

Research Article



Clinical, Prevalence and Molecular Studies of *Parascaris equorum* in Infected Horses in Al-Muthanna Province

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Abstract | Due to a lack of research on parasite infections in horses in Iraq, the aims of the current investigate is to assess the clinical manifestations linked with ascariasis in infected horses. Additionally, it seeks to assess the prevalence of ascariasis in horses in Al Muthanna province and evaluate the impact of various risk factors on the infection rate such as age, sex, and seasonality. Moreover, the PCR was used to confirm the presence of *P. equorum* in the horses. This study was conducted on 138 samples of horse feces, which gathered from various locations within the Al-Muthanna province during the period extended from January 2022 and the end of October 2022. The most typical clinical indications of ascariasis infection in horses were diarrhea 51.4%, increased heart rate 50.27%, respiratory rate 46.37%, coughing 50%, snoring during breathing 48.55%, nasal and ocular discharge 49.27%, and rough hair coat 52.89%. Also, results of epidemiological investigation discovered that out of 138 examined horses, 75 tested positives for ascariasis, with infection rate of 54.34% depended to flotation technique. A correlation was observed between climatic conditions and the occurrence of ascariasis, with the highest infection rates occurring in winter (73.1%) and the lowest in summer (26.6%). Regarding gender, infection rates were 66.6% for females and 45.67% for males. In contrast, the age group of <3 years had the highest infection rate at 72.41%. While, the infection rate had been lowest (32.75%) in the age group of >6 years (Old). On the other hand, PCR findings indicated that 36 (72%) out of 50 horses were positive to *p. equorum* in PCR. The current study concluded that PCR demonstrated higher specificity and sensitivity for diagnosing ascariasis in horses compared to the flotation technique. Additionally, the study found that, although ascariasis occurs throughout the year, it is most prevalent in winter. The survey also showed that males and horses older than 6 years are less susceptible to ascariasis.

Keywords | Clinical, Prevalence, Molecular analysis, *P. equorum*, Horses

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INTRODUCTION

The horse is an important animal that plays a vital role to resource-poor populations in both agricultural and urban locations, that they provide energy for agriculture, as well as transportation services in difficult terrain and on narrow streets (Bizuyehu and Bedada, 2018). They are also very important in the police services, crowd control, research, and pleasure riding and racing in the equestrian clubs (Idoko et al., 2021; Alaba et al., 2022). The number of equines worldwide is estimated to be 122.4 million, of

which 12% are mules, 20% zebras 33% donkeys and 35% are horses (Ali et al., 2018). However, since the 1980s, the number of horses in world has gradually decreased as a result of road development, decreased grassland and forest area, and inadequate attention and medical care given to horses (Devkota et al., 2021). Furthermore, traditional methods persist in horse care and management, involving antiquated farms or individual stables for each horse, along with insufficient veterinary attention. As reported by Ottman and Alzuheir (2019), these factors collectively contribute to the deterioration of animal welfare and pro-

ductivity, thereby amplifying the prevalence of infectious diseases, including internal parasites. Horses, like other domestic animals are susceptible to a variety of pathogenic includes parasites in the gastrointestinal tract such as *Parascaris equorum*, *Oxyuris equi*, *Strongylus spp.* which can significantly affect an animal's productivity and ability to reproduce and associated medical costs for control and treatment. The severity of the damage depends on the age, nutritional and immune status of the horse, as well as the number and species of parasites present (Lashari and Tasawar, 2011; Asefa and Dulo, 2017; Devkota et al., 2021).

