

Overview on Fish Mycobacteriosis with Special Reference to Pathology, Diagnosis and Human Health

Kibruyesfa Bayou

Wollega University, College of Medical and Health Science,
School of Veterinary Medicine, P.O. Box: 395, Nekemte, Ethiopia

Abstract: Mycobacterial diseases rank among the most common chronic diseases of bacterial etiology found in fish worldwide. In the food industry, particularly in tropical and subtropical countries, mycobacterial infections may significantly decrease fish production. In addition, certain mycobacterial species (Particularly *M. marinum*, *M. fortuitum* and *M. chelonae*) can cause contagious skin infections in humans (Under particular conditions) transmitted from fish. Diagnostic pathological techniques of mycobacteriosis are usually based on the examination of suspect fish. Gross and histologic lesions of necrogranulomas containing acid-fast bacilli give strong clues towards the involvement of *Mycobacterium*. Submissions of samples to a laboratory for diagnosis should include suitable specimens of fish, that is suspected to be involved and a full clinical history. Through a systematic examination of the whole body, organs and tissues (Necropsy), external pathological changes like skin ulcers, scale loss, nodular formations in the fin bases and unilateral or bilateral exophthalmia can be seen. Internally, the most significant macroscopic pathological change is the presence of miliary white to greyish nodules in the kidney, spleen and liver. Histopathological examination of spleen, kidney and liver reveals granulomatous lesions characterized by a central region constituted by epithelioid cells surrounded by mononuclear inflammatory cells, especially lymphocytes. However, most of the granulomatous lesions present a central necrotic region surrounded by an inflammatory tissue capsule rich in mononuclear cells. In some Ziehl-Neelsen staining sections, it is possible to observe acid-fast bacilli in the core of the granulomas. Because culturing the *Mycobacterium spp.* is difficult and time consuming, using molecular techniques (PCR-based techniques) allow rapid diagnosis and identify organisms to the species level on tissue and blood specimens.

Key words: Fish • Granuloma • Histopathology • Lesions • Mycobacterium

INTRODUCTION

Fish is one of the known aquatic animals used for human consumption as food. Aquatic animals in general do contain a high level of protein (17-29%) with an amino-acid profile similar to that of the meat of land animals. Aquatic animals are a source of minerals such as calcium, iron and phosphorus as well as trace elements and vitamins. Marine species are particularly rich in iodine [1].

One of the problems of the fishery sector in the fish population is bacterial diseases. Fish are susceptible to a wide variety of bacterial pathogens. Many of these bacteria capable of causing diseases in the aquatic environment. These bacteria become pathogens when fishes are physiologically unbalanced, nutritionally deficient, or there are other stressors, i.e., poor water quality, overstocking, which allow opportunistic bacterial

infections to proceed. Mycobacteria are among many organisms that can occur naturally in the aquatic environment and result in fish mycobacteriosis [2].

Mycobacteriosis is among the major diseases of concern in fish for several reasons, which is caused by many species of the genus *Mycobacterium*. It is a serious and often lethal disease of fish, affecting a wide range of species globally both in culture and wild settings [3]. The disease has received considerable attention in recent years because of the discovery of new species in piscine hosts, epizootics in wild fisheries and the ability to infect humans [4].

Therefore, based on the above facts, the objective of this seminar paper is to overview etiology, epidemiology, transmission, clinical signs, pathological lesions, diagnosis and impact on human health of fish mycobacteriosis.

Etiology: Mycobacteriosis is one of the various diseases that occur in fish and is caused by bacteria belonging to the family Mycobacteriaceae. These are visually demonstrated as straight or slightly curved bacilli which have aerobic, immobile and non sporulating characteristics. They are acid-alcohol fast and often considered to be Gram-positive. Various mycobacterial species are ubiquitous in the environment and can be found in water, soil and dust. Many different species of mycobacteria belonging to both fast and slow growing species have been isolated from fish [5].

However, those most frequently isolated like *Mycobacterium marinum*, *M. fortuitum*, *M. chelonae* and *M. avium* are considered pathogenic for fish [6]. *Mycobacterium marinum* forms the largest proportion of all mycobacteria isolated from fish. Both tropical freshwater and marine fish are susceptible to *M. marinum* infections. The isolation of *M. fortuitum* from fish has been less frequently documented than *M. marinum*, but the prevalence of infection is probably more widespread than generally suspected. Of the other species of mycobacteria, the following have been isolated: *M. aurum*, *M. gordonae*, *M. parafortuitum*, *M. poriferae* and *M. Triplex* [7].

