Department for Farm Animals and Veterinary Public Health,

University of Veterinary Medicine Vienna, Austria

University Clinic for Poultry and Fish Health

Division of Fish Health

(Head: Univ. Prof. Astrid Holzer)

***Ichthyophthirius multifiliis,* the causative agent of Ichthyophthiriose - An Update (literature thesis)**

***Ichthyophthirius multifiliis*, der Erreger der Ichthyophthiriose - ein Update (Litraturarbeit)**

Diplomarbeit

For obtaining the dignity of

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University of Veterinary Medicine Vienna, Austria

Prepared by

Christin Said

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Supervisor

Univ. Prof. em. Dr. Mansour El-Matbouli

Division of Fish Health
Department for Farm Animals & Veterinary Public Health
University of Veterinary Medicine Vienna, Austria

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ABSTRACT

Ichthyophthirius multifiliis is a widely distributed ectoparasite of fish and has a significant impact on commercial aquaculture worldwide. It is a ciliate protozoan, which causes Ichthyophthiriasis or "white spot disease". This parasite infects the skin, gills, fins, and eyes of fish and leads to severe economic losses in the aquaculture industry and high mortalities in ornamental-and food fish and epizootic in the population of wild fish. The closest living organism of *Ich* is *Cryptocaryon irritans,* but it affects marine fish.

The life cycle consists of four main stages and all stages are ciliated and are affected by temperature and salinity of the water. Each stage of the parasite depends on a certain temperature. It is reported that the suitable temperature for the development of *I. multifiliis* is between 14 and 17.5°C, encystment between 13.1 and 25°C and the division of the cyst takes place in 24 hours at 16.2 to 25°C.

There are several treatment trials to prevent and control the development of "Ich." This can be drug or non-drug intervention. Malachite green was one of the most effective treatments of *Ich* because it is always available, its cost is low, it is easy to dissolve in water and it does not spoil or be affected by storage, but it has prevented in EU member states, the United States, and other countries from use in food fish because of its teratogenic effect.

In this thesis, I will talk about the most important findings of researchers in terms of results and research on the ich parasite, what are the characteristics of this disease, how to control infection through some methods to prevent infection, and some treatments and immunizations necessary to control white spot disease.

Keywords *Ichthyophthirius multifiliis,* white spot disease, ornamental and food fish, prevention, and control.

ZUSAMMENFASSUNG

Ichthyophthirius multifiliis, der Erreger der Weißpünktchenkrankheit, Grieß-körnchenkrankheit oder Pünktchenkrankheit -Ein Update (Literaturarbeit)

Die Krankheit ist ein großes Problem sowohl für gezüchtete als auch für wilde Süßwasserfische in allen Regionen der Welt. Die meisten Arten von Süßwasserfischen gelten als anfällig. Die klinischen Anzeichen einer *I. multifiliis*-Infektion sind durch bis zu 1 mm große weiße Flecken gekennzeichnet, die häufig auf der Haut und den Flossen infizierter Fische zu sehen sind. Der Parasit hat einen direkten Lebenszyklus und kann sich mit der von der Umgebungstemperatur abhängigen Entwicklungsgeschwindigkeit zu Hunderten von infektiösen Theronten vermehren. Innerhalb von ein bis zwei Wochen kann der Parasit ganze Fischherden bei ungünstigen Witterungsbedingungen oder Wasserbelastung töten. Früherkennungs- und Behandlungsstrategien sind entscheidend, um die Ausbreitung dieser Krankheit einzudämmen. Ichthyophthiriose führt zu schweren wirtschaftlichen Verlusten und hohe Mortalitätsrate. Der erste Ausbruch der Krankheit wurde im Jahr 1876 von dem französischen Parasitologie Fouquet beschrieben.

Während des gesamten Lebenszyklus kann *I. multifiliis* in den Epithelien die Immunantwort seines Wirts aktivieren, während Theronts und Tomonts von einer Vielzahl von Faktoren in der aquatischen Umwelt beeinflusst werden.

Zu den Hauptkrankheitsmerkmalen von mit Ciliaten infizierten Fischen gehört die schwarze Färbung; erhöhte Schleimproduktion; Schäden an Schuppen; hämorrhagische, entfärbte Stellen auf der Haut; und dermale nekrotische Läsionen, die letztendlich Gewebe schädigen, was zu hohen Mortalitätsraten führt. Verschiedene Behandlungen werden verwendet, um den Parasiten mit Behandlungsregimen zu bekämpfen.

Gegenwärtige Behandlungen umfassen die Verabreichung von Formaldehyd, Natriumchlorid, Kupfersulfat und Kaliumpermanganat. Vermeintlich umweltfreundlichere Medikamente wie Huminsäure, Kaliumferrat (VI), Bronopol und die auf Peressigsäure basierenden Produkte wurden jedoch kürzlich getestet und stellen vielversprechende Alternativen dar. Weitere Untersuchungen sind erforderlich, um die Behandlungen zu

optimieren und genaue Protokolle zu erstellen, um die Menge des verwendeten Arzneimittels zu minimieren und gleichzeitig die wirksamste Leistung sicherzustellen. Gleichzeitig muss den nicht-medikamentösen Aspekten der Managementstrategien größere Bedeutung beigemessen werden, einschließlich des Einsatzes nicht-chemischer Interventionen, die sich auf die Entfernung freischwimmender Stadien und Tomozysten von *I. multifiliis* aus landwirtschaftlichen Kultursystemen konzentrieren. Der Einsatz solcher Strategien lässt auf umweltfreundlichere Alternativen zur Bekämpfung von *Ich* Infektionen hoffen.

1. INTRODUCTION

Undoubtedly, Ichthyophthiriasis, commonly known as Ich or white spot disease, stands out as the most pathogenic ailment impacting freshwater fish globally. Its prevalence has been documented in numerous countries (EL-DIEN et al., 1998; DICKERSON; FINDLY, 2014; LI et al., 2023) and appears obviously in aquarium fish due to the heavy mass of fish.

The outbreak and spreading of ichthyophthiriasis are controlled by using many ways of treatment and prevention like chemical treatment and vaccination. The most common treatment is malachite green. It has been used till the conformity of its carcinogenic effect on fish, which is consumed by humans (ALDERMANN 1985; WAHLI et al., 1993).

The life cycle of *I. multifiliis* is influenced by various environmental factors such as temperature, pH, water hardness, ion strength, and salinity (NOE and DICKERSON, 1995; J.Z. WEI et al., 2015). This cycle comprises several stages, each playing a crucial role in its completion (JIA-YUN YAO et al., 2017). Studies have shown that the optimal temperature for white spot disease outbreaks is around 20°C, particularly with Danish geographic strains (BUCHMANN and BRESCIANI, 1997). Higher water temperatures lead to increased theront levels (the first stage of the life cycle) and enhanced division rates (WAHLI-MOSER, 1985; SCHUMACHER et al., 2011).

Ichthyophthiriasis can affect a wide range of freshwater fish species, including rainbow trout (*Oncorhynchus mykiss*) (FORWOOD et al., 2014), chub (*Leuciscus cephaleus*) from the Cyprinidae family (ABDEL HAFEZ, 2011), and channel catfish (*Ictalurus punctatus*) (KLESIUS and ROGERS, 1995). A lot of studies are interested in studying whether parasites are one of the reasons for the increased mortality rate due to bacterial secondary infection. There is not enough information if parasites are carriers of pathogenic bacteria in fish (DE-HAI XU et al., 2012). Bacterial infections can occur due to the presence of micro lesion and wounds on the fish's body surface. That makes the fish more susceptible to ectoparasites such as *Gyrodactylus* spp. and *Dactylogyrus* spp. (J.Z. WEI et al., 2013).

