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Molecular characterization and associated risk factors of zoonotic cryptosporidiosis in bovine calves and humans in Menoufia governorate, Egypt

Gehad Ramzy[1], Walid Mousa[1], Osama K. Gaidan[2], Ahmed Zaghawa[1], Mohamed Nayel[1], Ahmed Elsify[1], Nourhan Eissa[3]*, Ashraf M. Abu-Seida[4] and Akram Salama[1]

[1]*Department of Animal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt*
[2]*Faculty of Veterinary Medicine, Omar Al-mukhtar University, Al Bayda, Libya*
[3]*Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt*
[4]*Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt*

ABSTRACT

Background: *Cryptosporidium* is a common intestine anthroponotic protozoan parasite that affects humans and other animals all over the world. Many different kinds of vertebrates have their gastrointestinal epithelium infected by the ubiquitous, obligatory parasites of *Cryptosporidium* spp. In humans, those with weakened immune systems, including those with acronym for acquired immunodeficiency syndrome, are most affected. Particularly prevalent in developing nations, cryptosporidiosis poses further difficulties for the underfunded public health system. Humans frequently harbor a number of *Cryptosporidium* species, whose prevalence varies according to socioeconomic level and level of animal husbandry.

Aim: The present study was conducted to ascertain the prevalence of *Cryptosporidium bovis* and associated relevant risk factors of contracting cryptosporidiosis in calves and humans in Menoufia governorate, Egypt. In addition, a phylogenetic analysis was performed for additional molecular identification in order to study the evolution of the parasite and comprehend the mechanism of cryptosporidiosis evolution in the selected governorate.

Methods: Using direct wet smear, sedimentation technique, simple fecal flotation technique, and modified Ziehl–Neelsen staining technique, the parasitological analysis was conducted on fecal samples from 156 diarrheic calves and 125 humans in Menoufia governorate; polymerase chain reaction and phylogenetic analysis were further used.

Results: Based on the microscopical examination, the prevalence of *Cryptosporidium* was 19.2% in humans and 64.1% in calves. A statistical correlation was found between the prevalence of cryptosporidiosis in calves of American breeds, their early age, and the predominant warm climate. In the analyzed calves, the study was unable to detect any statistically significant difference between the type of diarrhea and increased infection. In terms of people, the study found no significant correlation between the sex of the people under inquiry, whereas a significant association was revealed among contracting cryptosporidiosis in young people, during warm climates, and among diarrhea sufferers. PCR application produced a 4% positive result. Following phylogenetic analysis, the *Cryptosporidium parvum* species was identified from the PCR-positive samples.

Conclusion: Different transmission trajectories were proposed by cluster analysis, and it was possible to take into consideration the role of calves for the zoonotic transmission of cryptosporidiosis to humans.

Keywords: Calves, *Cryptosporidium*, Humans, Menoufia governorate, Risk factors.

Introduction

Zoonosis, also known as zoonoses for pleural, is an infectious disease that can transfer between animals and humans by a number of various pathways, including direct ingestion, respiration, skin-to-mucous membrane contact, penetration through wounds or abrasions, and even vectors (Elrashedy *et al.,* 2022; Zin Eldin *et al.,* 2023; Bedair *et al.,* 2024; Eissa, 2024; Salman *et al.,* 2024a,b). A significant intestinal zoonotic protozoan parasite that affects people and other animals

***Corresponding Author:** Nourhan Eissa. Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt. Email: *vet_noura@yahoo.com*

worldwide is called *Cryptosporidium* (Jang *et al.,* 2023). According to Xiao *et al.* (2004), *Cryptosporidium* is linked to illness in at least 150 mammalian species, including humans, as well as in fish, amphibians, bird species, and reptiles. When the World Health Organization listed cryptosporidiosis as a neglected illness in 2004, it became a global health concern (Gilbert *et al.,* 2023). *Cryptosporidium* parasite causes significant economic losses in farm animals similar to other infectious diseases such as Bovine ephemeral fever (Zaghawa *et al.,* 2016; 2017). *Cryptosporidium parvum* was classified as a "priority pathogen" by the National Institute of Allergy and Infectious Diseases in 2012, meaning that it is an organism or biological agent that poses a hazard to emerging pathogens in the United States (Sheehan, 2024). According to Xiao and Fayer (2008), five species—*C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, and *C. canis*—are mostly linked to cases of human cryptosporidiosis. The most frequent species in industrialized nations that cause infections in humans are *C. hominis* and *C. parvum* (Köster *et al.,* 2024). When *C. parvum*, which was previously thought to be an opportunistic infection, was linked to newborn diarrhea in calves, serious sickness in cattle first emerged, resulting in considerable financial losses, growth retardation, weight loss, and potentially even mortality (Jang *et al.,* 2023). The identification of *Cryptosporidium* as a public health concern was established by the well-documented initial human cases of the disease in immunocompromised people (Taherkhani *et al.,* 2007). In 1993, cryptosporidiosis began with the greatest known waterborne outbreak in history, which affected over 4,000,000 persons in Wisconsin, USA (Gradus, 2015). Growing evidence points to cryptosporidiosis as a significant pediatric diarrheal illness that can cause malnutrition and stunting in developing nations (Greigert *et al.,* 2024). The main zoonotic reservoirs for cryptosporidiosis include domestic animals, livestock, and humans. According to Köster *et al.* (2024), oocysts can spread *Cryptosporidium* from person to person, animal to person, or by the consumption of tainted food and drink. Because existing diagnostic methods cannot distinguish between distinct parasite species and genotypes, the relative relevance of transmission routes remains unclear (Ramirez *et al.,* 2004). The spread of cryptosporidiosis cases is predisposed in an environment, particularly in poor nations, with a high concentration of infectious oocysts and a high concentration of vulnerable animals and people (Köster *et al.,* 2024). As a potential threat to the world's water supply, *Cryptosporidium* oocysts may tolerate chlorine disinfection and water filtration and can survive for a variety of lengths of time in both soil and water under suitable environmental circumstances (Sheehan, 2024). Improved epidemiological data on the origins of infection, modes of transmission, and relative risk for various populations are necessary for the appropriate treatment of cryptosporidiosis in both animal and human populations. In impoverished nations, there is little and often inconsistent information available about *Cryptosporidium* (Helmy *et al.,* 2015).

