Research Article

First study of equine cryptosporidiosis in asymptomatic traction horses in Peshawar Khyber Pakhtunkhwa Pakistan

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Abstract

The present project was conducted to explore the prevalence of *Cryptosporidium* species in equines along with associated risk factors (age, sex, month wise). Overall 384 samples were collected randomly from apparently healthy horses from different stations in district Peshawar. As a result, the overall prevalence of equine cryptosporidiosis was 11.97% (46/384×100) screened by microscopic examination after acid fast staining. According to the age wise equine cryptosporidiosis, the highest prevalence (16.96%) was recorded in young equines at the age of (<1-5 years) while the lowest (9.92%) in adult equines at the age of (≥6-10 years) and observed significant (*p*<0.001) association. The highest sex wise equine cryptosporidiosis was recorded in male horses (13.76%) followed by female horses (10.97%) and statistically insignificant association (*p*<0.132) was recorded. According to the month wise prevalence, the highest prevalence was record in month of June (23.07%) followed by April (16.12%), July (15.62%), September (14.70%), October (13.79%), August (10.71%), January (10.71%), March (8.82%), February (8.69%) while the lowest in December (6.06%) and statistically significant association (*p*<0.05) was recorded. As a result, it was concluded that asymptomatic horses were responsible for high excretion of *Cryptosporidium* oocysts that continuously contaminate the environment as well as different sources of drinking water used for humans and animals in rural areas.

Keywords: Cryptosporidiosis; Equines; Risk factors; Prevalence; *Cryptosporidium*; Zoonotic

Introduction

Cryptosporidium species are major enteric protozoan parasites carrying both medical and veterinary importance affecting a wide range of humans and animals globally [1]. This protozoan has been considered as the primary enteric pathogen causing gastrointestinal problems in humans and

animals [2].Among different species of *Cryptosporidium*, C. *parvum* has been recognized as primary enteric pathogen causing huge economic losses in humans and animals [3]. *Cryptosporidium parvum* is the main specie affecting the humans and equines. The *Cryptosporidium* infections are commonly linked with sign of diarrhea in man and animals [4]. This organism has two form of life cycles such as "zoonotic and anthroponotic form" [5]. It is transmitted either by drinking of contaminated water or ingestion of contaminated vegetables or food.Cryptosporidiosis leads to enteritisresulting in diarrhoea. The severity of the diarrhoeic condition has been ranked from self − limiting to lethal diarrhea [6]. Equine cryptosporidiosis plays a vital role as a potential source of zoonotic infection to young and adults [7]. Clinically some animals are suffering from cryptosporidiosis but apparently look healthy with no clinical signs; such hosts play a potential role for spread of *Cryptosporidium* infection. All those animals suffering from *Cryptosporidium* infection mainly suffer from dysentery or presence of mucous in stool with heavy production of gas [8]. It has been estimated that *Cryptosporidium* infection causes 30-50% mortality in young patients and it has been recorded second most common cause of diarrhea as well as death after rotavirus infection in kids globally [9]. Currently a major morbidity and mortality has been reported in humans are suffering with diarrhea infected by *Cryptosporidium parvum* after drinking of contaminated water [10, 11]. The domestic and wild animals play a vital role in contamination of drinking water used for humans consumption. Among domestic animals equines access should be minimized to different sources of surface water such as rivers, streams, canals and ponds etc [10, 12, 13]. About equine cryptosporidiosis, limited informations are available in our country although it has been reported in different regions of the world, linked with diarrhoea in equines [13, 14]. The plan of the study was to explore the prevalence of equine cryptosporidiosis in apparently healthy equines responsible for human cryptosporidiosis.

Materials and methods

Ethical approval

This study was properly approved by University Ethical Committee (UEC).First took the permission from the horse owner then fecal materials were collected with the help of disposable gloves after proper lubrication and restraining. Necessary veterinary services were also provided free of cost along with free medication (deworming).

Study area

The Peshawar is the capital of Khyber Pakhtunkhwa, Pakistan. The latitude of Peshawar is 34.025917, and the longitude is 71.560135. The Peshawar coordinates at 34° 1' 33.3012" N and 71° 33' 36.4860" E. Peshawar is located at elevation of 317 meters height (1040ft) from sea level.

Selection criteria of animals

The horses were selected on the basis of simple random sampling technique. The horses were selected based on some clinical signs such as mild form of diarrhoea or without diarrhoea and poor response to routine antidiarrheal drugs. Both sexes and different age groups of horses were selected regardless to breed. Age was estimated by examination of teeth.

Study period

This study was continued for a period of12 months (January, 2018 to December, 2018). Horses were selected randomly from 20 different stations where horses were used for transport or carriage purpose.