Epidemiology and Fish Species Affected: *Mycobacterium marinum* is ubiquitous and is found worldwide in bodies of fresh water, brackish water and salt water. All species of fish are susceptible to mycobacteriosis and it has been described from a wide variety of aquarium fish [1]. One survey found that more than 67% of water specimens collected from natural, treated and animal contact sources contained mycobacteria, including *M. marinum* and it was reported from many marine and freshwater fish species and also fish families occurring in Africa (Cichlidae and Characidae) [8]. There are several case reports from African Cichlid species; *Sarotherodon andersonii* and *Tetras sparmanii* in the Okavango swamps in Botswana, farmed *Oreochromis niloticus* in Kenya and also from aquarium reared *Haplochromis multicolor*, *Hemichromis bimaculatus* and *Oreochromis mossambicus* [9].

Outbreaks are most common in tropical aquarium fish. Members of several freshwater families such as Anabantidae (Bettas and Gouramis), Characidae (Tetras and Piranhas) and Cyprinidae (Danios and Barbs) appear to be particularly susceptible. Mycobacteriosis has been found in wild stocks of fish including Cod, Halibut, Striped bass, North-East Atlantic mackerel and Yellow perch. Intensively culture warm water fish species are also very susceptible. There has been an increase in frequency

of *M. marinum* infection in cultured or hatchery-confined fish, such as Chinook salmon, Cultured striped bass, Freshwater ornamental fish and Sturgeon [1].

Transmission and Source of Infection: Ingestion of mycobacteria is probably the major source of infection, including fish that have recently eaten dead tank mates [8]. Cannibalism and eating contaminated feed and thereby ingesting the infectious agent are also the main routes for the transmission of mycobacterium in fish. Mycobacterial lesions in the digestive tract, gill and skin are the main source for releasing infectious materials into the water column. In addition, once the fish dies and decomposes, release of infective bacteria from infected tissues increases [10].

Another possible means of mycobacterial infection is through injured skin, to which numerous identifications of mycobacteria in skin lesions bear witness [11]. Mycobacteria were isolated from embryo of infected viviparous mothers and this posits the transovarial transmission of mycobacteria in viviparous fish. *Mycobacterium marinum* can remain viable in the environment (Soil and water) for two years or more or in carcass and organs up to one year. This can lead to the possible indirect transfer of the organism [12].

Clinical Signs: Signs of mycobacteriosis in fish are variable and often resemble other diseases. Some fish may show no external signs of disease. The chronic form of the disease is most commonly seen. In general, affected fish can be anorexic, emaciated, listless and lethargic; they may separate from other fishes and seek out corner of the holding facility. Additional signs may include corneal opacity and eventually cause exophthalmos (Bulging eyes), abdominal distention and skeletal deformities, such as spinal curvature or stunting defects and pale gills. Some fish may develop fin and tail rot. Fading of cutaneous pigmentation is also common. The acute form of the disease occurs rarely and it is characterized by rapid morbidity and mortality with few clinical signs [7].

When present in a population, infection rates can vary from 10% to 100%. Unfortunately, there is no non-lethal method available to identify infected individuals, especially those in early to mid stages of disease. Fish with late-stage disease often develop ulcerations and other external lesions that may have some diagnostic value. Therefore, if one fish in a population is diagnosed with the condition, then the entire population must be considered exposed and potentially infected. Methods for detection of infected individuals have yet to be

developed. In addition, factors that promote the establishment of mycobacteria within a given aquaculture system also need to be identified to decrease chance of exposure. The infection will smolder in infected fish, resulting in chronic health problems and mortality in the population [11].

Pathological Lesions

Gross Pathology: Scale loss, fin necrosis, abdominal distention, multiple skin ulcers (Figure 1A and Figure 2) and lesions were comprised of small nodules (Figure 1B). Dermal aggregates extended into the epithelial layer. Lymphocytes as well as pigment cells were aggregating around the lesions, resulting in heavy melanization of the skin. Spleen and kidneys usually contained the highest concentration of mycobacterial tuberculi (Visceral nodular/granulomatous lesions) in many species of fish (Figure 5) [8].

