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Molecular detection of *Cryptosporidium parvum* in sheep and goats in rural areas of Al-Muthanna Province, Iraq

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ABSTRACT

Background: Cryptosporidium species are widespread protozoa and are considered an important cause of diarrhea in humans and animals. *Aim:* The aim of this study was to identify the occurrence of *Cryptosporidium parvum* in diarrheic sheep and goats from the rural areas of the Al-Muthanna Province, southern Iraq, based on molecular screening using PCR. The outcomes would help in determining the distribution of *C. parvum* in sheep and goats in the area, as well as the risk factors for the infection. *Methodology:* Sixty fecal samples were collected from clinically ill sheep and goats. The samples were submitted to PCR and sequencing that targeted the 18S rRNA gene in the protozoan. *Results:* The PCR revealed a 60% (18 samples) and 76.67% (23 samples) positive identification of *C. parvum* in sheep and goats, respectively. The sequencing demonstrated nucleotide similarities with isolates deriving from Iraq (MG878399.1) and India (JN836324.1) for the sheep isolates, and with isolates deriving from South Korea (DQ054819.1) and the USA (AF159112.1) for the goat isolates. *Conclusion: C. parvum* is the dominant species in the sheep and goats of Al-Muthanna Province, in southern Iraq. Moreover, the phylogenetic analysis demonstrates that the current study's isolates may share the same ancestor with local and global isolates.

KEYWORDS

antiprotozoal, cryptosporidiosis, epidemiology, parasitic infection, protozoa

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1. INTRODUCTION

Cryptosporidium species are widespread protozoa and are considered an important cause of diarrhea in humans and animals. They belong to the order eucoccidia, subclass coccidia, class sporozoas, and phylum apicomplexa [1-5]. The most important zoonotic species responsible are *C. parvum*, *C. hominis*, and *C. canis*. Non-zoonotic pathogenic species of *Cryptosporidium* include *C. meleagridis, C. felis, C. bailey*, and *C. muris.* The oocyst shape, size, and host species are the most important things that differentiate the *Cryptosporidium* species [6,7].

The outer shell of the *Cryptosporidium* allows it to live outside the body for long periods of time.

Over the last two decades, *Cryptosporidium* has been found in public swimming pools and water playgrounds. Several outbreaks of *Cryptosporidium* have been linked to public waterparks. Once a waterpark or pool is contaminated, the *Cryptosporidium* is difficult to kill, and it may take a few days before a facility can be safe to use. A chlorine-resistant parasite, *Cryptosporidium* can live for more than 10 days in properly-treated recycled water. An average of 15 poop particles has 1,000,000 *Cryptosporidium* parasites. Health departments check swimming pools and water playgrounds for all pathogens, including *Cryptosporidium*, as part of their routine inspection program. Pool water is tested for proper pH and chlorine levels, and *Cryptosporidium* should never be present in properly-chlorinated drinking water [8].

Cryptosporidium can spread in several different ways, including through changing the diapers of an infected individual or drinking contaminated water. Recently, several multi-state outbreaks of cryptosporidiosis have been associated with contact with livestock. Outbreaks have been linked to dirt, fences, and gates within animal habitats, suggesting that the *Cryptosporidium* can spread when people touch these surfaces [9,10]. People are at increased risk for becoming infected with *Cryptosporidium* if they have frequent exposure to animals, such as visitors to petting zoos and owners of show animals. As a result, the United States are now working in order to improve the prevention strategies in place for *Cryptosporidium* in animals, including those on farms, at petting zoos, and during human / animal exhibitions [11]. *C. parvum* infects the intestinal tract of ruminant mammals, such as cattle, sheep, and goats [1-7,9,10,12].

The aim of this study was to identify the occurrence of *C. parvum* in diarrheic sheep and goats from the rural areas of the Al-Muthanna Province, southern Iraq, based on molecular screening and by using PCR.

2. METHODOLOGY

2.1. Samples

The study was undertaken by collecting 60 fecal samples from the rectum of clinically ill sheep and goats in the Al-Muthanna rural areas. The clinical signs sought after were abdominal pain, severe diarrhea, and depression. The study lasted from November 2023 to February 2024. The samples were processed at the Laboratory of Parasitology of the College of Veterinary Medicine of Al-Qasim Green University.

