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DETECTION OF CRYPTOSPORIDIUM SPECIES IN CATTLE AND HANDLERS BY MOLECULAR TECHNIQUE IN WASIT PROVINCE

Murtadha Kadhim Hasan
Department of Biology College of science for women University of Babylon ,Iraq

Zahraa Hamid Mohan
Department of Anatomy, Hammurabi College of Medicine, University of Babylon ,Iraq

Farah Mohammed Saeed
Department of Biology College of science for women University of Babylon ,Iraq

Oras Abdulsayed Mahdi
Department of Biology College of science for women University of Babylon ,Iraq

Rasha Hamzah Abdulhussein
Pediatric Department, Hammurabi College of Medicine, University of Babylon, Iraq

Sarah Hussein hamzah
Department of Biology College of science for women University of Babylon ,Iraq

Mustafa basim Kareem
Alkut hospital for Gynecology and Pediatrics Iraq wasit

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Abstract: I collected 300 cow feces samples (130 males - 170 females) and used molecular analysis to detect the parasite.

The results showed significant differences ($p \leq 0.05$) between the infection rate using particle technology of 38% (38/100) and 41% (41/100) in cows and breeders, respectively, while traditional microscopic methods showed 21% (21/100) and 17% (17/100) in cows and breeders, respectively.

DNA sequence analyzes of 10 isolates from infected cows identified four species of the Cryptosporidium parasite genus which included: C. parvum (6/10), C. andersoni (2/10), C. bovis (1/10) and C. ryanae (1/10. The species C. andersoni, C. parvum, C. bovis and C. ryanae have been genetically confirmed through DNA sequence analysis, and it is considered the first recording of these species in local cattle in Iraq.

In breeders, three types of the Cryptosporidium parasite were recorded through DNA sequence analysis in 10 isolates from infected individuals, which included: C. parvum (2/10), C. andersoni (5/10) and C. hominis (3/10). It is considered the first recording of this species among breeders in Iraq.

Keywords: Synergistic, Biofilm, E. Coli, Ascorbic Acid.

INTRODUCION

Cryptosporidium spp. is an intracellular extracytoplasmic coccidian-like protozoan parasite phylum Apicomplexa which causes diarrhea in humans and livestock worldwide (Zahedi et al., 2016). Cryptosporidium was discovered in 1907, however it remained mostly unknown until 1976 when the first human cases of cryptosporidiosis were reported (Nime et al., 1976; Tzipori and Widmer, 2008). The genus consists of multiple genetically distinct species and genotypes whose identification relies on molecular methods since oocysts are morphologically indistinguishable. Thirty-four Cryptosporidium species and over seventy genotypes have been determined based on DNA polymorphisms of the small subunit (SSU) rRNA gene, although only two are responsible for most human infections, including the anthroponotic species C. hominis and the zoonotic species C. parvum (Ryan et al., 2017; Zahedi et al., 2017).

Human cryptosporidiosis is frequently accompanied by abdominal pain, fever, vomiting, malabsorption, general malaise, weakness, fatigue, loss of appetite, nausea, chills and sweats and diarrhea that may sometimes be profuse and prolonged (Chalmers and Davies, 2010). Cryptosporidium is well adapted to infect human beings and animals through zoonotic, waterborne, foodborne, and person-to-person routes of transmission (Helmy et al., 2014)(Ali et al., 2023). These routes enable Cryptosporidium to be endemic in many low-income countries and potentially epidemic in high-income countries. Feco-oral transmission between domestic animals and humans may be an important mode of infection and it is likely that both serve as reservoirs of the disease (Xiao and Fayer, 2008)(Barrak et al., 2023).