In cases of spontaneous mycobacteriosis caused by *M. fortuitum* in *Oreochromis niloticus* under conditions of intensive culture in Kenya, the affected tilapias showed small focal granulomas in the kidney, liver and spleen with clear enlarged spleens and exophthalmia (Figure 2A) [9]. The infected Goldfish (*Carassius auratus*) revealed haemorrhagic skin ulcer on the back region, detachment of scales and frayed fins, spinal deformity, clear unilateral exophthalmia, abdominal ascitis, erected scales, emaciation, congestion and adhesion of the abdominal viscera (Figure 3, 4, 6) [13].

Histopathological Lesions: The predominant pathological hallmark of mycobacterial infections in fish is the infiltration of lymphoid cells and macrophages with granuloma formation. Both the granulomatous tissue and surrounding areas produce a positive reaction with periodic acid-Schiff method staining. The nodules are granulomas of varying sizes and degrees of development and are visible in infected tissue sections. The nodules consist of clumps of epitheloid cells surrounded by a connective capsule of varying thickness and areas of necrosis are often seen in the centre. In mycobacteriosis, the most severe granulomas are predominately located in the liver and the spleen of the fish [8].

In kidney, granulomatous lesions were characterized by a central region constituted by epitheloid cells surrounded by mononuclear inflammatory cells, especially lymphocytes. However, most of the granulomatous lesions presented a central necrotic region surrounded by an inflammatory tissue capsule rich in mononuclear cells (Figure 7). Structurally, the most affected organs were

strongly injured. In some Ziehl-Neelsen staining sections it was possible to observe acid-fast bacilli in the core of the granulomas [13]. Spleen of naturally infected Goldfish (*Carassius auratus*) showed necrosis and depletion of the haemopoietic tissues. Spleen of the experimentally infected at the day 60 post-infection with *M. fortuitum* showed considerable increase in the melanomacrophage centers numbers with excessive melanosis and filled with hard tubercles (Figure 8) [14].

Diagnosis: In addition to the clinical signs, diagnosis of mycobacteriosis of fish requires examination of any visible external and internal organs and tissues changes through appropriate necropsy techniques; sampling and the processing of diagnostic samples by an appropriately qualified laboratory. Typically, the disease is diagnosed based on microscopic evaluation of tissues processed for histopathology. The pathologist evaluates the tissues for the presence of granulomas. Part of this evaluation is to perform a special stain (Ziehl-Neelsen Acid Fast Stain) on the tissues. This stain specifically stains the mycobacteria due to the structure of the bacterial cell wall. Different biochemical evaluations of the infection can be done using the approved techniques. Some laboratories also culture the bacterium, although this requires a special medium and the bacterium may take several weeks to grow on the agar plate [6].

Primary isolation of fish mycobacteria is best achieved using Ogawa and Lowenstein-Jensen media. Subcultures develop at 28°C within 3-5 days on these media. On Ogawa medium, the cultures appear creamy in the dark but brilliant yellow color when exposed to light. Cultures may not always be obtained even from fish showing unequivocal evidence of infection. Mycobacteria may also be isolated occasionally on general-purpose bacteriological media such as tryptic soy agar, or brain heart infusion agar, provided that a large inoculum is used. All fish mycobacterium have been cultured at 20-30°C for 2 to 30 days. The isolates are strongly acid-fast, rod-shaped, weakly gram-positive, cord forming, non-motile and non-spore forming [15].

Immunohistochemistry (IHC) was found to be effective in the detection of mycobacterial antigens in tissue at the early stages of disease where conventional histological staining methods failed. The specific reaction by IHC was visible as a goldenbrown colour within the cytoplasm of phagocytic cells. The IHC method proved to be more sensitive than Ziehl-Neelsen (ZN) staining since positive regions were observed in the liver of infected fish by IHC which could not be seen or were only faintly

