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Cryptosporidium in Fish: Morphological Characterization, Prevalence and Molecular Epidemiology - A Review

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ABSTRACT

Species of the genus *Cryptosporidium* (phylum Apicomplexa) are zoonotic protozoan pathogens, able to infect the epithelium of the gastrointestinal tract of a wide range of invertebrate and vertebrate hosts including humans. There is limited knowledge regarding the geographical distribution, prevalence, and epidemiology of Cryptosporidium isolates infecting fish. In the past 20 years, several studies have focused on *Cryptosporidium* in fish. To date, four species (*Cryptosporidium molnari*, *Cryptosporidium huwi*, *Cryptosporidium scophthalmi* and *Cryptosporidium abrahamseni*) have been identified as piscine-host-specific, nine piscine genotypes and more than 29 unnamed genotypes have been described in fish hosts. In addition, other non-piscine-host-specific *Cryptosporidium* species (*C. parvum*, *C. hominis*, *C. scrofarum*, *C. xiaoi*) have been genetically characterized in fish. While the presence of Cryptosporidium zoonotic subtypes in edible fish increases the risk of fish-borne zoonotic infections, which is significant from the perspective of public health, the pathology of cryptosporidiosis is very important for the aquaculture industry since it causes mortalities in farmed fish. Understanding the dynamics and transmission channels of Cryptosporidiosis infection is critical; however, none of the laboratory diagnostic techniques such as acid fast staining and direct or indirect immunofluorescence microscopy can differentiate between the species or subtypes of the parasite. These days, the polymerase chain reaction (PCR) is used more often as a diagnostic technique to identify and classify species and track the parasite's numerous pathways of transmission. Thus, the history, biology, pathology, and clinical symptoms of *Cryptosporidium* in fish from freshwater and marine environments are gathered up in this review in conjunction with the prevalence, and molecular epidemiology of the disease. In addition, data on how piscine hosts may act as a reservoir for zoonotic *Cryptosporidium* species were included.

INTRODUCTION

The demand for fish food is rising in areas where people's incomes are low, particularly in developing nations since fish meat is a significant source of necessary nutrients, particularly poly-unsaturated fatty acids **(Soliman *et al.*, 2019)**. The second half

of the 20th century experienced a notable increase in fish output worldwide and in the last ten years, the Egyptians' per capita fish intake has more than doubled, rising from less than 7 to over 14kg **(Mahmoud *et al.*, 2019a)**. *Cryptosporidium* spp. are intracellular protozoan parasites that are recognized in the gastrointestinal epithelium causing severe enteric infections in a wide range of vertebrates including humans **(Squire & Ryan 2017; Aboelsoued *et al.*, 2023)**. The cryptosporidiosis is increasingly identified as an infection in a diverse range of wildlife species, including mammals, birds, reptiles, amphibians, and fish. Due to the zoonotic character of some of its species, it stands among the most relevant parasitic enteric agents in human and veterinary medicine **(Hassanain *et al.*, 2013; Shaapan, 2016)**.

Cryptosporidiosis can cause a variety of symptoms in humans and animals, ranging from asymptomatic to vomiting, severe diarrhea, and death, especially in young people **(Shaapan *et al.*, 2010; Certad *et al.*, 2019)**. The transmission of the parasite occurs through ingesting environmentally ubiquitous stable oocysts contaminated food or drinking water which can be acquired through several routes, including person-to-person contact, contact with companion or farm animals, and recreational water, all of which contribute to the faecal-oral contamination route **(Ghazy *et al.*, 2015a; Elfadaly *et al.*, 2018)**. In animal husbandry, cryptosporidiosis is not just an opportunistic disease that can cause significant financial losses, zoonotic consequences and control challenges **(Ghazy *et al.*, 2016)**. Children in impoverished countries are more likely to contract the parasite *Cryptosporidium* sp. infection since these countries have less access to clean drinking water **(Obateru *et al.*, 2017)**.

Cryptosporidiosis, the ensuing disease is typically self-limiting in healthy adult hosts and immune-competent subjects, however it can be fatal in immunocompromised individuals, such as AIDS patients, malnourished people, and children **(Shaapan *et al.*, 2015)**. Water is a major method of transmission of *Cryptosporidium* since the environmentally robust oocysts is resistant to disinfection including chlorine **(Zahedi & Ryan, 2020)**. The most reliable traditional diagnostic method is still the routine staining of tissue scraping smears or *Cryptosporidium* spp. oocysts in faeces using acid-fast staining **(Ghazy *et al.*, 2015b; Saad-Alla *et al.*, 2022)**. Effective strategies for detecting parasite antigen are enzyme immunoassays (EIA), yet there is a disagreement about how sensitive these immuno-detection techniques are **(Hassanain *et al.*, 2016)**. The polymerase chain reaction (PCR) is currently being used more and more as a diagnostic method for detecting *Cryptosporidium* DNA in tissues and faeces **(Adeyemo *et al.*, 2018)**. This takes place since none of the laboratory diagnostic procedures, such as acid fast staining and direct or indirect immunofluorescence microscopy can distinguish between *Cryptosporidium* species or subtypes, which is crucial for understanding the disease's dynamics and transmission pathways **(Shaapan *et al*., 2012; Costa *et al.*, 2021)**.

