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DETECTION AND MOLECULAR STUDY OF *CRYPTOSPORIDIUM* SPP. IN HORSES AT BAGHDAD CITY, IRAQ

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Abstract

This study was carried out to detection of *Cryptosporidium* spp.oocytes in the feces of horses by using traditional and molecular methods (PCR), also sequencing and phylogenetic analysis. A total of 180 horse fecal samples from equestrian club / Baghdad, from both sex, and different age groups, during the period from October 2019 - March 2020. The total rate of infection was 56.66% (102/180). Both sexes are subjected to the infection with *Cryptosporidium*, 57.40% (62/108) in males and 55.55% (40/72) in females, without significant differences. The infection was highest at age group >3-<6 years 65.51% (57/87%), while the lowest infection rate 35.48% (11/31) at age group 6-20 years with significant differences ($P \leq 0.01$). The highest infection rate was recorded in November 21/30 (70%), and December, while the lowest rate was in March 14/30 (46.66%) and January 13/30 (43.3%) with significant differences ($P \leq 0.01$). Using 18s rRNA gene, for PCR, the result revealed that 69% of horse fecal samples have *Cryptosporidium* positive. The Iraqi strains of parasite mainly appeared closely related to each other. However, two sequences appeared relatively divergent from the rest (MT476891 and MT476893). Importantly, sequence with accession number (MT476898) looks highly similar to that of Iranian origin. All the sequences appeared far distanced from the sequence of USA.According to the molecular study and phylogenetic tree, *Cryptosporidium parvum* are considered the main species that cause cryptosporidiosis in horses of Baghdad city which recorded for the first time in Iraq by using molecular technique.

Key words : *Cryptosporidium*, 18s RNA, horses, Baghdad.

Introduction

Cryptosporidium spp. A pathogenic parasite found in the digestive system of many hosts (Cunha *et al.,* 2019). The description of this parasite for the first time in 1907 by Ernest Edward Taser, in the intestinal epithelium of mice. Human infection was first described in 1976, in a child and in an adult in the same year. *Cryptosporidium* spp is the source of public health concern due to reports of disease outbreaks in day care centers, and patients with immunosuppression as well as in reports of transmission of water (Meireles, 2010).

Cryptosporidium are common types of food and water borne protozoa that affect a wide range of domestic and wild animals as well as humans. In horses, *cryptosporidiosis* was first described in immune-deficient Arab foals (Santín, 2013).

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Cryptosporidium spp is apicomplexan parasites living in the brush border of the intestinal epithelium and respiratory system. At first thought it was to be only a pathogen of young animals such as calves, lambs and foals, and is an important cause of diarrhea, enters colitis in humans and animals (Checkley *et al.,* 2015).

Diarrhea is very common clinical sign in newborn foals and can be a sign of infectious diseases or hypoxic gut injury or dietary changes or disturbances in the intestinal flora, which quickly lead to the emergence of systemic manifestations (Magdesian, 2005; Bernard and Barr, 2011). The most common causes of diarrhea in new-born foals are *Cryptosporidium* Rotavirus, *Clostridium perfringens* and *Salmonella* spp. (Oliver-Espinosa, 2018).

Due to high economic losses and the delayed growth that determined by diarrhea, more information has been

written about *cryptosporidiosis* in calves, lambs and kids (De Graaf *et al.,* 1999; Burton *et al.,* 2010).

In these animals, *Cryptosporidiumparvum* is the main cause of *cryptosporidiosis*, which has been described as a severe diarrheal disease, characterized by yellow faces and unpleasant odor, with a soft to liquid consistency associated with depression, abdominal pain and loss of appetite (Lanci *et al.,* 2018).

In horse, until 2003, only *C. parvum* was known as main cause of diarrhea (Bjorneby *et al.,* 1991; Veronesi *et al.,* 2010). In 2003, *Cryptosporidium horse* genotype was described for the first time in Przewalski adult horse and subsequently also isolated in healthy foals, less than 1 month old, in the New York State (Ryan *et al.,* 2003;Burton *et al.,* 2010).

