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MYCOBACTERIOSIS IN SHARPTOOTH CATFISH, CLARIAS GARIEPINUS

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SUMMARY

The aim of this study was to investigate piscine mycobacteriosis in wild sharptooth catfish, *Clarias gariepinus*. Out of 120 fish collected, *Mycobacterium SPP.* Were isolated from fish 5 (4.16%), *M. fortuitum* was isolated from 3 (2.5%), while *M. marinum* was isolated from 2 (1.67%) fish. Conventional and molecular methods were applied to identify suspected mycoacterial isolates. Experimental induction of mycobacteriosis in sharptooth catfish by intraperitoneal inoculation and $1.2X10^8$ and $1.6X10^8$ cfu of *M. fortuitum* (MF4) or *M. marinum* (MM31), respectively, resulted in acute infections with severe peritonitis and adhesions. Less severe to chronic cases resulted from intraperitoneal inoculation of $1.2X10^7$ and $1.6X10^7$ cfu of *M. fortuitum* and *M. marinum*, respectively. Sharptooth catfish with induced chronic *M. fortuitum* infections showed severe enlargement of the spleen and dark coloration of the liver and kidneys, while induced chronic *M. marinum* showed sanguineous granular ascites. Antibiograms of the isolates were also conducted. The fisherman dealing with sharptooth catfish had developed nodules on the dorsum of hand that could be a case of fish handler granuloma.

INTRODUCTION

Piscine mycobacteriosis is a typically chronic disease and affects over 160 species of marine and freshwater fish (Chinabut, 1999). The first report of a mycobacterial infection in fish has been puplished by Bataillon et al. (1897), who isolated acid-fast bacilli from a tuberculous lesion in common carp, *Cyprinus carpio*. The

authors named the carp isolate *Mycobacterium piscium* on the basis of its derivation (Bataillon et al., 1902). Currently, piscine mycobacteriosi is attributed principally to infections with *Mycobacterium marinum*, *Mycobacterium fortuitum* and *Mycobacterium chelonae* (Frerichs, 1993).

Mycobacterium marinum was originally isolated and identified from marine fish at Philadelphia Aquarium (Aronson, 1926). It was initially thought to infect only marine fishes, and was named accordingly; however, it is now known to be an ubiquitous species of both marine and freshwater fishes (Chinabut, 1999). The second fish mycobacterial pathogen was recovered initially from neon tetra, *Paracheirodon innesi* in the early 1950s and identified as *M. fortuitum* (Ross and Brancato, 1959). Mycobacteriosis in fish may occur in either an acute or chronic form. The acute form of the disease rarely occurs and is characterized by rapid morbidity and mortality with few clinical signs (Grady et al., 1992). Mycobacteriosis is however a typically chronic progressive disease that may take years to develop into a clinically noticeable illness (Chinabut, 1999).

Diagnoses of mycobacterial infections in fish is often based on observing acid-fast bacilli in either tissue sections or imprints. Therefore, the causative agents of most infections are not diagnosed beyond the genus level (Whipps et al., 2003). Further identification of the species depends on biochemical characteristics (Frerichs, 1993), which are, however, labor and time-consuming (Sanguinetti et al., 1998) and do not lead to a definitive identification of the type strains. Gómez et al., (1993) and Adams et al., (1996) introduced immunology-based techniques to characterize and detect mycobacteria in fish, but the results remained inconclusive. New approaches to the identification of bacterial species on the basis of comparative DNA sequence analysis hold great promises to improve diagnosis of mycobacterial infection (Tortoli, 1999).

Fish mycobacteriosis is an important zoonosis and poses a significant risk to all human beings working with the affected fish or the aquaria. Tuberculoid infections in people using public swimming pools were first reported in 1939 from Sweden and in 1951 from the US. It was identified in 1954 after 80 persons who had used the same public swimming pool were

diagnosed with granulomatous skin lesions (Durborow, 1999). These early findings led to the disease's once-common name of "swimming pool granuloma". The names "fish tank granuloma" and "fish handler's disease" are nowadays used because of the associations with home aquariums and water-related activities such as swimming, fishing and boating (Ang et al., 2000).