Cryptosporidiosis is an emerging disease in both wild and farmed fish in numerous countries worldwide. The stages of the parasite have been discovered on the stomach or

intestinal surface, or both, in both freshwater and marine fish species **(Golomazou *et al*., 2021; Shaapan *et al*., 2022)**. From the perspective of public health, fish-borne zoonotic potential danger from *Cryptosporidium* species is very significant. These parasites may be the etiological factor causing epidemics of fish-borne cryptosporidiosis when they are present in the edible fish. Infected fish may also exhibit a range of clinical symptoms, which are more noticeable in cases of severe illnesses **(Abbas *et al*., 2022; Hayes *et al*., 2023)**. Presently, DNA studies have identified approximately 29 unique genotypes in piscine species, revealing three *Cryptosporidium* species known to be present in fish: *Cryptosporidium molnari*, *Cryptosporidium scophthalm*i and *Cryptosporidium huwi* **(Certad *et al*., 2020)**. Although the survival of human species in fresh and saltwater has been established, cryptosporidiosis remains a common waterborne disease. Given that the species status of these novel fish genotypes is unknown, it is crucial to comprehend the evolutionary history and taxonomy of piscine *Cryptosporidium* **(Abd El-Halim *et al*., 2019)**. The pathology of cryptosporidiosis in cultured fish is important, followed by poor growth rates and increased mortality causing an economic impact in the aquaculture industry, which is expanding worldwide **(Couso-Perez *et al*., 2019)**. There have been extensive studies conducted on cryptosporidiosis; however, our knowledge of piscine cryptosporidiosis is much less complete. *Cryptosporidium* has been described in fresh and marine water fish, yet limited knowledge is found about the epidemiology, pathogenicity and zoonotic transmission of piscine species of *Cryptosporidium* **(Moratal *et al*., 2020)**. Therefore, the present review aimed to evaluate the prevalence and molecular epidemiology of *Cryptosporidium* spp. in fish.

1. Cryptosporidium parasitic agent

Cryptosporidium species parasite is a member of the Phylum *Apicomplexa*. At least 22 species of *Cryptosporidium* have been named and based on host occurrence, parasite morphology, host predilection, and site of infection. There are 16 valid named species of *Cryptosporidium* by most investigators (Table 1); undoubtedly, *C. parvum* is the major species responsible for disease in humans and domestic animals **(Smith & Nichols, 2010; Ghazy *et al*., 2015b)**. Introduction of DNA sequencing has provided the data to identify genetic variants affecting different or even same host species. It seemed logical to think that individual hosts have one unique host specific to *Cryptosporidium* spp., as well as species that are less host specific affecting a wider spectrum of hosts **(Hassanain *et al*., 2011)**. Molecular tools have provided the techniques for accurate diagnosis, and gene sequencing has generated data which allowed the construction of molecular phylogenies, mapping the evolutionary relationships between individual species and isolates **(Xiao *et al*., 2004; Mahmoud *et al*., 2021)**.

Table 1. *Cryptosporidium,* host type, site of infection, and oocysts measurements

Species	Host type	Site of infection	Oocyst size (µm)
C. parvum	Humans, Mice, Cattle, most livestock	Small intestine	4.5 × 5.5
C. hominis	Humans, sheep, Cattle	Small intestine	4.9 × 5.2
C. muris	House Mouse	Stomach	5.6 × 7.4
C. suis	Pigs, Humans	Small and large intestine	4.6 × 4.2
C. felis	Cat, Humans, Cattle	Small intestine	4.7 ×5
C. canis	Dog, Humans	Small intestine	4.7 × 4.9
C. meleagridis	Turkey, humans	Small intestine	5.2 × 4.6
C. galli	Chicken	Proventriculus	8.3 × 6.3
C. baileyi	Poultry. Quails, Ducks	Bursa, Cloaca, Trachea	6.2 × 4.6
C. bovis	Cattle, Sheep	Small intestine	4.9×4.6
C. andersoni	Cattle, Camel	Abomasum	7.4×5.6
C. wrairi	Guinea pig	Small intestine	5.4×4.6
C. serpentis	Snake, Lizards	Stomach	36 ×× 2.8
C.saurophilum	Snake, Lizards	Stomach and intestine	5.2 × 4.2
C. scophthalmi	Fish (turbot)	Stomach and intestine	4.4 × 3.9
C. molnari	Fish (sea-bream)	Stomach and intestine	4.7 × 4.5

2. Fish cryptosporidios

There is limted knowledge about the prevalence or geographical distribution of isolates of *Cryptosporidium* infecting fish. The first report of *Cryptosporidium nasorum* in fish was in a tropical marine fish (*Naso lituratus*) in 1981 **(Xiao *et al*., 2004)**. Recently, the species is named solely based on the presumed host specificity and genetically characterized in more than 25 species of both freshwater and marine fish. Over 20 piscine genotypes have been identified, including *Cryptosporidium molnari*, *C. scophthalmi*, *C. huwi*, *C. bollandi*, piscine genotypes 3–8, piscine genotype 9 , a *C. molnari*-like genotype, and five unnamed novel genotypes **(Golomazou & Karanis 2020)**. The *Cryptosporidium molnari* was mainly found in the stomach epithelium of teleost fish, in addition to the gilthead sea bream (*Sparus aurata* L.) and the European sea bass (*Dicentrarchus labrax* L.), whereas the *C. scophthalmi* was found specifically in the intestinal epithelium of cultured turbot (*Scophthalmus maximus*) (Table 2) **(Couso-Pérez *et al*., 2022)**.