According to the aforementioned anthroponotic significance of cryptosporidiosis in animals and humans, the current investigation was carried out to perform a survey on the prevalence and associated relevant risk factors of *Cryptosporidium* spp. among calves and humans in Menoufia governorate, Egypt. In addition, a phylogenetic analysis was performed as an ideal tool to investigate the parasite evolution, to determine the relatedness between global isolates, and even to understand the propagation mode of cryptosporidiosis.

Materials and Methods

Samples collection and processing

The current study was carried out on two sides: the first side concerned the animal samples, a total number of 156 fecal samples were randomly collected from calves (58 calves of German origin and 98 calves of American origin) aged from 4 days to 2 months. Of the 156, 100 of them were suffering from watery non-bloody yellowish to green diarrhea, while the other 56 were normal (with no diarrhea), all of them were living in localities belonging to Sadat City, Menoufia governorate, Egypt. On the other side, the human samples, a total of 125 fecal samples were collected from human breeders (59 males and 66 females) aged from 3 years to 74 years in close contact with the examined calves in Sadat City, Menoufia governorate, Egypt. A history questionnaire was reported from the examined people regarding their clinical signs in accordance with Byomi *et al.* (2019a,b). All animal and human samples were collected in the period from August 2021 to March 2022 in sterile plastic tubes, labeled, and sent immediately to the laboratory in the Department of Animal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Sadat City, Egypt, where they stored at 4°C till ready for further examinations.

Parasitological examination

All animal and human samples were subjected to parasitological examination ranging from simple fecal flotation technique, sedimentation technique, direct wet smear, and modified Ziehl–Neelsen staining technique using different reagents, according to Henriksen and Pohlenz (1981), such as saturated salt solution (SG 1.27), modified Ziehl–Neelsen stain, carbol fuchsine, malachite green 5%, and 70% ethanol.

Molecular diagnosis

In order to identify *Cryptosporidium* (The gene coding for the *Cryptosporidium* oocyst wall protein (COWP) gene), DNA extraction from positive stool samples was processed in accordance with Xiao *et al.* (2000); Bedair *et al.* (2024); Salman *et al.* (2024a) using the instructions for the QIAamp DNA Stool Mini Kit (Catalogue Number 51504). Polymerase chain reaction

(PCR) reactions were carried out using the T3 Thermal cycler (Biometra) in a final volume of 25 µl reaction, which was separated beside a reference positive control strain that was kindly obtained from the Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Dokki, Giza, Egypt, on a 1% agarose gel by electrophoresis, and then were visualized and analyzed using Alpha Innotech gel documentation system. PCR was performed targeting COWP gene (specific for genus *Cryptosporidium*) using primers Cry9 (of sequence: GGACTGAAATACAGGCATTATCTTG) and Cry 15 (of sequence: GTAGATAATGGAAGAGATTGTG), with the expected band product size of 553 bp according to Xiao *et al.* (2000).

Furthermore, concerning the cycling conditions (adjusted by thermal cycler) for the detection of the used gene in the current study, a primary denaturation cycle was performed at 94°C for 5 minutes followed by 35 cycles of repeated secondary denaturation at 94°C for 30 seconds, annealing at 55°C for 55 seconds, and extension at 72°C for 45 seconds. A final extension cycle was done at 72°C for 10 minutes.

