RESEARCH ARTICLE

IJVAR *International Journal of Veterinary and Animal Research*
E-ISSN: 2651-3609 4(3): 87-91, 2021

Direct Detection of *Cryptosporidium* spp. in Cattles in Karbala Province and its environs, Iraq

Firas Alali[1,a,*], Marwa Jawad[2,b], Kadhim kh.k.Al-khayat[3,c]
[1] Kerbala University, College of Veterinary Medicine, Department of Parasitology, Kerbala, Iraq
[2] Kerbala University, College of Science, Department of Biology, Kerbala, Iraq
[3]Ministry of agriculture, Agriculture Directorate. Department of Veterinary, Karbala Iraq.

[a]ORCID: 0000-0002-3438-6453; [b]ORCID: 0000-0003-2927-9220; [c]ORCID: 0000-0002-5815-2168

*Corresponding Author
E-mail: firas.o@uokarbala.edu.iq

Received: July 30, 2021
Accepted: October 02, 2021

Abstract

Gastrointestinal diseases are caused by many of the apicomplexan protozoan parasites and cause significant clinical diseases. Cryptosporidiosis is one of the most important diseases of young ruminant livestock, particularly neonatal calves. This study investigates the prevalence of *Cryptosporidium* infection in Karbala farm cattle. Totally, 1360 fecal samples were collected from cows and calves between (1 week to 5 years old) from both genders, from different regions of Karbala. Different fecal examination methods (direct fecal smear, fecal flotation with saturated salt solution and faecal smear stained by modified Ziehl-Neelsen were performed to determine *Cryptosporidium* spp. oocysts and other mixed parasites in the fecal samples. The results showed that 29.4% of the fecal samples were detected positive for cryptosporidiosis (400/1360). The highest infection rate appeared (41.9%) in the age group from 3-6 months old, and less rate was (15.1%) in 2 years ($P \leq 0.05$). Additionally, there was no significant variation found in infection rates between males (33.8%) and females (25.6%). Mixed infections have been reported with *Eimeria* spp. oocysts (7.3%), *Giardia lamblia* cysts (1.4%) and *Trichostrongylus* spp. eggs (5.1%) ($P \leq 0.05$). The results indicated the requirement for the eradication of the gastrointestinal parasites by deworming and a good management system in the sampled region.

Keywords: Cattle, *Cryptosporidium* spp., Internal parasites, Iraq, Mix infection.

INTRODUCTION

Cryptosporidium spp. is an intracellular protozoan parasite that lives intracellularly in the gastrointestinal tract of humans and many other vertebrates animals including mammals, reptiles, birds, and fish (Tzipori and Ward, 2002). *Cryptosporidium* have valid species (19) in mammals, birds, reptiles and amphibians was considered hosts. Many host are includes mammals, birds, reptiles and amphibians serve as hosts for 19 species of parasite. All 19 species have been detected by morphological, biological, and molecular diagnosis. All 19 species have been recognized by morphological, biological, and molecular technique (Fayer, 2010). Actually, 26 species are detected as valid on the basis of morphological, biological and molecular data again. Currently, 20 species and genotypes of *Cryptosporidium* that have been recorded in humans, *C. parvum* and *C. hominis* are cause of infections (Ryan et al., 2014). Cryptosporidiosis is one of the most prevalent infections in newborn calves. Cryptosporidiosis characterized by some clinical signs such as diarrhea, fever, loss of appetite, dehydration and abdominal pain in calves. Cryptosporidiosis is detected in calves by fecal examination and species identification is perform by use molecular methods (Santín et al., 2004; Díaz et al., 2021). Livestock, particularly cattle (domesticated and wild), are one of the most important reservoirs of zoonotic diseases. Cattle can be infected with various *Cryptosporidium* species. Cattle are infected with four major *Cryptosporidium* species (*C. parvum*, *C. bovis*, *C. andersoni* and *C. ryanae)* (Ryan et al., 2014). The prevalent species was *C. parvum* in pre-weaned calves especially with diarrhea while *C. ryanae* and *C. bovis* were commonly detect in post-weaned heifers and calves (Yildirim et al., 2020). *Cryptosporidium parvum* is consider amongst the common species diagnostic in calves (Aydoğdu et al., 2018). *Cryptosporidium parvum* can also infect humans and cause significant effects. These oocysts remain in the pasture, drinking water, soil, and environment. It is highly infectious, except ten oocysts can cause cryptosporidiosis in sensitive calves (Tzipori and Ward, 2002). *Giardia lamblia* and *Cryptosporidium* spp. have anthroponotic and zoonotic transmission, as well as foodborne and waterborne transmission. They are transmitted by the fecal-oral route, direct contact with an infected host, consumption of food or water contaminated with resistant infective stages (cysts or oocysts). The resistant stages (oocysts and cysts) can remain for a longtime extend from weeks to months in the environment (Abeywardena et al., 2014). In Iraq, many indexes referred to that ruminants are a reservoir of zoonotic *Cryptosporidium* spp. from where humans get infected by contaminated food and water or through direct contact with livestock, for example, animal handlers (Mahdi and Ali, 2002). *Eimeria* spp. is the more abundant and most common enteric protozoan of cattle that can cause a high economic setback to the farms and beef industry (Hassan and Barzinji, 2018). *Trichostrongylus* spp. is one of the most frequently gastrointestinal (GI) parasite infections in water buffaloes (Al-Jubury et al., 2020). The disease is highly common in humans and animals, but mostly employed traditional methodologies in all of Iraq among them Karbala province. *C. parvum* and *C. hominis* were detected in human beings and the latter in isolates from cattle, sheep, goats and birds (Alali et al. 2021). The goal