2.2. Molecular techniques and DNA extraction

The extraction was performed using 100 mg as a starting fecal material for each sample, and by employing the Qiagen kit and according to the instructions of the kit. The DNA was quantity- and quality-measured by a NanoDrop spectrophotometer.

2.3. PCR detection of the 18S rRNA gene

The gene of the small subunit of ribosomal RNA (SSU rRNA) and the 18S rRNA gene region are the most suitable loci for nested PCR amplification and other molecular diagnostic tools; the reason is because they are conserved sufficiently so as to allow the primers to detect nearly all *Cryptosporidium* spp. and their genotypes from most mammalian hosts.

A gradient PCR was performed on all isolates so as to confirm the species by detecting *C. parvum*. The PCR samples' preparation involved adding 1.5 µL of 10-pmol of the forward and reverse primers (F: AGTGACAAGAAATAACAATACAGG and R: CCTGCTTTAAGCACTCTAATTTTC) at 295-bp (KX151739.1), 5 µg of genomic DNA, 12.5 µL of Master Mix, and a nuclease free water for a 25-µL total volume. The PCR program was set with 95ºC for 5 min, 35 cycles of 94ºC for 30 sec, 50ºC for 30 sec, 72ºC for 45 sec, and a final extension at 72ºC for 7 min, with a final hold at 4ºC. The PCR products were transferred to a 1.5% agarose gel for electrophoresis. The electrophoresis was carried for 60 min, at 80 V. The DNA bands were stained with ethidium bromide, and the sizes of the 18S rRNA gene was evaluated by comparing against a 1,500-bp DNA ladder.

A purified *18S rRNA* PCR product amount of 40 µL was prepared and sent to Macrogen Inc. (Korea) for partial sequencing. The *18S rRNA* PCR gene product with genome sequences of *C. parvum* was identified using the NCBI BLAST engine. The obtained sequence data were analyzed by using biological evolutionary algorithms so as to construct a dendrogram generated by the MEGA X software, and to evaluate the genetic distances of the subject strains compared to other global strains.

3. RESULTS

The PCR amplified the gene in the samples of the feces obtained from sheep and goats (Figure 1), and revealed a 60% (18 samples) and 76.67% (23 samples) positive identification of *C. parvum* in sheep and goats, respectively. The sequencing

demonstrated nucleotide similarities with isolates deriving from Iraq (MG878399.1) and India (JN836324.1) for the sheep isolates (Figure 2), and with isolates deriving from South Korea (DQ054819.1) and the USA (AF159112.1) for the goat isolates (Figure 3).

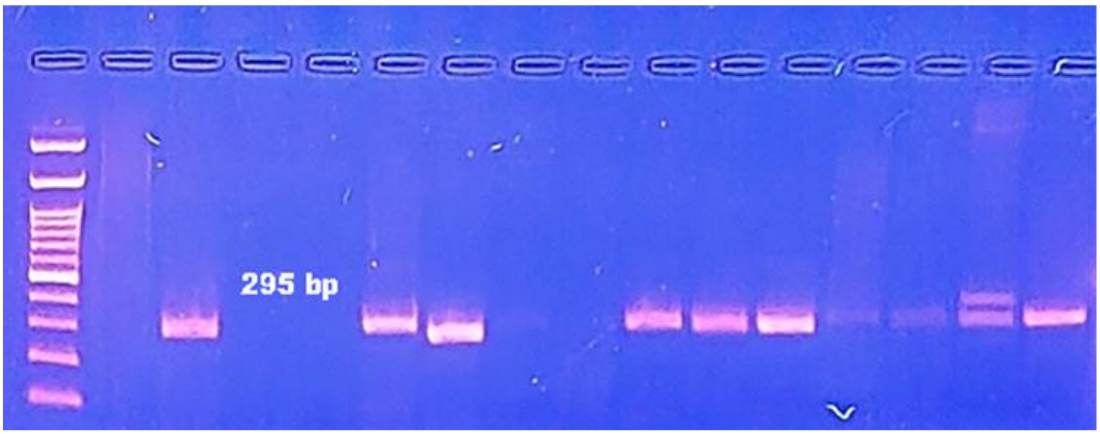

Figure 1. Representative image of the agarose gel electrophoresis of the 18S rRNA gene of the of *C. parvum* isolated from the fecal samples of sheep and goats. Bands represent positive samples.