Many attempts have been made to control the disease, such as immunotherapy and chemotherapy, but these methods will not turn out to be the desired success and until the date of writing this thesis, there is no effective vaccine proven to control *I.multifiliis* (JORGENSEN, 2017; JIA-YUN YAO et al, 2017; SALEH M et al., 2021).

Many types of treatments are used to control *Ich, including* oral treatments (SHINN et al., 2003), external chemical treatments (TIEMAN and GOODWIN, 2001), as well as non-chemical ones (FARLEY and HECKMANN, 1980). The goal of these types of therapeutics is to destroy the infective theront and the reproductive tomont in the water and prevent it from infecting the host. However, these treatments can be expensive, difficult, and sometimes ineffective in controlling *Ich* (TIEMAN and GOODWIN, 2001; SHINN et al., 2003)

In the United States, it was found that infection with parasites led to large and repeated losses. In 2002, 42% of channel catfish producers experienced a massive loss of more than 2,000 lbs. (HANSON et al., 2008). In 2009 *Ichthyophthirius* affected 4% of channel catfish producers (USDA, 2010). In Europe, the annual loss of fish farmers amounts to 140 million dollars per year (BUCHMANN, 2013; H.W DICKERSON, 2014).

2. MATERIAL AND METHODS

2.1 Data Basis

The data basis for this literature review draws on the extensive pool of existing publications on the key topics of this study: *Ichthyophthirius multifiliis*, the causative agent of ichthyophthiriosis. The foundation of the information is built on research from the past decades, combined with recent developments, to provide a comprehensive and up-to-date overview of the topics covered. The selection criteria for relevant literature included the suitability for covering the topics mentioned above and the publication date of the works.

2.2. Data Collection Methods

The databases Google Scholar, Science Direct, PubMed, Springer Link, ResearchGate, Wiley Online Library, and the "vetmed " search engine of the Library of the University of Veterinary Medicine, Vienna were utilized for data collection.

3. ETIOLOGY

The causative agent of "Ich" is *Ichthyophthirius multifiliis* ((Fouquet 1876), which is a ciliate protozoan that can rapidly become epizootic and an obligate parasite that causes" white spot disease" (NEGRELLI, 1976; DICKERSON,2006) due to the appearance of the parasite as white spots on the gills and skin of infected fish (SCHUMACHER et al., 2011). The parasite nests under the epidermis of the infected fish and feeds on blood and body fluids, so it leads to the destruction of skin cells. The fish counteract this by secretion of more mucus, which provides the parasite with more food and thus causes a mass multiplication of the parasite, which leads to the disease. It belongs to the phylum Ciliophora, the class Oligohymenophora, and the suborder Ophryoglenina (LOM and DYKOVA, 1992).

These white spots mean that the parasite is in the feeding stage named trophonts (NOGA, 2010; MACEDA-VEIGA et al., 2014). *I. Multifiliis* causes mass mortalities in juvenile and adult fish due to the presence of many trophonts on the surface of fish (VALTONEN and KERANEN, 1981). Each reproductive adult trophont produces up to two thousand infective theront (MacLENNAN 1937).

3.1. NOMENCLATURE

The parasite was described by the French parasitologist Fouquet in 1876 and listed in the index of ectoparasites. The name multifiliis means much plus daughter in Latin, and this meaning is because of the huge number of theronts (the infective stage) (J.Z. WEI et al. 2013). The name of the parasite translates as 'the fish louse with many children, it is based on its reproductive behavior, since many daughter cells can arise from a trophant (WAHLI-MOSER, 1985; BUCHMANN et al., 2001; MATTHEW, 2005).

3.2. TAXONOMY

It is listed under the phylum *Ciliophora*, which is distinguished by more cilia around the whole body (DICKERSON,2006). The class Oligohymenophora is characterized by a groove-like mouth with oral cilia (J.Z. Wei et al., 2013).

The classification of Ich according to (LOM and DYKOVA, 1992; BUCHMANN et al. 2001) is shown in (table 1)

Table 1: classification of *I. multifiliis*:

Infrakingdom	***Alveolata***
Phylum	***Ciliophora***
Class	***Oligohymenophorea***
Suborder	***Ophryoglenina***
Family	***Ichthyophthiriidae***
Genus	***Ichthyophthirius Fouquet,1876***

3.3. LIFE CYCLE AND MORPHOLOGY

The life cycle of *Ichthyophthirius multifiliis* is complicated because it includes stages in the host and in the environment (Michael K. et al. 2015). It requires 90-96 days at 3-5 °C, 20 days at 10 °C, 13-14 days at 13-15 °C, and 3-7 days at 20-25 °C. (HAWKE & KHOO, 2004; WISE et al.,2004).

It is a direct life cycle and requires no intermediate host (EWING and KOCAN, 1992) and consists of four main stages (SHINN et al., 2012; TANGE et al., 2020) **(Fig. 1)**

- **The theront (1)** – is pear-shaped or elliptical shape and transparent. Except for the anterior end, it is completely covered with cilia (BUSCHKIEL, 1910; WAHLI-MOSER, 1985). It is the infective stage of the parasite (MATTHEW, 1996), which is a free-swimming reproductive stage that penetrates the skin and the epithelia of the infected host (NIGRELLI et al., 1976; DICKERSON 1995) characterized by the presence of white spots all over the body surface and gills of the infected fish (MALLIK et al., 2015). Large numbers of theronts can harm the fish before the parasite becomes visible on the skin. In such cases, death is caused by extensive damage to the gill epithelia (LEMOS, 2007). The theront penetrates the epidermis and develops into a trophont. The development depends on the temperature and the salinity of the water (NOE and DICKERSON, 1995; AIHUA and BUCHMANN, 2001). It is important to kill the theronts to prevent or control the life cycle of *Ich.*

- **The trophont (1mm) (2)** – the vegetative growth parasitic stage, which lives and feeds on the skin of the infected fish especially mucous and tissue and is distinguished by numerous contractile vacuoles and is resistant to any chemical treatment as they hide underneath the mucous and epidermis (JAMES M. FORWOOD et al., 2014).
- **The tomont (3)** – which leaves the infected fish and secretes a thick gelatinous cyst wall to form a tomocyst in water. (LI et al., 2023) (Fig. 4)
- **In the tomocyst (4) –** reproduction and multiple division take place (5-8) forming a lot of tomites (daughter cells). The number of tomites, which are released from one tomont varies from 50 to a thousand (WAGNER, 1960; LI and BUCHMANN, 2001).
- The cyst ruptures (9) – and the theront released from it to attach the skin of the host to begin a new journey of the infection.

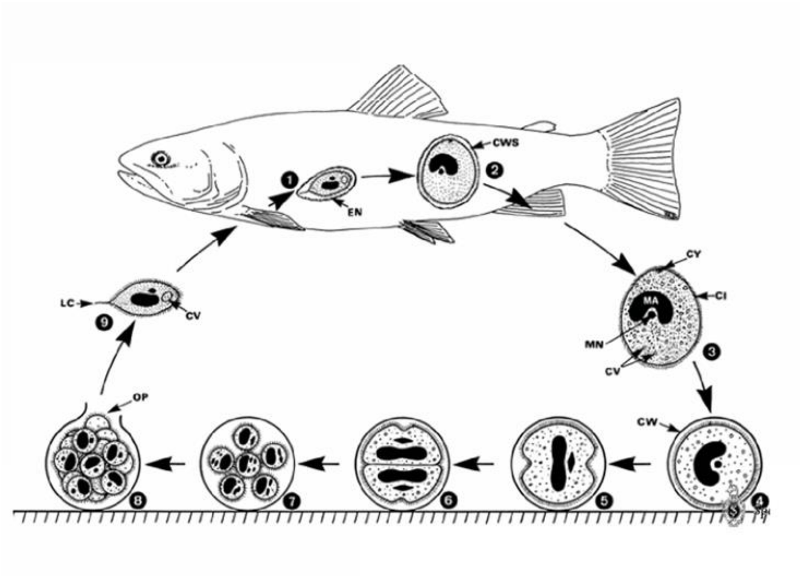

Fig. 1: Life cycle and morphology of *I. multifiliis* (MATTHEWS, 1994)

3.4. STABILITY OF *ICHTHYOPHTHIRIUS MULTIFILIIS*

Factors that significantly affect the life cycle of Ich in the non-parasitic stage, may be chemical or physical factors such as temperature, pH degree, and salinity. According to Garcia et al., (2011), it appeared that high hard water (120 mg l−1 CaCO3) and pH (9) was a suitable environment for the life of Ich.