Sequencing of the purified PCR product

Furthermore, purification of the PCR product was performed directly in accordance with Zaghawa *et al.* (2017) and Bedair *et al.* (2024) (Qiagen Inc. Valencia CA) kit in order to apply a Bigdye Terminator V3.1 cycle sequencing kit (Cat. No. 4336817, Perkinelmer, Foster City, CA) that was utilized with an Applied Biosystems 3130 genetic analyzer (HITACHI, Japan) to do gene sequencing. Finally, Jalview software (2.11.2.6), designed by Waterhouse *et al.* (2009), was used to visualize a comparative analysis of the sequences using MEGA11's CLUSTAL W multiple sequence alignment program. In addition, FigTree software (v1.4.4), designed by Tamura *et al.* (2021), was used to display phylogenetic analyses using maximum likelihood, neighbor-joining, and maximum parsimony in MEGA11, available at: http://tree.bio.ed.ac.uk/software/figtree/.

Statistical and epidemiological analysis

The chi-square test was used to compare the proportions of seropositive results between groups according to Zaghawa *et al.* (2016) where IBM SPSS Statistics 22.0 (IBM Corporation, Armonk, NY) was used for data analysis. A significance level of $p < 0.05$ was used.

Ethical approval

Under the supervision of the University of Sadat City's Faculty of Veterinary Medicine, all animal handling operations, samples collection, and disposal were carried out in accordance with the rules of the Institutional Animal Care and Use Committee (ethical permission number: VUSC-012-1-24). Furthermore, each human case gave consent for each sample to be taken under the condition that any data that might be used to identify them would be kept confidential.

Results

Parasitological identification of Cryptosporidium oocysts

Table 1 and Figure 1 represent that 64.10% (100/156) and 19.2% (24/125) of calves' feces and human feces, respectively, were positive for *Cryptosporidium* oocysts as detected by microscopical examination using oil immersion lens (X100).

Prevalence of Cryptosporidium oocysts according to various demographic factors and clinical signs in animals and humans

Tables 2 and 3 show the prevalence of *Cryptosporidium* oocysts in calves feces and human feces, respectively, in relation with demographic data and clinical signs of examined cases. Concerning calves, there were statistical differences between *Cryptosporidium* prevalence and breed, age, and season of samples collection, where the prevalence increased among calves of American origin 82.76% (48/58), calves of age group (2–3 weeks) 90.91% (10/11), and the prevailed warm season 82.76% (48/58), whereas there was no statistical significance declared regarding the clinical signs of examined calves. On the other side, there were statistical differences between *Cryptosporidium* prevalence and human age, season of samples collection, and clinical signs, where the prevalence increased with age group (0-20 years) 34.29% (12/35), prevailed warm condition 35.56% (16/45), and presence of diarrhea 63.64% (7/11), whereas no statistical difference was found between *Cryptosporidium* prevalence and sex of examined humans.

Molecular identification of Cryptosporidium

Molecular confirmation of identified Cryptosporidium by PCR

Molecular identification of all identified positive microscopic *Cryptosporidium* samples was done using PCR on the genus level for detection of COWP

Table 1. Prevalence of *Cryptosporidium* oocysts by microscopical examination in calves and human samples.

Species	Total number of fecal samples	Positive microscopical examination		Negative microscopical examination	
		No.	%	No.	%
Calves	156	100	64.10	56	35.90
Humans	125	24	19.2	101	80.8

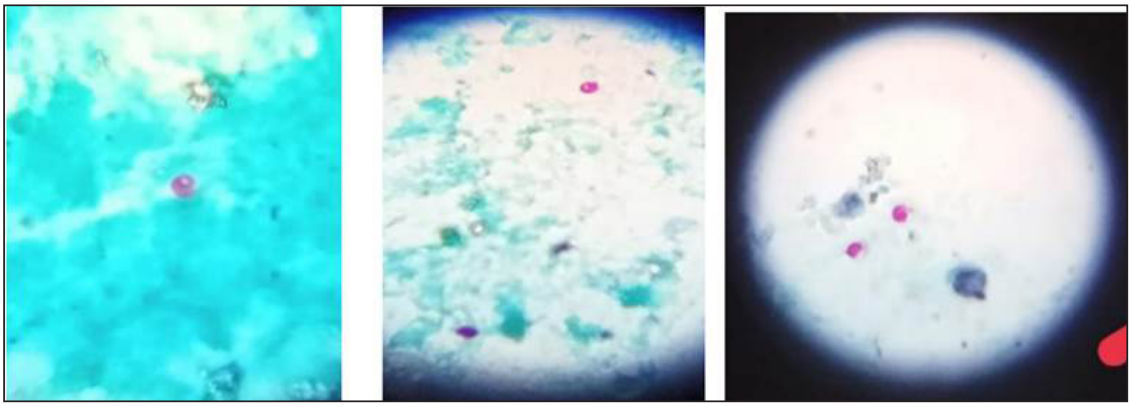

Fig. 1. *Cryptosporidium* oocyst by microscopical examination × 100 (oil immersion lens) in fecal samples.

