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Validation of FECPAK^{G2} equipment and prevalence of equine gastrointestinal parasites in Polo horses in Plateau State, Nigeria

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Abstract

Gastrointestinal (GI) parasites are a global concern and cause serious damage to the health of animals, including horses, because of their deleterious effects on the health of these animals. Owing to improperly diagnosing the parasitic load and the resulting incorrect treatment, anthelmintics are becoming a global problem. Although rapid diagnostic techniques such as McMaster and FECPAK^{G2} are available in Africa, concentration techniques, including flotation and sedimentation, are commonly used in screening for gastrointestinal parasites. In this study, we compared the sensitivity of diagnostic techniques, namely, McMaster and FECPAK^{G2}, in quantifying and determining the prevalence of gastrointestinal parasites as an alternative to the commonly used flotation and sedimentation techniques for equine fecal samples in Jos, Plateau State, Nigeria. Three hundred fecal samples were collected from different locations. The instructions provided by the manufacturers of FECPAK^{G2} and McMasters were used as the methodology for egg count per gram of fecal matter, while parasite egg/ova identification was performed through sedimentation and flotation techniques. In general, a prevalence of 66.3% was recorded, with a higher prevalence on Babale ranch (84%) than on Jos Polo Club (62%) and Jos mounted troop (53%). A total of nine gastrointestinal parasites were identified via both sedimentation and flotation techniques: *Strongylus* spp. (19.3%) was the most prevalent parasite identified via the flotation method, whereas *Gastrodiscus aegyptiacus* (25.3%) was the most prevalent parasite identified via the sedimentation technique. In general, FECPAK^{G2} had a sensitivity of 86%, whereas McMaster had a sensitivity of 64% in terms of egg counts per gram of fecal sample. This finding suggests that equines in Jos are highly infected with a variety of gastrointestinal parasites to varying degrees, with *Strongylus* spp. and *Gastrodiscus aegyptiacus* being more prevalent. Although the parasite load was found to range from low to moderate levels, the majority of the equine samples contained < 200 eggs per gram, suggesting a low infection rate via both the McMaster technique and the FECPAK^{G2} technique. On the basis of these findings, FECPAK^{G2} is recommended for rapid diagnosis because of its prompt outcome and infestation rate as well as ease of routine examination. Additionally, routine examination should be carried out to determine infestation by gastrointestinal parasites in all equine holdings and the effectiveness of the therapeutic agents used.

Keywords Gastrointestinal parasites, Prevalence, Polo horses, FECPAK^{G2}

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Background

Globally, the estimated equine population is 60 million (Grant 2024). Nigeria alone has approximately 200,000–240,000 recorded horses (Nwobi et al. 2023). These horses are used for various purposes; for example, in Plateau State, which is known for its unique tourism and rich entertainment culture, these horses are used for sporting activities, coronation ceremonies, movie production, and military and paramilitary activities (Joachim et al. 2021). A look back in history will show that horses have long been associated with humans and have been recognized to harbor parasites since ancient Roman times, thus serving as reservoirs of infection (Kaplan et al., 2010). According to a review, horses suffer considerable harm from helminth parasites, with more than 150 different types of internal parasites known to parasitize them (Tolossa et al., 2013). Most gastrointestinal parasites infecting horses result in diseases that adversely affect their health, resulting in poor body condition, diminished power output, slow development, poor reproductive success and a shortened lifespan (Mulwa et al. 2020).

Although various techniques are employed in the diagnosis of helminthosis, most of these methods focus on speciation while placing secondary concern on the severity of infection and level of parasitemia. Fecal egg count has been adopted as one of the methods for investigating the intensity of helminth infection. The different fecal egg count (FEC) methods rely on microscopic examination of fecal preparations using a floatation solution. Among the widely used FEC methods for livestock and horses include McMaster (MAFF 1986), Cornell–Wisconsin (Egwang et al., 1982), FLOTAC (Cringoli et al. 2010), mini-FLOTAC (Gobler et al., 2015) and FECPAK^{G1} (Presland et al. 2005).