Ascariasis is a global endoparasitic disease caused by *P. equorum* in equine, which is a member of the phylum Nematoda and the family Ascarididae. It has been determined to be the most severe pathogenic parasite infection in horses, with a major effect on health, performance and productivity, and they primarily affect young horses (Hautala et al., 2019; Scala et al., 2021). For horses, contaminated food and water with infective stage (egg containing L2) are likely the main sources of infection, and the eggs can live for at least a year outside in pastures and boxes (Reinemeyer and Nielsen, 2017). The most significant clinical signs in horses infected with ascariasis are nasal discharge, coughing, respiratory distress, weight loss, rough hair coat and rusty appearance due to mineral deficiency, poor growth, diarrhea, and colic. In addition, occasionally small intestine rupture occurs in infected animals due to small intestinal impaction after heavy infection (Cribb et al., 2006; Nielsen, 2016; Cain and Nielsen, 2022). On the other side, successful control of infectious diseases like ascariasis is largely dependent on diagnostic techniques. Traditionally, the diagnosis of parasitic infections in laboratories has relied on the microscopic identification and morphological detection of different stages in the parasite life cycle from clinical samples. However, these techniques have some limitations, including the inability to detect other parasitic stages including infections in the larval tissue stage and variable sensitivity and specificity (Ghafar et al., 2023). Besides, most parasites cannot be distinguished based solely on egg morphology (Lichtenfels et al., 2008). Consequently, a more sensitive methods, such as polymerase chain reaction (PCR) is the most commonly employed technique and the foundation for numerous additional molecular methods in horse parasitology. There has been little study conducted regarding the clinical and epidemiological aspects of ascariasis infection, despite the fact that disease poses an important risk to horses' health. Thus, the objective of this investigation was to describe the clinical manifestations linked to the infection and assess the influence of age, gender, and seasonal variation on the infection rate. Finally, PCR technique was used to confirm the diagnosis and differentiation of *P. equorum* in the horses.

ETHICAL CLEARANCE

It was obtained from the Faculty Scientific Committee (College of Veterinary Medicine, Al-Muthanna university, Iraq) numbered 202708 – Naer Abdulbari Madlool Al-kaabawi.

AREA OF STUDY AND COLLECTION OF SAMPLES

For this study, 138 horse feces samples that were randomly collected from villages located in Alkider, Rumathia and Samawah in Muthanna province, southern Iraq. Collection of samples spanned from January 2022 to the end of October 2022. Horse feces samples (5 grams) were gathered and stored separately in plastic containers. These containers were transported in a cool box to the Veterinary College parasitology laboratory at Al-Muthanna University for analysis. All samples were labeled with details concerning gender, age, and the month of collection and this was done as part of the current study's goal to determine the impact of specific factors that may affect the infection rate, such as age categories, sex, and seasons. The examined animals were divided into three age categories: < 3 years (young), 3-6 years (Adult) and > 6 years (Old). The dental formula has been utilized to determine their age (Mezgebu et al., 2013). The fecal sample were collected from about 86 female and 52 males. Furthermore, all horse underwent a thorough clinical examination. This includes evaluating their general health, body temperature, and external shape (nature of the skin), heart rate, respiratory rate and examining for superficial lymph nodes. All signs that have been observed were recorded.

EXAMINATION OF FAECAL SAMPLES IN THE LABORATORY

According to Devkota et al. (2021) the flotation technique with sheather's solution was used every day to examine fecal samples collected from all horses in order to identify *P. equorum* eggs. The eggs were recognized based on their morphology characteristics, as described earlier (Soulsby, 1982).

EXTRACTION OF DNA AND PCR ASSAY

The genomic DNA from eggs obtained from 50 fecal samples of horses infected with *P. equorum* was isolated using the DNA Stool Mini Kit/ QIAGEN, Germany. By using 1% agarose gel electrophoresis with 0.05% ethidium bromide, the DNA's integrity was evaluated. Partial DNA sequences of *P. equorum* were taken from GenBank (Accession No.MF678787.1) for primer design. PCR was conducted to identify *P. equorum* in infected horses using a primer designed to amplify a 231 bp-long fragment of the ITS-1 gene, as shown in Table (1). The PCR program involved an initial denaturation at 94°C for 5 mins., suc

Table 1: The primer design sequences utilized in the PCR assay.

Primer	Sequence	Product Size (bp)	Characterized
ParaF	5- CGTCTACGCTTCACCGAGTT-3	231 bp	<i>P. equorum</i>
ParaR	5-CATCGATCC ACGAACCGAGT-3		

ceeded by 35 cycles of denaturation at 94°C for 25 secs., annealing at 52°C for 25 secs., and extension at 72°C for 40 secs. After that, a final extension was done at 72°C for 5 mins. After amplification, all PCR results were analyzed. The amplification samples (5 µl) were loaded on a 1.5% agarose gel for electrophoresis, and the results (bands) were seen using a UV transilluminator (Jasim et al., 2015).

