Epizootiological and Histopathological Studies on Mycobacteriosis in Some Ornamental Fishes

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Abstract: A total number of 480 ornamental fishes of different species namely Goldfish (Carassius auratus), Swordtail (Xiphophorus helleri), Black molly (Poecilia latipinna) and Gambusia (Gambusia gaigei) was randomly collected from private ornamental fish breeders located at Giza governorate. During a period of 12 months. The clinical examination indicated the presence of excessive muocus secretion, ulcerations, detachment of the scales, exophthalmia and body deformity with external hemorrhages. The prevalence of fish mycobacteriosis in different seasons of the year and species susceptibility were detected. Bacteriological examination revealed isolation of 30 fish mycobacterium isolates. Twenty isolates were identified as M. Fortuitum, 8 as M. Marinum and 2 as unidentified mycobacteria for fish. The highest isolation rate was recorded in aged than younger. The experimental infection of Goldfish (Carassius auratus) with 2 isolates of M. fortuitum and 2 isolates of M. marinum induced clinical sings, gross pathological lesions and histopathological investigations of the naturally and experimentally infected fishes.

Key words: Mycobacteria • Ornamental fishes • Bacteriology • Histopathology • Epizootiology

INTRODUCTION

Breeding and culturing of ornamental fishes is a fast growing trade allover the world. Cultures of ornamental fish are usually exposed to several potential stressors such as un proper management and environmental condition, which compromises the fish's natural defenses so they cannot effectively protect themselves from invading pathogens [1]. Many disease causing organisms normally occur in the same environment as the fish, they usually only become a problem when present in significant quantities and/or after occurrence of stress. Fish mycobacteria are the most common chronic bacterial diseases affecting ornamental fish, affecting both temperate and tropical species both in freshwater and in the marine environment [2]. Besides causing mortality in fishes, they represent a potential hazard for man, being included among zoonoses [3]. Mycobacteriosis is caused by ubiquitous bacteria that are highly resistant in the aquatic environment and are difficult to control. Mycobacterium fortuitum, M. marinum and M. chelonae are the most common isolated species. They are all capable of inducing human cutaneous infections, with development of nodular lesions that are generally localized in the limbs. In fish, signs appear later on and are non-specific including slow growth, lethargy and anorexia. Typical whitish nodules in the viscera may be detected at necropsy [4]. The aim of this study was to investigate the prevalence of mycobacteriosis among some species of ornamental fishes during different seasons of the year. Also, studying the pathogenicity and histopathological alterations induced by different species of isolated fish mycobacteria in goldfish (Carassius auratus) as an experimental model was another target.

MATERIAL AND METHODS

A total number of 480 clinically diseased ornamental fishes of different species namely Goldfish (Carassius
auratus), Swordtail (Xiphophorus helleri), Black molly (Poecilia latipinna) and Gambusia (Gambusia gaigei) was randomly collected from some private ornamental fish breeders located at Giza governorate. During a period of 12 months where ten fish from each fish species were collected monthly to record the incidence of fish mycobacteriosis allover seasons of the year. A total number of 60 goldfish (Carassius auratus) of different ages (2, 4 and 6 months) were collected from a private goldfish hatchery with history of mycobacteriosis to investigate the role of age on the incidence of mycobacteriosis. All fishes were subjected to full clinical, post-mortem, bacteriological and histopathological examinations. Also, a total number of other 50 apparently healthy goldfish (Carassius auratus) was obtained from a private fish breeder in Giza Governorate. They were 20-30g in weight and 10 -15 cm. in total length. Fish were brought alive to the wet laboratory. They were kept in full glass aquaria, supplied with chlorine - free tap water, continuous aeration, filtration and the water temperature was adjusted at 28±2°C during the experimental work. A full clinical examination including the abnormal behaviours and abnormal clinical signs were carried out according to Austin and Austin [5]. Samples were taken under aseptic precautions from skin, liver, spleen, kidney, heart, gills and any visible external or internal lesions for isolation of mycobacterium species according to the method described by Cruickshank et al.[6].

Identification of the Isolated Bacteria: Identification of the isolated bacteria was carried out depending on morphological characters [7], rate of growth [8], pigmentation[9] growth at different temperature degrees (25, 30 and 37°C) up to 7 days.