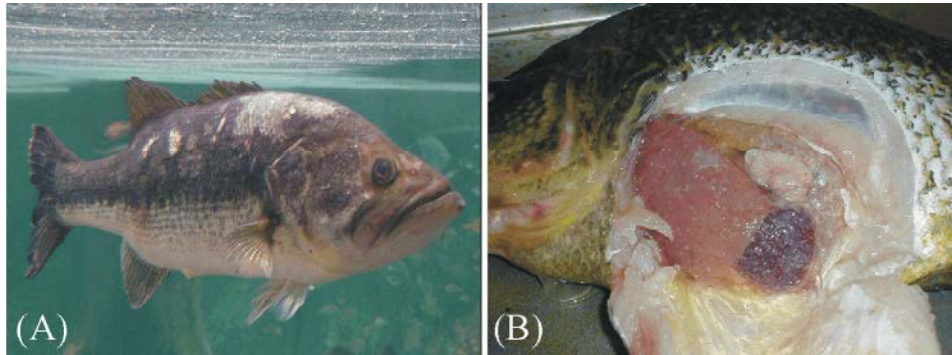


Fig. 1: Largemouth bass showing clinical signs and lesions consistent with mycobacteriosis. The fish is thin and has obvious scale loss and shallow ulcers visible on the skin (A). Small gray nodules visible within the spleen of a Black crappie during necropsy (B) [16].

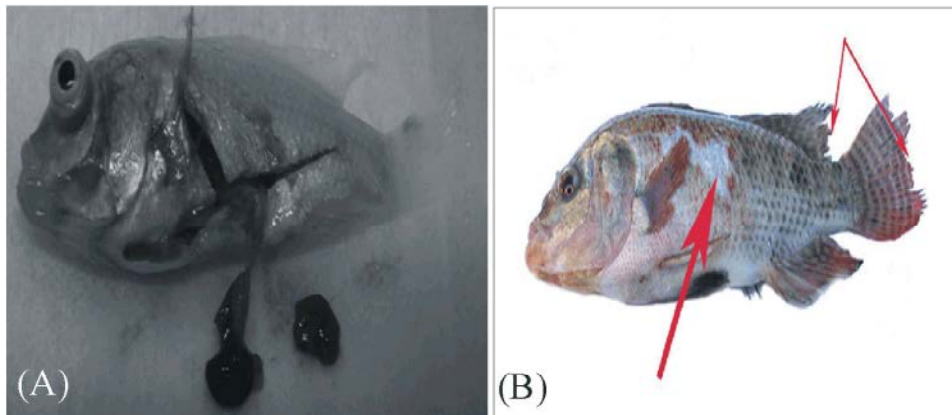


Fig. 2: Nile tilapia (*Oreochromis niloticus*) with grossly enlarged spleen and exophthalmia due to mycobacteriosis (A) [11]. Fin rots and scale loss of Nile tilapia with mycobacteriosis (B) [16].

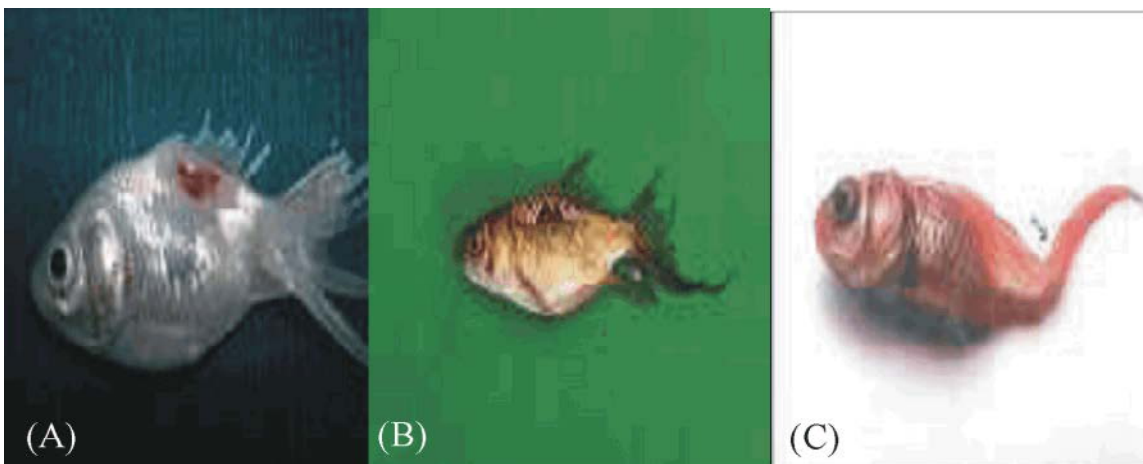


Fig. 3: Naturally infected Goldfish (*Carassius auratus*) showing haemorrhagic skin ulcer on the back region (A), detachment of scales and frayed fins (B) and spinal deformity (C) [15].