Few reports are available on the occurrence of mycobacteriosis in the wild population of fish and little is known about its prevalence and impact (Heckert et al., 2001). This study aimed to investigate piscine mycobacteriosis in wild sharptooth catfish. Conventional and molecular methods were used to identify suspected mycoacterial isolates. Experimental induction of the disease and antibiogram , of the isolates were also conducted.

MATERIAL AND METHODS

Fish:

A total of 120 alive sharptooth catfish, *Clarias gariepinus*, were collected from the small tributaries of El-Ibrahemia canal, Assiut City over a calendar year (10 fish/month). The body weight of examined fish ranged from 900 to 1000g with total length of 42 to 50cm. Fish were transported to the lab where clinical and bacteriological examination had been conducted.

Clinical and Bacteriological Examination of fish:

Fish were examined for clinical signs or external lesions according to Stoskopf (1993). Fish were incised according to Stoskopf (1993) to be internally examined for macroscopic granuloma or lesions. Mycobacteria isolation was conducted according to Kent and Kubica, (1985). Briefly, tissue samples were pooled from liver, kidneys and spleen for each fish, homogenized in a tissue-grinding mortar with 5ml of sterile saline. Tissue homogenates were centrifuged at 3000xg for 15 minutes. The supernatant fluid was discarded and the sedimens was treated with 2ml of 4% sulphuric acid for 15 minutes, and then washed twice with sterile saline and centrifugation at 3000xg for 15 minutes. The sediments were neutralized with 4% NaOH containing phenol red indicator and immediately inoculated onto Löwenstein-Jensen media, L-J, (Biolife, Milano, Italy). Incubation was at 25 to 37°C and cultures were observed for up to 6 weeks.

Conventional identification:

Smears of suspected colonies were stained with Ziehl Neelsen stain (UCCMA Diagnostics, United Co. for Chemicals and Medical preparations, Industrial Area, El-Salam) to assess acid fastness and morphology of bacteria. Colony morphology, pigment production under dark and light conditions and ability to grow at temperatures ranging from 25 to 37 °C were examined. Conventional biochemical tests were performed as previously described by Kent and Kubica (1985) and included niacin accumulation, nitrate reduction, Tween 80 hydrolysis, urease activity, iron uptake, tolerance to 5% sodium chloride, growth on Thiophen-2-Carboxylic Acid Hydrazide (T2H) and ability to grow on MacConkey agar.

Molecular identification:

DNA extraction, target amplification and sequencing: Crude DNA of MF4 and MM31 isolates were extracted as described by Plikaytis, et al. (1990) and purified using the QIAmp Blood Kit (Qiagen Inc., Valencia, CL, USA) according to the manufacturer instruction. The hypervariable region of the Internal Transcribed Spacer 1 (ITS-1) target was amplified by Polymerase Chain Reaction (PCR), as previously described (Mohamed et al., 2004), using 5 µl template DNA (10 ng/µl) with PCR buffer mix and 1.5 U of REDTaq DNA polymerase (Sigma-Aldrich, Inc.) in a total reaction volume of 50 µl. PCR amplification was performed in Techne thermocycler model TC-312 (Techne, Duxford, Cambridge, UK) starting with an initial denaturation step at 95°C for 10 min., followed by 35 cycles where each cycle consisted of a denaturation at 95°C for 1 min., an annealing at 64°C for 30 sec. and an extension step at 72°C for 1 min. A specific pan-mycobacterium primer set of a forward primer ITS-A1 (5'-GAAGTCGTAACAAGGTAGCCG-3') to amplify from outside the ITS-1 target at the 3' end of the 16S rDNA, and a reverse primer ITS-A6 (5'-ATGCTCGCAACCACTATCC-3') to amplify from within the conserved region of the ITS-1 target, was used. PCR products of expected size of 230 bp. (fig. 1) were detected on 2% Agarose gel according to the instructions of the manufacturer (UVP, Upland, CA, USA). PCR products were purified from gel for sequencing using the OMEGA gel extraction kit (OMEGA BIO-TEK, Doraville, GA, USA). Purified PCR products were sequenced using the above forward and reverse PCR

samplification primers at the Molecular Biology Core Laboratory (Egyptian Institute for Biological Products and Vaccine Production).