Diagnosis of *Cryptosporidium* spp. generally occurs by different methods, the common methods used for the identification and detection of oocysts are direct, concentration and staining methods (Henriksen and Pohlenz, 1981; Garcia *et al.,* 1983) these ways gave a good laboratory practice. *Cryptosporidium* parasites also can be discovered by electron microscopic examination in the intestinal mucosa of the hosts (Juranek, 1995). Also many diagnostic methods were used to diagnosed the parasite, including, immunological tests like IFAT, ELISA (Arrowood and Sterling, 1989; Casemore, 1989) and molecular technique (Polymerase chain reaction) have an active way to identification and genotype of the pathogen (Saramago Peralta *et al.,* 2016). In molecular techniques, the multiple species of *Cryptosporidium* parasite recognized in faecal samples (Xiao, 2010).

Materials and Methods

Sample collection

One hundred and eighty horse fecal samples (30-50 g) were collected from both sex and different age groups, during the pried extend from 1st /October 2019, to the end of March 2020 (30 samples from each month) from equestrian club, this club contain approximately 4000 horses and located in AL-Ameria / Baghdad. Each fecal sample were collected using sterile disposable latex glove, and placed into individual plastic box with ice, which were sealed, labelled, and transported immediately to the Parasitology laboratory, at College of Veterinary Medicine -University of Baghdad.

Sample preparation

Small amount from each fecal sample (1/2 tea spoon) of feces placed in Eppendorf tube then labelled and stored at -20°c used later for DNA extraction. Added a sufficient quantity of distilled water (20-30ml) to the remain fecal sample and mixed well, then filtered with four layer of gauze then examined by direct smear, flotation and stain methods, then we added 0.5 ml of potassium dichromate solution 2.5% as preservation for each sample and mixed, and kept in refrigerator at 4°C until used again. Direct smear As in (Anne M. Zajac, 2007), then Floatation Concentration Method by Sheather's sugar solution technique As in: (Anne Zajac, 2007; Makawi and. Al-Zubaidi,2017), Modified Ziehl-Neelsen staining (Street, 2015).

DNA extraction

DNA was extracted from one hundred fecal samples randomly collected and stored previously at -20°c by using addbio DNA extraction kit /Korea. The extraction was performed according to manufacturer's instruction of the addbio Company. The primer used was according to (Murphy and Arrowood, 2020) the forward 5'-GGAAGGGTTGTATTTATTAGATAAAG-3' and reverse 5'-CTCATAAGGTGCTGAAGGAGT-3' and the size 840bp, the primers were provided as lyophilized form (Macrogen /Korea).

Results

Result of prevalence

A total of 180 fecal samples from horses were examined for detection *Cryptosporidium* infection during period from 1st /October 2019 to end of March 2020 the total rate of infection was 56.66% (102/180).

Infection rate of *Cryptosporidium* according to sex

The study revealed that both sex were subject to infection with *Cryptosporidium* 57.40% (62/108) in males, 55.55% (40/72) in females without significant differences between both sex (P<0.05) (Table 1).

Table 1: Infection rate of *Cryptosporidium* in horses according to sex.

Sex	No. of examined	No. of infected	Percentage
Males	108	62	57.40%
Females	72	40	55.55%
Total	180	102	56.66%
P-value	.....	.....	0.0294 *

With significant difference (P<0.05).

Infection rate of *Cryptosporidium* according to age groups

The study showed that the infection rate with *Cryptosporidium* was high at age group >3-<6 years 65.51% (57/87) and 54.83% (34/62) at <1-3 years, while at >6-20 group was recorded less infection rate 35.48% (11/31) with significant differences (P≤0.01) between

three age group (Table 2).

Table 2: Infection rate of*Cryptosporidium* spp. according to age groups.

Month	No. of examined horses	No. of infected horses	Percentage
October	30 sample per month	15	50%
November		21	70%
December		21	70%
January		13	43.33%
February		18	60%
March		14	46.66%
Total	180	102	56.66%
P-value	.....	.....	0.0061 **

With significant differences (P<0.05).

Table 3: Infection rate of Cryptosporidium spp. according to months.

Age group	No. of examined	No. of infected	Percentage
< 1-3 years	62	34	54.83%
>3 - <6 years	87	57	65.51%
>6-20 years	31	11	35.48%
Total	180	102	56.66%
P-value	.....	.....	0.0001 **

with significant differences (P<0.05).

Infection rate of *Cryptosporidium* spp. according to months

The prevalence of *Cryptosporidium* were recorded all over the months of the study in horses. The highest infection rate was recorded in November and December 70% (21/30), while the lowest rate was recorded in March 46.66% (14/30) and January 43.3% (13/30) with significant differences (P≤0.01) between rate of infection (Table 3-4).