Table 2. Prevalence of *Cryptosporidium* oocysts in calves in relation to different demographic data and clinical signs.

Factor	Positive		Negative		*p* value
	No.	%	No.	%	
Breed					
Calves of American origin (no. = 58)	48	82.76	10	17.24	0.001[a]
Calves of German origin (no. = 98)	52	53.06	46	46.94	
Age					
1–2 weeks (no.=17)	12	70.59	5	29.41	
2–3 weeks (no. = 11)	10	90.91	1	9.09	0.003[a]
3–4 weeks (no. = 23)	17	73.91	6	26.09	
4–5 weeks (no. = 49)	36	73.47	13	26.53	
>5 weeks (no. = 56)	25	44.64	31	55.36	
Season					
Cold season (no.= 98)	52	53.06	46	46.94	0.0004[a]
Warm season (no.= 58)	48	82.76	10	17.24	
Clinical signs					
Watery diarrhea (no. = 85)	86	100	0	0	0.994[b]
Mucous diarrhea (no. = 15)	15	100	0	0	
Normal (no. = 56)	0	0	56	100	

[a]Highly significant.
[b]Non-significant.

gene; only 4 out of 100 (4%) positive calves' samples by microscopic examination were positive for *Cryptosporidium* spp., while no human samples were detected by PCR, as shown in Figure 2.

Phylogenetic analysis

The phylogenetic analysis was performed, as shown in Figures 3 and 4.

Discussion

Beside the zoonotic significance of cryptosporidiosis, it may progress into a serious, hard-to-control disease in many agricultural animals and result in significant financial losses, which piqued the interest of veterinary researchers (Gattan *et al.,* 2023). The goal of the present study was to determine the epidemiological risk factors and prevalence of cryptosporidiosis infection in humans and calves in the Menoufia governorate of Egypt. In addition to providing information on the potential for a particular disease to spread, epidemiological data on parasitic diseases are a crucial component in the future design and implementation of operational control measures (Tan *et al.,* 2017; Byomi *et al.,* 2018; Eissa

Table 3. Prevalence of *Cryptosporidium* oocysts in humans in relation to different demographic data and clinical signs.

Factor	Positive		Negative		*p* value
	No.	%	No.	%	
Sex					0.88[NS]
Males (no. = 59)	11	18.64	48	81.36	
Females (no. = 66)	13	19.70	53	80.30	
Age					0.049[a]
0–20 years (no. = 35)	12	34.29	23	65.71	
20–40 years (no. = 59)	9	15.25	50	84.75	
40–60 years (no. = 27)	3	11.11	24	88.89	
>60 years (no. = 4)	0	0	4	100	
Season					0.001[b]
Cold season (no. = 80)	8	10	72	90	
Warm season (no. = 45)	16	35.56	29	64.44	
Clinical signs					0.0001[b]
Liver problems (no. = 45)	8	17.78	37	82.22	
Diabetes mellitus (no. = 35)	5	14.29	30	85.71	
Diarrhea (no. = 11)	7	63.64	4	36.36	
Kidney problems (no. = 34)	4	11.76	30	88.24	

[a]Statistically significant.
[b]Highly significant.
[NS]Non-significant.

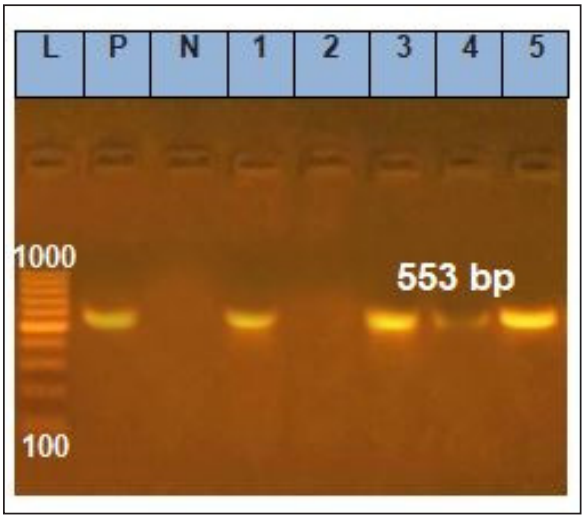

Fig. 2. A representative figure showing agarose gel electrophoresis of PCR for the detection of COWP gene of *Cryptosporidium* spp. Lane L: molecular weight marker, 100 bp. Lane P: positive control of COWP gene with a band of amplicon size at 553 bp. Lane N: negative control. Lanes 1, 3, 4, 5: positive samples. Lane 2: negative sample.

and Harb, 2023; Bedair *et al.,* 2024; Salman *et al.,* 2024a).