4. DISCUSSION

Cryptosporidium species infecting sheep and goats have previously been studied in various countries, including China, Spain, Poland, Portugal, Turkey, India, Jordan, and Egypt. Compared to other host animals, fewer studies have been conducted in small ruminants in recent years, and there are no nationwide data about the *Cryptosporidium* prevalence in sheep or goats in Iraq. As such, the present study was conducted in order to evaluate the prevalence and genetic diversity of *Cryptosporidium* in small ruminants in the Al-Muthanna Province, by using PCR and Sanger sequencing of the 18S rRNA gene. Sheep and goats are hosts for *C. parvum*, *C. ubiquitum*, and *C. xiaoi*, and they can be possible reservoirs for human cryptosporidiosis. Surveillance on cryptosporidiosis is necessary as it causes public health problems and severe economic loss to farmers in the Al-Muthanna Province. The prevalence of *Cryptosporidium* was 60% for clinically-ill sheep and 76.67% for goats.

Parasites of the genus *Cryptosporidium* cause diarrhea and severe dehydration, and can cause weight loss in livestock and humans. Bad animal management, poor sanitation, and the consumption of raw water, especially for rural residents, may contribute to the severity of the disease as well as its spread [12]. High seroprevalences of cryptosporidiosis were observed in the current study, raising concern particularly about the potential zoonotic risk factors of sheep and goats in some rural provinces in this country. The prevalence and diversity of *Cryptosporidium* species differ among different countries and regions [3]. This might be due to geographic, host, and source differences among regions. As lengthy clinical courses, clinic-microbiological diversity, high morbidity rates, and potential pandemic risks for human cryptosporidiosis have been attracting attention continually, the isolation and identification of what is the causative species is now vital for the timely diagnosis of human cryptosporidiosis, its therapy, and the prevention of outbreaks.

Previous studies have revealed prevalence rates in sheep and goats that range from 0.1% to 64.2%. This wide range highlights the need for more research in this area so as to better understand and manage *Cryptosporidium* infections in these animals [13]. It has also been found that certain strains of *Cryptosporidium* were more prevalent in sheep than in goats, thereby indicating potential species-specific differences in susceptibility [14-16]. Moreover, the phylogenetic analysis undertaken in our study demonstrates that the herein reported isolates may share the same ancestor with local and global isolates.

5. CONCLUSION

Our study reveals that *C. parvum* is the dominant species in clinically-ill sheep and goats of the Al-Muthanna Province, southern Iraq. The phylogenetic analysis undertaken in our study demonstrates that the reported isolates may share the same ancestor with local and global isolates.