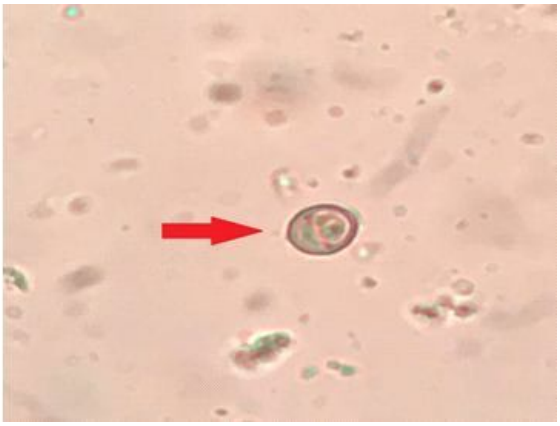

Fig. 1: *Cryptosporidium* oocyst appear (oval to spherical in shape) by using flotation with Sheather's sugar solution x 100.

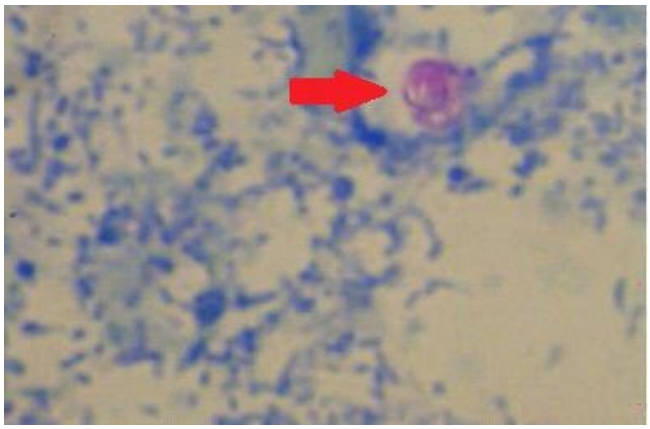

Fig. 2: *Cryptosporidium* oocyst (oval, round or spherical shapes with pink colour) by using staining with Modified Ziehl-Neelsen Stain x100.

Result of molecular study

Genomic DNA estimation

Genomic DNA was extracted from 100 fecal sample using addbio DNA extraction kit /Korea and they done according to company instruction. The extracted genomic DNA was estimated by using Nano-drop spectrophotometer (Thermos, USA), the measured purity of DNA through reading the absorbance at (1.5-1.6) and the concentration of the extracted DNA which ranged from 5 to 50 ng/µl.

Infection rate of *Cryptosporidium* by using PCR technique

The use of conventional PCR technique to identify *Cryptosporidium* spp. by using 18s rRNA gene and primer have 840 base pare. The result showed that from 100 horse fecal samples DNA sample of *Cryptosporidium* 69% (69 out of 100) was identified table.

Detection and genotyping of *Cryptosporidium* isolates

The molecular detection and genotyping of *Cryptosporidium* in the faecal samples were achieved through the Polymerase Chain Reaction (PCR) and the sequencing respectively. PCR analysis:

Phylogenetic analysis

Phylogenetic tree of *Cryptosporidium* 18s rRNA gene generated by maximum likelihood method from a nucleotide sequence alignment in MEGA7, with a bootstrap of 1000 replicates to provide support for individual nodes. The resulted pyelogram depicts splitting the sequences. Importantly, sample number 15 was closely related to the Iranian's isolate. Both were exhibited divergent from sample number 95. The rest samples were

revealed closely related to each other with exception of samples number 30 and 21. Phylogenetic tree analysis of *Cryptosporidium parvum* of the currently identified sequences (18S ribosomal RNA) referred as red triangle with their corresponding accession numbers. As can be seen, the Iraqi strains mainly appeared closely related to each other. However, two sequences appeared relatively divergent from the rest (MT476891 and MT476893). Importantly, sequence with accession number (MT476898) looks highly similar to that of Iranian origin. All the sequences appeared far distanced from the sequence of USA (Fig. 4-5).