Temperature is one of the most important factors affecting the life cycle of the parasite. The appropriate temperature for the spread of Ich in Denmark was 20°C (BUCHMANN and BRESCIANI 1997) and it was proven that high temperatures of up to 30°C led to the elimination of the trophont. This method may be used to treat fish that can tolerate high degrees of temperature. It was reported that temperature has a strong and noticeable effect on the rate of Ich growth, whether in the reproductive or parasitic stage. Aihua and Buchmann (2001) recorded this connection and relationship, as it was found that at low temperatures (5°C), division of tomocyst requires eight to nine days with the largest size and littlest number of theronts. While at higher temperatures (30°C) required 18 hours to split, with smaller sizes but fewer theront. At medium temperatures, tomocyst produced the largest number of theront.

An investigation by (TANG et al., 2020), studied the effect of pH on tomont survival, reproduction, size, and survival of theronts of a Nordic parasite strain. The study investigates the impact of pH on various aspects of the parasite's free-living stages, including tomont and theront survival, tomocyst reproduction, and theront size, at room temperature. Results show that tomonts in acidic pH levels (2-5) exhibit high mortality within the first 6 hours, while those at pH 6-9 survive and successfully encyst. However, at extreme pH levels (10-11), tomonts display premature division and increased mortality. Overall, pH 7 is the control, with significantly lower mortality than acidic and extreme pH levels. The experiment was replicated three times, yielding consistent findings.

4. INFECTIVITY AND PATHOGENICITY

Due to the presence of the parasite on the surface of the skin and the gills of the host, this appears in the form of white spots of different shapes and sizes (DICKERSON, 2006), and ulcers also occur in the skin that leads to the shedding of dead skin.

In the beginning, the fish usually still look unchanged but show behavioral problems (HINES and SPIRA, 1973; DICKERSON, 2006). The first signs are general restlessness and increased rubbing of the fish on the bottom or objects, the so-called "flashing" (WAHLI-MOSER, 1985). These symptoms are due to the itching caused by the stages growing under the epidermis. And because the injury to the gills is more severe than the skin, this leads to difficulty breathing (MAJEED et al., 1984) and can also lead to entry of secondary invaders like fungi or bacteria which induce the pathological effect on the host (EWING et al., 1994; TUMBOL et al., 2001) such as loss of the excretory and osmoregulatory functions leading to death of the fish (HINES and SPIRA 1973; DICKERSON 2006).

As mentioned before the theront penetrates the epithelia of the skin and gills by moving between two cells and from there to the main layer of the skin (EWING et al., 1985; KOZEL, 1986; BUCHMANN, 2001), where the presence of mucous cells through which the infective stage reaches to epidermis (BUCHMANN et al., 1999). Here it is noted that the theront is strongly attracted to the mucous cells (BUCHMANN and NIELSEN, 1999) due to the existence of components of the serum in the mucus.

The parasite spreads rapidly from one host to another, and the tomont can produce from hundreds to thousands of the infective stage in less than a day (MAC.LENNAN 1935; MATTHEWS 2005)

5. FISH SPECIFICITY AND SUSCEPTIBILITY

I. multifiliis is one of the most prevalent protozoan parasites that infects all species of freshwater fish, because of this diversity, the disease is difficult to be controlled from the point of ecological view (NIGRELLI et al., 1976; WURSTBAUGH and TAPIA 1988; VALTONEN and KOSKIVARA 1994; TAVARES-DIAS et al., 2010).

The infection of *I. multifiliis* in freshwater fish is largely nonspecific and can even affect tadpoles (*Limnodynastes peronii*) under experimental conditions (GLEESON, 1999). However, different fish species or subspecies exhibit varying levels of susceptibility to *I. multifiliis*. For instance, recently domesticated goodeid species such as *Ameca splendens* and *Ilyodon xantusi* are more susceptible to *I. multifiliis* compared to the long-domesticated poeciliid species like *Xiphophorus maculatus* and *Xiphophorus variatus* (CLAYTON and PRICE, 1992). Additionally, within the common carp (*Cyprinus carpio*), susceptibility to *I. multifiliis* varies based on scale pattern genotypes: fully scaled carp show greater resistance compared to mirror carp, while scaleless domestic carp are even more susceptible (PRICE and CLAYTON, 1999). Furthermore, there is evidence of heterosis in resistance to *I. multifiliis* infections (CLAYTON and PRICE,1994).

5.1. SUSCEPTIBLE FISH SPECIES:

Table 2: Fish species which are susceptible to infection with *I. multifiliis* according to Price and Bone (1984).

	Scientific Name	**Common Name**
1	*Acipenser baerii*	Siberian sturgeon
2	*Anguilla japonica*	Japanese eel
3	*Aristichthys nobilis*	Bighead carp
4	*Betta splendens*	Siamese fighting fish
5	*Bidyanus bidyanus*	Silver perch
6	*Carassius auratus*	Auratus goldfish

7	*Cichlasoma urophthalmum*	Mayan cichlid
8	*Colossoma macropomum*	Tambaqui
9	*Ctenopharyngodon idella*	Grass carp
10	*Cyprinodontidae*	
11	*Cyprinus carpio*	Common carp
12	*Dorosoma cepedianum*	
13	*Fundulus notatus*	
14	*Helostoma temminckii*	Kissing gourami
15	*Ictalurus punctatus*	Channel catfish
16	*Micropterus dolomieu*	Smallmouth bass
17	*Micropterus salmoides*	Largemouth bass
18	*Oncorhynchus mykiss*	Rainbow trout
19	*Oncorhynchus nerka*	Sockeye salmon
20	*Oncorhynchus tshawytscha*	Chinook salmon
21	*Oreochromis aureus*	Blue tilapia
22	*Paralichthys olivaceus*	Bastard halibut
23	*Perca flavescens*	Yellow perch
24	*Poecilia reticulata*	Guppy
25	*Salmo salar*	Atlantic salmon
26	*Salmo trutta*	Sea trout
27	*Xiphophorus maculatus*	Southern platyfish

6. EPIDEMIOLOGY

Ich infection spreads in the presence of conditions suitable for the rapid reproduction of the parasite, such as an appropriate environment and a susceptible host. A prerequisite for the extension of the disease and the further growth of trophonts is the temperature of the water. Stress can also cause disease outbreaks among fish (MacLennan, 1937, 1942; NIGRELLI et al., 1976; NOE and DICKERSON, 1995).

In China, there were outbreaks of the disease as early as the 10th century (HINES & SPIRA, 1974) and in Europe white spot disease, as this ectoparasitic disease is also known, was well known in the Middle Ages, and was probably introduced by cyprinids imported from Asia (NIGRELLI et al., 1976; MATTHEWS, 2005).