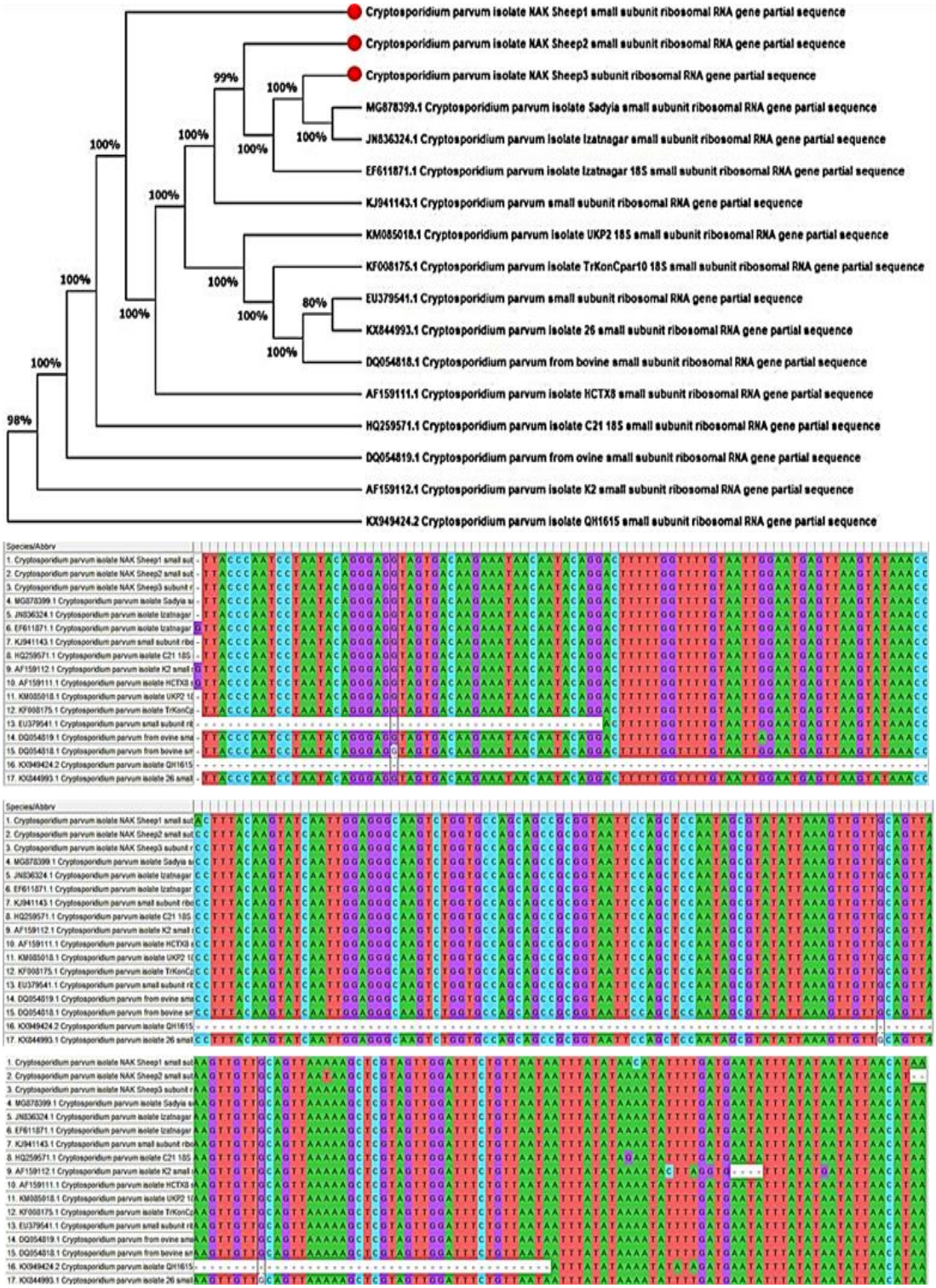

Figure 2. Phylogenetic analysis of the 18S rRNA gene of *C. parvum* isolated from the fecal samples of sheep.

