observed on more than 50% of positively infected horses with helminths, a number of signs were observed on others infected with helminths. These signs are shown in Table 4 with a corresponding frequency in horses. Most seen signs according to frequency were the cough followed by emaciation. Colic, itching and nasal discharge were of lesser frequencies.

Table 4
Frequencies and percentages of clinical signs associated with helminths infections in examined horses.

Helminth	Clinical Sign						
	Colic	Emaciation	Cough	Itching	Nasal discharge	No Obvious Sign	Total
Strongyle	0	3 (75.00%)	0	0	0	1 (25.00%)	4
P. equorum	2 (5.26%)	4 (10.52%)	9 (23.00%)	0	1 (2.63%)	22 (57.00%)	38
D. arnfield	0	1 (14.28%)	1 (14.28%)	0	0	5 (71.42%)	7
O. equi	0	0	0	1 (100.00%)	0	0	1
Total	2	8	10	1	1	28	50

Shedding of gastrointestinal parasites during two different seasons from repeated cases.

Repeated examination of the horses was not possible on all animals due to the absence of some horses imposed by management necessities and therefore, about 62% of the horses were available as repeats. Detailed data are shown in Table 5. It could be noticed that most of the helminths and protozoa in positive repeated cases were more prevalent in warm season. Nonetheless, it could be noticed that all of the helminths in total positive of the repeated cases were shed during the cold season while the protozoa (*T. equi*) were more prevalent in the warm season. However, there were no significant differences ($p>0.05$) between effects of cold and warm months on the prevalence of the gastrointestinal parasites in repeated horses.

Table 5
Shedding of gastrointestinal parasites during worm and cold seasons from repeated cases.

	Worm Season				Cold Season			
	E. leukarti	*T.equi*	*D. arnfieldi*	*P. equorum*	*E. leukarti*	*T equi*	*D. arnfieldi*	*P. equorum*
Repeated Positives	1	17	1	11	0	4	0	3
%	1.30	22.10	1.30	14.30	0	5.20	0	3.90
Mean Count	99 ***	6 **	100 *	581 *	0	3	0	466

* (ova) ** (cyst) *** (oocyst)

DISCUSSION

Horses are exposed to a complex of gastrointestinal parasitic infections which compromises their health and welfare. These parasites have a high prevalence and are an important cause of morbidity and mortality (Matthews *et al.,* 2004). In the current study, six types of gastrointestinal parasites were present in the feces of horses from farms with an overall prevalence of 30.46%. These are *T. equi*, *P. equorum*, *D. arnfieldi, O. equi, E. leuckarti* and strongyles as diagnosed in the feces of examined horses during a one year study. The prevalences of *T. equi* and *P. equorum* were the highest amongst the parasites found (13.6% and 12.6% respectively). These were followed by the prevalence of 2.32% for *D. arnfieldi*. The prevalences of other parasites were 1.3% for strongyles which contradict with other findings in tropical countries probably as a result of environmental conditions (Lem *et al.,* 2012), 0.33% for *O. equi* and 0.33% for *E. leuckarti*.

The significant association that had been detected between the warm season and the occurrence of *T. equi* ($p<0.008$) in horses, was probably due to the increased relative humidity in the farms as irrigation becomes more frequent. This gives better chances for the parasitic transmission by flies as epidemiological studies suggest that flies may serve as mechanical vectors (Soulsby, 1986, and Garcia, 2001). Diarrhea has not been significantly associated with *T. equi* infection such clinical observation has been described (Damron, 1976). In fact, a debate had been taken place which supported the idea that considers enteric trichomonads do not precipitate a frank disease (Soulsby, 1986).