The disease occurs less frequently in wild fish populations due to the low prevalence of the parasite (EWING and KOCAN, 1992). The financial damage in fish farms is primarily due to the massive loss of stocks, but reduced fish growth and the resulting reduced production output also led to losses. When keeping ornamental fish, it is not only financial aspects that are important, but often emotional aspects as well, since individual fish have an individual value for the fish keeper.

The occurrence of ichthyophthiriasis is seasonal. Epidemics are most common in spring and early summer when water temperatures are rising (WAHLI-MOSER, 1985), but cases of white spot disease can occur all year-round (BAUER, 1958). Outbreaks of the disease always occur when the conditions are favorable for the rapid multiplication of the parasite and at the same time, the fish's defense mechanisms are weakened. An important criterion is the water temperature, as this has a decisive influence on the development cycle (MACLENNAN, 1937, 1942; SCHÄPERCLAUS, 1954; NIGRELLI et al., 1976; WAHLI-MOSER, 1985).

It is known that *Ich* is widespread worldwide in all freshwater fish (NEGRILLI et al., 1976). The parasite is not host-specific and no freshwater fish species have been found to completely resist the disease (WAHLI-MOSER, 1985). However, there are indications of different susceptibilities of different fish species (HOFFMAN, 1967) as well as individual differences in fish of the same species (VENTURA & PAPERNA, 1985).

6.1. GEOGRAPHICAL DISTRIBUTION

Table 3 illustrates, that Ich is a globally distributed parasite affecting fish (NIGRELLI et al., 1976; VALTONEN and KERÄNEN, 1981). It has been found in fish farming regions worldwide, from tropical to subarctic climates, and in wild fish populations across most continents (NIGRELLI et al., 1976; VALTONEN AND KERÄNEN, 1981). The organism was first described by Fouquet in France in 1876 (STILES, 1894). According to HILDENDORF and PAULICK (1989), they published observations on a fish disease caused by a ciliate, likely *I. multifiliis*, in Hamburg, Germany. These reports were the first detailed accounts of the parasite and its life cycle. However, the disease was known in Europe during the Middle Ages (HOFFMAN, 1999). Evidence suggests it originated in Asia as a parasite of carp (HINES and SPIRA, 1974a).

I. multifiliis has been found to infect more than 190 freshwater fish species across 26 countries. In Iran, it has been isolated from 51 fish species across 57 different localities (BARZEGAR et al., 2023), while in Iraq, this parasite has been recorded in 40 fish species (MHAISEN, 2023). In Turkey, Özer (2021) documented infections in six wild freshwater fish species, four cultured freshwater fish species, and 14 aquarium fish species.

Table 3: the geographical distribution of *I. multifiliis* around the world (NIGRELLI et al., 1976; VALTONEN and KERÄNEN, 1981)

Continent/Country/Region	Reference
Africa	
South Africa	Bragg (1991)
Uganda	Paperna (1972)
Asia	

China	Hines and Spira (1974)
Europe	
Finland	Valtonen and Keranen (1981)
France	Stiles (1894)
Germany	Stiles (1894)
Russia	Bauer (1953)
North America	
Canada	CABI
British Columbia	Traxler et al., (1998)
United States	CABI
Alabama	Allison and Kelly (1963)
Kentucky	Kozel (1976)
Maryland	Eiser (1955)
South America	
Bolivia	Wurtsbaugh and Tapia (1988)
Peru	Wurtsbaugh and Tapia (1988)

7. IMMUNOLOGICAL RESPONSE OF *I. MULTIFILIIS*

In the past, studies on the immune response to *I. multifiliis* have been carried out mainly on rainbow trout (*Oncorhynchus mykiss*), *channel catfish* (*Ictalurus punctatus*) and carp (*Cyprinus carpio*). It was generally assumed that the immune responses were the same in all species, but this has not always proved to be the case. For example, channel catfish lack IgT immunoglobulins, which are important mucosal antibodies in rainbow trout, and instead have IgM secreting cells in their skin, with IgD being prominent. Antibody titers have been observed to rise as early as 4 hours after immunization with *I. multifiliis*, and species-specific responses vary due to species-specific characteristics. This emphasizes the importance of conducting studies at the species level. Consequently, this review categorizes the current state of knowledge into primary and secondary immunological responses based on species-specific findings (JORGENSEN, L. V. G., 2017).

Recently, research has expanded to other fish species and novel immunological analytical tools have been applied. These developments contribute significantly to the understanding of the complex interactions between hosts and parasites.

7.1. IMMUNOLOGICAL TISSUES AND CELLS

Invasion of the host surface is followed by a series of immune responses involving a variety of immune-related molecules (WANG et al., 2019). Teleost fish have mucosal tissues that contain lymphoid cell clusters similar to those found in the gut of higher vertebrates (XU et al., 2013). These accumulations are named differently depending on their location (KOPPANG et al., 2010). However, unlike discrete tissues such as the head kidney and spleen, these cells lack an organizational structure, which has led to the proposal to replace 'T' with 'C' in their abbreviations.

The initial response to parasite invasion is the expression of genes encoding inflammatory cytokines and acute phase reactants (GONZALEZ et al., 2007). As the infection progresses, genes associated with adaptive responses, including both B and T cells, become visible. Exposure to parasites triggers the upregulation of genes encoding chemokines and cytokines, reflecting innate and adaptive responses (JORGENSEN et al., 2018; SYAHPUTRA et al., 2019). Proteomic (SALEH et al., 2019) and transcriptomic (SYAHPUTRA et al., 2019) analyzes reveal intricate physiological responses triggered by the parasite at the site of infection, some of which are directed towards pathogen elimination and others towards tissue restoration. As the trophont matures, it is able to consume epithelial cells and evade host defenses, including the uptake of neutrophils (JORGENSEN, 2016). Even after the trophont has left the epidermis, a large number of genes of the innate immune system remain upregulated, possibly creating an unfavorable environment for parasites.

Nevertheless, adaptive immune responses appear to be essential for protection, as shown by immunized fish that exhibit increased expression of immunoglobulin genes and Th2-associated cytokines when challenged, facilitating antibody production (JORGENSEN , 2018).

8. PATHOGENESIS

A characteristic and reliable means of identifying ichthyophthiriosis is the appearance of white spots on the fins and the entire body surface of the fish (DICKERSON, 2006). However, this clinical picture is only found in the advanced stage of the disease.

At the beginning, the fish usually look unchanged, but show behavioral problems (HINES & SPIRA, 1973; DICKERSON, 2006). The first signs are general restlessness and increased rubbing of the fish against the ground or objects, the so-called "blinking" (WAHLI-MOSER, 1985). These symptoms are due to the itching caused by the stages growing under the epidermis. As the disease progresses, the general condition of the fish deteriorates. As a defense reaction against the parasite infestation, an increased layer of mucus forms on the skin (HINES & SPIRA, 1973), and infestation with fungi of the genus Saprolegnia, which have good growth conditions due to the previous damage to the skin by *I. multifiliis*, often occurs on the second day (DICKERSON, 2006). The parasite stages on the gills cause oxygen deficiency by damaging the gill epithelium (SCHÄPERCLAUS, 1990). As the disease progresses, the fish become increasingly apathetic, refuse to feed, and spend most of their time at the water inlet in order to get as close as possible to oxygen-rich water (WAHLI-MOSER, 1985; DICKERSON, 2006).

Ultimately, the death of the fish occurs due to the interaction of several factors, such as the disturbance of the homeostatic balance, secondary infections by bacteria and fungi (NIGRELLI et al., 1976; KOZEL, 1986), and oxygen deficiency due to the massive destruction of the gill epithelium (SCHÄPERCLAUS, 1990; SMITH, 1990).