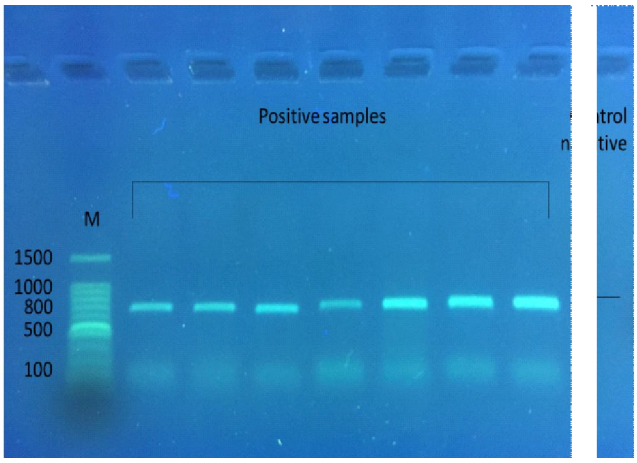

Fig. 1: Gel electrophoresis image using (1%) agarose shows the amplification of Cryptosporidium gene at 840bp. Amplicon size 800bp. M is molecular marker.

Discussion

Cryptosporidium spp. is a pathogenic protozoan parasite present in the gastrointestinal tract of several hosts (Cunha *et al.,* 2019). The investigation about *Cryptosporidium* parasite in horses was conducted in Baghdad city during period 6 months. The total infection rate with *Cryptosporidium* in present study was 56.66%, the result was agree with Moosa, (2019) in Mosul which record infection rate of *cryptosporidium* (30%) in horses, and Wannas, (2012) in Al Diwaniyah government who record infection rate 20.45% in horses, also agree with Olson *et al.,* (1997) which recorded 17% infection rate in Canadian horses. Also Paper, (2007) record infection rate 15.8% in Urmia area, northwestern Iran, Khan, (2020) in Pakistan, show that the prevalence rate was 11.97%.

This study was disagree with Johnson *et al.,* (1997) in California were the infection rate of *Cryptosporidium* was zero, and Deng *et al.,* (2017) found the infection rate was 1.8% were detected of *Cryptosporidium* from Southwestern China by PCR amplification of the partial

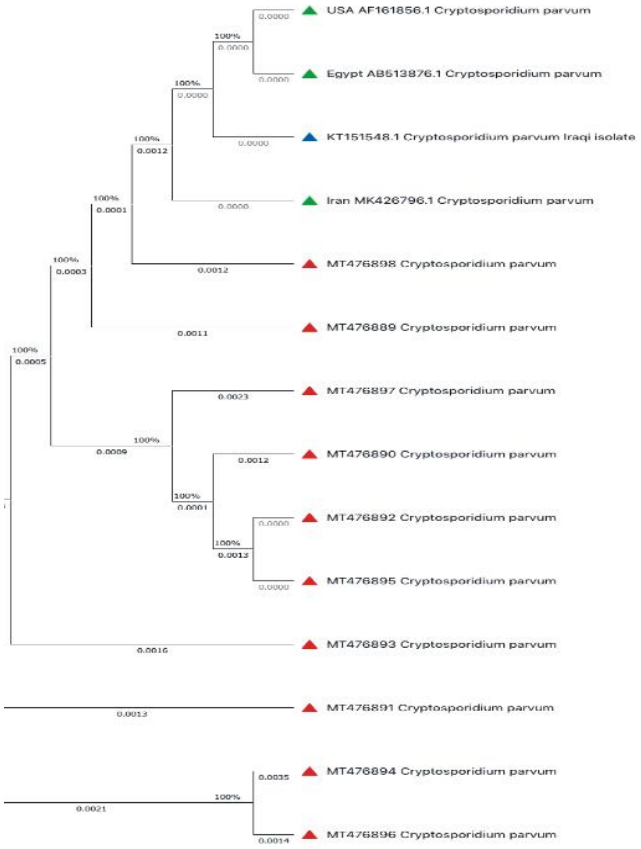

Fig. 2: Phylogenetic tree analysis of Cryptosporidium parvum.

SSU rRNA gene, Wagnerová *et al.,* (2015) record infection of *Cryptosporidium* spp. in the Czech Republic and Poland was 3.4%, (Piva *et al.,* 2016) in Italy record 8% infection of *Cryptosporidium* in horses, also in Italy the prevalence of *Cryptosporidium* was 8% (Veronesi *et al.,* 2010), the infection rate in western Poland was ranged from 0% to 11.5% in horses (Majewska *et al.,* 2004).