The FECPAK^{G1} fecal egg counting (FEC) technique (Presland et al. 2005) is similar in principle to the McMaster method (MAFF 1986) but differs in that the FECPAK^{G1} technique uses a larger microscope slide, providing an improved lower limit of detection. Although it was first developed for the diagnosis of ruminant samples, FECPAK^{G1} has also been found to be useful in other sectors. Since its launch in the 1990s, it has been utilized in the diagnosis of ruminant samples, but recently, the FECPAK^{G1} technique has been used for equine samples since 2004 (Presland et al. 2005).

More recently, an improvement in the earlier technique FECPAK^{G1} (Upjohn et al. 2010) resulted in the invention of FECPAK^{G2}, which has thus far been used for sheep, cattle, and other ruminants (Rashid et al. 2018). Relying still on the flotation principle, the FECPAK^{G2} technique allows for the floating of helminth eggs in a flotation solution, which is then viewed microscopically. The advantage of the FECPAK^{G2} technique over the other methods is that the results can be viewed with the aid of a digital camera rather than a microscope, and the test can be set up by a lay operator in the field with little difficulty, thus reducing the cost and maximizing the time required to process large sample sizes. In addition, the captured images can still be viewed afterwards.

Although FECPAK^{G2} has been used for a variety of animal groups, its use for equine samples has not yet been reported. Therefore, this study aimed to assess the sensitivity of FECPAK^{G2} in the detection of helminth ova in equines in comparison with the commercially available McMaster technique.

Table 1 Prevalence of parasite eggs/oocysts detected via sedimentation and flotation techniques from some stables in the Jos metropolis

Location of stable	No examined	No infected	% infected
Jos Mounted Troops	100	53	53
Jos Polo Club	100	62	62
Babale Ranch	100	84	84
Total	**300**	**199**	**66.33**

$\chi^2 = 2567.31$, df = 2, $P = 0.001$

Table 2 Prevalence of parasite eggs/oocysts detected via the floatation technique

Parasite	No encountered	% encountered
Oxyuris equi	16	5.3
Trichonema species	29	10
Anoplocephala perfoliata	21	7
Strongylus species	58	19.3
Triodontophorus species	19	6.3
Strongyloides westeri	24	8
Parascaris equorum	27	9
Total	**194**	**64.7**

$\chi^2 = 2743.02$, df = 7, $P = 0.001$

Results

A total of 300 horse samples were diagnosed with gastrointestinal parasites via two different methods in this study. Overall, 199 horses were found to carry different parasites, resulting in a prevalence of 66.33%. The prevalence of infection was highest in Babale Ranch (84%), followed by Jos Polo Club (62%) and Jos Mounted Troops (53%) ($P < 0.05$), as shown in Table 1.

Table 2 shows the parasites that were encountered in the study via the floatation method. Seven parasites were identified. Among these species, *Strongylus* species were the most prevalent (19.3%), followed by *Trichonema* species (10%). The least common species was *Oxyuris equi* (5.3%). With sedimentation techniques (Table 3), five

Table 3 Prevalence of parasite eggs/oocysts detected via the sedimentation technique

Parasite	No encountered	% encountered
Oxyuris equi	2	0.6
Trichonema species	24	8.0
Anoplocephala perfoliata	1	0.3
Strongylus species	57	19
Gastrodiscus aegyptiacus	76	25.3
Total	**160**	**53.3**

$\chi^2 = 640.0$, df = 4, $P = 0.001$

parasites were encountered. The most common species were *Gastrodiscus aegyptiacus* (25.3%) and *Strongylus* species (19%), with the least common being *Anoplocephala perfoliate* (0.3%). These findings indicate that there was a significant difference ($P < 0.05$) in the prevalence of parasites encountered when both diagnostic methods were used.

Assessment of the parasite species encountered at each study location (Table 4) revealed that *Strongylus* species and *Trichonema* species were the most prevalent parasites encountered in Jos Mountain Troops (28.3% and 22.6%, respectively) and Babale Ranch (31% and 23.8%, respectively). In Jos Polo Club, *Strongylus* species (27.4%) and *Gastrodiscus aegyptiacus* (17.7%) were the most prevalent ($P < 0.05$).