9. DIAGNOSIS

9.1. FIELD DIAGNOSIS

9.1.1. CLINICAL SIGNS

a. Damaged, irritated, and ulcerated epithelia, due to the presence of the trophont on the gills and skin (ABDEL HAFEZ, 2011; J.Z. WEI, 2013).
b. Deformation in the lamellae of the gills, due to the thickening of the epithelial layer of the gills. (DURBOROW et al., 1998; ABDEL HAFEZ, 2011)
c. Appearance of white spots on the skin of the infected fish (Fig. 2) and increasing mucous production (Fig. 4) (DICKERSON and DAWE, 1995).
d. The presence of white spots (trophont) on the eyeball of fish can lead to blindness (EGUSA, 1992).
e. Secondary bacterial and fungal infections (DICKERSON, 2006).
f. Fish infected with the parasite have some pathological characteristics such as their black coloring, the presence of bleeding in some places of the skin, and the production of mucus increases, thus causing damage to tissues, which in turn leads to an increase in the death rate (SALEH M, et.al, 2021)

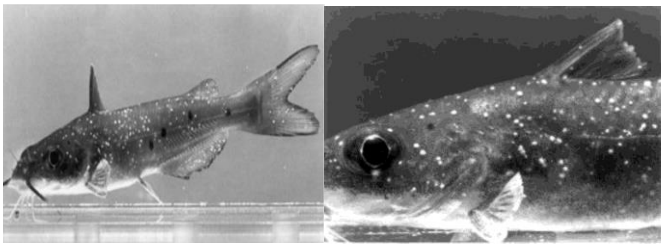

Fig.2 Infected channel catfish with *Ichthyophthirius multifiliis*. The infection appears as white spots on the skin and gills. (H.W DICKERSON, 2006)

9.1.2. BEHAVIORAL CHANGES

a. **Loss of Appetite:** Fish affected by *I. multifiliis* may exhibit a reduced appetite or completely stop feeding. This loss of appetite is often associated with the discomfort and stress caused by the parasite's presence (HEDRICK et al, 1993; DICKERSON, 2006).

b. **Gasping at the Water Surface:** The infected fish start to swim quickly to reach the surface of the water to gain some oxygen. This behavior is often indicative of respiratory distress caused by gill damage inflicted by the parasite. (BUCHMANN & LINDENSTROM, 2002; DICKESON , 2006; RAHIM et al., 2020).

c. **Increased Scratching or Flashing**: Fish infected with *I. multifiliis* often exhibit increased scratching or flashing behavior. They may rub their bodies against objects within the aquarium, such as rocks, plants, or tank walls. This behavior responds to the irritation caused by the parasite's presence on the skin and gills. (MATTHEWS, 2005; RAHIM et al., 2020)

9.2. CLINICAL DIAGNOSIS

9.2.1. MACROSCOPICAL OBSERVATION

Observation of trophont on the skin and gills of the infested fish. Pathological lesions of the skin and gills lead to localized lymphocytic infiltration, epithelial proliferation, and focal necrosis (MAKI et al., 2001).

Although most authors emphasize that Ich can be diagnosed with relative certainty with the naked eye, microscopic examination should be performed whenever possible; especially since this can usually be done very easily by simply examining a skin swab.

9.2.2. MICROSCOPICAL EXAMINATION

I. multifiliis can be diagnosed by the examinations of biopsy material from the skin and gills of the infected fish (MICHAEL, 2015) by removing some white spots and putting them on a slide then adding a few drops of water and covering them with a glass.

The parasite is identified by its horseshoe shape under microscopical examination (KLINGER and FLOYD, 2009) (Fig. 3).

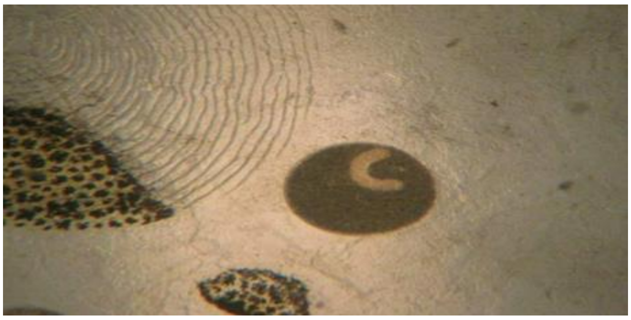

Fig. 3 Trophont with the typical horseshoe-shaped nucleus of *I. multifiliis* (Jour.Myan.Acad.Arts &SC. 2013; REKLINGER and RF FLOYD, 2009)

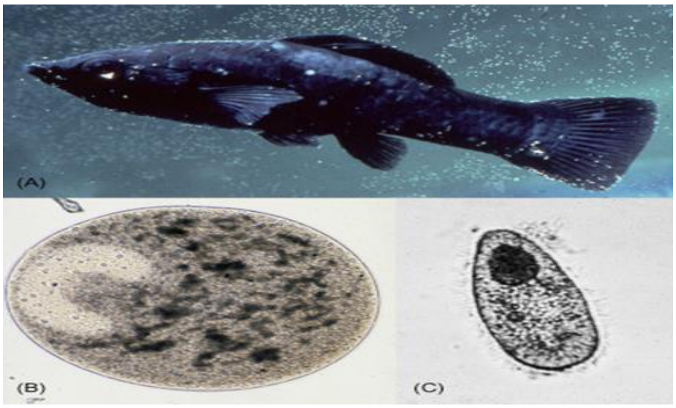

Fig. 4 (A) Black molly with multiple white spots. (B) tomont stage, which is characterized by its horseshoe-shaped nucleus. (C) theront, the free-swimming ciliated stage (MICHAEL , 2015)

9.3. ISOLATION AND IDENTIFICATION

Accurately identifying and isolating a pathogen from an afflicted fish holds paramount significance. This process facilitates a definitive diagnosis of the underlying cause of clinical disease and furnishes material for subsequent investigations into the morphology, life cycle, and genetic makeup of parasites. Given the pervasive presence of many parasites and their often intimate relationship with their hosts, establishing a direct link between a particular parasite and a disease can be challenging.

In the case of *I. multifiliis*, widely recognized as the causative agent of the often fatal white spot disease in freshwater fish, there exists a scarcity of unequivocal reports demonstrating the direct association between this specific parasite and the disease it causes. Considering the diverse array of Ichthyophthirius strains infecting various fish species, isolating this ciliate from infected tissue stands as the initial crucial step in investigating the role of I. multifiliis in precipitating disease across multiple hosts.

The isolation and identification of *I. multifiliis* involve several steps, including sample collection, microscopic examination, and confirmation of the parasite's identity. Here's a general outline of the process (WANG et al., 2002; XU et al., 2012):

Step 1: Sample Collection

1. **Fish Sampling:**
    - According to Dickerson and Clark (1998), infected fish are collected from aquaculture or aquarium environments showing clinical signs of Ich, such as white spots on the skin and gills.
    - Fish should be handled carefully to prevent additional stress and damage.
2. **Environmental Sampling:**
    - Water samples from tanks or ponds with infected fish can also be collected to capture free-swimming theronts (GUANG et al., 2024)

Step 2: Laboratory Techniques

1. **Microscopic Examination:**
    - Skin and gill scrapes from infected fish are examined under a microscope (PICONCAMACHO et al., 2012)
    - Trophonts (feeding stage) are identified by their large size, horseshoe-shaped macronucleus, and movement (GUANG et al., 2024)
2. **Culture Methods:**

- Attempting to culture tomonts (reproductive stage) and theronts (infective stage) in the laboratory can help isolate the parasite (MATTHEWS, 2005 and GILBERT et al., 2017)
- Infected fish are placed in separate containers to allow tomonts to fall off and encyst. These cysts can be collected and incubated to release theronts (LOM and DYKOVA , 1992)

3. **Use of Host Fish:**

- Healthy fish can be experimentally infected with collected theronts to maintain the parasite's life cycle in the laboratory.
- This method helps in obtaining fresh samples for study and further identification.