The total prevalence rates of *Cryptosporidium* oocysts in the current investigation as detected by microscopical examination were (100/156, 64.1%) and (24/125, 19.2%) among calves and humans examined, respectively, in Sadat City, Menoufia governorate, Egypt. The obtained findings were nearly agreed to those identified in previous Egyptian research as 68.3% in calves in Qalubia governorate (Abd-El-Wahed, 1999), 19% in humans in Beni-Suef governorate (Ibrahim *et al.,* 2016), and 19.5% in humans in Cairo governorate (El-Badry *et al.,* 2017).

On the contrary, lower prevalence rates were represented in calves in several Egyptian governorates as follows: 9.7 % in Dakahlia (Naguib *et al.,* 2018), 43.2% in Behera (Bessat *et al.,* 2019), 38.27% in Assuit (Elmahallawy *et al.,* 2022), 24.67% in Kafr El Sheikh, 14.29% in Qalubia, and 17.14% in Gharbia as mentioned by Gattan *et al.* (2023). In addition, regarding humans, lower prevalence rates were declared as follows: 6.7% in Ismailia governorate (Helmy *et al.,* 2015), 10.93% in Qena governorate (Elshahawy and Abou Elenien, 2019), and 1.5% in Gharbia governorate (Elmonir *et al.,* 2021).

Concerning the international level, many authors in separate localities reported lower prevalence rates in calves as follows: 7.17% in Iran (Shafieyan *et al.,* 2016), 12.5% in Malaysia, and 33% in Kuwait (Majeed *et al.,* 2022). On the other hand, Singh *et al.* (2006) reported a high prevalence rate of 79.41% among calves in India, while Zintl *et al.* (2009) notified a very high prevalence rate of 94% among humans in Ireland.