The difference in the infection of *Cryptosporidium* in various regions may be related to feeding conditions, different local climatic conditions, detection methods, sampling time, sample size, animal husbandry practices as well as the different susceptibility to different strains of horses. The nature of breeding with restricted movement, limited grazing and low density may have played a role in raising the infection rate compared to other parts of the world (Putignani and Menichella, 2010).

The study revealed that both sex were subject to infection with *Cryptosporidium*. The total rate of infection in males was 62 (57.40%) out of 108 males examined while in females was 40 (55.55%) out of 72 females examined with significant differences between both sex ($P<0.05$).

The result was agree with (Wagnerová *et al.,* 2015)

in Czech Republic and Poland which Statistical analyses did not show any association between sex in horses, (Paper, 2007) in Iran also record no significant differences between the rate of infection in sexes (17.1%) males and (13.6%) females horses. This study was disagree with Khan, (2020) in Pakistan were the highest prevalence was recorded in male horses (13.76%) followed by female horses (10.97%) statistically significant ($p<0.132$). (Burton *et al.,* 2010) in New York State showed that the infection was higher in female than male.

The study showed that the infection rate with *Cryptosporidium* was high at age group >3-<6 years 57 (65.51%) out of 87 examined and 34(54.83%) at <1-3 years out of 62 examined, while at >6-20 group was recorded less infection rate 11 (35.48%) out of 31 horses examined with significant differences ($Pd\leq0.01$) between three age group.

This result was agree with Khan, (2020) in Pakistan where the highest prevalence (16.96%) was determined in young equines at the age of (<1-5) years while lowest infection (9.92%) was observed in adult equines at the age of (≥6- 10) years as presented by and statistically significant ($p<0.001$), (Li *et al.,* 2019) in china identified in all three age groups: <6 (1.4%), 6-12 (3.7%) and >12 (12.5%).

(Moosa, 2019) in Mosul showed an increase in the percentage of infection of foals compared to adult horses (26%, 4%) respectively, (Veronesi *et al.,* 2010) in Poland show that the higher infection rate (26.66%) was observed in foals younger than <8 weeks of age. (Olson *et al.,* 1997) in Canada showed that the infection was (21%) in >6 months of age and (10%) in <6 months. The results of this study confirmed that *Cryptosporidium* infection is common in foals (Xiao and Herd, 1994), (Burton *et al.,* 2010) / USA show that the infection in foals was higher than adult.

Wagnerová *et al.,* (2015) there is no significant differences between the rate of infection in different ages (Paper, 2007) also show o significant differences between the rate of infection in different ages.

The prevalence of *Cryptosporidium* were recorded all over the months of the study in horses. The highest infection rate was recorded in November 21/30 (70%), December 21/30 (70%) February 18/30 (60%), October 15/30 (50%), while the lowest rate was recorded in March 14/30 (46.66%) and January 13/30 (43.3%) with significant differences ($P\leq0.01$).

This result was disagree with (Khan, 2020) 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 ($p<0.05$)

The study showed according to the molecular technique and phylogenetic tree, *Cryptosporidium parvum* was considered the main species that cause *cryptosporidiosis* in horses of Baghdad city, which recorded for the first time in Iraq by using molecular technique.

This study was disagreement with Wagnerová *et al.,* (2015) in Czech Republic and Poland which found more than one spp. of *Cryptosporidium* by Analysis of partial sequences of the SSU gene showed the presence of C. parvum, *Cryptosporidium* horse genotype and *C. muris*, also this study was disagree with Li *et al.,* (2019) in china which identified Four *Cryptosporidium* species/ genotypes in horses, including C. parvum, Cryptosporidium horse genotype. Hijjawi *et al.,* (2016) detect six species; *C. xiaoi, C. andersoni, C. ryanae, C. parvum. C. baileyi* from horses.

References

Anne, M. and G.A.C. Zajac (2007) *Veterinary Clinical Parasitology, 7th ed.*, *Can. Vet. J.*

Arrowood, M.J. and C.R. Sterling (1989) "Comparison of conventional staining methods and monoclonal antibody-based methods for Cryptosporidium oocyst detection. *J. Clin. Microbiol.*, **27(7):** 1490–1495.

Bernard, W. and B. Barr (2011) "Alimentary Tract Disorders," in Equine Pediatr. Med. CRC Press, 91–137.