STATISTICAL ANALYSIS

The statistical program SPSS was used to analyze the variance of the data in this study (Chi square). Significance was determined for mean differences at a value of P≤0.05.

RESULTS

Total number of horses clinically examined is 138 horses of various ages and sexes. Table (2) shows the clinical manifestations that have been observed during the horses' examinations. Also, the parasitological examination findings demonstrated that *P. equorum* eggs have been recognized in horse feces samples, as shown in Figure (1). The epidemiological investigation results revealed that only 75 of 138 horses examined were positive for *P. equorum* during the study period from January 2022 to the end of October 2022 with an infection rate of 54.34% based on the laboratory examination by using the floatation technique. The findings of this investigation demonstrate the prevalence rates of ascariasis infection and the variations between males and females. The results indicate that highest percentage of infection with ascariasis were recorded in females in 66.6%. While, the percentage of infection with ascariasis were recorded in male is lower than female in 45.67%, as shown in Table (3). The survey results were showed that ascariasis able to produce the infection in all age groups, which recorded highest percentage of infection with ascariasis in horses with age group < 3years (young) 72.41 %, followed by age group 3-6 years (Adult) in 68.62%. Whereas, the current study's findings showed that the age group older than six years (Old) had a decreased infection rate at 32.75%, as indicated in Table (3). In addition, according to the survey study, the highest proportion of ascariasis infections was observed in winter at 73.1%, whereas, the lowest infection rates were recorded in summer at 26.6%, as presented in Table (4). On the other hand, the present study was revealed that genomic DNA of eggs was successfully extract and purified from 50 faecal samples obtained from horses infected with ascariasis using the DNA Stool Mini Kit/ QIAGEN, Germany. Following that, agarose gel electrophoresis was used to evaluate the

Table 2: The most important clinical signs associated with horses infected with ascariasis.

Clinical sings	No. of horses examined	Percentage %
Diarrhea	71	51.4
Increase Respiratory rate	64	46.37
Coughing	69	50
Snoring during breathing	67	48.55
Increase Heart rate	70	50.72
Nasal and Eye discharge	68	49.27
Rough hair coat and rusty appearance	73	52.89
Colic	54	39.13
Emaciation (weight loss)	74	53.62
Congestion of mucous membranes	53	38.4
Fever	15	10.86

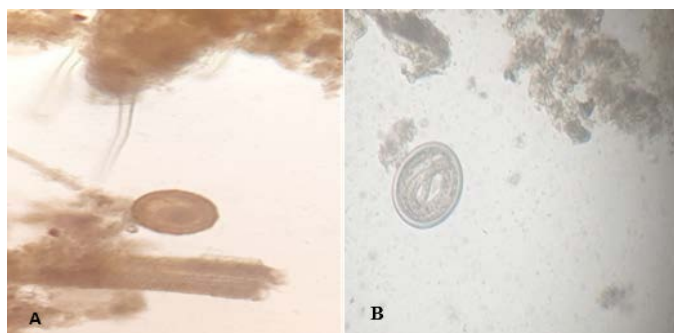


Figure 1: Eggs of *P. equorum* unstained isolated from infected horse, A- *P. equorum* uncellular egg, B- *P. equorum* egg contain Larvae (10X).

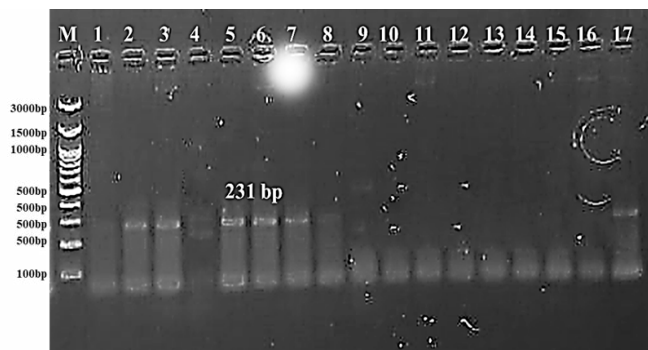


Figure 2: *P. equorum* amplified DNA utilizing primers (ParaF, ParaR) from the ITS-1 region, on an agarose gel. Lanes 2, 3, 5, 6, 7 and 17 positive product; Lane M: Marker (DNA ladder).

Table 3: The percentage of horses infected with ascariasis based on age and sex.