Table 2. *Cryptosporidium* species and genotypes currently recognized in piscine hosts

Species/Genotype	Fish host	Origin	Habitat	Prev. (%)
Cryptosporidium molnari	*Chromis viridis*	Ornamental	Marine	15.4
	Dicentrarchus labrax	Cultured	Marine	57.9
Oocyst size: (4.7×4.5) *μm*	*Exos lucius*	Wild	Fresh water	40.0
Location: stomach	*Clarias gariepinus*	Wild	Fresh water	64 .0
***Cryptosporidium molnari*-like**	*Amphiprion percula*	Ornamental	Marine	9.1
	Cyprinus carpio	Ornamental	Fresh water	20.0
	Maccullochella peelii	Cultured	Fresh water	95.4
Cryptosporidium huwi	*Paracheirodon innesi*	Ornamental	Fresh water	50.0
	P. reticulata	Ornamental	Fresh water	1.9
Oocyst size: (4.6×4.4) *μm*	*Puntigrus tetrazona*	Ornamental	Fresh water	4.5
Location: stomach				
Cryptosporidium bollandi	*Astronotus ocellatus*	Ornamental 5.0–75.0	Fresh water	75.0
Oocyst size: (3.1×2.8) *μm*	*Mugil cephalus*	Wild	Marine	0.5
Location: stomach	*Paracheirodon innesi*	Ornamental	Fresh water	50.0
Cryptosporidium abrahamseni	*Moenkhausia sanctaefilomenaea*	Ornamental	Fresh water	62.5
Oocyst size: (3.8×3.2) *μm*	*Paracheirodon innesi*	Ornamental	Fresh water	27.3
Location: intestine				

3. Life cycle of piscine *Cryptosporidium*

The life cycle of *Cryptosporidium* spp. in fish can be assumed to involve the following different stages: (1) Excystation and release of sporozoites, (2) schizogony or merogony, (3) gamogony, (4) zygote formation, (5) oocyst wall formation, and (6) sporulation (Fig. 1). The sporozoites reach the apical surface of the epithelial cells and are enveloped inside a parasitophorous vacuole in the microvilli. Within the PV, the sporozoite is differentiated into a trophozoite, which undergoes nuclear division through merogony, producing a type I meront, followed by type II meronts. Type II merozoites invade other cells and undergo gamogony, forming microgametes and macrogametes. After fertilization, a zygote is formed, followed by sporulated oocysts that contain four naked sporozoites (sporogony). The thick-walled oocysts are released with the feces of the host, while thin wall is reinitiated through endogenous autoinfection **(Bolland *et al.*, 2020)**.

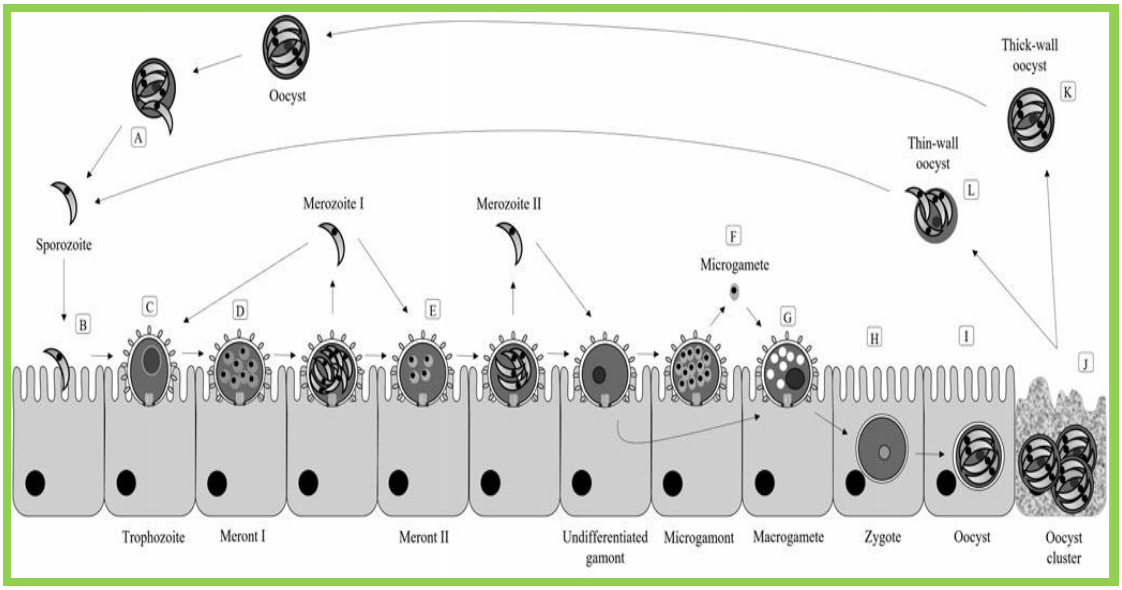

Fig. 1. Life cycle of piscine *Cryptosporidium*

4. Infection mechanism and transmission

Cryptosporidium pathogenic oocysts spread through the fecal–oral pathway, and water serves as an effective medium for this process. Among the most commonly found infectious pathogens in water, *Cryptosporidium* is documented in a variety of global water types, including rivers, recreational, drinking and wastewater **(Omarova *et al.*, 2018)**. This aquatic protozoan parasite can develop in surface waters as a result of animal or human feces (from both domestic and wild animals) being contaminated. The oocysts can enter water bodies either directly or indirectly by runoff from contaminated land that has been contaminated by animal dung. Furthermore, wastewater treatment plant effluents and inadequate or inefficient sewage treatment systems might damage the aquatic environment **(Vermeulen *et al.*, 2019)**.