Step 3: Identification and confirmation of *I. multifiliis*

1. **Morphological Identification**

Microscopic Characteristics:

- *I.multifiliis* trophonts are large, ranging from 0.5 to 1 mm in diameter (MICHAEL K., 2015)
- Distinctive features include a ciliated surface and a horseshoe-shaped macronucleus (JOUR.MYAN et al., 2013)
- The presence of ciliary rows and contractile vacuoles helps in distinguishing it from other protozoan parasites.

2. **Molecular Methods**

a. **DNA Extraction and PCR:**

- According to Jousson et al., (2005) DNA is extracted from isolated parasites.
- Polymerase Chain Reaction (PCR) is used to amplify specific genetic markers unique to *I. multifiliis*.
- Primers targeting the internal transcribed spacer (ITS) regions or other specific

genes are employed.

b. Sequencing and Phylogenetic Analysis:

- Sequencing the amplified PCR products helps in confirming the identity of *Ich*.
- Phylogenetic analysis can further validate the species identification by comparing the sequences with known genetic databases (PEKMEZCI et al., 2022)

3. Serological Methods

a. Antibody Development:

- Antibodies specific to *I. multifiliis* antigens are developed for use in diagnostic tests.
- These antibodies can be used in various assays to detect the presence of the parasite (WANG and DICKERSON, 2002)

b. Immunofluorescence and ELISA:

- Immunofluorescence involves labeling antibodies with fluorescent dyes to visualize the parasite under a microscope (XU et al., 2016)
- Enzyme-linked immunosorbent Assay (ELISA) detects parasite antigens in fish tissues or water samples, providing a quantitative measure of infection.
- Advances in non-invasive sampling methods to reduce stress on fish and improve sample quality (XU et al., 2003)

10. PREVENTION AND CONTROL OF ICH

It is much easier to prevent the disease than to treat it. Incoming fish should be isolated for at least three days at a temperature between 75- and 83 degrees Fahrenheit (23 und 28 C°). The temperature should be high to match the length of Ich's life cycle, and in order not to spread other diseases, the quarantine period can be prolonged up to three weeks (FLOYD et al., 2018). There are many methods for preventing the infestation. Disinfectants have been used for many years to control and prevent Ich. (PICONCAMACHO et al. 2012).

According to Alderman (1985), a new environmental-friendly program must be reached in combating the parasite, especially after banning the use of malachite green by some agencies like the FDA (Food and Drug Administration) of the EU and USA because of its carcinogenic effect (FEI LING et al., 2013).

Many types of research have proven that some extracts from medical plants have an effective effect in controlling Ich, especially the theront stage, due to their proven efficacy and low environmental impact (YAO et al., 2011; LING et al., 2012, 2013; FU et al. 2014a, b; SONG et al. 2015).

10.1. ULTRAVIOLET LIGHT CONTROL

Ultraviolet light is used to prevent the production of "Ich" in closed fish cultures. Larger dosage of UV radiation is important because of the enormous size of the pathogen (HOFFMAN, 1974), which is also enough to control all other bacteria and viruses in the aquaria (GRATZEK et al. 1983).

10.2. CHLOROPHYLLIN

Chlorophyllin, a photodynamic substance, has been shown to effectively eliminate all life stages of the parasite *I. multifiliis* (Richter et al., 2012). A study by Richter and Häder (2012) confirmed that chlorophyllin significantly aids in preventing the Ich parasite.

10.3. MEDICATED FOOD CONTAINING QUININE.

It has been reported that quinine has a strong effect in preventing "Ich," as it kills the trophont stage in ornamental fish (SCHMAHL et al., 1996). Many groups have evaluated the efficiency of quinine (SCHÄPERCLAUS 1979; KABATA 1985; WITZKE et al., 1988;

SCHMAHL et al., 1989b). After assessing a medicated food containing quinine on the infected host with *I. multifiliis*, it was found that the trophozoites were damaged within the skin of fish (SCHMAHL et al., 1996). When quinine was injected at a dose of 60mg/kg body weight, it was found that the trophont amount was reduced (SCHUMACHER et al., 2011).

10.4. PERACETIC ACID (PAA)

PAA is an antimicrobial disinfectant used for preventing and controlling the infestation of *I. multifiliis* in aquaculture because of its acute toxicity against the parasite (MEINELT et al., 2009). It is supposed to be a more environmentally friendly drug (PICÓN-CAMACHO et al., 2011)

10.5. PLANT OF ARTEMISIA ANNUA

In a study conducted by Zhibin et al., (2016) on the effect of *Artemisia annua* plant against Ich infection. The study says that when the plant is taken orally at a concentration of 20g/kg for 45 days, the results proved a strong effect in protection against the parasite. Also, the number of trophonts on the fins was significantly reduced. However, this method provides little protection against high doses of infection.

10.6. METHANOL EXTRACT OF *PSORALEA CORYLIFOLIA*

In a study by Song et al., (2015) it was determined that two compounds, psoralidin, and isopsoralen, were isolated from the methanol extract of *P. corylifolia* using bioassay-guided fractionation based on their activity against anti-ich encysted tomonts. Their in vitro and in vivo efficacies were assessed against the free-living and parasitic stages of Ich. Psoralidin was found to effectively kill theronts, protomonts, and encysted tomonts, and also negatively affected *I. multifiliis* trophont in situ, indicating its potential as a lead compound for developing a commercial drug against Ich.

10.7. CURCUMIN ISOLATED FROM CURCUMA LONGA :

According to Liu, Y. et al., (2017) Curcumin demonstrated significant in vitro and in vivo antiparasitic efficacy against *I. multifiliis* and has strong potential as a safe and effective treatment against the parasite in the fish farming industry.

In acute toxicity tests of curcumin on grass carp, mortality increased with higher concentrations of curcumin. At 56 mg/L, 30% of fish died within 48 hours, and at 58 mg/L, 76.7% died within 72 hours. At 60 mg/L, there was 100% mortality within 12 hours, while no fish died at concentrations ≤54 mg/L. Curcuma longa (Zingiberaceae), commonly known as "jianghuang" in Chinese, is widely used in food and in traditional Chinese medicine to treat some diseases such as bleeding, menstrual difficulties, colic and hematuria (LABBAN, 2014).

11. TREATMENT OF ICHTHYOPHTHIRIASIS

Whenever white spots are observed on the skin and gills of fish, the treatment must be applied quickly to save the infected fish. Eliminating the infective theront or the reproductive trophont can interrupt the parasite's reproductive cycle, thus limiting the spread of the disease (TUCKER and ROBINSON 1990, SCHÄPERCLAUS 1991). Treatment can be obtained in two ways:

11.1. CHEMOTHERAPY

Chemical treatment of the aquatic culture can help on destroying of the free-living stages of Ich before infestation takes place (FARLEY and HECKMANN, 1980) and that leads to interrupting of the life cycle (FEI LING et al., 2013)

A single treatment cannot be effective so it must be repeated to affect the disease because the life cycle of the parasite contains sensitive phases (theronts and trophont) and non-sensitive phases (tomonts) (TOJO et al., 1994; STOSKOPF, 1993).