A diagnosis of cryptosporidiosis is based on the identification of Cryptosporidium spp. oocysts in the fecal sample by conventional and immunodiagnostic methods (Fayer and Xiao, 2008)Several conventional techniques such as flotation by Sheather's sugar/zinc sulfate solution, formal ether concentration method, formal ethyl acetate sedimentation technique, medefied Ziehl–Neelsen (mZN), and some of negative staining methods using Nigrosin, light green, malachite green and carbol fuchsin are used for the diagnosis of cryptosporidiosis (Current and Garcia, 1991). Molecular techniques (more sensitive and specific) like polymerase chain reaction (PCR) are widely used nowadays for the genotyping of cryptosporidiosis (Rekha et al., 2016). Several studies agree on the higher sensitivity of PCR targeting the 18S rRNA gene. Nested PCR has been put forward as a means of improving the sensitivity of detection (Jothikumar et al., 2008)(Al-Rubea et al., 2021). We report prevalence of most important species of Cryptosporidium amongst cattle handlers in Baghdad province, Iraq by microscopy and nested PCR(Mohammed et al., 2021).

MATERIALS AND METHODS

The study included one hundred stool samples of 5-10g each collected from cattle handlers of both sexes and different age groups in different regions of Wasit province. The study was performed from the beginning of January 2018 to the end of September 2018.

Molecular diagnosis: A nested PCR was performed to detect Cryptosporidium spp. based on 18S ribosomal rRNA gene in volunteer stool samples as described by Ruecker et al., (2013). Primary PCR master mix preparation by using (MaximeTM PCR PreMix Kit (i-Taq)) included the first primer pair: forward (5–AGACGGTAGGGTATTGGCCT –3) and reverse (5-TACGAATGCCCCCAACTGTC-3), then placed in PCR Thermocycler. Secondary PCR master mix was prepared by using

(MaximeTM PCR PreMix Kit (i-Taq)) included second primer pair: forward (5-ATTGGAGGGCAAGTCTGGTG -3) and reverse (5 -TACGAATGCCCCCAACTGTC-3), then placed in PCR Thermocycler.

The two PCR rounds were done under the same conditions: Nested PCR mixtures contained 1x PCR buffer, 5 mM MgCl2, 200 lμ each deoxynucleoside triphosphate, 100 nM each primer and 1.25 U Hot Start Taq polymerase. Cycling conditions consisting of a hot start at 94˚ C for 5 min followed by 30 cycles with de-naturation at 94˚ C for 30 seconds, annealing at 58˚ C for 30 seconds, and at 72˚ C for 30 seconds, and a final extension at 72˚ C for 5 minutes then holding at 4 ˚ C forever (Ruecker et al., 2013).

We constructed a phylogenetic tree for our Cryptosporidium versus NCBI-Blast-GenBank. Positive PCR 18S rRNA gene were analyzed for DNA sequencing (Molecular Evolutionary Genetics Analysis version 6.0) and Multiple sequence alignment analysis (ClustalW). Evolutionary distances were computed by Maximum Composite Likelihood as described by Tamura et al., (2013). Data was analyzed using SPSS version 17 and Chi-square test as described by Petrie and Watson, (2006).

RESULTS

Results are shown in Tables 1-4 we found Cryptosporidium spp. oocysts in 17% stool samples (17/100). However, by nested PCR we found Cryptosporidium infection in 41% (41/100). PCR confirmed microscopic findings in all cases. BY PCR all stool samples exhibited a distinct band of 318 bp on agarose gel for Cryptosporidium sppas depicted in Figure 1, below.

Table 1. *Cryptosporidium spp.* (%) by microscopy and Nested PCR in stools of 100 cattle handlers.

Host	Stool samples	Conventional microscopy		Molecular-Nested PCR	
Handlers	Total No.	No.	%	No.	%
	100	17	17 % B	41	41 % A

Different letters in same row are significantly different (P 0.05)