The intensity of helminth infection was assessed via the FECPAK^{G2} technique (Table 5), which revealed that most of the infections observed (70%) were low-intensity (< 200 EPG). The majority were Strongyles (33%). Moderate-intensity (501–600) infection was also observed in 32 Strongyles (10.67%) ($p < 0.05$). Inconsistency, the conventional McMaster technique (Table 6) significantly ($P < 0.05$) detected low-intensity (< 200 EPG) infections (44%), with most infections coming from Strongyles (26%) and moderate-intensity (501–600) infections (approximately 5%).

Consequently, the study revealed that (Table 7) the FECPAK^{G2} technique is more sensitive (86%) than the conventional McMaster technique (with a sensitivity of 64%) in detecting the intensity of infection ($P < 0.05$).

Table 4 Prevalence of parasite eggs/oocysts according to location

Location	Parasite	No encountered	% encountered	χ^2	*P*- value
Jos Mounted Troops	*Oxyuris equi*	4	7.55		
	Anoplocephala perfoliata	5	9.43		
	Strongylus species	15	28.30	224.54	0.001
	Strongyloides westeri	4	7.55		
	Parascaris equorum	4	7.55		
	Gastrodiscus aegyptiacus	6	11.32		
	Trichonema species	12	22.64		
	Triodontophorus species	3	5.66		
	Total	**53**	**100**		
Jos Polo Club	*Oxyuris equi*	6	9.67		
	Trichonema species	4	6.45		
	Strongylus species	17	27.42		
	Triodontophorus species	5	8.06		
	Strongyloides westeri	4	6.45	328.58	0.001
	Parascaris equorum	8	12.90		
	Paranoplocephala mamillana	7	11.29		
	Gastrodiscus aegyptiacus	11	17.74		
	Total	**62**	**100**		
Babale Ranch	*Oxyuris equi*	2	2.38		
	Trichonema species	20	23.81		
	Anoplocephala perfoliata	3	3.57		
	Strongylus species	26	30.95	696.45	0.001
	Triodontophorus species	8	9.52		
	Strongyloides westeri	4	4.76		
	Parascaris equorum	5	5.95		
	Gastrodiscus aegyptiacus	16	19.04		
	Total	**84**	**100**		

Table 5 Intensity of infection based on the FECPAK^{G2} technique

Parasite	EPG range (level of infection)						
	0 (none)	**< 200 (low)**	**200–500 (mild)**	**501–600 (moderate)**	**601–1000 (high)**	**> 1000 (severe)**	**Total**
Strongyles	84 (28%)	98 (32.67%)	36 (12%)	32 (10.67%)	0 (0%)	0 (0%)	250 (83.34%)
Parascaris equorum	0 (0%)	8 (2.67%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	8 (2.67%)
Strongyloides westeri	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	84 (32.56%)	186 (70.09%)	36 (13.95%)	32 (12.40%)	0 (0%)	0 (0%)	**258 (100%)**

$\chi 2 = 459.67; P = 0.001$

Table 6 Intensity of infection based on the conventional McMaster technique

Parasite	EPG range (level of infection)						
	0 (none)	**< 200 (low)**	**200–500 (mild)**	**501–600 (moderate)**	**601–1000 (high)**	**> 1000 severe)**	**Total**
Strongyles	73 (24.33%)	79 (26.33%)	18 (6%)	16 (5.33%)	0 (0%)	0 (0%)	186 (62%)
Parascaris equorum	0 (0%)	6 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (2%)
Strongyloides westeri	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	73 (38.02)	85 (44.27)	18 (9.38)	16 (8.33)	0 (0)	0 (0)	**192 (100%)**

$\chi 2 = 266.01; P = 0.001$

Table 7 Comparative sensitivity of the FECPAK^{G2} and McMaster techniques

Method	No. examined	No. infected (%)	Sensitivity (%)
McMaster	300	192	**64%**
FECPAK^{G2}	300	258	**86%**