Bjorneby, J.M., D.R. Leach and L.E. Perryman (1991) "Persistent cryptosporidiosis in horses with severe combined immunodeficiency. *Infect. Immun*., **59(10):** 3823–3826.

Burton, A.J., D.V. Nydam, T.K. Dearen, K. Mitchell, D.D. Bowman and L. Xiao (2010). "The prevalence of Cryptosporidium, and identification of the Cryptosporidium horse genotype in foals in New York State". *Vet. Parasitol. Elsevier B.V.*, **174(1–2):** 139–144.

Casemore, D.P. (1989). "Sheep as a source of human cryptosporidiosis". *J. Infect.*, **19(2):** 101–104.

Checkley, W., A.C. White, D. Jaganath, M.J. Arrowood, R.M. Chalmers, X.M. Chen, R. Fayer, J.K. Griffiths, R.L. Guerrant, L. Hedstrom, C.D. Huston, K.L. Kotloff, G. Kang, J.R. Mead, M. Miller, W.A. Petri, J.W. Priest, D.S. Roos, B. Striepen, R.C.A. Thompson, H.D. Ward, W.A. Van Voorhis, L. Xiao, G. Zhu and E.R. Houpt (2015). "A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium". *Lancet Infect. Dis*., **15(1):** 85–94.

Cunha, F.S., J.M. Peralta and R.H.S. Peralta (2019). "New insights into the detection and molecular characterization of Cryptosporidium with emphasis in Brazilian studies: A

review". *Rev. Inst. Med. Trop. Sao Paulo*, **61(April):** 1–12.

Deng, L., W. Li, Z. Zhong, C. Gong, X. Cao, Y. Song, W. Wang, X. Huang, X. Liu, Y. Hu, H. Fu, M. He, Y. Wang, Y. Zhang, K. Wu and G. Peng (2017). "Occurrence and Genetic Characteristics of Cryptosporidium hominis and Cryptosporidium andersoni in Horses from Southwestern China". *J. Eukaryot. Microbiol.*, **64(5):** 716–720.

Garcia, L.S., D.A. Bruckner, T.C. Brewer and R.Y. Shimizu (1983). "Techniques for the recovery and identification of cryptosporidium oocysts from stool specimens". *J. Clin. Microbiol*., **18(1):** 185–190.

De Graaf, D.C., E. Vanopdenbosch, L.M. Ortega-Mora, H. Abbassi and J.E. Peeters (1999). "A review of the importance of cryptosporidiosis in farm animals". *Int. J. Parasitol.*, **29(8):** 1269–1287.

Henriksen, S.A. and J.F. Pohlenz (1981). "Staining of cryptosporidia by a modified Ziehl-Neelsen technique". *Acta Vet. Scand*., **22(3–4):** 594–596. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6178277.

Hijjawi, N., R. Mukbel, R. Yang and U. Ryan (2016). "Genetic characterization of Cryptosporidium in animal and human isolates from Jordan". *Vet. Parasitol. Elsevier B.V*., **228:** 116–120.

Johnson, E., E.R. Atwill, M.E. Filkins and J. Kalush (1997). "The prevalence of shedding of Cryptosporidium and Giardia spp. based on a single fecal sample collection from each of 91 horses used for backcountry recreation". *J. Vet. Diagnostic Investig*., **9(1):** 56–60.

Juranek, D.D. (1995). "Cryptosporidiosis: sources of infection and guidelines for prevention". *Clin. Infect. Dis*., **21(Supplement_1):** S57–S61.

Khan, N.U. (2020). "First study of equine cryptosporidiosis in asymptomatic traction horses in Peshawar Khyber Pakhtunkhwa Pakistan". *Pure Appl. Biol*., **9(1):** 396–402.

Lanci, A., J. Mariella, E. Iacono, M. Caffara, S. Piva, R. Galuppi and C. Castagnetti (2018). "Observational Study on Cryptosporidiosis in an Equine Perinatology Unit". *J. Equine Vet. Sci. Elsevier Ltd*, **71:** 51–56.

Li, F., J. Su, B. Chahan, Q. Guo, T. Wang, Z. Yu, Y. Guo, N. Li, Y. Feng and L. Xiao (2019). "Different distribution of Cryptosporidium species between horses and donkeys". *Infect. Genet. Evol*., **75:** 103954.

Magdesian, K.G. (2005). "Neonatal foal diarrhea". *Vet. Clin. North Am. - Equine Pract.*, **21(2):** 295–312.