5. Diagnosis

5.1. Morphological characterization of Cryptosporidium oocysts

Fine smears from the stomach and intestine epithelial layers of fish samples were preserved in methanol and stained with a modified Ziehl-Neelsen stain, following the method of **Elaadli *et al.* (2023)**. At higher-magnification under a light microscope, a 100X objective lens with a stage micrometre coupled with an eyepiece micrometre was used to confirm the presence of and measure *Cryptosporidium* spp. Oocysts, as outlined by **Shaapan *et al.* (2021)**. The mean was computed using around 20- 50 oocysts with the range in parenthesis as the standard unit of measurement (m= 0.001mm), following the method of **Ghazy *et al.* (2015b)**. The *Cryptosporidium* spp. oocysts were characterized by a spherical to ovoid form with smooth wall, an incomplete suture line of the oocyst wall and an acid-fast (red-pink) appearance on green back. The diameter of the oocysts ranged from 3.20– 4.5 x 3.90– 6.05m, with a mean (3.9 x 5.0) m in diameter and a shape index of 1.4– 1.6, which was morphologically comparable to *Cryptosporidium molnari* oocysts (Fig. 2), as determined by the method outlined by **Shaapan *et al.* (2022)**.

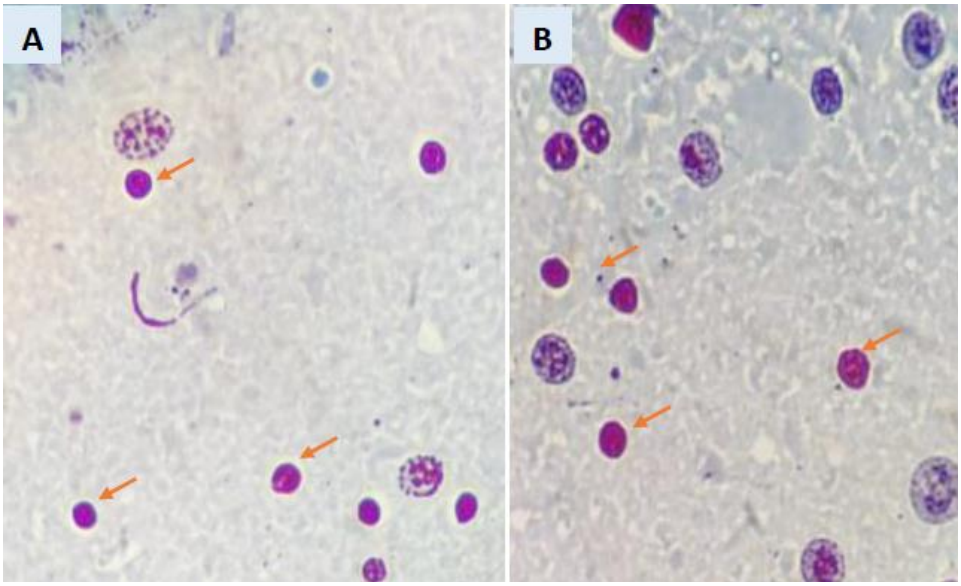

Fig. 2. *Cryptosporidium molnari* oocysts in *Clarias gariepinus* showing: Stomach **(A)**, and intestine **(B)**, (Red arrow) stained with Modified Ziehl-Neelsen stain (mZN) (X100)

5.2. Serological assays

5.2.1. Enzyme linked immunosorbent assay (ELISA)

ELISA technique was carried out for the detection of *cryptosporidium* antibodies in the sera of the infected fish. Using procedures based on Sheather's flotation, the isolated contaminant-free Cryptosporidium oocysts from scraped stomach and intestinal mucosa were utilized for antigen preparation, as outlined by **Hassanain *et al.* (2016)**. A controlled checkerboard titration was employed to identify the optimal antigen, serum,

and conjugate concentrations. The ELISA test procedures were carried out following the method outlined by **Abd El Wahab *et al.* (2018)**.

5.2.2. Direct fluorescence assay (DFA)

Direct fluorescent-antibody staining assays, considered the gold standard for diagnosing gastrointestinal cryptosporidiosis due to their high sensitivity and specificity, were routinely employed, following the outlines of **Abdalhamed *et al.* (2019)**. Fluorescein isothiocyanate (FITC)-conjugated anti-*Cryptosporidium* sp. monoclonal antibodies in direct fluorescent-antibody staining assay were used for the diagnosis of cryptosporidiosis in humans, domestic mammals, and fish (Fig. 3), following the method outlined by **Barugahare *et al.* (2011)**.