Examples of chemotherapy:

11.1.1. FORMALDEHYDE SOLUTION (FORMALIN):

Formalin and sodium percarbonate affect the free-swimming stages of *Ichthyophthirius* (theront) (BAUER 1970 and STOSKOPF 1993; SHINN et al., 2005; LAHNSTEINER and WEISMANN, 2007; HEINECKE and BUCHMANN, 2009). Formalin is the only approved treatment for controlling protozoan parasites, but its use is limited due to its high cost and potential risks to human safety (NOGA,2010), but to achieve satisfactory results, it must be highly concentrated. For example, in salmon farms, 100 mg per liter for 30 minutes to one hour for 10 days. There are side effects of using formaldehyde in aquatic life such as reducing the amount of oxygen in the water by 1 ppm for each 5 ppm of formaldehyde (CROSS, 1972; PILLAY and KUTTY, 2005). This can lead to a big problem especially during summer because of the increase in the water temperature, which quickens the life cycle of Ich and reduces the ability of water to retain oxygen.

11.1.2. SALT “NACL”:

NaCl is one of the most common products used to treat Ich. Its efficacy was evaluated in tanks and aquaria to control and prevent the infestation with *I. multifiliis* (BAUER 1970;

STOSKOPF 1993). It has proven that the application of a minimum of 2.5 g NaCl, leads to a reduction of tomont and theront survival (AIHUA AND BUCHMANN, 2001; SHINN et al., 2005). Parasites, existing as free-living invertebrates, exhibit varying degrees of tolerance to fluctuations in salinity. Consequently, some are categorized as euryhaline, while others are considered stenohaline. Changes in salinity can induce osmotic stress, potentially leading to the mortality of various protozoans (BUCHMANN, 2022). Elevated salinity levels inhibit the development of tomonts, including the transition through the tomocyst stage to become infective theronts. This interruption in the life cycle leads to the exhaustion of the parasite population (LI and BUCHMANN, 2001).

11.1.3. COMBINATION OF MALACHITE GREEN AND FORMALIN:

It has been used for the treatment of "*Ich*" and was found highly effective for eliminating of theront, trophont, and tomont stages and is not expensive (AMLACHER, 1961; HOFFMAN and MEYER, 1974; JOHNSON 1976; ALDERMAN 1985; SCHMIDT et al., 1993 and WAHLI et al., 1993). Also, fish for human consumption had been treated with malachite green until the early 1990s (CALLINAN and ROWLAND 1995), until it was proved that malachite green has a mutagenic, teratogenic, and carcinogenic effect on fish for human consumption (ALDERMAN, 1985; SRIVASTAVA et al., 2004; SUDOVA et al., 2007).

11.1.4. POTASSIUM PERMANGANATE (KMNO4):

Potassium Permanganate is also used to treat Ich infection, mainly in farm pond systems (Brown and GRATZEK, 1980; NOGA, 2010). For the elimination of theront stage in the water, low concentration (0·8–1·0 mg l−1) from KMnO4 and exposure for a short period (30 min - 4 h) (STRAUS & GRIFFIN, 2002). Using high concentrations (10–20 mg l−1) for 30 min was proved to be toxic for the treatment of infected fish (BALTA et al., 2008). It is extensively used in aquaculture, with abundant data available on its applications (Duncan 1978). As a strong oxidizer, its effectiveness depends on the amount of easily oxidizable material present in the water (Marking and Bills 1975). It can eliminate the free-living stages of *I.multifiliis*, but it has limited effectiveness against the trophont (MATTHEWS, 2005)

11.1.5. COPPER SULPHATE:

Is widely used in aquaculture, where it is considered an algaecide, and is also used as a treatment for parasites, including Ich. It is known for its lethal impact on ectoparasites and external infective stages (LASEE, 1995; NOGA, 2012). The chemistry of water has a strong influence on the toxicity of copper sulfate, as the toxicity decreases as the hardness and alkalinity increase. According to Straus and Tucker (1993) and Straus et al., (2009), it is believed that the form of copper most toxic to aquatic organisms and to algae is Cu++.

11.1.6. POTASSIUM FERRATE (VI):

In a study conducted by Fei Ling et al., (2009) aimed at evaluating the toxicity of Potassium ferrate (VI) and the appropriate concentration to prevent infection with Ich, 500 Theront were exposed to different concentrations of potassium ferrate (VI) and observed for 4 hours. When the results were obtained, it was found that exposure of theronts to a concentration of 4.80 mg/L or more of pot. Ferrate leads to 100 % mortality in 4 hours.

11.1.7. Medicines:

The traditional approach to parasite control involves the application of various medications as antiparasitic agents (PICON-CAMACHO et al., 2012). However, strict adherence to regulations and legislation is imperative when treating parasitic infections in fish with medications. This applies to both initial investigative and validation studies before licensing, as well as the administration of licensed products (SOMMERVILLE et al., 2016; BUCHMANN, 2022). Prior to initiating treatments at the farm level, a specific diagnosis must be established, and a prescription issued by a veterinarian. Moreover, the medication should be licensed in the specific country where the treatment is intended. It's worth noting that several medications with known antiparasitic effects have been prohibited in animal production for various reasons.

11.2. ELECTROTHERAPY:

The application of an electrical field (table 4) reduces the amount of the parasite for example: The alternating current (AC): Although it is effective and reduces the number of the parasite (COOMBS, 1968), it can be dangerous to the fish and destroy it (SPENCER, 1967).

Table 4:

(A strategy is regarded as being partially effective if it kills 50–80%, and effective if it kills ≥80% of the stages under test. Mortality refers to the parasite stages unless otherwise stated.)

Electrotherapy							
In vitro – Trophozoides*							
	Electrode type	Volts per 2·5 cm separation	Current	Duration (s)		Efficacy	Reference
	Carbon	55–150	150–350	5		Not effective –14·35% mortality after 24 h	Farley and Heckmann (1980)
	Carbon	104–150	200–350	5		Not effective –7·09% mortality after 24 h	
	Carbon	150		3		Effective – 100% mortality	
	Carbon	150		3		Effective – 100% mortality	
	Carbon	250		3		Effective – 100% mortality	
	Carbon	350		3		Effective – 100% mortality	
	Carbon	350		3		Effective – 100% mortality	
	Copper	88–115	135–200	5		Effective – 100% mortality after 24 h	
	Copper	115	135	5		Effective – 100% mortality after 24 h	
	Steel hardware cloth	150–240	160–400	5		Not effective –2·99% mortality after 24 h	
	Steel hardware cloth	150	340	5		Not effective –0·87% mortality after 24 h	
Mechanical filtration							
In vitro – protomonts							
	Mesh size (μm)					Efficacy	
	500					Not effective – 0% protomonts filtered out	Heinecke and Buchmann (2009)
	300					Not effective – 6% protomonts filtered out	
	160					Not effective – 22% protomonts filtered out	
	80					Effective – 100% protomonts filtered out	
Mechanical removal of the tomocysts							
In vitro – protomonts							
	Lining surface					Efficacy	
	Crystal polysterin					Not effective – 9·8% mortality	Shinn *et al*. (2009)
	Polypropylene – based plastic					Effective – 90·2% mortality	
	Polyethylene – based plastic					Partially effective – 76·5% mortality	
	Chlovar chlorinated rubber					Not effective – 46·6% mortality	
In vivo – commercial raceways in *O. mykiss* hatchery (Suction head + lining of the bottom of the raceways)							
	Visit number					Efficacy	
	1 (after 2 weeks)					No infection in control and experimental raceways	Shinn *et al*. (2009)
	2 (after 4 weeks)					No infection in control and experimental raceways	
	3 (after 6 weeks)					Low infection levels in both control and experimental raceways	
	4 (after 8 weeks)					Effective – 92% reduction in trophont numbers compared to the control	
	5 (after 10 weeks)					Effective – 99% reduction in trophont numbers compared to the control	
(Suction head stopped, only lining of the bottom of the raceways)							
	6 (after 12 weeks)					Partially effective – 54% reduction in trophont numbers compared to the control	
UV light							
In vivo- fish species not specified							
	Number of UV bulbs used (UV light generated)					Efficacy	
	0					Not effective – 82·81% fish mortality	Gratzek *et al*. (1983)
	1 (91 900 μW s cm^{-2})					Effective – 1·33% fish mortality	
	2 (183 800 μW s cm^{-2})					Effective – 0·7% fish mortality	
Water flow							
In vivo – experimental raceways of *I. punctatus* fingerlings							
	Fish density (no. L^{-1})	Flow rate (L min^{-1})	Velocity (cm min^{-1})	Turn-over (no. h^{-1})	Efficacy		
	0·33	5		4·1	0·5	Not effective – 100% mortality of infected fish	Bodensteiner *et al*. (2000)
	0·25	15		12·2	1·5	Not effective – 52% mortality of infected fish	
	0·25	25		20·3	2·5	Effective – 14% mortality of infected fish	
	0·33–0·66	5		4·1	0·5	Not effective – 100% mortality of infected fish	
	0·33–0·66	25		20·3	2·5	Effective – 9% mortality of treated fish	
	0·33–0·66	45		36·5	4·5	Effective – 7% mortality of treated fish	
In vivo – production raceways of *I. punctatus* fingerlings							
	0·89–1·29	>2800		>85	>2·1	Effective – no trophonts observed	
	0·71–1·40	>2800		>85	>2·1	Effective – no trophonts observed	