P. equorum with a prevalence of 12.6% was found in diagnosed horses. Similar ranges of prevalence were found in Hungary (15%), Iran (13.8%) and Poland (13.4%) (Szell *et al.,* 1999, Eslami *et al.,* 2005, and Kornas *et al.,* 2007). In the American continent, higher rates were reported in Kentucky that ranged between 22% and 39% (Lyons and Tolliver 2004, and Lyons *et al.,* 2006). The intensity of *P. equorum* infection was inversely related with the age of horses. Distribution of *P. equorum* in infected horses in the farms at Al-Ahsa area was found to be

significant ($p<0.023$) which paralleled the value of the prevalence of *P. equorum* that was more than those in Al-Dammam and Al-Qatief areas. It is worth mentioning that the number of examined foals in Al-Ahsa was greater than those examined in other regions (Al-Dammam and Al-Qatief), which was behind the greater registered values. Seasonality does not seem to be an important factor in the prevalence of *P. equorum.* The variation in the prevalence between cold and warm months did not show any significance ($p<0.117$). The ova of *P. equorum* were detected in the feces of horses throughout the year but the rate of infection was slightly higher during warm months. Transmission of equine parascarisosis is seasonal (Rodostitis *et al.,* 2002), but the eggs are very resistant to adverse environmental conditions and can be transmitted over the winter. Thus, the horses may in the absence of good hygiene become infected all year round (Rodostitis *et al.,* 2002). The clinical manifestations of this parasite were highly significant ($p<0.001$) and associated with specific signs including cough, emaciation, colic and nasal discharge which were described earlier (Ryu *et al.,* 2004, and Koudela and Bodecek 2006).

The prevalence of *D. arnfieldi* was 2.3% in horses which is similar to that reported in the USA by Lyons *et al.,* 1985a and b. However higher rates were detected in certain states like Kentucky (Lyons 1985). The discrepancy amongst those prevalence rates in different areas could be a result of the use of ivermectin. In fact, the use of ivermectin had reduced the prevalence of *D. arnfieldi* over the last 20 years (Boyle and Houston 2006). It seemed that seasonality had lesser effect on *D. arnfieldi* since infection rates in both warm and cold months had not shown any significant difference ($p<0.424$). This is in accordance with earlier reports proving that infection with *D. arnfieldi* were prevalent in horses and donkeys throughout the year (Pandey, 1980, and Soulsby, 1986). In this work, despite the fact that the ova of *D. arnfieldi* were detected in the feces of horses at higher rates in months that are warmer than in those colder, a significance in the difference was not found. It is generally believed that donkeys are the natural hosts of *D. arnfieldi* and that horses become infected following grazing with donkeys (Round, 1976). In this study, the *D. arnfieldi* positive animals were detected in Al-Ahsa area where the prevalence was 3.47%. This may be due to the mixed rearing of horses and donkeys that are raised on the same premises in this region. *D. arnfieldi* infection in horses is characterized by a chronic cough (Rodostitis *et al.,* 2002). This was evident in the current work in which cough was associated with *D. arnfieldi* in the examined horses. The prevalence of these parasites was 1.3% in the examined horses. The prevalence reported in this study is significantly less than the prevalence that was reported in other studies such as in Macedonia and Thessalia (42.5% and 45.6% respectively) (Sotiraki *et al.,* 1997), Brazil (80.5%) (Barbosa *et al.,* 2001), and Kentucky (27.6%) (Lyon and Tolliver 2004). Western Turkey (68.4%) (Cirak *et al.,* 2005), In the current work, the reported low prevalence rate may be due to the arid nature of the weather in the Eastern Province of the Kingdom of Saudi Arabia.

Strongyle parasites require an optimum level of herbage to successfully complete their life cycle (Smith, 2002). Low prevalence percentage found in this work may be also due to the high efficacy of treatment with ivermectin used by owners in most of the examined animals or to environmental factor (Lyon *et al.,* 2006, and Umar *et al.,* 2013). A work that has been conducted in Louisiana, USA showed that the effectiveness of modern anthelmintics such as macrocyclic lactones against the species of strongyles has substantially reduced their prevalence (Klei and French, 1998). Variation in the season had a significant impact ($p<0.021$) on the infection with strongyles that was manifested in cold season only. The infective larvae survive the winter in the soil beneath the fecal pats which may lead to infection of horses with these parasites during the cold season