Sequence analysis: The ITS-1 sequences of the MF4 and MM31 isolates were compared with the *Mycobacterium* species sequences available at both GenBank database (National Center for Biotechnology Information [NCBI], Washington, D.C.) using the BLAST analysis program (http://www.ncbi.nlm.nih.gov/blast/) and the custom *MycoAlign* database (Mohamed et al; 2004).

Pathogenicity of *Mycobacterium marinum* and *Mycobacterium fortuitum* to *Clarias gariepinus*:

Fish: Apparently healthy sharptooth catfish with an average body weight of 100±5g and total length of 19±1cm were obtained from ponds of cultured sharptooth at ElMinia Governorate and randomly examined for mycobacterial infections as described above. Fish were acclimated to laboratory conditions for 2 weeks according to Ellsaesser and Clem (1986).

Bacterial strains: *M. fortuitum* (MF4) and *M. marinum* (MM31) isolates were passed through sharptooth catfish three times via intraperitoneal inoculation and used for determination of pathogenicity. Isolates MF4 and MM31 were grown on L-J Media and suspended in distilled water to be used for experimental infection.

Bacterial counts and dilutions: Bacterial suspensions were prepared by harvesting the mycobacterial growth and suspension in 2 ml distilled water. Two concentrations of isolates MF4 and MM31 were made in distilled water as ten fold serial dilution of the original bacterial suspension. Using standard plate count method (Elkamel and Thune., 2003), counts of colony forming units (cfu) of the bacterial dilutions were determined on Middlebrook 7H10 agar (Difco, Becton Dickinson, Sparks, MD, USA) with OADC enrichment (BBL, Becton Dickinson, Sparks, MD, USA). Counts of the two MF4 dilutions were $2.4X10^8$ and $2.4X10^7$ cfu/ml, while the two MM31 dilutions were $3.2X10^8$ and $3.2X10^7$ cfu/ml.

Experimental infection: Acclimated sharptooth catfish were divided into groups of 5 fish each. Fish of each group were injected intraperitoneally (I/P) with either 0.5ml of one bacterial dilution or distilled water, while another control group remained un-injected as shown in table (1). Clinical signs and mortalities were recorded daily over 28 days. Moribund fish were examined to record clinical signs and isolate the bacteria from internal

organs. Tissue impressions were made from liver, spleen and kidneys and stained with Ziehl Neelsen stain. By the end of the 28th day all fish alive were euthanized and examined as described above.

Table 1. Experimental infection of sharptooth catfish, *Clarias gariepinus*, with *Mycobacterium fortuitum* (MF4) and *Mycobacterium marinum* (MM31).

Bacterial isolate	Dose	Route	No. of injected fish
MF4	$1.2X10^8$ cfu	IP	5
	$1.2X10^7$ cfu	IP	5
MM31	$1.6X10^8$ cfu	IP	5
	$1.6X10^7$ cfu	IP	5
Control (distilled water)		IP	5
		Un-injected	5

Antibiogram:

Antimicrobial susceptibility test was done using the standard macrodilution method with radiometric broth and evaluated on a BACTEC 460 instrument (Becton Dickinson, Sparks, MD.) (Siddiqi et al. 1993). Tested antimicrobial agents were rifampicin, ethambutol, isoniazid and streptomycin.

RESULTS

Clinical Examination:

Examined fish did not show specific clinical signs of mycobacterial infection. Some fish, however, showed ulcerative lesions on the body surface. Liver was pale in some cases, while spleen and kidneys were apparently healthy in all cases.