of this study was survey for *Cryptosporidium* parasites by use of coprological techniques and investigation of Cryptosporidium in calves in Karbala city.

MATERIALS AND METHODS

Specimens collection

Fecal specimens (~5 g each) were sampled from 1360 calves and heifers (aged between 1 week to 5 years old) through the period from November 2020 until 30th April 2021. The fecal specimens were sampled directly from rectum of the each animal and put in a separate container with information about age and gender, and they transferred to the Parasitology Laboratory at College of Veterinary Medicine-University of Karbala.

Direct smear

A thin smear of the 0.1 g fecal material was liquefied in an isotonic saline solution made on a glass slide and rolling it lightly with a drop of water and covered with a cover slide and examination in the microscope (Cebra and Stang, 2008).

Detection of oocysts

Modified Ziehl-Neelsen stain technique was used to realize the *Cryptosporidium* oocysts in the fecal specimens. Thin smears of feces had been made on a glass slide and air-dried. Accordingly, the smears were fixed with 70% methanol, and stained with a carbol-fuchsin solution for 5 minutes. After staining, by methyl alcohol 50% and then the slides were washed in running tap water for 1-2 minutes, after that decolorized in 1% sulphuric acid for 30 seconds. In last, the slides were washed in tap water for 1-2 minutes and counter stained with methylene blue for 1 minutes. Finally, the smears had been washed in tap water and air-dried and examined microscopically beneath oil immersion (100 ×), according to (Sevinc et al., 2003).

Flotation method

Fecal samples were examined for the presence of other intestinal parasites by floatation method with saturated salt solution. The cover slide was examined by using a microscope (Cebra and Stang, 2008).

Statistical Analysis

The Chi-square (χ^2) test was applied for the measurement and compare between all results. P≤0.05 were considered significant statistically.

RESULTS

The prevalence of cryptosporidiosis was studied based on the detection of oocysts in the fecal materials collected from different areas in the province of Karbala. Out of 1360 fecal samples screened 400 (29.4%) animals were found positive for cryptosporidiosis. All of males samples was 33.8% (210/620) of the males compared with, 25.6% (190/740) of the females were positive for Cryptosporidiosis (Table 1, Figure 1). The prevalence was studied in five different age groups. The highest prevalence was 130/310 (41.9%) in the 3-6 month age group and the lowest rate was 50/330 (15.1%) in the >2 years age group (Table 2, Figure 2). Mixed infections with *Eimeria* spp. oocysts (7.3%), *Giardia lamblia* cysts (1.4%) and *Trichostrongylus* spp. eggs (5.1%) were detected in the fecal samples examined (Table 3, Figure 3, Figure 4).