$\chi 2 = 450.00, P = 0.001$

Discussion

In this study, two different methods of helminth parasite detection were compared (the McMaster method and the updated FEDPACK method). In addition, the floatation and sedimentation techniques for parasite detection were also used in the detection of helminth infection in equine samples. Among the 300 horses sampled, 199 (66%) were infected, indicating a high prevalence. A similar finding was reported by Ola-Fadunsin et al. (2018) in the states of Kwara and Niger, Nigeria. Other authors, such as Saeed et al. (2010), Regassa and Yimer (2013) and Tesfu et al. (2014), reported prevalences of 62.86%, 65.5% and 60.80%, respectively, which were similar to what we found in this study. In contrast to our findings, however, Bulgaru and Tudor (2015), in Romania, as well as Matto et al. (2013), in a work done in India, reported prevalences of 28.57% and 20.63%, respectively, which were much lower than our findings. In Nigeria, there are reports of higher prevalence rates than those reported in this study: 70.8% in Kaduna (Umar et al. 2013) and 76.1% in Abuja (Wosu & Udobi 2014). In Ethiopia, Mezgebu et al. (2013) reported a prevalence of 80.95%. These variations in the observed prevalence across the different studies may have arisen from factors such as variation in climatic conditions, hygienic conditions of the stables as well as the pasture fed to the horses, breed-related conditions and the management system adopted.

Notably, infection was greater in Babale Ranch, which is a privately owned facility, than in the Jos mounted troop and Jos Polo Club, which are government-owned facilities. Perhaps the purpose for which the horses are kept in both Jos mounted troops (security-wise) and Jos Polo Club (sports) necessitates their being properly taken care of compared with Babale Ranch. In addition, as a government facility, the animals might have been taken care of by well-trained personnel other than those in Babale Ranch. Another reason could be that Babale Ranch is located in the city outskirt, and since the horses there are exposed to paddock sessions, this could have predisposed them more to infection. Furthermore, the management system in which horses in the Jos mounted troops and Jos Polo Club are fed more grain-by products instead of pasture, which may have been exposed to contamination with the eggs/oocysts of these helminths from the previous season, could have led to the reduced prevalence recorded.

The float technique was able to detect seven parasites with a total prevalence of approximately 65%, among which *Strongylus* species 1 (9.3%) and *Trichonema* species (10%) were more dominant. However, the sedimentation

technique detected only 5 parasites, with a prevalence of 53%, *Gastrodiscus aegyptiacus* (25.3%) and *Strongylus* species (19%) being the most dominant. Although the float technique appeared to detect most of the parasites (65%), the sedimentation method appeared to be more specific for parasite detection. Overall, nine (9) different gastrointestinal parasites were detected in this study. In another study, Ola-Fadunsin et al. (2018) recorded a greater number of cases. Other authors, such as Umar et al. (2013), Ehizibolo et al. (2012) and Romero et al. (2020), recorded fewer cases. These differences could be due to the two diagnostic techniques adopted in this study. The parasites encountered in this study included *Strongylus* spp., *Gastrodiscus aegyptiacus, Trichonema* spp., *Parasacaris equorum, Triodontophorus* spp., *Strongyloides westeri, Anoplocephala perfoliata, Oxyuris equi* and *Paranoplocephala mamillana*. *Strongylus* spp. are the most prevalent among all the parasites encountered and could be due to their direct mode of transmission, requiring no intermediate host, thus facilitating their transmission among horses in a stable state. Assessment of the parasite species encountered in each study location revealed that *Strongylus* species and *Trichonema* species were the most prevalent parasites encountered in Jos mounts (28.3% and 22.6%, respectively) and Babale Ranch (31% and 23.8%, respectively). In Jos Polo Club, *Strongylus* species (27.4%) and *Gastrodiscus aegyptiacus* (17.7%) were the most prevalent. Among the *Strongylus* spp. associated with equine infection, *Strongylus edentatus, S. vulgaris,* and *S. equinus* are implicated. Among the remaining members of the genus, infection with *S. equinus* is least common (Ola-Fadunsin et al., 2018). Factors such as sex, breed, access to pasture, anthelminthic use, season, geographic area and emergence of drug-resistant strains (Romero et al. 2020) may facilitate the transmission of these parasites.