Biochemical Identification: For full identification of the isolated fish mycobacterium species the following different biochemical reactions were carried out: Niacine test, Hydrolysis of tween 80, Aryle sulfatase test, Growth on MacConkey agar [10], Nitrate reduction test [11], 5% sodium chloride tolerance [12], Sensitivity to Thiophene-2 carboxylic acid hydrazide[13], Iron uptake[11] and Urease test [14].

Experimental Inoculation: To study the pathogenicity of the isolated mycobacterium species, a total number of 50 goldfish were divided into 5 groups of 10 fish each. The fish in the group 1 to 4 was inoculated I/P with 0.5 ml of mycobacterium suspension isolates No. 1,2,3 and 4 respectively of density equal to 10⁸ CFU according to Talaat et al. [15], the fish in the group 5 was inoculated I/P with 0.5 ml of sterile physiological saline and kept as the control group. The inoculated fish were daily observed for any clinical abnormalities and mortalities were recorded for two months post-inoculation. Reisolation of inoculated mycobacterium isolates were carried out during and at the end of 2 months observation period.

Histopathological Examinations: Tissue samples were collected from different organs of naturally infected and experimental fish after inoculation (skin, gills, heart, liver, spleen, kidneys and eyes),and prepared according to Bancroft and Stevens [16].

RESULTS

In this study, the clinical examination of randomly collected ornamental fishes Goldfish (Carassius auratus), Swordtail (Xiphophorus helleri), Black molly (Poecilia latipinna) and Gambusia (Gambusia gaigei) indicated the presence of various clinical signs including excessive mucous secretion and ulcerations (Fig,1). Also detachment of the scales (Fig,2), exophthalmia (Fig,3) and body deformity (Fig,4) with external hemorrhages (Fig,5).

The prevalence of fish mycobacteriosis in different species of ornamental fishes randomly collected during different season were recorded in Table 1.

<table>
<thead>
<tr>
<th>Seasons</th>
<th>The prevalence of fish mycobacteriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>1.4%</td>
</tr>
<tr>
<td>Spring</td>
<td>12.5%</td>
</tr>
<tr>
<td>Summer</td>
<td>23.3%</td>
</tr>
<tr>
<td>Autumn</td>
<td>3.1%</td>
</tr>
</tbody>
</table>

The epizootiological studies of mycobacteriosis in the randomly collected ornamental fishes were detected in Table 2.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>% of Species susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldfish</td>
<td>83.3%</td>
</tr>
<tr>
<td>Gambusia</td>
<td>3.3%</td>
</tr>
<tr>
<td>Swordtail</td>
<td>6.7%</td>
</tr>
<tr>
<td>Black molly</td>
<td>6.7%</td>
</tr>
</tbody>
</table>
Table 3: Result of identification of the isolated mycobacteria from different species of ornamental fishes