Table 1. *Cryptosporidium* species prevalence in concerning the genders

		Infection			Total
		Non-infection	Infection	%	
Gender	Male	410	210	33.8	620
	Female	550	190	25.6	740
Total		960	400	29.4	1360

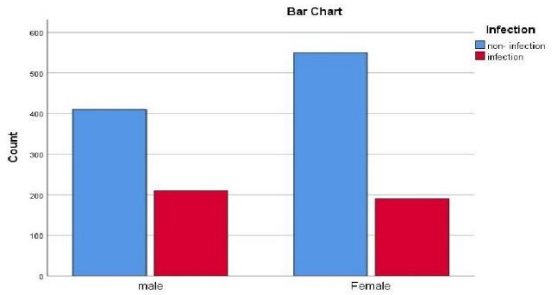

Figure 1. *Cryptosporidium* species prevalence in concerning the genders.

Table 2. Infection prevalence in concerning the different age groups.

		Infection			
		Non-infection	Infection	%	Total
Age	<3 months	130	60	31.5	190
	3-6 months	180	130	41.9	310
	7-12 months	210	50	19.2	260
	1-2 years<	160	110	40.7	270
	2 years<	280	50	15.1	330
	Total	960	400	29.4	1360

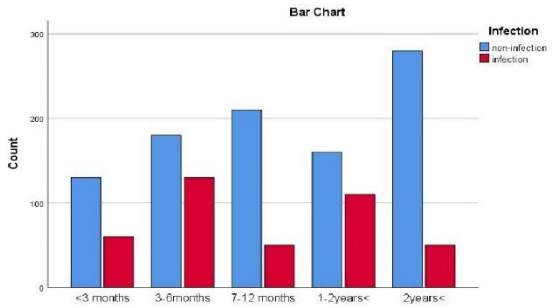

Figure 2. Infection prevalence in concerning the different age groups.

Table 3. Prevalences of mix internal parasites.

		Infection					
		Single infection	**Double infection**	**Three infection**	**Four infection**	**%**	**Total**
Parasite	*Cryptosporidium parvum*	201	50	115	34	29.4	400
	Eimeria spp. *oocyst*	46	28	17	9	7.3	100
	Giardia lamblia cyst	20	0	0	0	1.4	20
	Trichostrongylus spp. eggs	40	15	10	5	5.1	70
	Total	307	93	142	48		590

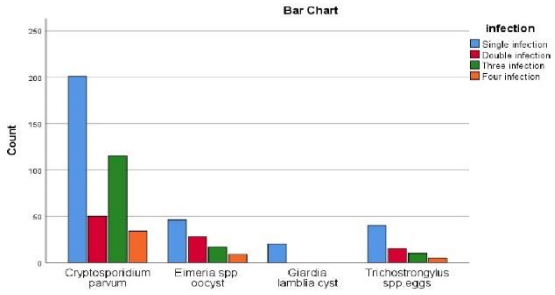

Figure 3. Prevalences of mix internal parasites.

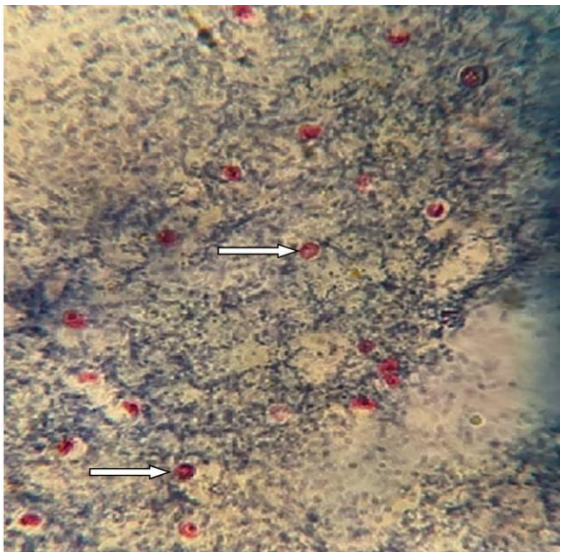

Figure 4. *Cryptosporidium* spp. oocyst in feces of animals (MZN stain). Both arrows are pointed to oocysts (x100).