The intensity of infection was assessed via the FECPAK^{G2} technique and the conventional McMaster technique. In both techniques assessed, there were more low-intensity infections, particularly for Strongyles. The FECPAK^{G2} technique was more effective and detected 70% low-intensity (< 200 EPG) infections, with the majority being Strongyles (33%). Since the FECPAK technique has been regarded previously as a more sensitive technique in terms of high infection (Cringoli et al. 2004; Rinaldi et al. 2014), the results obtained in this study could suggest a high intensity of infection in the sampled horses. Although most of the infections observed were of low intensity, mild and moderate infections were also recorded. The results obtained via the McMaster technique were much lower than those obtained via the FECPAK^{G2} technique. Prevalence rates of 62% and 2% were recorded for Strongyles and *Parascaris equorum,* respectively, via the McMaster technique, whereas 44.7% and 5.3%, respectively, were reported by Mulwa et al. (2020) for the same parasites in donkeys. This variation could be a result of varying degrees of exposure to pastures and climatic conditions as well as the season in which the study was carried out. This study recorded an EPG of 0–599 for Strongyles. In contrast, Mulwa et al. (2020) and Vercruysse et al. (1986) reported higher ranges (0–1,900 and 100–9,100, respectively, for Strongyles and *Parascaris equorum*). Most likely, since this study was performed in the rainy season, it accounted for the parasites detected as well as the burden of the infection, in contrast to the findings of Mulwa et al. (2020), who worked in the dry season and detected other parasites in addition to those we reported.

Our results showed that the FECPAK^{G2} technique is more sensitive (86%) than the conventional McMaster technique (with a sensitivity of 64%) in diagnosing equine samples. In contrast, Mohammed et al. (2018) reported lower sensitivity (77% and 64%, respectively) in *Huacaya alpacas,* and Rinaldi et al. (2014) reported a sensitivity of 66.7% in cattle than was found in this study. This variation observed in sensitivity could be due to the different animals used as well as the floatation medium (for example, Mohammed used a sugar and salt solution). However, it has been reported previously that FECPAK^{G2} is more sensitive than the McMaster technique. In addition, Presland et al. (2005) reported a high percentage of error in McMaster's technique compared with the FECPAK technique at low egg densities. Since the FECPAK is a product derived on the basis of improvements made in McMaster's technique (Presland et al. 2005; Rinaldi et al. 2014), it is most likely that this should have a higher degree of efficiency.

Conclusion

FECPAK^{G2} equipment, which is a modification of the McMaster egg counting technique, is designed to determine the degree of infection, especially with respect to nematode parasites in individual animals or a herd/flock. Owing to the high sensitivity of this technology, it is highly recommended for the quantitative detection of nematode parasites, with less emphasis on the speciation of such parasites. In this test, human errors in parasite egg counting are overcome with automation. The parasites encountered in this study included *Strongylus* spp., *Gastrodiscus aegyptiacus, Trichonema* spp., *Parasacaris equorum, Triodontophorus* spp., *Strongyloides westeri, Anoplocephala perfoliata, Oxyuris equi* and *Paranoplocephala mamillana*. *Strongylus* spp. was the most prevalent among all the parasites encountered and could be due to its direct mode of transmission, requiring

no intermediate host, thus facilitating its transmission among horses in a stable state.

Methods

Study area

The study area is the plateau state, the fifth largest state in Nigeria by land mass. The plateau state is located around central Nigeria and has a topography that ranges from hills surrounding the Jos Plateau. Two local government areas (Jos North and Jos South) were selected for the study. Jos South, which may be referred to as the de facto capital of Plateau State, has its headquarters in Bukuru and houses the governor's office in Rayfield. Jos South has coordinates of 9°48′00″N 8°52′00″E with a population of 306,716 at the 2006 census and occupies an area of 510 km^2. On the other hand, the Jos north local government area had a population of 429,300 at the 2006 census and covered a land area of 291 km^2.

Sample collection

The horses sampled for this study were from both government and privately owned establishments in the two selected local government areas of study. Sampling was performed between March and October 2021. The sample consisted of three hundred (300) horses of different ages, breeds and genders. Approximately 5 g of fecal material was collected from the rectum of each horse or soon after defecation into a zip lock bag, which was properly labeled and transported in cool cases (Stoltenow & Purdy 2003) immediately to the Veterinary Parasitology and Entomology Teaching and Research Laboratory, University of Jos, Jos, Nigeria. The laboratory diagnosis was carried out.