Bacteriological isolation and identification:

Bacteriological examination resulted in the recovery of 11 isolates from the 120 fish examined. Out of the 11 isolates, only 5 isolates (45.5%, n=11) were acid-fast, non-motile and non-spore-forming bacilli, and suspected to be *Mycobacterium* species. Mature colonies developed at a range of 1-3 weeks on L-J media as some isolates developed within 7 days, while

others did not grow until the 3rd week. The growth temperature range was 25-30°C. Mature colonies were smooth and creamy in appearance, while other colonies were smooth and with yellow to orange pigmentation sometimes with photoinduction and other times under both dark and light conditions. Results of the biochemical analysis of suspected

isolates revealed the presence of two different groups of *Mycobacterium* species (table 2), however, the phenotypic characteristics were not sufficient to provide a definite identification of isolates. The ITS-1 target-sequence analysis using both the BLAST and *MycoAlign* database search analysis resulted in the precise identification of the isolate MF4 from group 1 as *Mycobacterium fortuitum* and MM31 from group 2 as *Mycobacterium marinum*. Out of the 120 fish collected, *M. fortuitum* and *M. marinum* were isolated from 5 (4.16%) fish, where *M. fortuitum* was isolated from 3 (2.5%) fish, while *M. marinum* was isolated from 2 (1.67%) fish.

Table 2. Phenotypic characteristics of suspected *Mycobacterium* species isolates.

Phenotypic tests	**Group One**	**Group Two**
Growth at:		
25 °C	-	+
30 °C	+	V
Pigment production*	N	P/S
Niacin accumulation	-	-
Nitrate reduction	V	-
Iron uptake	+	-
Tween 80 hydrolysis	-	+
Growth in presence of:		
T2H (1mμg/ml)	+	+
5% NaCl	+	-
MacConkey agar	-	-

Reactions are scored as: -, negative; +, positive; or V, variable.
Abbreviations: S, scotochromogenic; P, photochromogenic; N, non-photochromogenic

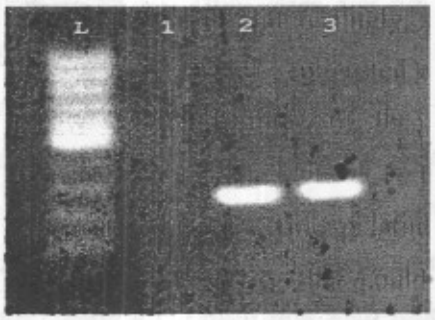

Fig 1. Amplification of ITS-1 target using ITS-A1/ITS-A6 primer set from MF4, and MM31 isolates of suspected *Mycobacterium* species showing expected product size of 230 bp.; (L) 100 pb.-DNA ladder, (1) negative control, (2) isolate MF4, and (3) isolate MM31.

Experimental infection

Fish inoculated with $1.2X10^8$ cfu of *M. fortuitum* isolate MF4 did not show specific external signs of mycobacteria infection, however, they were sluggish in movement and easily caught by hand. Internally, signs of severe inflammation and peritonitis were evident. Massive adhesions of the internal organs to the extent that all viscera appeared as one unit. Liver was congested in 3 fish and, showed pale areas in the other 2 fish. Kidneys and spleen were congested. Acid fast bacilli were seen in tissue impressions of liver, spleen and kidneys. All fish of this group died by the fast bacilli. No macroscopic granulomatous , however, were observed. Out of this group, one fish died within 10 days post inoculation, one died within the third week and three fish remained alive till the 28th day.

end of the first week post inoculation. In contrast, inoculation of fish with $1.2X10^7$ cfu of isolate MF4 resulted in emaciation and poor body condition. Internally, adhesions of the internal organs were evident, but signs of inflammation were less severe. Liver was pale in color in most fish. Interestingly, spleen was, in some cases, severely congested and greatly enlarged occupying most of the abdominal cavity (fig. 2). Also, kidneys were severely congested. There were white gelatinous masses in the peritoneal cavity that could be easily mistaken for fat depositions, but staining with Ziehl Neelsen revealed numerous acid