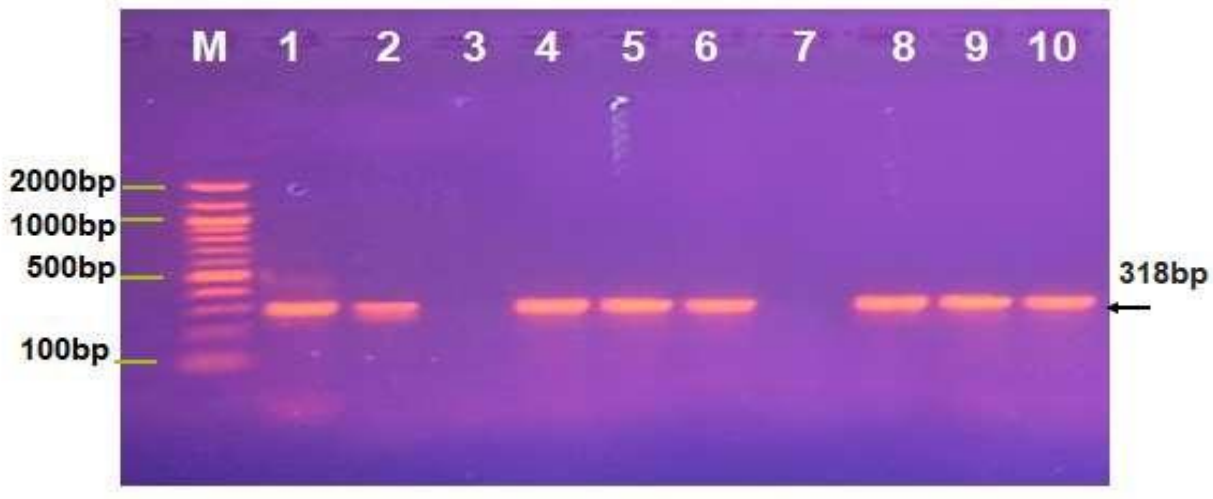

Figure 1. Agarose gel electrophoresis of a Nested PCR product subunit ribosomal RNA gene in Cryptosporidium spp. from 10 animal handlers stool samples. M: Marker (2000-100bp), lanes 1-10 show Cryptosporidium spp. at 318bp Nested PCR product size.

There was no difference between genders at 41.6% in males and 40% in females (Table 2).

Table (2): *Cryptosporidium* by nested PCR and gender.

	Number	Positive	
		No.	%
Male	60	25	41.6 % [a]
Female	40	16	40 % [a]

Different letters in same row are significantly different (P 0.05)

Results in Table 3 below show significantly (<0.05) higher (69.9%) incidence in children < 10 years old compared to handlers aged 10-19 and 20-29 years ~32%, 30-39 with ~18% and older than 40 ~15%.

Table (3): *Cryptosporidium* by nested PCR and age.

Age groups	Number	Positive	
		No.	%
10 Years	33	23	69.96 % [a]
10-19 Years	28	9	32.14 % [b]
20-29 Years	15	5	33.33 % [b]
30-39	11	2	18.18 % [c]
40	13	2	15.38% [c]

Different letters in same column are significantly different (P 0.05)

Ten positive PCR stool samples with *Cryptosporidium* species strains were compared with gen-bank revealing *C. andersoni* in 5, *C. hominis* in 3 and *C. parvum* in 2 (Table 4).

(Table 4): *Cryptosporidium* strains in cattle handlers and NCBI BLAST homology sequences.

Local Human *Cryptosporidium* sp. No.	Gen-Bank accession No.	NCBI BLAST Homology sequence identity		
		NCBI BLAST *Cryptosporidium* sp.	Gen-Bank accession No.	Identity (%)
1	MH885549	*C. andersoni* [a]	KX710086.1	100%
2	MH885550	*C. parvum* [c]	MH341586.1	100%
3	MH885551	*C. andersoni* [a]	KX710086.1	100%
4	MH885552	*C. andersoni* [a]	KX710086.1	98%
5	MH885553	*C. hominis* [b]	KT123173.1	100%
6	MH885554	*C. andersoni* [a]	KX710086.1	98%
7	MH885555	*C. parvum* [c]	MH341586.1	100%
8	MH885556	*C. hominis* [b]	KT123173.1	100%
9	MH885557	*C. andersoni* [a]	KX710086.1	98%
10	MH885558	*C. hominis* [b]	KT123173.1	100%

Different letters in same column (P0.05)

Figure 2 shows a Phylogenetic tree of *C. parvum, C. hominis* and *C. andersoni* referenced against those of Gen-Bank which highlight differences by DNA STAR (Tamura *et al.*, 2013). *Cryptosporidium* spp. 1, 3, 4, 6 and 9 were closely related to NCBI-Blast *Cryptosporidium andersoni* (KX710086.1) 5, 8 and 10 to NCBI-Blast *Cryptosporidium hominis* (KT123173.1), and 2 and 7 to NCBI-Blast *Cryptosporidium parvum* isolates (MH341586.1) with a genetic difference of 0.01-0.04%. Datasets suggest strong genetic distinctiveness amongst species.