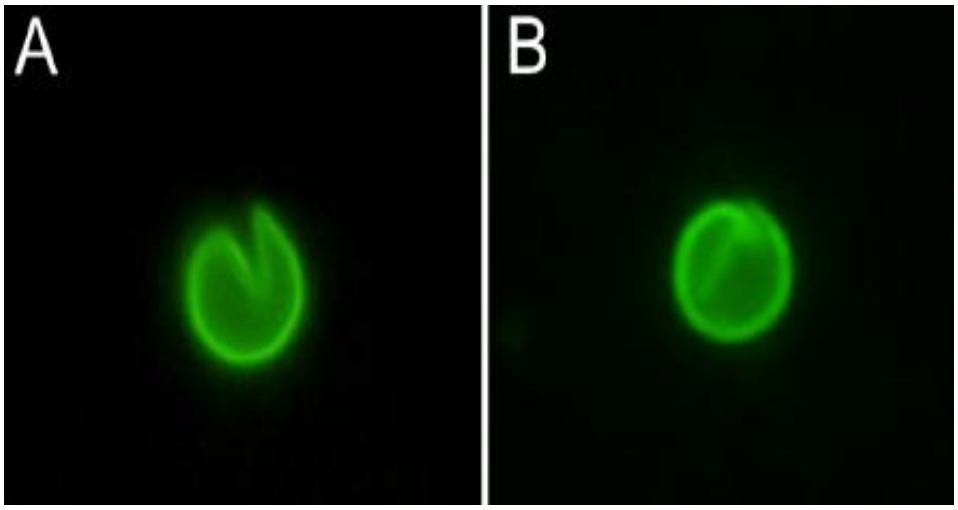

Fig. 3. Direct fluorescent-antibody staining of *Cryptosporidium molnari* oocysts showing: A fresh oocyst **(A)**, and An oocyst fixed in formalin for 7 days **(B)**. FITC-conjugated anti-*Cryptosporidium* sp. MAbs monoclonal antibodies were used. The oocysts exhibited the typical semicircular longitudinal suture in the oocyst wall

5.3. Molecular identification

Molecular studies have revealed the considerable genetic distance between piscine *Cryptosporidium* and the remaining species of the genus infecting other host classes **(Hassanain *et al.*, 2011)**. Genomic DNA was extracted from *Cryptosporidium* oocysts, and the cycling conditions of the primers during conventional polymerase chain reaction cPCR were followed by agarose gel electrophoreses. The gel was photographed using a gel documentation system, and the data were analyzed through computer software (Fig. 4). PCR product was sequenced in the forward and/or reverse directions. The genotypes/assemblages were aligned with homologous sequences available in the GenBank database using CLUSTAL W, and the sequences were submitted to a BLAST® analysis (Basic Local Alignment Search Tool), following the directions outlined by **Hassanain *et al.* (2019)**. Phylogenetic analysis of piscine-derived *Cryptosporidium*

species/genotypes showed that the piscine clade has a basal position relative to all other *Cryptosporidium* species, which form two main broad branches: intestinal and gastric species (Fig. 5).

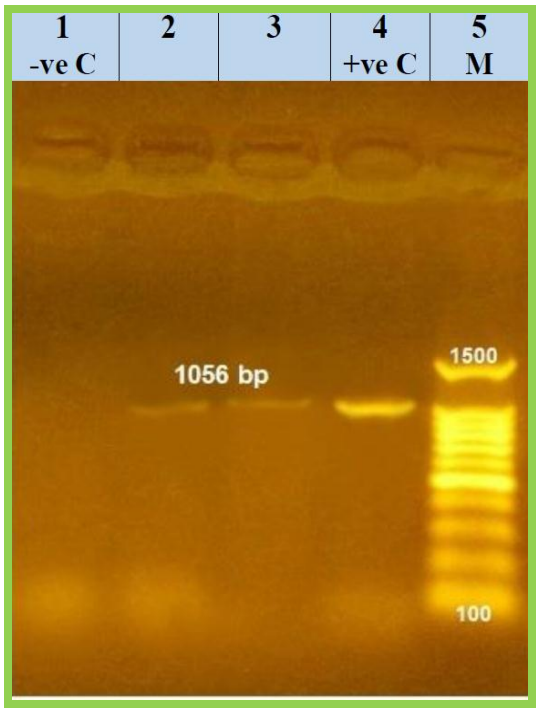

Fig. 4. PCR analysis for *Cryptosporidium* spp. showing: A negative control sample (lane 1), +ve tested fish samples (lanes 2, 3), positive control sample (lane 4), and DNA markers (lane 5)