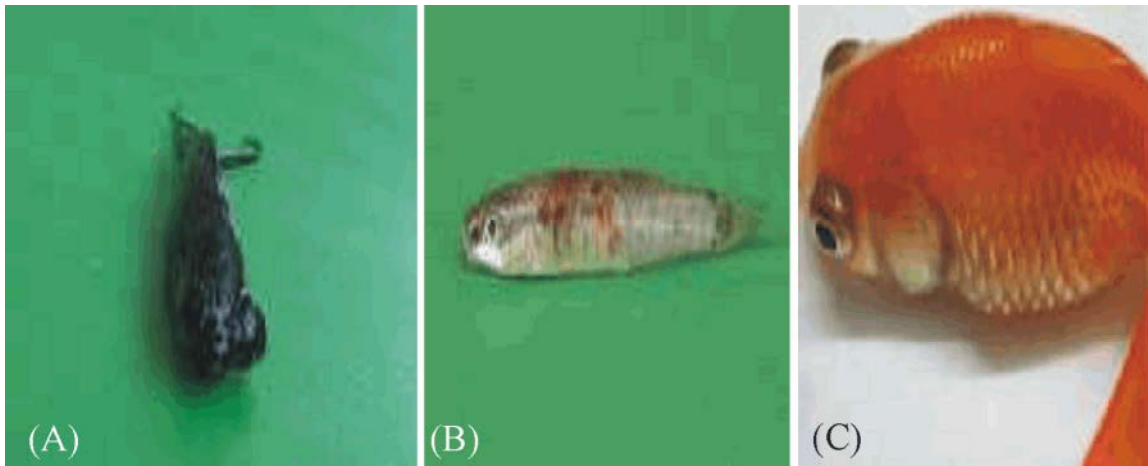


Fig. 4: Naturally infected Goldfish (*Carassius auratus*) showing clear unilateral exophthalmia (A). Gambusia (*Gambusia gaigei*) showing external hemorrhages (B). Experimentally infected Goldfish (*Carassius auratus*) showing abdominal ascitis and erected scales (C) [15].

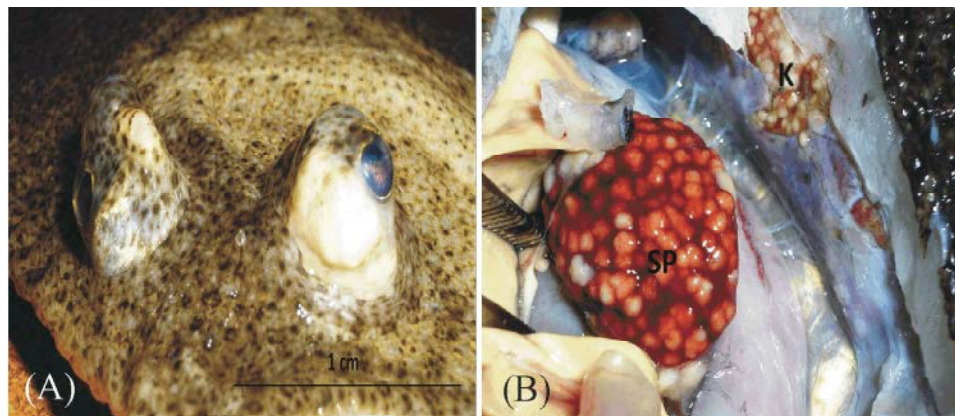


Fig. 5: Bilateral exophthalmia caused by large whitish nodules (A). Miliary white to greyish nodules in kidney (K) and spleen (SP) (B) in farmed turbot [17].