Management strategies tested against infections of *I. multifiliis* Fouquet, 1876 (PICÓN-CAMACHO, S. et al., 2011)

11.3. VACCINATION:

A vaccine is a biologically prepared compound designed to stimulate immunity, primarily acquired and indirectly non-specific, against a specific disease or group of diseases (ABBAS et al., 2023). It is regarded as an effective method for preventing and controlling Ichthyophthiriasis. However, the lack of sufficient effective antigens for mass production makes large-scale applications impractical (JORGENSEN, 2017). To combat the pathogen, disinfectants and drugs are used for eradication. Prolonged use of ineffective drugs can lead to drug resistance (PICON-CAMACHO et al., 2012), compelling farmers to increase the dosage. This escalation can result in environmental pollution and issues with medication residues.

Given fish's capability to develop immunity against *I. multifiliis*, vaccination emerges as a superior strategy compared to recurrent treatments for disease management. Vaccination minimizes fish handling and stress, thereby significantly improving animal welfare. Additionally, since vaccinated fish remain disease-free, there is a notable reduction in the release of environmentally harmful substances typically used during disease outbreaks (JORGENSEN, 2017)

According to Ling et al., (1993), Goldfish can be vaccinated against Ich using a free-living protozoan and Tetrahymena pyriformis by injection intraperitoneal or by immersion. The results confirmed that the goldfish formed a protective immunity not only against Ich but also against some ectoparasites, that reside in the fish environment. Immunization was related to the antibody titer present in the host’s skin and mucus.

Dickerson and Clark (1988) confirm the failure of many attempts to protect catfish against Ich by using tetrahymena as a vaccine, either by injection or by surface exposure. The use of live and dead cell preparations ((DICKERSON et al., 1984; BURKART et al., 1990). The same negative results were proven by Houghton et al., (1992)

12. ECONOMIC IMPORTANCE

I. multifiliis presents a considerable economic challenge to the aquaculture industry, ornamental fish trade, and wild fish populations. Implementing effective management and preventive strategies is crucial to reducing its impact and ensuring the sustainability and profitability of fisheries and aquaculture operations.

12.1. ECONOMIC IMPACTS

12.1.1. MARKET IMPLICATIONS

Frequent outbreaks and poor disease management can damage the reputation of aquaculture businesses and ornamental fish suppliers, leading to decreased demand and market share (NOGA, 2010; WOO, P.T.K., and BRUNO, 2011).

12.1.2. TREATMENT COSTS:

Costs associated with treating Ich include purchasing chemicals or medications, labor for administering treatments, and potentially more expensive, less harmful treatments to avoid affecting non-target organisms in the tank or pond (HINES, R. S., and SPIRA, D. T., 1973; R. FRANCIS and P. REED, 1991). Loss of Revenue due to fish deaths and reduced growth rates directly result in financial losses for commercial aquaculture operations. The ornamental fish trade also suffers as diseased fish become less marketable, and treatment expenses can cut into profits (SHINN et al., 2015)

12.2. ECOLOGICAL IMPACTS

12.2.1. ECOSYSTEM DISRUPTION:

Outbreaks in wild fish populations can disrupt food webs and ecological interactions. Predatory fish that rely on affected species for food may also suffer, leading to cascading ecological effects (GUANG et al., 2024)

12.2.2. ENVIRONMENTAL CONTAMINATION:

The use of chemicals to treat Ich can lead to environmental contamination. Chemicals such as formalin, malachite green, and copper sulfate, commonly used in treatments, can have toxic effects on non-target organisms and the environment (WRIGHT and WELLBOURN, 2002; SHIVAJI et al., 2004)

12.2.3. BIODIVERSITY THREATS:

In natural water bodies, *I. multifiliis* (Ich) poses a threat to wild fish populations, especially those already under stress from environmental factors. Such threats can precipitate declines in biodiversity and disturbances in local ecosystems (MATTHEW, 2005). Native fish species lacking resistance to Ich are particularly susceptible, potentially resulting in shifts in species composition and disrupting ecosystem equilibrium (TAVARES and MARTINS, 2017).

13. DISCUSSION AND CONCLUSION

Ichthyophthirius multifiliis, a freshwater fish parasite with a widespread distribution, poses a significant threat to fish populations due to its highly pathogenic nature. This ciliate parasite exhibits broad host compatibility, infecting nearly all species of freshwater fish and targeting epithelial tissues, leading to the formation of characteristic white spots, commonly known as "white-spot" disease or ichthyophthiriasis (LI et al., 2023). In aquaculture, Ich outbreaks can cause substantial economic losses, including reduced fish growth, increased mortality rates, and the cost of treatment and management, leading to financial setbacks for fish farmers and aquaculture businesses. Additionally, *I. multifiliis* has the potential to cause widespread mortality, particularly in densely stocked aquaculture systems or crowded ornamental fish tanks, exacerbating economic losses and environmental impacts.

The parasite follows a direct life cycle comprising four distinct stages: the trophont, tomont, tomocyst, and theront, with some scholars proposing a fifth stage, the protomont, to characterize the parasite during its detachment from the fish epidermis (LI et al., 2023; EWING et al., 1985). Environmental factors such as salinity, water temperature, pH, and water hardness play crucial roles in the development of *I. multifiliis* (AIHUA and BUCHMANN, 2001; MIRON et al., 2003; GARCIA et al., 2011; TANGE, MATHIESSEN, and JØRGENSEN, 2020).

Numerous studies have explored chemical treatments as a means to control and prevent Ich infections, utilizing medications like formalin, copper sulfate, and malachite green (only for ornamental fish). However, the use of chemical treatments raises concerns about the development of drug-resistant strains and negative impacts on water quality and ecosystem health. Balancing the effectiveness of these treatments with their potential risks to fish health, environmental integrity, and economic viability is crucial for sustainable *Ichthyophthirius* management.

Advancements in understanding Ich immunology have spurred efforts in vaccine development, with studies investigating different antigens and vaccine formulations to induce protective immunity against Ich infections.

In conclusion, future research and intervention efforts should prioritize the development of effective vaccines, sustainable management practices, interdisciplinary approaches, environmental risk assessment, and public education to address the multifaceted challenges posed by *I. multifiliis* in aquatic ecosystems and ensure the long-term health and resilience of fish populations and their habitats.

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