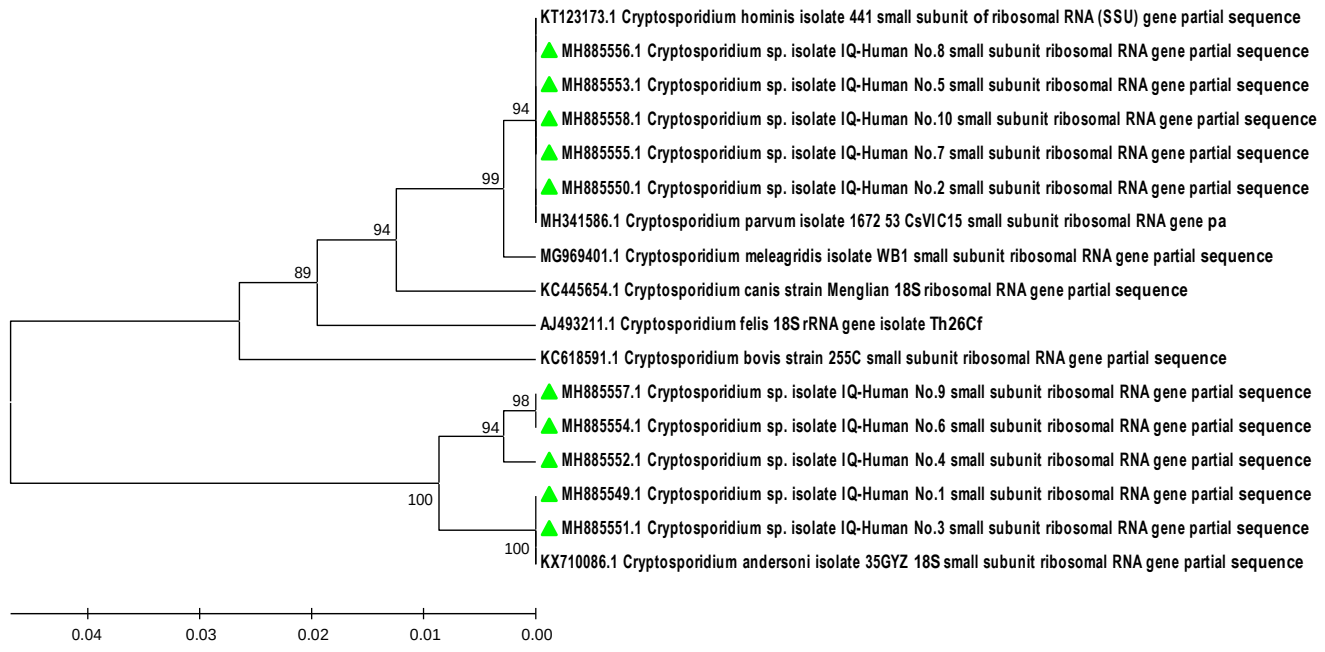

Figure 2: Phylogenetic tree analysis based on the partial sequence Small subunit rRNA gene in *Cryptosporidium* spp. isolated from stools of cattle handlers and *Cryptosporidium* species genetic identification analysis.