Fish inoculated with $1.6X10^8$ cfu of *M. marinum* isolate MM31 did not develop specific external signs of infection. Internally, sanguineous, granular, thick ascetic fluid was filling the abdominal cavity in all fish. Also, signs of peritonitis and inflammation were observed. Liver was pale than normal and

friable. Spleen and kidneys were congested and slightly enlarged. Tissue impressions and ascetic fluid showed enormous amount of acid fast bacilli. Four fish died within a week post inoculation, while the last fish died by the 10th day. On the other hand, fish inoculated with $1.6X10^7$ cfu of *M. marinum* isolate MM31

showed less severe signs. Only 3 fish showed sanguineous, granular ascetic fluid in the peritoneal cavity. No granulomatous lesions, however, were observed. All fish of this group were alive by the 28th day.

Antibiogram:

Both MF4 and MM31isolates were sensitive to rifampicin and ethambutol, however they were resistant to isoniazid and streptomycin.

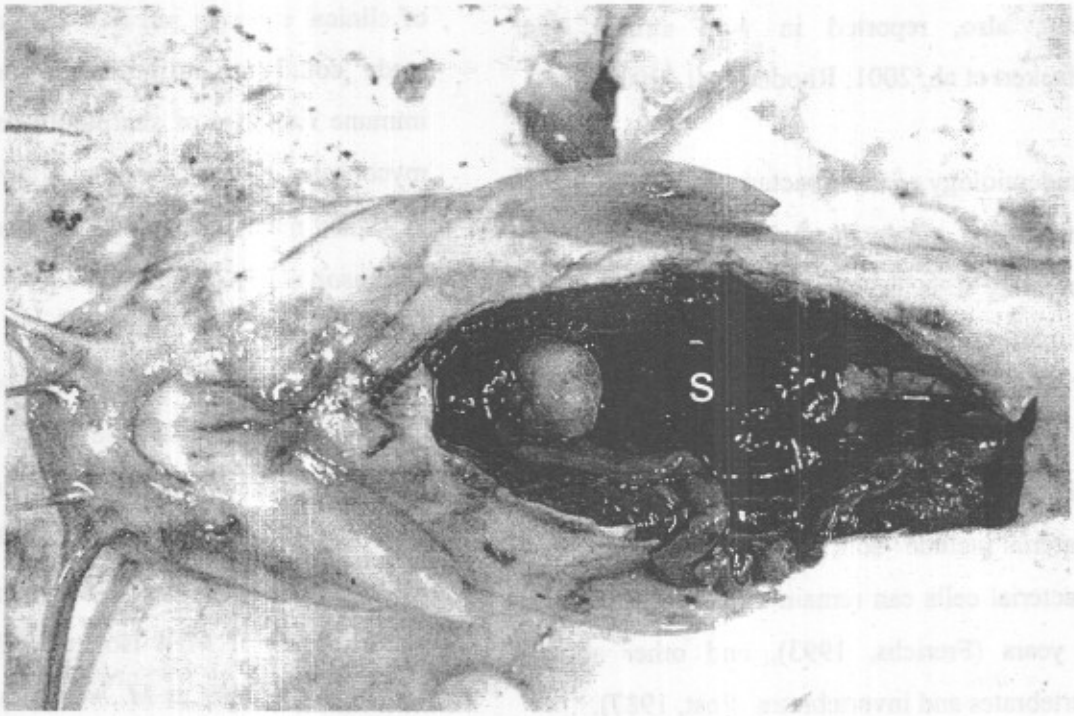

Fig 2. Sharptooth catfish, *Clarias gariepinus*, intraperitoneally inoculated with $1.2X10^7$ colony forming units of *Mycobacterium fortuitum* (MF4) and showing severely congested and enlarged spleen (s) and congested liver and kidneys.