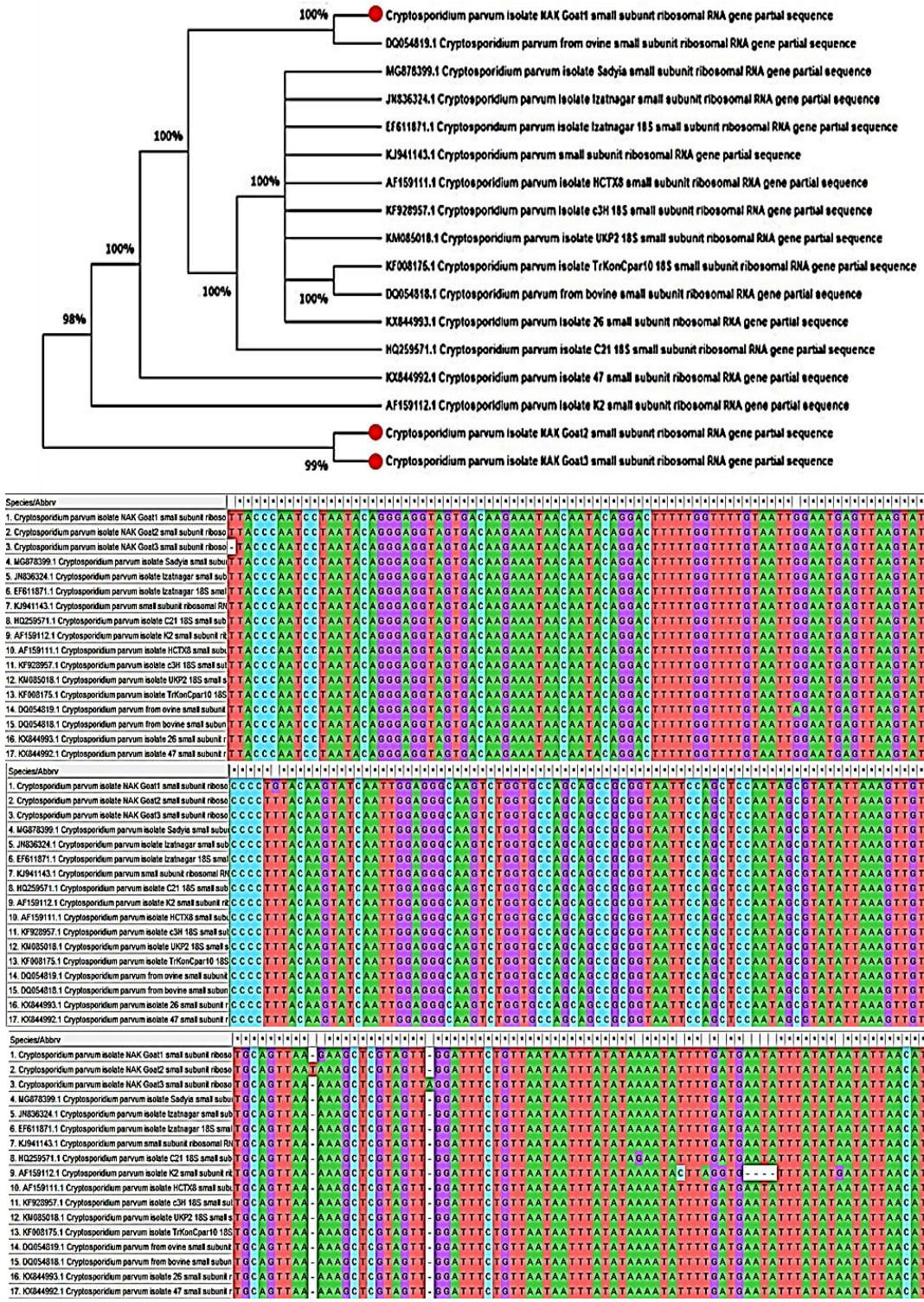

Figure 3. Phylogenetic analysis of the 18S rRNA gene of *C. parvum* isolated from the fecal samples of goats.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

REFERENCES

1. Benhouda D., Hakem A., Sannella A. R., Benhouda A., Cacciò S. M.: First molecular investigation of *Cryptosporidium* spp. in young calves in Algeria. *Parasite* 24: 15 (2017).
DOI: 10.1051/parasite/2017014

2. Khan N. U., Usman T., Sarwar M. S., Ali H., Gohar A., Asif M., *et al.*: The prevalence, risk factors analysis and evaluation of two diagnostic techniques for the detection of *Cryptosporidium* infection in diarrheic sheep from Pakistan. *PLoS One* 17(7): e0269859 (2022).
DOI: 10.1371/journal.pone.0269859

3. Elmahallawy E. K., Sadek H. A., Aboelsoued D., Aloraini M. A., Alkhaldi A. A. M., Abdel-Rahman S. M., *et al.*: Parasitological, molecular, and epidemiological investigation of *Cryptosporidium* infection among cattle and buffalo calves from Assiut Governorate, Upper Egypt: current status and zoonotic implications. *Front. Vet. Sci.* 9: 899854 (2022).
DOI: 10.3389/fvets.2022.899854