DISCUSSION

Lal (2014) and Lal *et al.*, (2016) did a major survey with sheep, poultry, pig, deer and dairy cattle density and concluded that only dairy cattle density was associated with a risk of cryptosporidiosis for handlers. Water may be the major route of transmission for *C. hominis*, *C. parvum* and *C. andersoni* genotypes, which accounts for high incidence of *C. parvum* and *C. andersoni* during the rainy season (Zahedi *et al.*, 2018). We found a much higher incidence of *Cryptosporidium* by PCR (41%) compared with microscopy (17%). The superior sensitivity of PCR in detecting *Cryptosporidium* infection has been reported by Uppal *et al.*, 2014 and Omoruyi *et al.*, 2014 in India and South African handlers. We presume the high prevalence is due to direct contact with cattle, unsanitary conditions and lack of parasite treatments in cattle. Our results are similar to those of Altaee *et al.*, (2014) who recorded an infection rate by microscopy of 47.72% in animal handlers in Wasit.

Wide variations in infectivity in cattle handlers may be due to hygiene, age, gender, rural or urban, environment, sampling and sample size, diagnostic techniques and/or direct contact with cattle. We found no difference in prevalence between males (41.6%) and females (40%). These results were in agreement with other studies conducted worldwide (Rahi and Raheem, 2013; Salman *et al.*, 2015) which indicates that both genders have equal chance of being infected. However because are more likely to be in contact with cattle/contaminants they may a higher change of infection (Tairsh *et al.* (2017) and Adler *et al.* (2017)

In our study lower age was correlated with higher prevalence. We found a very high rate of infection (69%) in children under 10, compared with previous findings (~10%) (Abdul Razak and Jasm, 2011; Sadek, 2014; Abdul-Sada, 2015; Salman *et al.*, 2015 and Tairsh *et al.*, (2017). However, Saeed and Khair (2014) reported 39.76% in children at Al-Ressafa Baghdad and Lal et al (2016) found a prevalence of 59% in children under 5 years in areas with high dairy cattle densities in New Zealand. The high infection rate in children is probably due to immature immunity, contact with soil and/or contaminated materials, livestock and poor sanitary conditions as noted by Al-Omashi, (2014) and Al-Ward (2010). AL-Gelany, 2003; Adler *et al.*, 2017 and Kaminsky and Garcia, (2017) maintained that maximum infection rates were in age groups (<1-5) and (6-12) years, and Yoder and Beach, (2010) in 1–9 year olds.

For nested PCR we used Small Subunit rRNA gene as it covers the major region of interspecies/genotype variability in the gene, allowing identification of nearly all *Cryptosporidium* spp. and genotypes by sequence analysis and is highly conserved with the abundance of multi-copy hyper-variable regions (Coupé *et al.*, 2005). Using 10 PCR positive sasmples we detected *C. hominis, C. parvum* and *C. andersoni*. Guyot *et al.* (2001) found *C. parvum, C. meleagridis, C. felis* and *C. andersoni* (*C. muris*) in humans in France, whereas Leoni *et al.* (2006) detected *C. hominis, C. parvum, C. meleagridis, C. andersoni, C. felis, C. canis, C. suis* and *C. cervine* type in 2414 humans with diarrhea in England. Morse *et al.* (2007) reported *C. hominis, C. parvum, C. hominis/C.*

parvum, C. meleagridis and *C. andersoni* in Malawi. Hijjawi *et al.* (2016) *C. hominis* and *C. parvum* in Jordan and Hussain *et al.* (2017) *C. parvum* and *C. andersoni* in India. Our findings are similar to those of Hussain *et al.* (2017) who reported *C. andersoni* in 6.97% and *C. parvum* in 1.7% in patients with diarrhea from India. While Leoni *et al.* (2006) and Morse *et al.* (2007) reported lower prevalence of *C. andersoni* than other *Cryptosporidium* species. We believe that to our best knowledge, this might be the first report on the prevalence of *C. andersoni* in Baghdad province/Iraq, using molecular techniques (Nested PCR and sequencing).

CONCLUSION

The study discovers that livestock handlers in Iraq have a high frequency of Cryptosporidium infection, which is better identified by PCR than by microscopy. This frequency is attributed to many factors, including direct livestock contact, filthy circumstances, and a lack of parasite treatments. Water transfer and the density of dairy animals are important danger concerns, particularly in the rainy season. Higher infection rates are seen in younger age groups, especially in children under 10. In the province of Baghdad, nested PCR effectively identifies Cryptosporidium species, including C. andersoni, maybe for the first time. Overall, the study highlights how crucial it is for cow handlers to improve hygiene and sanitation measures in order to lower their chance of contracting Cryptosporidium infections.