DISCUSSION

The current study is the first to report piscine mycobacteriosis in wild sharptooth catfish in Upper Egypt. Two *Mycobacteria* species were isolated from 5 (4.16%) out of 120 fish examined over a year. Piscine mycobacteriosis outbreaks had been reported in wild fish with prevalence ranging from 8% up to 100% (Abernethy and Lund, 1978; Sakanari et al., 1983; MacKenzie, 1988; and Daoust et al., 1989). High prevalence of the disease has been, also, reported in wild striped bass (Heckert et al., 2001; Rhodes et al., 2004).

Epidemiology of mycobacteriosis in wild fish is not fully understood (Chinabut, 1999); however, it is established that the disease is primarily transmitted through the consumption of contaminated feed or cannibalism (Chinabut et al., 1990; Grady et al., 1992; Post, 1987). In this respect, potential sources of infective material include soil, water, in which the bacterial cells can remain viable for more than 2 years (Frerichs, 1993), and other aquatic vertebrates and invertebrates (Post, 1987).

Although mycobacterial strains were successfully isolated from the internal organs of wild fish examined in this study, no typical signs of infection were evident. Similar findings were reported in zebrafish, *Danio rerio*, where several fish were presented with some typical clinical signs of mycobacteriosis, while others showed only general malaise as lethargy and emaciation (Kent et al., 2004). In addition, delta smelt, *Hypomesus transpacificus*, infected with mycobacteria rarely exhibited signs of the infection; however, their capacity to sustain high activity was impaired (Swanson et al., 2002). The absence of clinical signs in infected fish in the current study could be attributed to the distinctive immune response of sharptooth catfish toward mycobacterial infections or, alternatively, due to subclinical infection as suggested by (Swanson et al., 2002).

Two different species of *Mycobacterium* were isolated in the current study, but could not be identified based only on the conventional methods. Sequence analysis of the amplified target, ITS-1 sequence, has confirmed the identification of MF4 isolate as *M. fortuitum* and MM31 isolate as *M. marinum*. The study clearly showed that definite identification of

suspected isolates has been only achieved after implementing molecular identification. The increasing number of newly defined mycobacteril species and the "difficult-to-identify" variants of known species represents a significant challenge for conventional approaches (Floyed et al., 2000) and explains the inability of the conventional phenotypic tests, used in the current study, to reach definite identification of the suspected isolates.

Molecular identification of *Mycobacterium* species has two primary advantages when compared to phenotypic identification: rapid turn-around time and improved accuracy. Sequence dependant identification has been shown to be an especially effective molecular tool that provides rapid and accurate differentiation of *Mycobacterial* species (Mohamed et al, 2004). Most molecular approaches have focused on the conserved 16S small subunit rDNA sequence which may be inadequate to reach a definitive identification among closely related *Mycobacterium spp.* or strains. Alternatively, sequence analysis of the ITS-1 rDNA has been successfully used to elucidate this problem (Mohamed et al., 2004).

Results of the current study showed that prevalence of infection was 2.5% and 1.67% for *M. fortuitum* and *M. marinum*, respectively. Such findings are in accordance with (Puttinaowarat et al., 2000) who reported that *M. fortuitum* was the most common species identified in snakehead, *Channa striatus*, and siamese fighting fish, *Betta splendens*, farms and water samples, while *M. marinum* was less frequently found. On the contrary, Frerichs (1993) stated that the isolation of *M. fortuitum* has been less frequently documented than *M. marinum* and known to occur in both tropical and temperate waters.