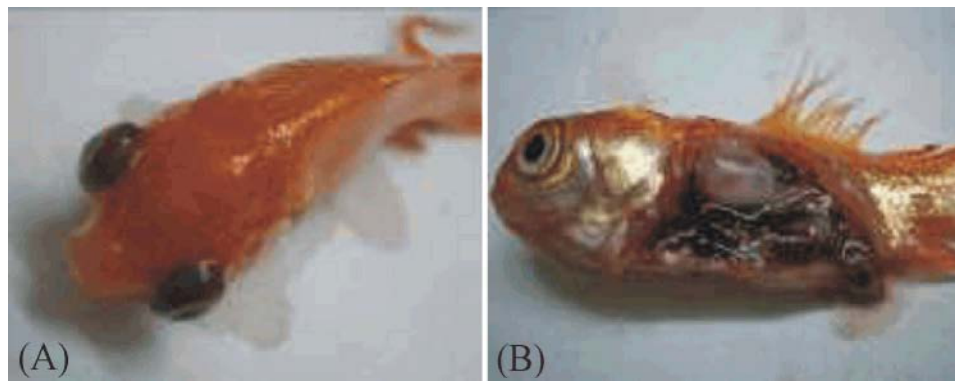


Fig. 6: Experimentally infected Goldfish (*Carassius auratus*) showing bilateral exophthalmia (A), emaciation, congestion and adhesion of the abdominal viscera at the end point of the experiment (60 days of post-infection with *M. fortuitum*) (B) [15].

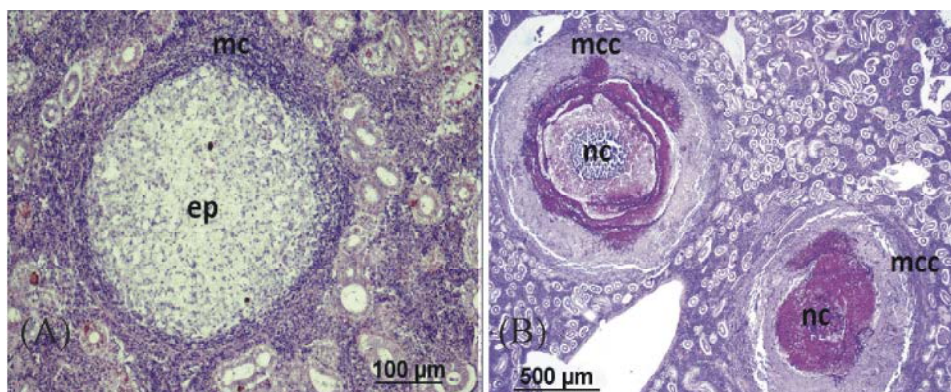


Fig. 7: Early kidney granulomatous lesions with epithelioid cells (ep) and mononuclear cells (mc) (A) and with central necrotic material (nc) and mononuclear cells capsule (mcc) (B) [18].

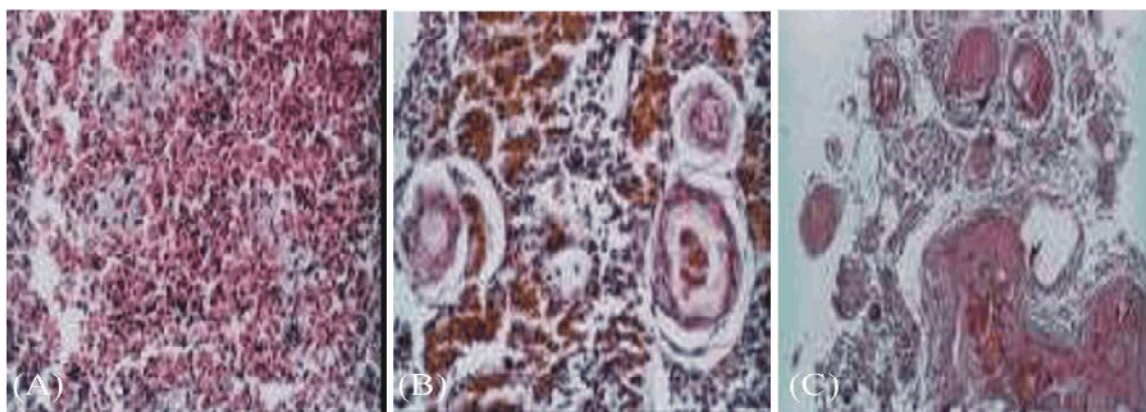


Fig. 8: Spleen of naturally infected Goldfish (*Carassius auratus*) showed necrosis and depletion of the haemopoietic tissues (A). Spleen of experimentally infected Goldfish at the day of 60 post-infection with *M. fortuitum* showed considerable increase in the melanomacrophage centers with excessive melanosis and filled with hard tubercles (B). Spleen of experimentally infected Goldfish with *M. marinum* at the end point of the experiment (60 days) appeared to be filled with tubercles (C) [15].