Sample size

The sample size was calculated according to the following formula designated by [15].

n = Z ^{2} (P) (1-P)/ d2

Where:

n = stands for sample size, Z means Z value indicating (confidence level 95%); P means prevalence (i.e. 50%, p = 0.5) where d stands for precision (i.e. 5%, d = 0.05) n = 384

Epidemiological exploration

Questionnaire

Prepared a questionnaire where the different informations were entered at the time of visiting for the purpose of sampling. These informations were include name of the owner with address, age of the horse (≤1-5 years, ≥ 6- 10 years), sex of the horse (Male or Female), different months for sampling, previous treatment history of deworming, source of drinking water either deep or surface water such as well water and river water, month of sampling, diarrheic or non-diarrheic, stall feeding or grazing and stable is hygienic or non- hygienic.

Collection and shipping of fecal materials

A total of 384 samples were collected from 20 different stations of horses in Peshawar. Approximately 8-12 g fecal samples were either directly taken either from the rectum or fresh passed feces. After collection the feces were stored in clear plastic bottles containing 10% formalin (1 part feces: 4 parts formalin). All the samples were properly closed and labeled after collection.

Experimental site

All the samples were shipped to Parasitology Laboratory where stored at $4C^0$ till examination.

Laboratory analysis of fecal samples

Fecal samples were processed by a technique known as centrifugation-sedimentation technique, reported by [16]. 03 gram feces were weighed by electric balance. After weighing, dissolved in water to obtain homogenized solution and centrifuged at 1500rpm for 2 minutes. Removed supernatant after centrifugation and re-suspended in flotation solution (44%ZnSO4).Again the solution was centrifuged, removed supernatant and examined sediment under microscope after staining by modified Ziehl- Neelsen technique

Modified zeihl- neelsen staining technique

Made a thin smear from the sediments and stained by modified Zeihl-Neelsen staining technique as reported by [17].

Microscopic examination of stained slides

All the slides were examined with the help of light microscope at 40 x and 100 x magnification for detection of *Cryptosporidium* oocysts as mentioned by [17]. The oocysts appeared bright red in color. All the samples were considered positive where even one oocyst was identified. *Cryptosporidium* oocyst appeared as bright red granules on a blue-green background in MZN stained fecal smears.

Identification of *Cryptosporidium* oocysts

Cryptosporidium oocysts were recognized on the basis of size, shape and the identification key mentioned by [18].

By using the following formula the infection rate was calculated;

$$\text{Prevalence \% Rate} = \frac{\text{Total number of horses infected} \times 100}{\text{Total number of horses examined}}$$

Prepared a single slide from each fecal sample and examined under microscope. If at least single oocyst was seen per slide then such animal was considered a positive case for cryptosporidiosis.

Statistical analysis

The collected data were analyzed by conventional percentage and average analysis and chi square analysis to achieve the objectives of the study.

Results
Overall prevalence
In the present study, the overall prevalence of equine cryptosporidiosis was 11.97% (46/384×100) under field conditions screened by microscopic examination after staining in district Peshawar, KPK Pakistan as presented in (Table 1).

Table 1. Overall prevalence of cryptosporidiosis in equines

Factors	Total horses examined	Total number of positive cases	Overall Prevalence (%)
Equine cryptosporidiosis	384	46	11.97

Age wise prevalence
In the present study, the age wise equine cryptosporidiosis was also reported where the highest prevalence (16.96%) was determined in young equines at the age of (<1-5) years while lowest prevalence (9.92%) was observed in adult equines at the age of (≥6-10) years as presented by (Table 2) and statistically significant (p<0.001) association was observed.

Table 2. Age wise prevalence of cryptosporidiosis in equines

Factor(Age)	Total horses examined	Positive cases	Prevalence (%)	P. Value
Young(<1-5 years)	112	19	16.96[a]	P<0.001[*]
Adult(≥6-10 years)	272	27	9.92[b]	

[a, b] are theme an values if they are carrying similar superscript within the same row then differ non-significantly (P>0.05) while carrying different superscripts, are differ significantly at (P< 0.05).

Sex wise prevalence
Similarly sex wise equine cryptosporidiosis was also studied where the highest prevalence was recorded in male horses (13.76%) followed by female horses (10.97%) as shown by (Table 3) and statistically insignificant association (p<0.132) was observed.

Table 3. Sex wise prevalence of cryptosporidiosis in equines

Factor (sex)	Total horses examined	Positive cases	Prevalence (%)	P. value
Male	138	19	13.76[a]	p<0.132
Female	246	27	10.97[ab]	

[a, ab] are theme an values if they are carrying similar superscript within the same row then differ non-significantly (P>0.05) while carrying different superscripts, are differ significantly at (P< 0.05)

Month wise prevalence
Presently the month wise prevalence was also recorded where the highest prevalence was record in the month of June (23.07%) followed by April (16.12%), July (15.62%), September (14.70%), October (13.79%), August(10.71%), January(10.71%), March (8.82%), February (8.69%) while the lowest in the month of December (6.06%) and statistically significant association (*p*<0.05) was found (Table 4).