(Kuzmina *et al.,* 2006). Variation in the pre-patent period (the time from ingestion of L3 to the excretion of eggs in the feces) differs among species of strongyles being from 2 to 11 months (Round, 1969, McCraw and Slocombe 1974, and McCraw and Slocombe 1985,). Therefore, horses probably became infected with these parasites during summer season without the presence of eggs in the feces. Strongyles were detected in Al-Ahsa and Al-Dammam. Nonetheless, district variation in strongyle infection was not significant ($p<0.231$). There were common symptoms which were associated with strongyle worms in horses at levels which are highly significant ($p<0.001$). These signs included emaciation 75% that has been reported earlier (Rodostitis *et al.,* 2002). However, subclinical infection must be considered since strongyles may infect horses without showing overt clinical signs (DiPietro *et al.,* 1997).

In this study, the prevalence of *O. equi* was considerably low (0.3%) compared to the prevalence of other helminths. Low prevalence has been reported (Epe *et al.,* 1993) in Germany and in Iran (Eslami *et al.,* 2005). In Vietnam and Poland, the prevalence of *O. equi* was considerably higher reaching 50% and 36% respectively (Dung *et al.,* 2001, and Gawor, 1995) studies described various prevalence rates, where in Pakistan, it was 12% (Mahfooz *et al.,* 2008), and in Macedonia and Thessalia 4.1% (Sotiraki *et al.,* 1997). In the current work, *O. equi* eggs were found in horse feces during the cold season. It is with no doubt, that the wet season may have played an important role in increasing the prevalence of *O. equi* (Rodostitis *et al.,* 2002). Noted signs on the infected animals were perineal pruritus and broken hair. Oxyuriosis is known to cause such clinical manifestations.

The prevalence of *E. leukarti* was 0.33%. In Kentucky, USA a prevalence of *E. leukarti* of 100% was reported (Lyon *et al.,* 1988). This huge discrepancy may be due to the insufficient number of foals used in this study. Oocysts of *E. leuckarti* occur in foal feces as early as 15 days after birth and continue for as long as 4 months (Lyon *et al*., 1988, and Bowman *et al.,* 2003).

In this study, the repeated samples that were collected during two different seasons (warm and cold seasons) revealed a decrease in the number of infected animals in the cold season. It is unclear whether this is a result of a change in the weather factor or as a result of treatment. However, the total of positive repeated cases in both warm and cold seasons did not show significant differences ($p>0.05$) between effects of cold and warm months on the prevalence of the gastrointestinal parasites in horses. This may be due to the recovery of some animals that were infected in the warm season and the appearance of newly infected animals in the cold season on the level of repeated herds. In conclusion, most of the gastrointestinal parasites that were detected in horses in the Eastern Province of the Kingdom of Saudi Arabia were nematodes and protozoa.

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انتشار الطفيليات المعدمعوية في الخيول في المنطقة الشرقية من المملكة العربية السعودية

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الملخص

الطفيليات المعدمعوية لها تأثير على صحة الخيل كما تسبب تحديات اقتصادية لملاك الخيل. هناك معلومات محدودة عن وجود هذه الطفيليات في الخيل في المملكة العربية السعودية. الهدف من هذه الدراسة توثيق مدى انتشار الطفيليات الداخلية في الخيل في المنطقة الشرقية من المملكة العربية السعودية. تم فحص 302 حصان من خلال فحص عينات البراز باستخدام تقنيات المسحة المباشرة والطفو والترسيب. تم تشخيص طفيليات الترايكوموناس، البارسكاريس اكيواريم، ديكتوكوليس ارنفيلدي، سترونجايل، اوكزورياس اكواي، ايميريا ليوكارتي. لوحظ شيوع الطفيليات في الأحساء أكثر من الدمام كما لوحظ حالات إيجابية أكثر في فصل الصيف.

الكلمات المفتاحية: الخيل، الطفيليات الداخلية، مدى انتشار، المملكة العربية السعودية.