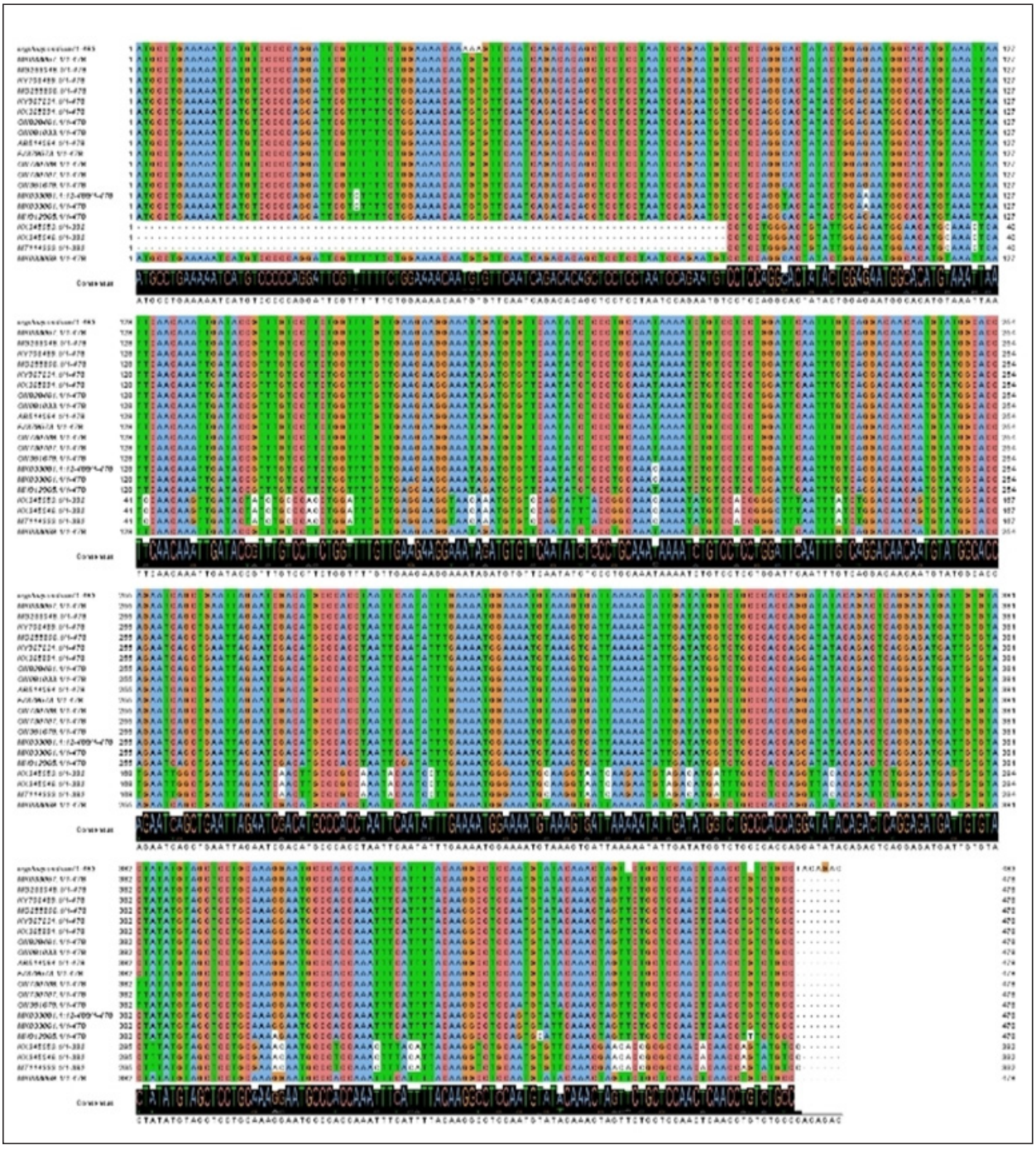

Fig. 3. The multiple sequence alignment between 21 sequences of orthologous COWP gene of *Cryptosporidium* species, and it revealed that there were almost similarities. The black line represented the consensus of each nucleotide, which occurs most frequently at that site in the different sequences.

Variations in ecology, ambient conditions, research design, climatic changes, management method, age, herd size, hygienic practices, and laboratory test used could all be responsible for the variations in total *Cryptosporidium* prevalence between the studies. The diagnostic techniques employed in this study were less accurate and may have led to falsely negative results. This could be the reason for the difference in the reports (Gattan *et al.,* 2023).

In the present research, it was possible to identify four risk factors in association with increasing the risk of *Cryptosporidium* infection in calves, including climatic

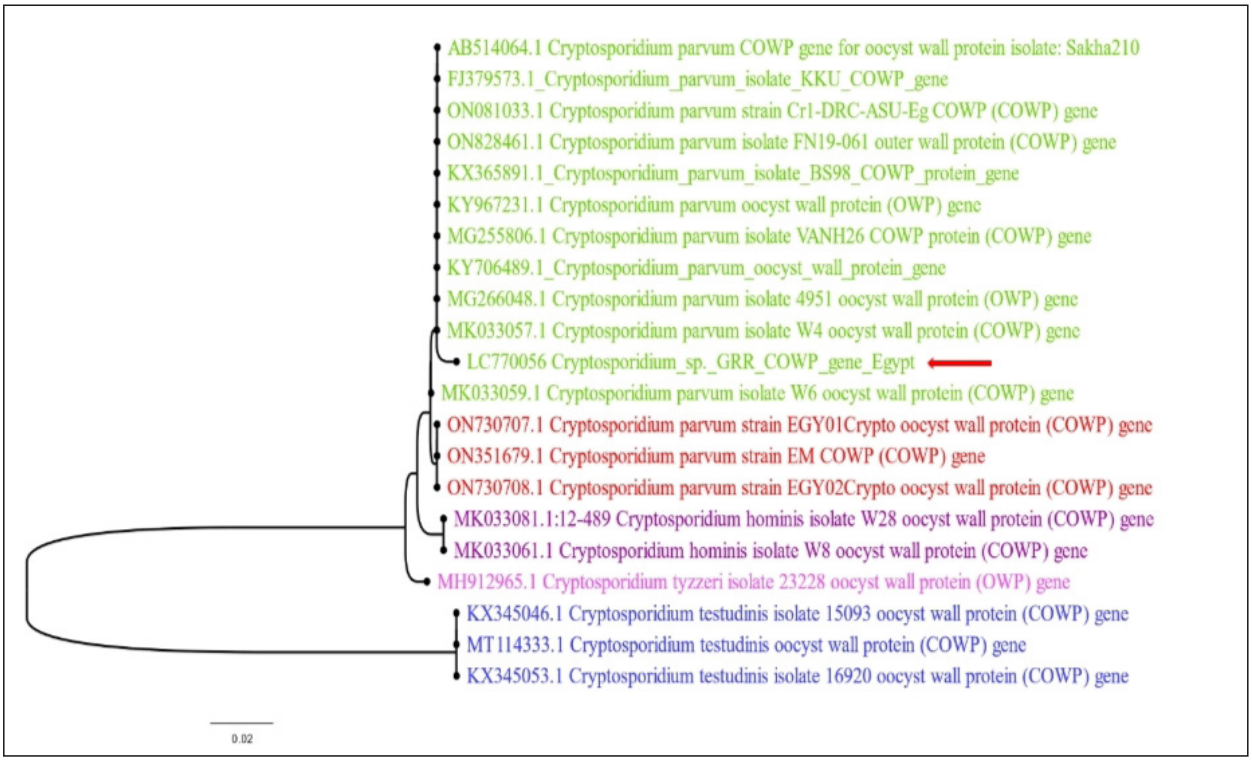

Fig. 4. Maximum-likelihood phylogenetic tree between 21 sequences of orthologous COWP gene of *Cryptosporidium* species from Egypt and global world. There were five clades represented in different colors. The study *Cryptosporidium* strain GRR from calf in Egypt is pointed with red arrow (LC770056), which clustered with *Cryptosporidium parvum* isolates and close to the other isolates from Egypt.

changes, age, breed, and type of diarrhea, whereas in humans, factors included sex, age, climatic changes, and different clinical signs.