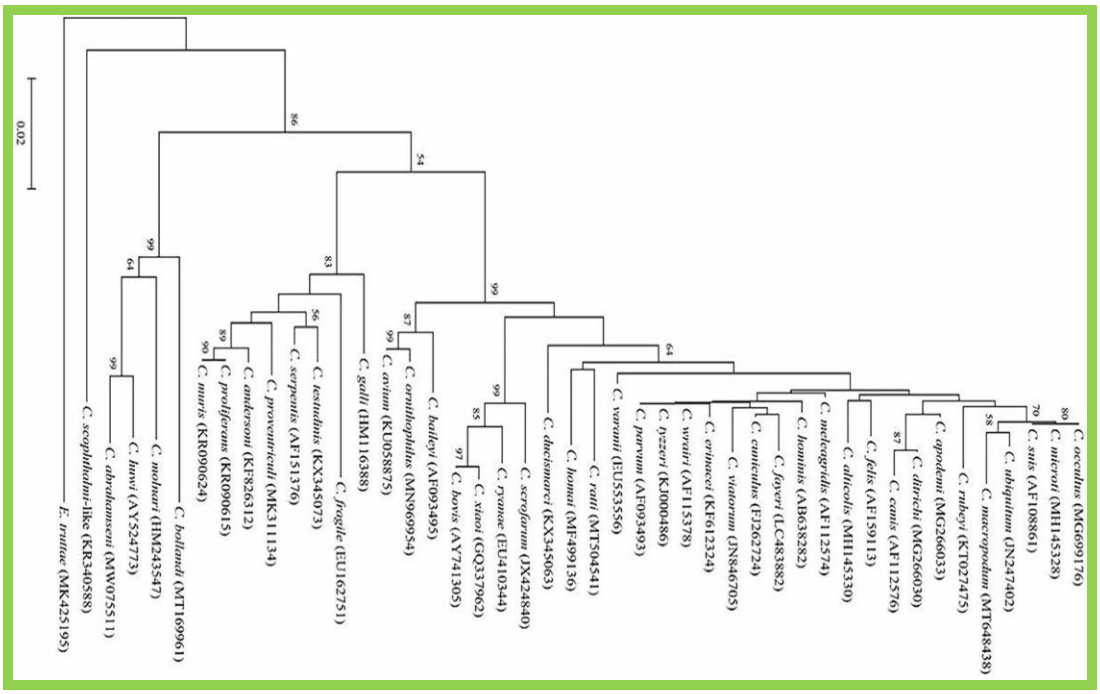

Fig. 5. Phylogenetic relationships in the genus *Cryptosporidium* inferred by neighbor-joining analysis of the small subunit ribosomal RNA (18SrRNA) gene

5.4. Histopathological examination

Cryptosporidium is an obligate intracellular protozoan parasite that mainly infects the microvillus border of the gastrointestinal epithelium. In contrast with the epicellular location of *Cryptosporidium* species from other vertebrates, in case of piscine *Cryptosporidium* species sporulation takes place deep within the epithelium **(Hassan *et al.*, 2012)**. For the histopathological study, specimens were collected from different parts of the stomach and small intestine of fish. Stained sections were microscopically examined for the presence of *Cryptosporidium* oocysts, pathological changes, and inflammation of intestinal mucosa, following the directions outlined by **Mahmoud *et al.* (2019b)**. The histopathological alterations in stomach and small intestinal mucosa including shortening and broadening of intestinal villi indicate a marked degree of villous atrophy and desquamation of epithelial lining layer in most villi. These changes were associated with a marked decrease in the number of goblet cells and compensatory crypt hyperplasia (Fig. 6), as suggested by **Aboelsoued *et al.* (2023)**.

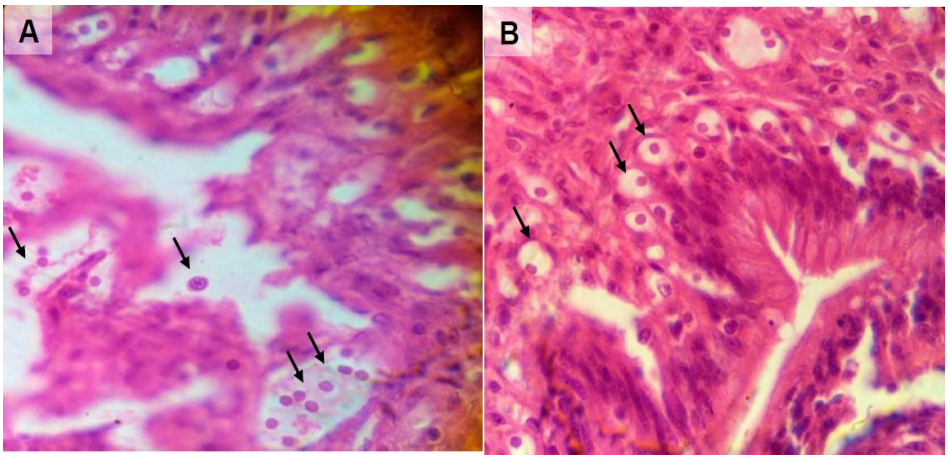

Fig. 6. The histopathology of fish intestinal epithelia stained with H&E (X 400) showing: *Cryptosporidium* oocysts in the intestinal lumen **(A)**, and *Cryptosporidium* developmental stages embedded in the mucosal layer crypts **(B)** (black arrows)

5. 5. Ultrastructural studies

Transmission electron microscopy (TEM) of intestinal epithelial mucosa of infected fish revealed the morphological characteristics, with the parasite maturing trophozoite completely enveloped by parasitophorous vacuoles situated between the elongated host cell microvilli. *Cryptosporidium* trophozoites and other parasite mature stages, especially meront stages, were present in large numbers in infected, and non-treated mice epithelium, with observed mucous secretion in the intestinal lumen (Fig. 7), as indicated by **Abd El Wahab *et al.* (2022)**.