Majewska, A.C., P. Solarczyk, L. Tamang and T.K. Graczyk (2004). "Equine Cryptosporidium parvum infections in western Poland". *Parasitol. Res*., **93(4):** 274–278.

Meireles, M.V. (2010). "Cryptosporidium infection in Brazil: implications for veterinary medicine and public health". *Rev. Bras. Parasitol. Veterinária*, **19(4):** 197–204.

Moosa, D.A. (2019). "Drought Horses Detection of Cryptospridium sp. Oocysts in Drought Horses in Mosul, Iraq", 11–16.

Murphy, J.L. and M.J. Arrowood (2020). *Cell Culture Infectivity to Assess Chlorine Disinfection (chapter 2)*. Edited by J.R. Mead and M.J. Arrowood. New York, NY: Springer New York (Methods in Molecular Biology).

Oliver-Espinosa, O. (2018). "Foal Diarrhea: Established and Postulated Causes, Prevention, Diagnostics, and Treatments". *Vet. Clin. North Am. - Equine Pract. Elsevier Inc.*, **34(1):** 55–68.

Olson, M.E., C.L. Thorlakson, L. Deselliers, D.W. Morck and T.A. McAllister (1997). "Giardia and Cryptosporidium and Canadian farm animals". *Vet. Parasitol*., **68(4):** 375–381.

Paper, S. (2007). "A Survey on Cryptosporidial Infection in Horse in Urmia Area, Northwestern Iran". *Iran. J. Vet. Res*., **8(118):** 86–90.

Piva, S., M. Caffara, F. Pasquali, C. Castagnetti, E. Iacono, E. Massella, R.G. Zanoni and R. Galuppi (2016). "Detection and quantification of Cryptosporidium oocysts in environmental surfaces of an Equine Perinatology Unit". *Prev. Vet. Med. Elsevier B.V*., **131(1):** 67–74.

Putignani, L. and D. Menichella (2010). "Global Distribution, Public Health and Clinical Impact of the Protozoan Pathogen Cryptosporidium". *Interdiscip. Perspect. Infect. Dis.*, **2010:** 1–39.

Ryan, U., L. Xiao, C. Read, L. Zhou, A.A. Lal and I. Pavlasek (2003). "Identification of novel Cryptosporidium genotypes from the Czech Republic". *Appl. Environ. Microbiol.*, **69(7):** 4302–4307.

Santín, M. (2013). "Clinical and subclinical infections with Cryptosporidium in animals". *N. Z. Vet. J*., **61(1):** 1–10.

Saramago Peralta, R.H., J. Néstor Velásquez, F. de Souza Cunha, M. Laura Pantano, F. Campos Sodré, S. da Silva, O. Germán Astudillo, J. Mauro Peralta and S. Carnevale (2016). "Genetic diversity of Cryptosporidium identified in clinical samples from cities in Brazil and Argentina". *Mem. Inst. Oswaldo Cruz*, **111(1):** 30–36.

Street, C. (2015). "Modified Ziehl-Neelsen" Uk Neqas.

Veronesi, F., F. Passamonti, S. Cacciò, M. Diaferia and D. Piergili Fioretti (2010). "Epidemiological survey on equine Cryptosporidium and Giardia infections in Italy and molecular characterization of isolates". *Zoonoses Public Health*, **57(7–8):** 510–517.

Wagnerová, P., B. Sak, J. McEvoy, M. Rost, A. Perec Matysiak, J. Ježková and M. Kváè (2015). "Genetic diversity of Cryptosporidium spp. including novel identification of the Cryptosporidium muris and Cryptosporidium tyzzeri in horses in the Czech Republic and Poland". *Parasitol. Res*., **114(4):** 1619–1624.

Wannas, H.Y. (2012). "Prevalence of Gastro-intestinal Parasites in Horses and Donkeys in Al Diwaniyah Governorate". *Al-Qadisiyah J. Vet. Med. Sci*., **11(1):** 148.

Xiao, L. (2010). "Molecular epidemiology of cryptosporidiosis: An update". *Exp. Parasitol. Elsevier Inc*., **124(1):** 80–89.

XIAO, L. and R.P. Herd (1994). "Epidemiology of equine Cryptosporidium and Giardia infections". *Equine Vet. J*., **26(1):** 14–17.