Regarding demographic data and clinical signs in the examined calves, the prevalence of cryptosporidiosis was shown to be increased among American breeds (82.76%, 48/58) (p = 0.001), among calves of age group (>2–3 weeks) (90.91%, 10/11) (p = 0.003), prevailing of warm climate (82.76%, 48/58) (p = 0.0004), and among calves suffered from watery and mucous diarrhea (100% each) than those showed normal diarrhea (0%) (p= 0.994).

Regarding breeds, Ayele *et al.* (2018) and Gattan *et al.* (2023) found no significant (p > 0.05) impact of breed on increasing the prevalence of cryptosporidiosis, while Urie *et al.* (2018) and Abdullah *et al.* (2019) found high-quality studies that examined breed as a risk factor (Holsteins vs. Jerseys) and came to the conclusion that there was no difference in the susceptibility to *Cryptosporidium* spp. between the main breeds of dairy cattle. It could be related to the fact that in addition to other elements such as environmental pollution and infection sources, climate fluctuations also have an impact on the viability and length of oocysts.

Moreover, an examination of the age data obtained was consistent with the findings of Ayele *et al.* (2018), Li *et al.* (2019), and Díaz *et al.* (2021), which reported that the incidence of cryptosporidiosis reduced with age and those young calves had the highest incidence. However, Wegayehu *et al.* (2016), Gattan *et al.* (2023), and Jang *et al.* (2023) concluded that the age-related nature of cryptosporidiosis infection was supported by the higher prevalence rates observed in calves older than 3 months.

This may be because young calves' immune systems are still developing and therefore have a reduced tolerance. According to Brook *et al.* (2008), newborns are more vulnerable to developing a *Cryptosporidium* infection. This result is in line with the findings of Kváč *et al.* (2006), who noted that immunological development over time can cause infection resistance to change with age.

Furthermore, in terms of climatic changes, the results aligned with those of Atwill *et al.* (1999), who discovered that calves were particularly vulnerable to cryptosporidiosis in May due to the warm climate. They attributed this to the highest level of contact with the infection source, a decrease in animal resistance, or environmental factors that could facilitate the spread of the infection during this month. Conversely, studies conducted by Lefay *et al.* (2000) and Elmahallawy *et al.* (2022) revealed that calves were more likely to

contract cryptosporidiosis in the winter than in the summer. The findings indicate that the prevalence of *Cryptosporidium* infection is correlated with both the existence of calves at risk and the availability of an appropriate climate for the parasite's viability and spread. Furthermore, it is unknown how season affects the prevalence of *Cryptosporidium* spp.; however, it appears that each country's farming practices may have an impact (Byomi *et al.,* 2019a,b). Notably, this variance may be influenced by breeding density, temperature, humidity, sunshine, and precipitation levels. One possibility is that in addition to the dry weather in the Sadat City region, most calves are born between the spring and the summer, which increases the density of calves within the herd and makes calves more susceptible to *Cryptosporidium* spp. oocysts shed by older calves that were born earlier in the season (Yang *et al.,* 2022).

Finally, a strong correlation between diarrhea (either watery or mucoid) and cryptosporidiosis was found, supporting the findings of Abebe *et al.* (2008) and Ayele *et al.* (2018) that the infection was three times higher in calves with diarrhea than in calves with normal feces ($p < 0.001$). This research was conducted to help with early diagnosis of the disease in the future. This may be because of the infection producing crypt hyperplasia and villous atrophy, which decrease the amount of the intestine's surface area that is accessible for absorption. As a result, cause diarrhea by interfering with the absorption of salt, carbohydrates, and water (Foster and Smith, 2009). Furthermore, the parasite could be able to lower the activity of the disaccharidase enzyme, which would lessen the quantity of sugars that are broken down. The resulting bacterial proliferation, volatile fatty acid synthesis, and osmotic pressure changes would produce severe watery diarrhea (Rickard, 2001), with diarrheal animals shedding more oocysts than regular-feces-producing calves.

On the other side of the current investigation, regarding demographic data and clinical signs in the examined humans, the prevalence of cryptosporidiosis was shown to be increased among women (19.70%, 13/66) ($p = 0.88$), among individuals of age group (0–20 years) (34.29%, 12/35) ($p = 0.049$), prevailing of warm climate (35.56%, 16/45) ($p = 0.001$), and among diarrheic cases (63.64%, 7/11) ($p = 0.0001$).

The rate of women who tested positive for *Cryptosporidium* at a little higher level than men was comparable to the findings of Laubach *et al.* (2004) and Elshahawy and Abou Elenien (2019). However, Egberongbe *et al.* (2010) and Saneian *et al.* (2010) showed a higher level of infection in males, which is contrary to the current data. This discrepancy revealed that lifestyle risk variables that raised female exposure to untreated water in the research location also affected male exposure to *Cryptosporidium* infection. For example, women in the agricultural study region spend more time than men on farmlands growing vegetables, which is linked to not washing their hands after touching dirt; they also take care of the majority of household duties, such as cleaning the pit latrines that are common in rural areas. Because of their living circumstances, women are consequently frequently exposed to unsanitary environments (Elshahawy and Abou Elenien, 2019; Elmonir *et al.*, 2021).