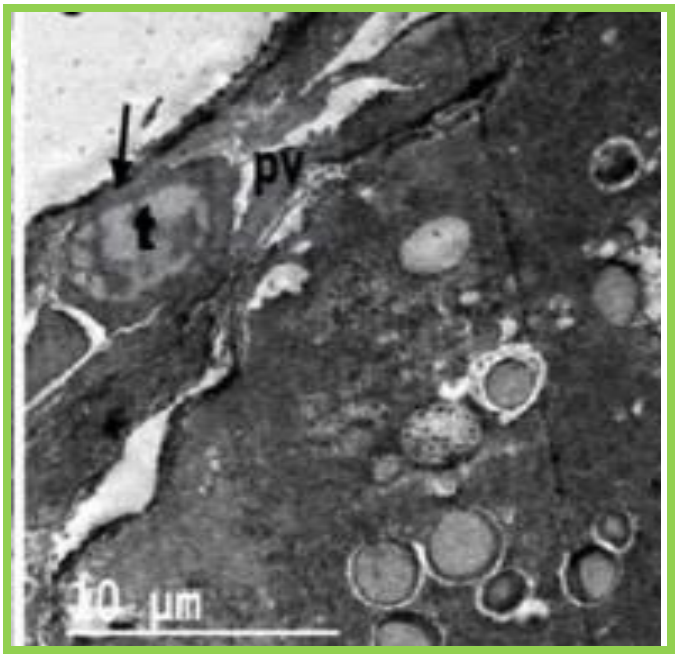

Fig. 7. The transmission of an electron microscopy intestinal epithelia infected with *Cryptosporidium* parasitic trophozoites (**t**) enveloped by the parasitophorous vacuole (**pv**), and electron-dense zone feeder at close contact to the host cell cytoplasm (**arrow**). Bar= 10 µm

6. Prevention and control

Though all the *Cryptosporidium* infections begin with the intake of highly resistant oocysts from the environment, controlling this stage is the single most essential aspect in restricting the disease's progress. The environment will continue to contaminate by the infected animals and humans, and eliminating these sources is very difficult, as reported by **Hassanain *et al.* (2021)**.

6.1. Hygienic measures

Preventive sanitary measures in the fight against cryptosporidiosis are the most important tools. From the standpoint of disease control, the goal is destroying the external parasite forms and preventing their transfer among animals and from the environment to the host. The most efficient way to control this parasite is to take preventative steps, as suggested by **Elfadaly *et al.* (2017)**.

6.2. Chemical and physical factors to reduce oocyst viability

To date, over 35 disinfectants have been tried, only five have been determined to be effective after only a few hours of exposure to 5% ammonia, 3% hydrogen peroxide, and 10% formalin. Steam heat sterilization and formaldehyde or ammonia gas fumigation have also been suggested as effective decontamination methods, as stated by **Mahmoud *et al.* (2021)**. Methods such as exposure to hot water at $+60^0$C for 6 min and -20^0C for 24hr have proven effective, while air-drying of *Cryptosporidium* oocysts has been shown to reduce infectivity by 97% after 2hr and 100% after 4hr **(Moriarty *et al*., 2005)**. Furthermore, the oocysts that are exposed to a 20-kGy dose exhibited a reduction of just 50%. A significant inactivation of *Cryptosporidium* oocyst in water was observed using ozone (O3) at concentrations greater than 3.0mg/ L and contact times of up to 7 minutes. According to **Ghazy *et al.* (2016**), the UV system is one of the most effective disinfection treatments for bacteria, viruses, and parasites found in drinking water and wastewater

6.3. Drinking and recreational water sources

Unfortunately, *Cryptosporidium* spp. is a chlorine-resistant germ that can survive for up to ten days in a well-chlorinated pool **(Olson *et al.* 2004)**. Furthermore, an appropriate disinfection and water treatment technique must be identified as soon as possible. Since polluted water supplies are the primary source of infection, it is vital to take steps to stop parasite oocysts from spreading in the environment. As a result, recognizing infection risk variables in livestock will aid in the development of oocysts shedding management strategies **(Abbas *et al.* 2022)**.

6.4. Prospects for vaccination

Several investigations have indicated that passive immunotherapy with hyper-immune serum or colostrums against *C. parvum* antigens is effective to improve clinical

indications of disease **(Ryan *et al.* 2014)**. In vaccination, protective immunity appears to involve CD4+ T-cells and related cytokines, such as IFN- and IL-12, as indicated by research conducted on both people and animals. $CD8^+$ T cells might contribute to the cell-mediated immune response against *Cryptosporidium* via direct cytolysis of the infected intestinal epithelial cells along with IFN-γ-mediated protection and clearance **(Aboelsoued *et al*., 2023)**.

6.5. Treatment

Several drugs either singly or in combinations were evaluated for treatment, including paromomycin, fluoroquinolone, azithromycin, as well as spiramycin with variable efficiency **(Diptyanusa & Sari, 2021)**. Nitazoxanide is the only FDA-approved drug for treating cryptosporidiosis. However, it showed limited efficacy in severely immunocompromised, such as AIDS patients and malnourished infants **(Caravedo & White, 2023)**. Other plant extracts, such as garlic, onion, ginger, ginseng, sage, curcumin, and black seeds, were also investigated on *Cryptosporidium* infected experimental animals and exerted anti *Cryptosporidium* effect through significant reduction in oocysts count and protecting intestinal epithelium **(Abu El Ezz *et al.*, 2011; Asadpour *et al.*, 2018; Aboelsoued *et al.*, 2020)**. Recently, there has been a growing interest in developing new antiparasitics from medicinal plants, such as *Citrus sinensis* **(Abd El Wahab *et al.*, 2022)**.