DISCUSSION AND CONCLUSION

Gastrointestinal parasites are more common in ruminant livestock and especially apicomplexan parasites may be leads to death of animals, particularly neonatal calves. Global distribution of *Cryptosporidium* and *Giardia* are the two pathogenic protozoans causing diarrhea in animals and humans in all over the world. Some Cryptosporidium species including *C. parvum, C. andersoni, C. bovis, C. occultus, C. xiaoi, C. ryanae* and *C. suis* could be infect in cattle (Abeywardena et al., 2014; Hooshyar et al., 2019; Díaz et al., 2021). Microscopically, *C. parvum* oocyst is too small and similar to *C. bovis* and *C. ryanae*. The sensitivity and specificity of modified Ziehl-Neelsen staining were detected as 88.5% and 100.0%, respectively compared to nested PCR-RFLP of SSU rRNA and TaqMan qPCR for the detection of Cryptosporidium in fecal materials (Yildirim et al., 2021). The combination of microscopy with stool concentration methods is fundamentally depend on determine of microscopic cyst or trophozoite in *Giardia* spp. samples using for the lab diagnosis due to high economics and sensitivity and should be remain a golden standard (Hooshyar et al., 2019). In the current study, light microscopy and staining methods which only based on morphological characterization have been used for identifying of *Cyrptosporidium* spp. oocysts in the sampled cattle.

The highly prevalent cryptosporidiosis in humans and animals in Iraq, and commonly applied routine procedures for the diagnosis of cryptosporidiosis. The different environmental matrices harbor drinking tap water, which allowed in transferring to humans and animals. *C. hominis* and *C. parvum* are detected in humans, cattle, sheep, goats and birds (Alali et al. 2021). There are some reports on cattle cryptosporidiosis in Iraq and cryptosporidiosis has been recorded as 20-35.44% in cattle (Mahdi and Ali 2002; Farhood, and Al-Idreesi, 2020; Al-Zubaidi, 2012; Al-Robaiee and Al-Farwachi, 2014). In the present study, cryptosporidiosis was detected as 29.4% in the sampled animals, which agrees with previous reports from Iraq (Mahdi and Ali 2002; Farhood, and Al-Idreesi, 2020; Al-Zubaidi, 2012; Al-Robaiee and Al-Farwachi, 2014). The results of the present study are not compatible with some studies (Hassan and Barzinji, 2018) in which cryptosporidiosis have been recorded as 69.16% in cattle in Kirkuk province, Iraq (Hassan and Barzinji, 2018). These differences may be related to some factors included sampled region, selected method for diagnosis, age of sampled animals etc.

Many epidemiological surveys have been performed to detect cryptosporidiosis in cattle in the world (Alali et al. 2021; Díaz et al., 2021; Yildirim et al., 2021). Cryptosporidiosis has been reported in all over the world, in the range from 0–100% (Santín et al., 2009; Lassen and Järvis, 2009; Swai and Schoonman, 2010; Li et al., 2021; Peng et al., 2021). The results of the present study, the infection was detected as 29.4% in the sampled cattle and it is obvious from this study that there is no effective difference in the infection rate between males and females in case they are exposed to similar condition and bred in the same place (Table 1, Figure 1). Clinically, calves with cryptosporidiosis are couldn't differentiate from calves with non-*Cryptosporidium* infection. Because *Cryptosporidium* infection interfere with other parasites

can cause diarrhea and enteritis including (*Eimeria* spp., *Giardia lamblia* and *Trichostrongylus* spp.) in case of single or mixed infection. In the current study, the high rate of age group in the (3-6 months) were 41.9% (13/31), (Table 2, Figure 2). Generally, prevalence infection of Cryptosporidium versus with increasing age (Santín et al., 2004). The continuous infection or exposing to stress factors may be explain doesn't decline the infection in high ages. The high rate infection and concentration of shedding the oocysts were in small calves manifestation. So, the small animals can shed the parasite which has a wide host and is regarded to be possibility zoonotic (Abeywardena et al., 2014).

The present study shows varying in the age-correlated patterns. Although, *C. parvum* was the more predominate species and prevalent in calves less than 1 month, while *C. bovis* was the more frequent in the all age groups (Fayer, 2010). The other dominant species like *C. andersoni* being in adults and (*C. bovis* and *C. ryanae*) being dominant in post-weaned calves (Díaz et al., 2021). The signs of Cryptosporidium and Giardia infections can be cause mild to severe or asymptomatic gastrointestinal disease in both humans and animals (Santin, 2020). The current results were detected that animals in all the study field excrete oocysts with other parasites and may be participate to the contamination of the environment carrying the oocysts to food, soil, raw water.

Mixed infection in this study was recorded and ranged (5.1%-29.4%) for all intestinal parasites that agreement with (Bărburaș et al., 2021) who recorded intestinal parasites of buffalo calves: *Cryptosporidium* spp. and *Giardia duodenalis*, and *Eimeria bareillyi* (11.1-38.5%) from Romania. While, *Trichostrongylus* infection was 70 (5.1%) in our study and corresponded with the result of Alim et al., (2012) who examined by traditional (routine) methods and recorded *Trichostrongylus* spp. (4.86%) infections.