Table 4. Month wise prevalence of cryptosporidiosis in equines

Factors(Months)	Examined cases	Positive cases	Prevalence (%)	P. Value
January	28	3	10.71[n]	
February	23	2	8.69[nm]	
March	34	3	8.82[nm]	
April	31	5	16.12[b]	
May	41	4	9.75[n]	
June	26	6	23.07[a]	p<0.05*
July	32	5	15.62[bc]	
August	37	4	10.81[n]	
September	34	5	14.70[bcd]	
October	29	4	13.79[bcdf]	
November	36	3	8.33[nm]	
December	33	2	6.06[ml]	

[a, ab, abc, bcd, bcdf, n, nm, ml] are themean values if they are carryingsimilar superscript within the same row then differ non-significantly (P>0.05) while carrying different superscripts, are differ significantly at (P< 0.05)

Discussion

Overall prevalence of equine cryptosporidiosis

In the present study, the overall prevalence of equine cryptosporidiosis was 11.97% (46/384×100) under field conditions screened by microscopic examination after staining in district Peshawar, KPK Pakistan. Our findings are in line with [19] who reported 3.5 to 11.5% equine cryptosporidiosis. [20] reported 15.8% cryptosporidial infection after examination 221 fecal samples from 18 villages of Urmia, NW. Iran. Our results are lower than [20]. Some researcher recorded little higher prevalence than our findings such as Butty reported 27.10(%) prevalence in horses. .However some researchers reported lower prevalence than our findings in different countries such as Poland, Germany and USA, ranging from 0.33 to 9.4%.In our study, the overall rate of cryptosporidial infection in horses was higher than that reported by other authors from Germany, Poland and Texas and Colorado, USA, which ranged from 0.33–9.4% [21].

However our results are lower than the findings reported by other authors such as 17 to 100% reported by [3, 8].The differences in the percentage of infection in the months may be related to different factors such as number of samples, environmental condition, age, sex, immunity status, stress factor etc.

Equine cryptosporidiosis associated with age

In the present study, the age wise equine cryptosporidiosis was also reported where the highest prevalence (16.96%) was determined in young equines at the age of (≤1-5) years while lowest prevalence (9.92%) was observed in adult equines at the age of (≥6-10) years as presented in(Table 2) and statistically significant (p<0.001) association was observed. Similar findings were also reported by [20] who observed highest prevalence (20%) at the age of <5 while lower at the age of ≥ 5 years (13.69%).Our results are lower than reported by [22] who reported highest prevalence at the age of 5-6 years(34.38%) than 7-8 years(27.59%) however highest prevalence was recorded at early age .The high prevalence in young animals may be due to immature immune status, poor hygienic conditions, sampling technique and nature of life either domestic or feral.

Equine cryptosporidiosis associated with sex

In the present study sex wise equine cryptosporidiosis was also studied where the highest prevalence was recorded in male horses (13.76%) followed by female horses (10.97%). and statistically insignificant association ($p >$ 0.05) was observed. Our results are like [20] who also reported highest prevalence in male (17.14%) than female (13.58%).Furthermore, our gender wise prevalence results are also close to the study of [20]. Our results are higher than [23] who reported 3.7% prevalence in male and 1.7% in female. However the highest prevalence was reported in male horses than female.

Equine cryptosporidiosis associated with months

Presently the month wise prevalence was also recorded where the highest prevalence was record in the month of June (23.07%) followed by April (16.12%), July (15.62%), September (14.70%), October (13.79%), August (10.71%), January (10.71%), May (9.75%), March (8.82%), February (8.69%) while the lowest in the month of December (6.06%) and statistically significant association ($p <$0.05) was found. Our results are close to the findings reported by [22] where similar findings were found in some months of the year such as January (14.29%: 10.71%), February (10%:8.69%), June (25%: 23.07%) and November (12.5%: 8.33%).However our results are differ in some other months of the year from [22] such as in March (36.36%:8.82%), April (55.56%:16.12%), May (23.08%: 9.75%), July (25%: 15.62%), August (20%: 10.71%), September (50%:14.70%), October (42.86%: 13.79%) and December (15.38%: 6.06%). It has been concluded that these variations may be due different hygienic conditions, sample size, immune status of the animals, study design and diagnostic techniques.

Conclusion

It has been concluded that asymptomatic horses are responsible for high excretion of *Cryptosporidium* oocysts that continuously contaminate the environment and act as a possible potential source of infection to their handlers (coachmen), children playing in a streets and different sources of water used for drinking purpose where they bath the horses. This study explores the importance of equine cryptosporidiosis and involved in control and prevention of protozoosis.

Authors' contributions

Conceived and designed the experiments: NU Khan & S Sultan, Performed the experiments: A Ali, Analyzed the data: T Usman, MS Sarwar & H Ali, Contributed materials/ analysis/ tool: A Razzaq, I Ullah & F Rabbani, Wrote the paper: AU Khan & M Hussain.

Acknowledgments

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