7. Public health significance of zoonotic *Cryptosporidium* spp. in fish

Fish-borne zoonotic potential risk from *Cryptosporidium* species is of major importance from a public health point of view **(Toaleb *et al.*, 2014)**. The presence of these zoonotic *Cryptosporidium* subtypes in edible fish may be the etiological agent responsible for outbreaks of fish-borne cryptosporidiosis **(Reid *et al.*, 2010)**. Although *C. parvum* oocysts from a human source were reported to be infectious for fish, multiple attempts to experimentally infect guppies (*P. reticulate)* and bluegills with *C. parvum* oocysts infectious for suckling mice were unsuccessful **(Graczyk *et al.*, 2004)**. Apparently, a slow passage of the inoculum oocyst in the fish gut or a prior undetected infection resulted in misdiagnosis of an experimental infection. In addition, the attempts to infect the rainbow trout (*Oncorhynchus mykiss*) with *C. parvum* oocysts were unsuccessful **(Freire-Santos *et al*., 2002)**. *Cryptosporidium molnari* was experimentally transmitted to gilthead sea bream (*S. aurata*) and European sea bass (*D. labrax*) through oral infection with infected stomach scrapings. The infection was also transmitted from infected gilthead sea bream to sea bass by cohabitation and transmission of *C. molnari* favored by cannibalism among cohabiting fish (Table 3) **(Sitja-Bobadilla *et al*., 2005)**.

Table 3. Mammalian *Cryptosporidium* species and genotypes detected in fish

Species/genotype	Fish host	Origin	Habitat	Prev. (%)
Cryptosporidium hominis	*Carassius auratus*	Ornamental	Fresh water	4.6
Cryptosporidium Parvum	*Carassius auratus*	Ornamental	Fresh water	0.9
	Clupea harengus	Wild	Marine	0.9
	Coregonus lavaretus	Wild	Fresh water	45.5
	Coregonus lavaretus	Wild	Fresh water	45.5
	Decapterus macarellus	Wild	Marine	6.9
	Engraulis encrasicolus	Wild	Marine	0.7
	Lates calcarifer	Cultured	Fresh water	20.0
	Oreochromis niloticus	Cultured	Fresh water	2.4
	Perca fluviatilis	Wild	Fresh water	33.3
	Sardina pilchardus	Wild	Marine	1.3
	Scomber japonicus	Wild	Marine	6.5
Cryptosporidium scrofarum	*Sillago vittata*	Wild	Marine	3.6
Cryptosporidium xiaoi	*Sillago vittata*	Wild	Marine	1.8
Rat genotype 3	*Carassius auratus*	Ornamental	Fresh water	5.3

Humans are susceptible to a wide range of *Cryptosporidium* spp. and according to a recent evolutionary genomic survey of anthroponotic *Cryptosporidium* species, *C. hominis* and *C. parvum* are the main species infecting humans globally **(Robertson *et al*., 2020)**. The presence of zoonotic subtypes in fish is probably related to water contamination by animal and human wastes and during the last decade, it was detected in 18 freshwater and marine fish species, including edible fish, both wild and farmed. *C. parvum* developmental stages are primarily detected in the digestive tract, including the stomach, pyloric caeca, and intestine **(Elfadaly *et al*., 2018)**. There is a potential zoonotic risk of transmitting infective stages from the fish digestive tract to the fish fillet in case of *C. parvum* infected edible fish. *Cryptosporidium* spp. can be transmitted either through the consumption of undercooked fish, or by contact with fish during preparation and handling **(Golomazou & Karanis, 2020)**.

CONCLUSION AND RECOMENDATIONS

Cryptosporidium species have been recorded in a wide range of piscine hosts worldwide. Research on *Cryptosporidium* in piscine hosts has increased in recent years,

reaffirming the ubiquitous nature of this protozoan parasite which has been detected in a large number of free-living, cultured, and ornamental fish species worldwide from both marine and freshwater environments. Cryptosporidiosis can be considered a great threat to fish. Fish can act as carriers and infection sources for other hosts, including humans. The presence of *Cryptosporidium* zoonotic subtypes in edible fish, commonly consumed raw or slightly processed, increases the fish-borne zoonotic potential risk. More research is needed to see if eating or handling an edible marine fish highly infected by these cryptosporidium species/genotypes poses a risk of zoonotic transmission, or if drinking water contaminated with fully sporulated oocysts shed in fish feces poses a danger of zoonotic transmission. Furthermore, the pathology of cryptosporidiosis in fish is very important. Intensive aquaculture practices, stressful conditions, high host density, water supply, and temperature, in combination with the immunological immaturity of the host, increase the prevalence, mortality rate, and interactions with other pathogens in farmed fish, having a significant economic impact on the aquaculture industry. Finally, the identification of zoonotic *Cryptosporidium* species in edible fish extends the range of foodstuffs potentially involved in the transmission of cryptosporidiosis, representing a risk to public health, although further risk assessment studies are required to confirm this possibility.

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