Gastrointestinal nematodes (GIN) and *Eimeria* spp. infections lead to significant economic losses in Indian cattle due to decline in quality and milk production. While, mixed infection rates of GIN and *Eimeria* spp. were (8.5-12.2%) higher in rural areas (Pinto et al., 2021).

Animals can be infected with different parasite species and showed different clinical signs. *C. parvum*, *Eimeria* spp., *G. lamblia* and *Trichostrongylus* spp. are more prevalent species in cattle. Generally, some of infected cattle do not almost offer clinical symptoms correlated with the internal parasitic diseases. Importantly, the variation in infection with different internal parasites may be lead to show different clinical signs in animals (Thanasuwan et al., 2021).

Cryptosporidium spp. are wide prevalent parasites in healthy cattle from Karbala in middle Euphrates, Iraq. Adult cattle may also play a role in the distribution of Cryptosporidium infection in humans and calves. The presence of one or more infected cattle in all farms were referred to endemic of cryptosporidiosis and mixed infection in the research field. Additionally, the distribution rate in all ages especially small ages may be life-threatening. Molecular studies should be performed to determine the species responsible for infection in this area and importance of zoonotic transmission.

Acknowledgements
The authors would like to thank the dairy farm owners.

Conflict of Interest
The authors declare that they have no conflict of interest.

REFERENCES

Abeywardena H, Jex AR, Koehler AV, Rajapakse RJ, Udayawarna K, Haydon SR, Stevens MA, Gasser RB. 2014. First molecular characterization of Cryptosporidium and Giardia from bovines (*Bos taurus* and *Bubalus bubalis*) in Sri Lanka: unexpected absence of *C. parvum* from pre-weaned calves. Parasites & Vectors, 7(1):1-0.

Alali F, Abbas I, Jawad M, Hijjawi N. 2021.Cryptosporidium infection in humans and animals from Iraq: a review. Acta Tropica, 5:105946.

Alim MA, Das S, Roy K, Sikder S, Mohiuddin MM, Hossain MA. 2012. Prevalence of gastrointestinal parasites in cattle of Chittagong division, Bangladesh. Wayamba Journal of Animal Science, 4:1-8.

Al-Jubury A, Jarullah B, Al-Jassim K, Badran M, Mahmmod Y. 2020.Prevalence and diffusion of gastrointestinal parasite infections in swamp water buffalo (Bubalus Bubalis) populations from marshlands of Iraq. Journal of Buffalo Science, 9:38-47.

Al-Robaiee I, Al-Farwachi M. 2014. Direct ELISA aided coprological diagnosis of *Cryptosporidium parvum* infection in diarrheic neonatal calves in Mosul city, Iraq. Journal of Advanced Veterinary and Animal Research, 1(1): 8-10.

Al-Zubaidi MTS. 2012. Prevalence of some Cryptosporidium species in cattle in Baghdad, Iraq. Al-Qadisiyah Journal of Veterinary Medicine Sciences, 11(2): 177-182.

Aydoğdu U, Işık N, Ekici ÖD, Yıldız R, Şen İ, Coşkun A. 2018. Comparison of the effectiveness of halofuginone lactate and paromomycin in the treatment of calves naturally ınfected with Cryptosporidium parvum. Acta Scientiae Veterinariae, 46(1):1524.

Bărburaș DA, Cozma V, Ionică AM, Abbas I, Bărburaș R, Mircean V, Gyorke A. 2021. Intestinal Parasites of Buffalo Calves from Romania: Molecular Characterization of Cryptosporidium spp. and Giardia Duodenalis, and the first Report of Eimeria Bareillyi.1-14. https://doi.org/10.21203/rs.3.rs-151344/v1

Cebra CK, Stang BV. 2008. Comparison of methods to detect gastrointestinal parasites in llamas and alpacas. Journal of the American Veterinary Medical Association, 232(5):733-741.

Díaz P, Navarro E, Remesar S, García-Dios D, Martínez-Calabuig N, Prieto A, López-Lorenzo G, López CM, Panadero R, Fernández G, Díez-Baños P. 2021.The Age-Related Cryptosporidium Species Distribution in Asymptomatic Cattle from North-Western Spain. Animals, 11(2):256.