4. Firoozi Z., Sazmand A., Zahedi A., Astani A., Fattahi-Bafghi A., Kiani-Salmi N., *et al.*: Prevalence and genotyping identification of *Cryptosporidium* in adult ruminants in central Iran. *Parasit. Vectors* 12(1): 510 (2019).
DOI: 10.1186/s13071-019-3759-2

5. Kaupke A., Michalski M. M., Rzeżutka A.: Diversity of *Cryptosporidium* species occurring in sheep and goat breeds reared in Poland. *Parasitol. Res.* 116(3): 871-879 (2017).
DOI: 10.1007/s00436-016-5360-3

6. Utaaker K. S., Chaudhary S., Kifleyohannes T., Robertson L. J.: Global goat! Is the expanding goat population an important reservoir of *Cryptosporidium*? *Front. Vet. Sci.* 8: 648500 (2021).
DOI: 10.3389/fvets.2021.648500

7. Li X., Vodovoza T., Atwill E. R.: Diverse genotypes of *Cryptosporidium* in sheep in California, USA. *Pathogens* 11(9): 1023 (2022).
DOI: 10.3390/pathogens11091023

8. Bairami Kuzehkanan A., Rezaeian M., Zeraati H., Mohebali M., Meamar A. R., Babaei Z., *et al.*: A sensitive and specific PCR based method for identification of *Cryptosporidium* sp. using new primers from 18S ribosomal RNA. *Iran. J. Parasitol.* 6(4): 1-7 (2011).

9. Baroudi D., Hakem A., Adamu H., Amer S., Khelef D., Adjou K., *et al.*: Zoonotic *Cryptosporidium* species and subtypes in lambs and goat kids in Algeria. *Parasit. Vectors* 11(1): 582 (2018).
DOI: 10.1186/s13071-018-3172-2

10. Wegayehu T., Karim R., Anberber M., Adamu H., Erko B., Zhang L., *et al.*: Prevalence and genetic characterization of *Cryptosporidium* species in dairy calves in central Ethiopia. *PLoS One* 11(5): e0154647 (2016).
DOI: 10.1371/journal.pone.0154647

11. Gaddy H. G.: Using local knowledge in emerging infectious disease research. *Soc. Sci. Med.* 258: 113107 (2020).
DOI: 10.1016/j.socscimed.2020.113107

12. Mahami Oskouei M., Fallah E., Ahmadi M., Safaiyan A., Bakhtiyari S., Naserifar R., *et al.*: Molecular and parasitological study of *Cryptosporidium* isolates from cattle in Ilam, west of Iran. *Iran. J. Parasitol.* 9(3): 435-440 (2014).

13. Abdou N. M. I., AlAzemi M. S., Al-Sayegh M. T., Majeed Q. A. H.: Performance of diagnostic assays used to detect *Cryptosporidium* oocysts in faecal samples of cattle in Kuwait and genotyping of *Cryptosporidium* species. *BMC Vet. Res.* 18(1): 336 (2022).
DOI: 10.1186/s12917-022-03435-w

14. Yang X. Y., Gong Q. L., Zhao B., Cai Y. N., Zhao Q.: Prevalence of *Cryptosporidium* infection in sheep and goat flocks in China during 2010-2019: a systematic review and meta-analysis. *Vector Borne Zoonotic Dis.* 21(9): 692-706 (2021).
DOI: 10.1089/vbz.2020.2713

15. Zhao Q., Qi M., Jing B., Jian F., Gong P., Lu C., *et al.*: *Cryptosporidium* spp. in large-scale sheep farms in China: prevalence and genetic diversity. *Sci. Rep.* 14(1): 11218 (2024).
DOI: 10.1038/s41598-024-62110-2

16. Lang J., Han H., Dong H., Qin Z., Fu Y., Qin H., *et al.*: Molecular characterization and prevalence of *Cryptosporidium* spp. in sheep and goats in western Inner Mongolia, China. *Parasitol. Res.* 122(2): 537-545 (2023).
DOI: 10.1007/s00436-022-07756-5