REFERENCES

Abdul Razak A.M. and Jasm Gh.A. (2011). Prevalence of Cryptosporidiosis in calves and child in Al Diwania city. Iraqi J.Vet.Med. 35 (1): 190-194.

Abdul-Sada K.M. (2015). Molecular and Epidemiological Study of *Cryptosporidium spp*. in Mid-Euphrates Area. Kufa Journal for Nursing sciences Vol.5 No. 1.

Adler S.; Widerström M.; Lindh J. and Lilja M. (2017). Symptoms and risk factors of *Cryptosporidium hominis* infection in children: data from a large waterborne outbreak in Sweden. Parasitol Res DOI 10.1007/s00436-017-5558-z.

AL-Gelany, B. A. (2003). An epidemiological and diagnostic study of *Cryptosporidium* in the man and animal in AL-Thahab AL-Abiydh Village. Ph.D thesis, University of Baghdad. pp. 192.

Al-Omashi Gh. (2014). Identification of *Cryptosporidium* Antigens in Stool Specimen Using Enzyme Linked Immunosorbent Assay (ELISA) in Al- Diwanyia Province- Iraq. QMJ VOL.10 No.17: 204-213.

Altaee I.A.; Al-Ani J.M. and Al-Rubaie H.M. (2014). Prevalence of *Giardia* spp. and *Cryptosporidium* spp. in horses and animal handlers in some areas in Baghdad city. Al-Anbar J. Vet. Sci., Vol.: 7 No. (2): 18-27.

AL-Warid H.S. (2010). Study in Epidemiology and PCR Detection of Cryptosporidiosis in North of Baghdad. Ph.D. thesis, (In Zoology /(Parasitology). College of Science, University of Baghdad pp.195.

Cama V. et al. (2006). Mixed Cryptosporidium infections and HIV. Emerg. Infect. Dis. 12:1025–1028.

Chalmers R. M. and Davies A. P. (2010). Minireview:Clinical cryptosporidiosis. Exp.Parasitol.124:138-146.

Coupé S.; Sarfati C.; Hamane S. and Derouin F. (2005). Detection of *Cryptosporidium* and identification to the species level by nested PCR and restriction fragment length polymorphism. Journal of Clinical Microbiology. 43 (3): 1017-1023.

Current W.L. and Garcia L.S. (1991). Cryptosporidiosis. Clin Microbiol Rev 4, 325-358.

Fayer R. and Xiao L. (2008). Cryptosporidium and cryptosporidiosis, 2nd edn. CRC press, Boca Raton, U.K. pp. 1-560.

Guyot K.; Follet-Dumoulin A.; Lelièvre E.; Sarfati C.; Rabodonirina M.; Nevez G.; Cailliez J.C.; Camus D. and Dei-Cas E. (2001) Molecular characterization of *Cryptosporidium* isolates obtained from humans in France. J. Clin. Microbiol 39(10):3472–3480.

Helmy Y.A.; Samson-Himmelstjerna G.V.; Nockler K. and Zessin K.H. (2014). Frequencies and spatial distributions of *Cryptosporidium* in livestock animals and children in the Ismailia province of Egypt. Epidemiol. Infect. Page 1-11. doi:10.1017/S0950268814001824.

Hijjawi N.; Mukbel R.; Yangc R. and Ryanc U. (2016). Genetic characterization of *Cryptosporidium* in animal and human isolates from Jordan.Vet.Parasitol. http://dx.doi.org/10.1016/j.vetpar.2016.08.015.

Hussain G.; Roychoudhury S.; Singha and Paul J. (2017). Incidence of *Cryptosporidium andersoni* in diarrheal patients from southern Assam, India: a molecular approach. Eur J Clin Microbiol Infect Dis. DOI 10.1007/s10096-016-2887-2.