Fig. 9: Superficial skin lesions can appear on the hands and arms of workers who handle fish infected with *M. marinum* (A). Lesions present on the skin of farmers of Siamese fighting fish: hand lesions (B) and ankle lesions (C) [27].

observed stained with ZN stain. Definitive identification of the type strains is however may not possible using these conventional methods mentioned above. An alternative approach to the identification of mycobacteria is the application of molecular techniques (Like PCR using 16SRNA primer) [16].

Human Health Concerns: In contrast to the notorious pathogens *Mycobacterium tuberculosis* and *M. leprae*, the majority of the mycobacterial species described to date are generally not considered as obligate human pathogens. The natural reservoirs of these non-primary pathogenic mycobacteria include aquatic and terrestrial environments. Under certain circumstances, e.g., skin lesions, pulmonary or immune dysfunctions and chronic diseases, these potentially pathogenic mycobacteria (PPM) may cause disease [17]. The most frequently isolated PPM from fish and the aquatic environment are *M. marinum*, *M. avium*, *M. abscessus*, *M. chelonae*, *M. aurum*, *M. poriferae*, *M. fortuitum*, *M. gordonae* and *M. Triplex* [18].

Humans become infected while working with the contents of aquaria with infected fish [19]. The consumption of insufficiently heat treated fish foods is a presumed source of potentially pathogenic mycobacteria for patients infected with HIV. For example, *M. marinum* has been isolated from patients with skin lesions and infected lymph nodes, which has also been identified in infected fish [20]. In humans, *M. marinum* causes granulomatous inflammation and nodular or diffuse granulomas of the skin, subcutaneous tissues and tendon sheaths of fingers and hands and is referred to as 'swimming pool granuloma', 'Fish tank granuloma', 'Fish handlers' disease', or 'Fish TB'. Invasive septic arthritis and osteomyelitis may occur in immunocompromised hosts, causing chronic skin lesions, congestion of the whole finger and hand (Figure 9) and tenosynovitis [21].

The Centers for Disease Control and Prevention (CDC) in the USA included *M. marinum* on their list of 'Emerging infectious diseases' from 2008. To reduce the risks of topically acquired infection, people should avoid direct contact with potentially contaminated fresh or salt water if they have open cuts, scrapes or sores on their skin. People with compromised immune systems should avoid handling fish or cleaning fish tanks or they should wear heavy, waterproof gloves when handling or processing fish and cleaning home aquariums or fish tanks. Everyone should wash their hands thoroughly with soap and water after contact with fish or processing fish.

It is also important to ensure the regular and adequate chlorination of swimming pools and fish tanks to kill any bacteria that may be present [22].

CONCLUSION

Mycobacteriosis has been reported to affect a wide range of freshwater and marine fish species, suggesting a ubiquitous distribution and has the potential to profoundly impact the fishery sector as a whole and its economical consequences include mortality, morbidity and effects of subclinical infection such as decreased feed efficiency, decreased growth rates and decreased marketability of fish. It has also a zoonotic importance and in humans can cause cutaneous lesions (Piscine granuloma), commonly associated with professional and recreational activities around aquatic environments. Diagnosis of fish mycobacteriosis mainly depends on clinical and histological signs and identifying the strains of bacteria responsible for causing the disease. Presence of gross and histologic lesions of necrogranulomas having acid-fast bacilli indicates the presence of the *Mycobacterium*. Therefore, based on the above conclusion the following recommendations are forwarded:

- Continuous investigations should be done to know the epidemiology and the impact of the disease.
- Appropriate diagnostic, treatment and prevention protocols should be set.

REFERENCES

1. Enzensberger, R., 2002. Disseminated cutaneous *Mycobacterium marinum* infection in a patient with non-Hodgkin's lymphoma. *Infection*, 30: 393-395.
2. Ayotunde, E.O., S.T. Change and I.R. Okey, 2007. Parasitological examination and food composition in the gut of Feral African carp, *lebeocoubie* in the Cross River, south Eastern, Nigeria. *Afr. J. Biotechnol.*, (6): 625-630.
3. Bragg, R.R., H.F. Huchzermeyer and M.A. Hanisch, 1990. *Mycobacterium fortuitum* isolated from three species of fish in South Africa. *Onderstepoort. Journal of Veterinary Research*, 57: 101-102.
4. Anderson, D.P. and P.J. Barney, 1991. The role of the diagnostic laboratory in fish disease control. *Annual Review of Fish Diseases*: pp: 41-62.
5. Wayne, L.G. and G.P. Kubica, 1986. Genus *Mycobacterium*: *Bergey's Manual of Systematic Bacteriology*, 2. The Williams & Wilkins Co., Baltimore. 1436-1457.