Concerning age, the obtained results were consistent with those of Yu *et al.* (2004) and Derouin *et al.* (2010), who found that young people (6–19 years old) and children under 6 years old had high seropositivity of *Cryptosporidium* infection, respectively. Although the precise cause of this greater level is unknown, Elshahawy and Abou Elenien (2019) suggest that it may be related to immuno-physiological and ethological variations (more exposed owing to water play games together with unawareness).

Seasonal variations in the incidence of human cryptosporidiosis could be caused by several factors, such as agricultural practices, temperature or humidity changes, increased contact with animals, or attendance at childcare centers. These factors can also increase the number of oocysts in the environment and the survival of oocysts when exposed to more of them. The results found were consistent with Painter *et al.* (2015), who showed that warm climates had higher seropositivity of cryptosporidiosis than cold climates. In contrast, King *et al.* (2017) found that people who were tested in the winter had higher seropositivity than those who were tested in the summer. People are more likely to come into contact with *Cryptosporidium* spp. during warm seasons due to increased human field activity being linked to increased activity of animal reservoirs that release large numbers of the parasite into the environment.

Furthermore, the results of the higher seropositivity of *Cryptosporidium* among patients with diarrhea were in line with the findings of Youssef *et al.* (1998), Gatei *et al.* (2006), and Helmy *et al.* (2015). One of the most prevalent intestinal parasites linked to diarrhea is *Cryptosporidium*. However, testing for this parasite is not usually carried out when diagnosing ova and parasites (Gatei *et al.*, 2006).

Additionally, PCR was performed on all positive human and animal samples, and the results showed that only 4% (4/100) of the positive animal samples were positive for the COWP gene. The sensitivity of molecular assays created to identify *Cryptosporidium* spp. is affected by the nucleic acid extraction method and the stage of the parasite's life cycle (Claudel *et al.,* 2021). Negative identification of *Cryptosporidium* spp. by PCR despite visible oocysts in flotation may be related to PCR inhibitors that are common in fecal samples and/or a failure break of oocyst walls during DNA extractions (Raj *et al.,* 2013). Furthermore, PCR results were impacted by differences in genetic loci, sampling sizes, and molecular techniques (Zhao *et al.,* 2020).

In the present study, Basic Local Alignment Search Tool (BLAST) search of *Cryptosporidium* COWP gene indicated that the sequence was 98.95%–99.13% identical to *C. parvum*. Phylogenetic analysis additionally demonstrated how *Cryptosporidium* identified in the current investigation clustered with *C. parvum*. The *C. parvum* sequences shared 98.74% of their similarities with earlier isolates obtained from buffalo, (ON730708.1, ON730707.1), dairy calves (AB514064.1), and Camel (ON351679.1) in Egypt, while it was 98.95% identical to (KX365891.1) and sheep (ON081033.1) in Egypt, (FJ379573.1) in Korea, (ON828461.1) in Canada, cucumber (KY967231.1) in India, human (MK033059.1, MK033057.1) in Egypt, and mollusca (KY706489.1) in Italy. According to the results, young calves may be a potential source of human cryptosporidiosis. Additionally, because of the diversity of this parasite, calves are more susceptible to contracting the disease from a range of different species as well as from its own parasites (El-Alfy *et al.,* 2019).

Conclusion

With rates of 64.1% and 19.2% among the calves and humans under examination, respectively, the incidence of *Cryptosporidium* infection was rapidly increasing among calves in the analyzed governorate. According to the current research, feminine gender in humans and animals, early age in relation to warm climates, and the occurrence of mucoid and watery diarrhea in addition to American breeds of cattle were all risk factors for *Cryptosporidium* infection. To limit and lessen the spread of disease from animals to humans, it is crucial to increase the knowledge of risk factors, infection sources, and mechanisms of transmission. Epidemiology and infection source identification are aided by additional genomic research and phylogenetic analysis, which has shown anthroponotic transmission of cryptosporidiosis from animals to humans. In order to stop the anthroponotic infection chain that leads to both animal and human cryptosporidiosis, the proper control measures must be put in place.

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Conflict of interest

No conflict of interest.

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Authors' contributions

GR and NE contributed to the sampling process, laboratory work, the scientific writing process, and the analysis of the data. WM, AE, and AS contributed to the study design, laboratory work, editing, and revising the manuscript. AZ and MN contributed to the conception, study design, data curation, editing, and revising the manuscript. AMA and OKG contributed to data analysis, writing and revising the manuscript. The final paper was read and approved by all authors.

Data availability

All data and materials generated or analyzed during this study are included in this published article.

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