Jothikumar N.; Da-Silva A.J.; Moura I.; Qvarnstrom Y. and Hill V.R. (2008). Detection and differentiation of *Cryptosporidium hominis* and *parvum* by dual TaqMan assays. J. Med. Microbiol. 57:1099-105.

Kaminsky R.G. and Garcia S.Z.R. (2017). Update of Human Infections Caused by *Cryptosporidium* spp. and *Cystoisospora belli*, Honduras. Clin Microbiol 6: 289. doi:10.4172/2327-5073.1000289.

Lal A. (2014) Evaluating the Environmental and Social Determinants of Enteric Disease in New Zealand, Doctor

of Philosophy: Univesity of Otago.
Lal A.; Dobbins T.; Bagheri N.; Baker M.G.; French N.P. and Hales S. (2016). Cryptosporidiosis Risk in New Zealand Children under 5 years old is greatest in areas with high dairy cattle densities. EcoHealth DOI: 10.1007/s10393-016-1187-8.
Leoni F.; Amar C.; Nichols G.; Pedraza-Díaz S. and McLauchlin J. (2006). Genetic analysis of *Cryptosporidium* from 2414 humans with diarrhea in England between1985and2000. JMedMicrobiol 55(Pt 6):703–707.
Makawi Z.A. and Al-Zubaidi M.T. (2017). Parasitic contamination of drinking water and its prevalence among handlers and sheep. Iraqi J. Vet. Med., 41(2): 7-14.
Morgan U.M.; Constantine C.C.; Forbes D.A. and Thompson R.C. (1997). Differentiation between human and animal isolates of *Cryptosporidium parvum* using DNA sequencing and direct PCR analysis. J. Parasitol. 83:825–830.
Morse T.D.; Nichols R.A.; Grimason A.M.; Campbell B.M.; Tembo K.C. and Smith H.V. (2007). Incidence of cryptosporidiosis species in paediatric patients in Malawi. Epidemiol Infect 135(8):1307–1315.
Nime F.A.; Burek J.D.; Page D.L.; Holscher M.A. and Yardley J.H. (1976). Acute enterocolitis in a human being infected with the protozoan *Cryptosporidium*. Gastroenterol 70, 592-598.
Omoruyi B.E.; Nwodo U.U.; Udem C.S. and Okonkwo F.O. (2014). Comparative diagnostic techniques for *Cryptosporidium* infection. Molecules. (19): 2674–2683. doi: 10.3390/molecules19022674.
Petrie A. and Watson P. (2006). Statistics for Veterinary and Animal Science, Second Edition. Ames: Blackwell Publishing, Pp: 24-112.
Rahi A.A. and Raheem H.H. (2013). Prevalence of *Cryptosporidium parvum* Among Children at Wasit Province. Journal of Wassit for Science & Medicine ISSN: 58161992. V. 6 (1): 105-111.
Reed C.; Sturbaum G.D.; Hoover P.J. and Sterling C.R. (2002). *Cryptosporidium parvum* mixed genotypes detected by PCR-restriction fragment length polymorphism analysis. Appl. Environ. Microbiol. 68:427–429.
Rekha H.K.M.; Puttalakshmamma G.C. and D'Souza P.E. (2016). Comparison of different diagnostic techniques for the detection of cryptosporidiosis in bovines, Veterinary World, 9 (2): 211-215.
Ruecker N.J.; Matsune, J.C.; Lapen D.R.; Topp E.; Edge T. and Neumann N.F. (2013). The detection of *Cryptosporidium* and the resolution of mixtures of species and genotypes from water. Infect Genet Evol. 15: 3-9.
Ryan U.; Lawler S. and Reid S. (2017). Limiting swimming pool outbreaks of cryptosporidiosis, the roles of regulations, staff, patrons and research. J. 550 Water Health. 15, 1–16.
Sadek G. (2014). Use of nested PCR-RFLP for genotyping of *Cryptosporidium* parasites isolated from calves and children suffering from diarrhea. Parasitol. United J. 7: 129–137.
Saeed A.R. and Khairi N.M. (2014). Study the prevalence of Giardiasis and Cryptosporidiosis among children at Al-Ressafa side of Baghdad by comparison between the efficiency of some diagnositic methods. AL-Taqani , Vol . 27, No 2: 82-93.
Salman Y.J.; Sadek W.S. and Rasheed Z.K. (2015). Prevalence of *Cryptosporidium parvum* among Iraqi displaced people in Kirkuk city using direct microscopy, flotation technique and ELISA-copro antigen test. Int.J.Curr.Microbiol.App.Sci. 4(11): 559-572.
Tairsh H.R.; Abdullah Ab.; Al-A-asady R.A.; AL-zeyadi M.J.; Alsherees H.A. and AL-Baldawy A.N. (2017). Identification of *Cryptosporidium* spp. infections in children with persistent diarrhea by modified Ziehl-Neelsen stain method and PCR technique. European Journal of Pharmaceutical and Medical Research. Ejpmr, 4(2): 208-215.
Tamura K.; Stecher G.; Peterson D.; Filipski A. and Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725-2729.
Tzipori S. and Widmer G. (2008). A hundred-year retrospective on cryptosporidiosis. Trends Parasitol. 24: 184–189.
Uppal B.; Singh O.; Chadha S. and Jha A.K. (2014). A Comparison of Nested PCR assay with conventional techniques for diagnosis of intestinal cryptosporidiosis in AIDS Cases from Northern India. J Parasitol Res.
Xiao L. and Fayer R. (2008). Molecular characterization of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. Int. J. Parasitol. 38: 1239–1255.
Yoder J. S. and Beach M. J. (2010). *Cryptosporidium* surveillance and risk factors in the United States. Exp. Parasitol. 124: 31-39.
Zahedi A.; Durmic Z.; Gofton A.W.; Kueh S.; Austen J.; Lawson M.; Callahan L.; Jardine J. and Ryan U. (2017). *Cryptosporidium* homai n. sp. (*Apicomplexa: Cryptosporidiiae*) from the guinea pig (*Cavia porcellus*). Vet. Para. 245: 92-101.
Zahedi A.; Monis P.; Gofton A.W.; Oskam C.L.; Ball A.; Bath A.; Bartkow M.; Robertson I. and Ryan U. (2018). *Cryptosporidium* species and subtypes in animals inhabiting drinking water catchments in three states across Australia, Water Research, doi: 10.1016/j.watres.2018.02.005.
Zahedi A.; Paparini A.; Jian F.; Robertson I. and Ryan U. (2016). Public health significance of zoonotic