Age	No. of horses examined	No. of horses infected	%	Male			Female		
				No. of horses examined	No. of horses infected	%	No. of horses examined	No. of horses infected	%
< 3years (young)	29	21	72.4	15	9	60	14	12	85.7
3-6 years (Adult)	51	35	68.6	31	20	64.5	20	15	75
>6 years (Old)	58	19	32.7	35	8	22.8	23	11	47.2
Total	138	75	54.3	81	37	45.6	57	38	66.6

Chi square =13.00

P value =0.011

Table 4: The proportion of horses infected with ascariasis in relation to seasons of study.

Season	No. of horses examined	No. of horses infected	%
Spring	32	19	59.3
Summer	30	8	26.6
Autumn	35	18	51.4
Winter	41	30	73.1
Total	138	75	54.34

Chi square = 9.00

P value = 0.035

extracted DNA's quality and purity by loading the genomic DNA. There was no indication of degradation in the gel image, which displayed a distinct and continuous band of genomic DNA. For the identification of *p. equorum* in the infected horses, the PCR technique was utilizing specific primers (ParaF, ParaR), which showed that 36 (72%) of the 50 horses tested positive for *P. equorum* as shown in Figure (2).

DISCUSSION

Ascariasis disease is considered one of the most common problems that cannot be ignored and that confronts horses in many regions of the world, because it is responsible for the poor condition of the body, which limits its performance and efficiency (Al-Biatee et al., 2024). The findings of this investigation proved that the horses had ascariasis, confirming the disease's prevalence in the parts of Iraq where horses are found. Moreover, the clinical signs of horse's ascariasis in have been described only in a few numbers of investigations. The present study's findings revealed clinical manifestations that agreed with the findings described by Ananzi and Alyousif (2011), Umar et al. (2013), and Kompi et al. (2021). Larval migratory pathway is responsible for the observed clinical signs in the study. Firstly, traveling through the portal veins to the liver, larvae then enter the bloodstream and go from the heart to the pulmonary veins. The third-stage larvae (L3)

subsequently enter the alveoli after emerging from the pulmonary venules and capillaries. Following that, they pass via the bronchioles and travel toward the trachea before being expelled by coughing into the pharynx and returning to the gastrointestinal tract. They undergo numerous molting stages inside the gut in order to reach maturity, which can result in intestinal obstruction or rupture, especially in cases where the infection is intense (Clayton, 1986; Reine-meyer and Nielsen, 2017). Besides, mature worms actively move through their intestinal fluids and feed using a sucking mechanism made possible by the pharyngeal or esophageal muscles (Clayton and Duncan, 1978; Clayton et al., 1980). The low growth rate of the affected animals was possibly caused by their lowered food intake, which also led to a decrease in their protein consumption (Sri-hakim and Swerczek, 1978; Clayton et al., 1980). Also, the parasite's capacity to suppress the immune system and increase the animals' exposure to other infections may result in clinical symptoms. (Abdul-Majeed, 2004). On the other hand, according to the flotation technique, only 75 out of the 138 horses that were examined had an overall rate of ascariasis infection of 54.34%. These results were very close to the earlier researches with (Epe et al., 1993; Alamma-ra et al., 2008; Alhaitami, 2023), and disagreement with (Al-Alousi et al., 1994; Tavassoli et al., 2010; Othman and Alzuheir, 2019), which found that the ascariasis infection rates were 10.52%, 12.2% and 15.6% respectively. Various environmental factors within the experimental area, such