Farhood NN, Al-Idreesi SR. 2020. Identification of *Strongyloides papillosus* and other gastrointestinal parasites of Cattle in Basrah province. Basrah Journal of Veterinary Research, 19(1):155-168.

Fayer R. 2010. Taxonomy and species delimitation in Cryptosporidium. Experimental Parasitology, 124(1):90-7.

Hassan HF, Barzinji AKR. 2018. Prevalence of ruminants gastro-intestinal parasites in Kirkuk province, Iraq. Kirkuk University Journal /Scientific Studies, 13(3): 96-108.

Hooshyar H, Rostamkhani P, Arbabi M, Delavari M. 2019. Giardia lamblia infection: review of current diagnostic strategies. Gastroenterology and Hepatology from bed to Bench, 12(1): 3-12.

Lassen B, Järvis T. 2009. Eimeria and Cryptosporidium in Lithuanian cattle farms. Vet Med Zoot, 48(70):24-8.

Li S, Zou Y, Wang P, Qu MR, Zheng WB, Wang P, Chen XQ, Zhu XQ. 2021. Prevalence and multilocus genotyping of Cryptosporidium spp. in cattle in Jiangxi Province, southeastern China. Parasitology Research, 120(4):1281-1289.

Mahdi NK, Ali NH. 2002. Cryptosporidiosis among animal handlers and their livestock in Basrah, Iraq. East African Medical Journal, 79(10):551-4.

Manyazewal A, Francesca S, Pal M, Gezahegn M, Tesfaye M, Lucy M, Getachew T. 2018. Prevalence, risk factors and molecular characterization of Cryptosporidium infection in cattle in Addis Ababa and its environs, Ethiopia. Veterinary Parasitology: Regional Studies and Reports, 13: 79-84.

Peng H, Tang L, Wu C, Li J, Tao L, Li C, Chen Z, Xie Y. 2021. Recombinant Expression And Indirect ELISA For COWP And HSP70 Proteins From Cryptosporidium andersoni. Acta Tropica, 214:105767.

Pinto A, May K, Yin T, Reichenbach M, Malik PK, Roessler R, Schlecht E, König S. 2021. Gastrointestinal nematode and Eimeria spp. infections in dairy cattle along a rural-urban gradient. Veterinary Parasitology: Regional Studies and Reports. 18:100600.

Ryan UNA, Fayer R, Xiao L. 2014. Cryptosporidium species in humans and animals: current understanding and research needs. Parasitology. 141(13): 1667-1685.

Santín M, Trout JM, Xiao L, Zhou L, Greiner E, Fayer R. 2004. Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. Veterinary Parasitology, 122(2):103–117.

Santín M, Trout JM, Fayer R. 2009. A longitudinal study of *Giardia duodenalis* genotypes in dairy cows from birth to 2 years of age. Veterinary Parasitology, 162(1-2): 40-45.

Santin M. 2020. Cryptosporidium and Giardia in ruminants. Veterinary Clinics of North America: Food Animal Practice, 36(1): 223-238.

Swai ES. Schoonman L. 2010. Investigation into the prevalence of Cryptosporidium infection in calves among small-holder dairy and traditional herds in Tanzania. Veterinary medicine international, 2010:1-5.

Thanasuwan S, Piratae S, Tankrathok A. 2021. Prevalence of gastrointestinal parasites in cattle in Kalasin Province, Thailand. Veterinary World, 14 (8): 2091-2096.

Tzipori S.Ward H. 2002.Cryptosporidiosis: biology, pathogenesis and disease. Microbes and infection, 4(10):1047-1058.

Yildirim A, Adanir R, Inci A, Yukari BA, Duzlu O, Onder Z, Ciloglu A, Simsek E. 2020. Prevalence and genotyping of bovine Cryptosporidium species in the mediterranean and central Anatolia region of Turkey. Comparative Immunology, Microbiology and Infectious Diseases, 69:101425.

Yildirim A, Sevinc F, Önder Z, Düzlü Ö, Ekici O, Isik N, Çiloğlu A, Şimşek E, Yetişmiş G, Inci A. 2021. Comparison of three diagnostic methods in the diagnosis of cryptosporidiosis and gp60 subtyping of *Cryptosporidium parvum* in diarrheic calves in Central Anatolia Region of Turkey. The EuroBiotech Journal, 5(2):63-69.