Experimental infection of sharptooth catfish with intraperitoneal inoculation of $1.2X10^8$ and $1.6X10^8$ cfu of *M. fortuitum* and *M. marinum*, respectively, resulted in acute infections with severe peritonitis and adhesions. It was stated that experimentally induced acute mycobacteriosis may result in severe peritonitis and necrosis and increased susceptibility to parasitic infection (Talaat et al., 1998). In another study, experimentally infected fish showed whitish membranes around the mesenteries, and various organs were fused (Ashburn, 1977). Talaat et al., (1998)

concluded that acute mycobacterosis is induced by the injection of 10^8 to 10^9 cfu per fish, while chronic case is induced by the injection of 10^2 to 10^7 cfu. Such findings are met by the results of the present study where less severe to chronic cases resulted from inoculation of $1.2X10^7$ or $1.6X10^7$ cfu of *M. fortuitum* or *M. marinum*, respectively. Sharptooth catfish with induced chronic mycobacterial infections showed severe enlargement of the spleen and dark coloration of the liver and kidneys as previously described in hatchery-confined chinook salmon, *Oncorhynchus tshawytscua*, (Ashburn,1977) and in snakehead (Chinabut et al., 1990). Furthermore, Gómez (1998) found white gelatinous masses in the abdomen in mountain minnow, *Tanichthys albonubes*, with chronic mycobacterial infection as demonstrated by sharptooth catfish.

Clearly, chronic systemic disease with granuloma formation is not the only possible presentation of fish mycobacteriosis, as currently demonstrated in the induced chronic mycobacterial infection of sharptooth catfish. Absence of granulomas in internal organs of fish does not exclude active mycobacterial infections as severe systemic mycobacteriosis without typical granuloma formation was diagnosed in frogfish *Antennarius striatus* (Yanong et al., 2003). Gómes et al., (1993), also, reported that nodules were not always evident in fish infected with mycobacteria and hypothesized that morphological variability of the lesions probably represents different stages of the disease.

Mycobacterial strains used to induce infection did not stimulate the sharptooth catfish immune systems in a typical manner to produce granuloma typical of mycoacterial infection. Development of granulomas depends upon both the specific *Mycobacterium spp.* involved and degree of immune reactivity and the species of the fish host (Chinabut, 1999). Sharptooth catfish may differ from other fish in their immune response to mycobacterial infections, or mycobacterial strains used in this study may differ in antigenicity or pathogenicity from other strains. This suggestion is supported by the data obtained by ELISA and reverse cross blot PCR that provided evidence that many of the *M marinum* isolates from different locations such as Greece, Israel, Thailand and Germany are different in their antigenic make-up (Puttinaowarat et al., 2000). Furthermore, granuloma formation requires variable lengths of time and mycobacteria can be present without the presence of granulomas in internal

organs because it is too early in the infection. Another explanation of absence of granuloma is that inoculation of fish with high doses of mycobateria may have resulted in immuno-suppression as suggested by (Talaat et al., 1998). Yanong et al., (2003) hypothesized that immunosupperssoin may hinder the formation of granuloma and alter the typical chronic immune response.

Antibiotic agents that have been shown to be active against *M. marinum* in vitro include ethambutol, rifampicin, streptomycin, trimethoprim-sulfamethoxazole, tetracyclines, clarithromycin, azithromyin and some of the quinolones (Kullavanijaya et al., 1993; Edelstein, 1994; Alloway et al., 1995; Ekerot et al., 1998; Bhatty et al., 2000; Aubry et al., 2002). No single agent, or combination of agents, has clearly been shown to be the treatment of choice.

Interestingly, the fisherman who supplied the sharptooth catfish to our lab has nodules on the dorsum of both of his hands (fig.3). He acknowledged that those nodules developed when he got stung with the spine of fish pectoral fin few months ago. There is a high possibility that those nodules are cutaneous lesions of infection with fish mycobacterial agent. The case was not, however, confirmed, and further investigation should be done. *M. marinum* has frequently been isolated from skin lesions of human (Philpott et al., 1963; Lawler 1994 and Ucko and Colorni, 2005). In human infections, *M. marinum* gains access through skin abrasions (Bhatty et al., 2000; Jernigan and Farr, 2000) and usually confined to the extremities and cooler parts of the body such as hands, forearms, elbows, and knees because of its inability to grow at 37°C (Chinabut, 1999). *M. marinum* cutaneous disease in human can appear as papulo-nodular, nodulo-ulcerative, granulomatous, plaques and lesions or deep tissue infections of the tendon and bone (Kullavanijaya et al., 1993; Holmes et al., 1999; Zenone et al., 1999).