Laboratory diagnosis

FECPAK^{G2} technique

Following the manufacturer's instructions, approximately 3 g of the fecal material was dispensed in a Fill-FLOTAC device container, to which tap water was added to make up the 40 mL volume, which was mixed by moving the conical collection in an up and down and right and left motion. The fecal suspension was then homogenized and moved to the FECPAK^{G2} sedimenter container, which was then rinsed with another 40 mL of tap water into the sedimenter. Water (210 mL) was then added to the waterline beneath the top of the sedimenter, which separated the debris in the supernatant from the helminth eggs and other debris that formed the sediment. The sedimentation step was followed by closing the sedimenter and inverting it three times for proper mixing. This mixture was then left standing for 30 min. Thereafter, the supernatant was decanted through the A-side of the sedimenter, and the helminth eggs were left behind in the sedimenter (approximately 15 mL). The flotation solution was then added to the sediment to achieve a total volume of 95 mL, and after mixing, the mixture was collected through the B-side of the sedimenter into the FECPAK^{G2} filtration unit. After the samples were mixed properly, aliquots were pipetted into two corresponding wells of the FECPAK^{G2} cassette and inserted into the MICRO-I for imaging. After each imaging step, the cassette was expelled from the MICRO-I, emptied and rinsed with tap water for reuse. The images captured were then uploaded to FECPAK^{G2} software to enable viewing, identification and counting of the parasite eggs online.

McMaster technique

This technique utilized the filtrate that was previously prepared as described in the FECPAK^{G2} technique and drawn into McMaster chambers after agitation and allowed to stand for 30 s for the eggs to float underneath the engraved areas before counting under a microscope with × 4 and/or × 10 objective lenses. The number of eggs per gram (EPG) of feces was estimated by multiplying the total number of eggs seen within the two chambers by 100. Since every chamber is 0.15 cm deep and has an etched area that is 1 cm × 1 cm, the total volume under the engraved area is 0.3 ml, as described by the manufacturer.

Sedimentation technique

For this technique, the fecal slurry used in the FECPAK above (3 g) was used. The slurry was resuspended in tap water for 30 min to sediment. The mixture was then filtered and centrifuged for 10 min at 3,000 rpm. Thereafter, the supernatant was discarded, the sediment was viewed under a microscope at × 10 and × 40 objectives, and the parasite ova were classified according to the keys provided by Ballweber (2014) and Cheesebrough (2006).

Flotation technique

This technique utilized the same fecal salt mixture, which was made for FECPAK.

The solution was transferred into test tubes and brought to full to the brim via a saturated salt solution. A coverslip was then gently placed over the test tube to make contact with the convex meniscus formed by the floatation solution. The slides were set up for 10 min before they were viewed via × 10 or × 40 objective lenses. Standard keys (Soulsby 1982; Cheesbrough, 2006; Taylor et al. 2016) were then used for the identification of parasites (helminth ova and *Eimeria* oocysts).

Statistical analysis

The collected data were imported into J. M. P. 13.0 statistical software for analysis. The data are presented in Tables as simple frequencies and percentages. A chi-square goodness-of-fit test was used to determine if the prevalence of infection differed significantly across locations, and the parasites were observed via different diagnostic methods (floatation and sedimentation). In addition, a chi-square test of associations was performed to ascertain if there was a significant association between the intensity of infection and the two diagnostic techniques (FECPAK^{G2} and the conventional McMaster). The results were considered significant when $p < 0.05$.

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Authors' contributions
G.J.G. and N.N. conceived the project. G.J.G., N.N.; E.E.R., P.M.N.; O.E.O. and O.D.O. performed the experiments and organized the data. I.G.N., L.L.H., D.C.H., and D.A.A. offered helpful discussions and technical assistance. N.N., I.G.N. and L.L.H. revised the manuscript. All the authors have read and approved the final version of the manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Verbal and written consent to conduct the study was obtained from the horse owners.

Consent for publication
All the authors approved and provided their consent for publication of the manuscript.

Competing interests
The authors declare that they have no competing interests.

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