6. Sanders, G.E. and L.E. Swaim, 2001. Atypical piscine mycobacteriosis in Japanese medaka (*Oryzias latipes*). *Com. Med.*, 51: 171-175.
7. Perez, A.T., D.A. Conroy and L. Quinones, 2001. Presence of acid-fast bacteria in wild and cultured silver mullets (*Mugil curema* VAL., 1836) from Margarita Island Venezuela. *Interciencia*, 26: 252-256.
8. Noga, E.J., J.F. Wright and L. Pasarell, 1990. Some unusual features of Mycobacteriosis in the cichlid fish *Oreochromis mossambicus*. *J. Comp. Pathol.*, 102: 335-344.
9. Roberts, R.J. and C. Sommerville, 1982. Diseases of Tilapias. In: Pullin, R.S.V. and R.H. Lowe-McConnel, (Eds.), *The Biology and Culture of Tilapia*. ICLARM Conference Proceeding, Manila, Philippines, pp: 247-263.
10. Decostere, A., K. Hermans and F. Haesebrouck. 2004. Piscine mycobacteriosis: a literature review covering the agent and the disease it causes in fish and human. *Vet. Microbiol.*, 99(3-4): 159-166.
11. Herbst, L.H., S.F. Costa, L.M. Weiss, L.K. Johnson, J. Bartell, R. Davis, M. Walsh and M. Levi, 2001. Granulomatous skin lesions in moray eels caused by a novel *Mycobacterium* species related to *Mycobacterium triplex*. *Infect. Immun.*, 69: 4639-4646.
12. Conroy, G. and D.A. Conroy, 1999. Acid-fast bacterial infection and its control in guppies (*Lebistes reticulatus*) reared on an ornamental fish farm in Venezuela. *Vet. Rec.*, 144: 177-178.
13. Prophet, E.B., B. Mills, J.B. Arrington and L.H. Sobin, 1994. *Laboratory Methods in Fish Histopathology*. American Registry of Vet. Pathology, Washington, DC, pp: 279-280.
14. Kenawy, M. and M. Doaa, 2009. *Epizootiological and Histopathological Studies on Mycobacteriosis in Some Ornamental Fishes*. Department of Fish Diseases and Management, Faculty of Vet. Medicine, Cairo University, Egypt.
15. Diamant, A., A. Banet, M. Ucko, A. Colorni and W. Knibb, 2000. Mycobacteriosis in wild rabbitfish *Siganus rivulatus* associated with cage farming in the Gulf of Eilat, Red Sea. *Dis. Aquat. Org.*, 39: 211-219.
16. Puttinaowarat, S., 1995. *Detection of Mycobacterium spp. in culture by enzymelinked immunosorbent assay (ELISA) and polymerase chain reaction (PCR)*. MSc dissertation, University of Stirling, Stirling, UK.
17. Falkinham, J.O., G. Nichols, J. Bartram, A. Dufour and F. Portaels, 2009. *Natural ecology and survival in water of mycobacteria of potential public health significance. Pathogenic mycobacteria in water: a guide to public health consequences, monitoring and management*. London: IWA Publishing, pp: 15-25.
18. Gauthier, D.T. and M.W. Rhodes, 2009. *Mycobacteriosis in fish: a review*. *The Veterinary Journal*, 180: 33-47.
19. Antonio, D.B., C. Swanson, J.J. Cech, R.C. Mager, S. Doroshov and R.P. Hedrick, 2000. Prevalence of *Mycobacterium* in wild and captive delta smelt. *Calif. Fish Game*, 86: 233-243.
20. Seiberras, S., D. Jarnier, S. Guez and C. Series, 2000. *Mycobacterium marinum nodular lymphangitis*. *Presse. Med.*, 29: 2094-2095.
21. Durborow, R.M., 1999. *Health and safety concerns in fisheries and aquaculture*. *Occupat. Med. (Oxford)*, 14: 373-406.
22. Shoemaker, C.A., 2009. *Overview of zoonotic infections from fish and shellfish. Zoonotic infections from fish and shellfish*. In Program, abstracts and report of European Association of Fish Pathologists (EAFP) Workshop. Proc. EAFP International Conference, 14-19 September, Prague Czech Republic, pp: 6.