Although less common than *M. marinum*, *M. fortuitum* is also capable of infecting humans (Westmoreland et al., 1990 and Collina et al., 2002). *M. fortuitum* was first isolated from a cold abscess in man in 1938 (da Costa Cruz 1938). Puttinaowarat et al., (2000), however, concluded that it is more common than *M. marinum* in fishermen working in snakehead and siamese fighting fish farms. In addition, Escalonilla et al., (1998) have isolated out of 13 patients with mycobacterial cutaneous lesions nine *M. fortuitum* isolates and only one *M. marinum* isolate.

In this respect, sharptooth catfish may pose a threat to public health as apparently healthy fish could be subclinically infected with those potential zoonotic agents. Further studies should investigate the relationship between the corded fish handler lesions and mycobacterial agents isolated in the present study. Additionally, prevalence of mycobaterial infections among wild population of other fish species and impact of the disease on the ecology of natural resources of water should be studied.

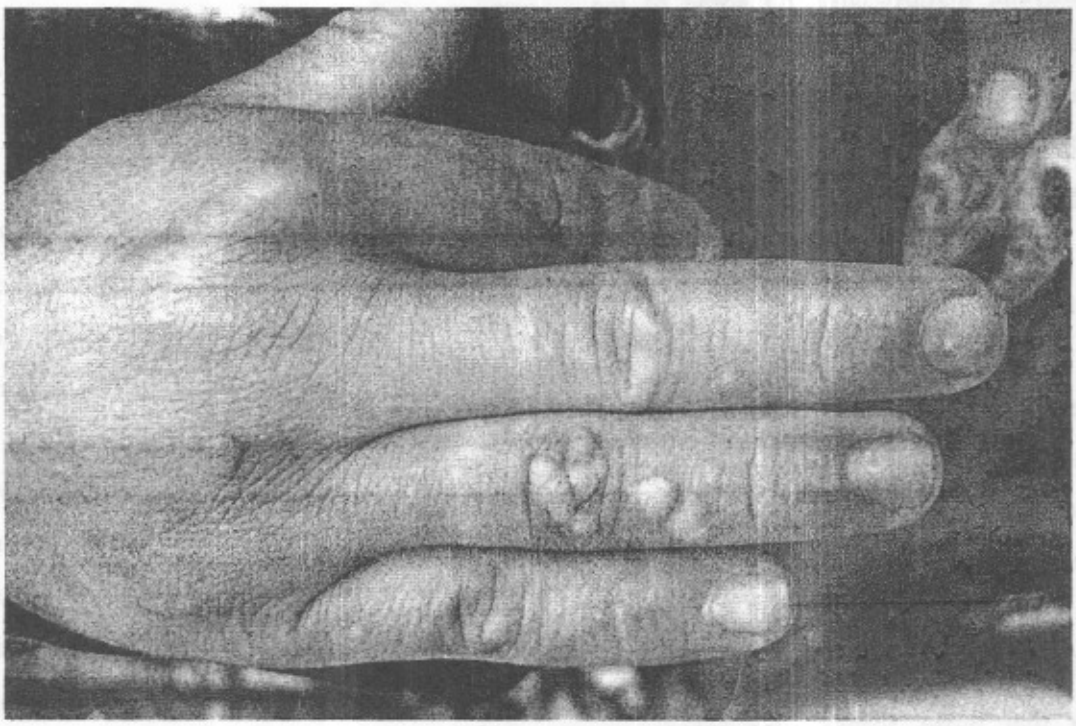

Fig 3. A suspected case of fish handler's disease showing nodules on the dorsum of the right hand of a fisherman who supplied our lab with sharptooth catfish, *Clarias gariepinus*

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