Cryptosporidium species in wildlife: critical insights into better drinking water management. Int. J. Parasitol Parasites Wildl 5:88–109.

Barrak, M. M., Bonyadi, M., Hami, H. J., Al Ali, M. D., Muttair, R. Y., & Abd, R. A. (2023). Exploring the Heterogeneity of Non-Syndromic Hearing Loss: A Comprehensive Review of Implicated Genes and the Role of Whole Exome Sequencing. *University of Thi-Qar Journal of Science*, *10*(2), 192-199.

Ali, M. D., Barrak, M. M., Salman, R. I., & Sa'doon, N. M. (2023, September). The combined effect of artemisia absinthium methanolic extract and vinblastine chemotherapy on apoptosis and decreasing chemotherapy drug concentration. In *AIP Conference Proceedings* (Vol. 2845, No. 1). AIP Publishing.

Al-Rubea, F. M., Mohammed, R. A. K., Barrak, M. M., & Aboob, A. J. (2021). Molecular Detection Of Bifidobacterium Dentium From Patients With Tooth Caries. *NVEO-NATURAL VOLATILES & ESSENTIAL OILS Journal| NVEO*, 5423-5430.

Mohammed, R. A. K., Barrak, M. M., Hussein, H. H., & Hasan, M. S. (2021). Molecular Diagnosis Of Some Anaerobic Bacteria From The Root Canals. *NVEO-NATURAL VOLATILES & ESSENTIAL OILS Journal| NVEO*, 5415-5422.