as temperature, moisture, rainfall season, type of pasture and horse rearing and grazing methods, could be the cause of the variations in infection rates (Oli and Subedi 2018; Alaba et al., 2022; Alhaitami, 2023). Moreover, variations may be attributed to the possibility that the prevalence of ascariasis was influenced by intermittent use of anthelmintics as well as ineffective control and management programs (Belete and Derso, 2015). In addition, different sample number, sample selection criteria, animal breeds, study duration and location, and the detection technique used to identify infection are all responsible for the findings' differences from previous investigations (Belete and Derso, 2015; Devkota et al., 2021). Moreover, the results of this study support previous studies by showing that gender has a significant impact on the prevalence of ascariasis (Yadav et al., 2014; Zelpina et al., 2022), and conflict with (Wannas et al., 2012; Othman and Alzuheir, 2019; Alhaitami, 2023), who indicated that males were more likely than females to get ascariasis. Variation in sensitivity to ascariasis infection between males and females could be as a result of differences in their breeding systems, Where females are usually utilized in order to get pregnant and reproduce and these may have suppressed their immune system result of stress caused by cyclical hormonal changes, this is due to a well-established correlation between immune suppression factors such as pregnancy and lactation, and an increased susceptibility to infection (Ademola and Onyiche 2013; Alaba et al., 2022). The study discovered that 72.41% of horses under the age of three (young) have Ascariasis infection, whereas 32.75% of horses over the age of six (old) have the lowest rate. The results agreed the findings of numerous earlier investigations (Anazi and Alyousif, 2011; Scala et al., 2021; Alhaitami, 2023) that found that horses younger than one year old had a higher infection rate. Furthermore, Bucknell et al. (1995) discovered that this parasite was restricted to horses younger than 2 years old. While, our results are different from those of Ali et al. (2018), who recorded that the higher prevalence rate among old equines. This discrepancy could be attributed to deteriorating body condition and immune suppression in old horses or young horses have probably had minimal to no anthelmintic treatment (Sapkota, 2009; Alhaitami, 2023). Besides, the results of our study revealed that the incidence of infection with ascariasis was related to the season. These findings were in agreement with several earlier investigations, including those conducted by Anazi and Alyousif, (2011), and disagree with the findings indicated by Aromaa et al. (2018); Scala et al. (2021), which observed the highest percentage of infection during spring and lowest in the winter. A probable explanation for the seasonal variation can be attributed to the fact that the disease is closely linked to the effects of climatic condition that involved temperature and rainfall, any few changes on these factors may directly affect the life cycles, as well as

the infective eggs sensitive to dehydration (Mfitlodze and Hutchinson, 1989; Anazi and Alyousif, 2011). On the other hand, a molecular technique is required for the diagnosis and differentiation of *P. equorum* particularly polymerase chain reaction (PCR) with sequencing and phylogenetic analysis in order to overcome the problems encountered with conventional and serological techniques (Bowman, 2020; Ghafar et al., 2023). The PCR results demonstrated that the specific primers (ParaF, ParaR) successfully amplified a 231 bp fragment of the ITS-1 rDNA gene specific to *P. equorum*. The PCR analysis recorded that 36 out of 50 horses (72%) tested positive for *P. equorum*. This finding agree with (Al-Biatee et al., 2024). The application of this technique to detect *P. equorum* is based on previous studies findings, which indicated that diagnosing parasite species alone by their morphological characteristics can yield imprecise results due to significant variations within and between species. Consequently, molecular techniques such as PCR have been employed for more accurate identification (Zhou et al., 2023; Ghafar et al., 2023). Furthermore, these primer sets' selection can be linked to the first Internal Transcribed Spacers (ITS1) of nuclear ribosomal DNA is highly conserved, making it a useful tool for molecular investigations. Therefore, the ITS region could be an excellent target for diagnostic purposes (Zhou et al., 2023; Ghafar et al., 2023; Al-Biatee et al., 2024).

CONCLUSION

The results of the current study concluded that the PCR technique is more sensitive and specific for diagnosing ascariasis compared to traditional methods, such as the flotation technique. The survey study also showed that ascariasis is a significant veterinary disease affecting horses throughout the year, with a concentration in the winter months. Furthermore, the study found that females and horses under 3 years of age are more susceptible to ascariasis than males and horses older than 6 years. Clinically, the study revealed that horses infected with ascariasis exhibit the following signs: diarrhea, increased heart rate, elevated respiratory rate, coughing, snoring during breathing, nasal and ocular discharge, and a rough hair coat.

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CONFLICT OF INTEREST

There are no conflicts of interest declared by the contributors.

The novelty of the study lies in its focus on determining the clinical presentation of horses infected with ascariasis and evaluating the impact of various risk factors, such as age, sex, and seasonality, on the infection rate in horses in Al Muthanna Province. Additionally, the study introduces the use of modern diagnostic methods, specifically PCR with specially designed primers, to detect *Parascaris equorum*. This study is the first of its kind in Iraq.

AUTHORS CONTRIBUTION

Contributions from each author were equal.

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