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Colonial morphology</th>
<th>Growth at diff.temp</th>
<th>Pigmentation</th>
<th>Dark</th>
<th>Fig</th>
<th>Growth rate</th>
<th>Niacin uptake</th>
<th>Iron reduction</th>
<th>Tween 80 Hydrolysis</th>
<th>Arylsulfatase</th>
<th>Urease</th>
<th>MacConkey TCH 5% NaCl</th>
<th>Identified species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25ºC</td>
<td>30ºC</td>
<td>37ºC</td>
<td>Dark</td>
<td>Fig</td>
<td>Growth rate</td>
<td>Niacin uptake</td>
<td>Iron reduction</td>
<td>Tween 80 Hydrolysis</td>
<td>Arylsulfatase</td>
<td>Urease</td>
<td>MacConkey TCH 5% NaCl</td>
<td>Identified species</td>
</tr>
</tbody>
</table>
| 1        | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | -  | +  | +  | +  | M.fortuitum  
| 2        | +    | +    | -    | S    | Buff | Yellow | slow after 10 days | -  | -  | +  | +  | +  | +  | -  | +  | +  | M.marinum  
| 3        | +    | +    | +    | R    | Buff | Buff | Rapid within 4 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 4        | +    | +    | -    | S    | Buff | Orange | slow after 8 days | +  | -  | -  | +  | -  | +  | -  | +  | +  | M.marinum  
| 5        | +    | +    | -    | S    | Buff | Yellow | slow after 12 days | +  | +  | -  | +  | -  | +  | -  | +  | +  | M.marinum  
| 6        | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 7        | +    | +    | +    | R    | Buff | Buff | Slow after 15 days | -  | -  | +  | +  | -  | +  | +  | +  | +  | slow grower unidentified atypical mycobacteria  
| 8        | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 9        | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 10       | +    | +    | +    | R    | Buff | Buff | Rapid within 4 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 11       | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 12       | +    | +    | -    | S    | Buff | Orange | slow after 8 days | +  | -  | -  | +  | -  | +  | -  | +  | +  | M.marinum  
| 13       | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 14       | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 15       | +    | +    | +    | R    | Buff | Buff | Slow after 15 days | -  | -  | +  | +  | -  | +  | +  | +  | +  | slow grower unidentified atypical mycobacteria  
| 16       | +    | +    | -    | S    | Buff | Yellow | slow after 10 days | -  | -  | +  | +  | -  | +  | -  | +  | +  | M.marinum  
| 17       | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 18       | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 19       | +    | +    | +    | R    | Buff | Buff | Rapid within 4 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 20       | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 21       | +    | +    | -    | S    | Buff | Orange | slow after 8 days | +  | -  | -  | +  | -  | +  | -  | +  | +  | M.marinum  
| 22       | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 23       | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 24       | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 25       | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 26       | +    | +    | -    | S    | Buff | Yellow | slow after 10 days | -  | -  | +  | +  | -  | +  | -  | +  | +  | M.marinum  
| 27       | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 28       | +    | +    | -    | S    | Buff | Orange | slow after 8 days | +  | -  | -  | +  | -  | +  | -  | +  | +  | M.marinum  
| 29       | +    | +    | +    | R    | Buff | Buff | Rapid within 5 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  
| 30       | +    | +    | +    | R    | Buff | Buff | Rapid within 3 days | -  | +  | +  | -  | +  | +  | -  | +  | +  | M.fortuitum  

Fig. 1: Naturally infected Goldfish (*Carassius auratus*) showing haemorrhagic skin ulcer on the back region.
Fig. 2: Naturally infected Goldfish (*Carassius auratus*) showing detachment of scales and frayed fins.
Fig. 3: Naturally infected Goldfish (*Carassius auratus*) showing spinal deformity.
Fig. 4: Naturally infected Goldfish (*Carassius auratus*) showing clear unilateral exophthalmia.
Fig. 5: Gambusia (*Gambusia gaigei*) showing external hemorrhages.
identified as *M. fortuitum* (Fig.7), 8 were identified as *M. marinum* (Fig. 8) and 2 isolates were slow grower unidentified mycobacteria. The biochemical identification of the isolated mycobacterium species were detected in (Table 3).

The relationship between fish age and the isolation rate of the mycobacteria from fish indicated that the highest recovery rate of fish mycobacteria was recorded in aged fish than the younger ones. The recovery rate was 25 % in 6 months old Goldfish followed by 5 % in 4 months old Goldfish and not recorded at all in the 2 months old Goldfish.

Studying the epizootiology of mycobacteriosis in the randomly collected ornamental fishes revealed that there were species susceptibility for fish mycobacteriosis. Goldfish was the most susceptible species to be infected with mycobacteria where the infection rate reached (83.3%), while the lowest susceptibility rate was recorded in Gambusia (3.3%). It was noticed that Swordtail and Black molly had the same susceptibility rate (6.7%).

Results of the experimental inoculation of Goldfish (*Carassius auratus*) with *M. Fortuitum* and *M. marinum* revealed different clinical signs namely ascitis (Fig.9), erected scales, exophthalmia (Fig.10), emaciation, ...
Exhaustion, loss of balance and abnormal swimming behavior

Results of the experimental infection revealed that the mortality rate was very low during the first month and increased towards the end of the experiment (2 Months), where it reached 90, 70, 60 and 90% in groups 1, 2, 3, and 4 respectively.

The gross pathological findings in the experimentally infected Goldfish (*Carassius auratus*) revealed the presence of severe congestion and adhesion of the abdominal viscera (Fig.11).

The histopathological investigation of the naturally infected ornamental fishes revealed the presence of tubercles in the renal tissue consists of epithelioid cells, lymphocytes, and melanomacrophages surrounded by connective tissue capsule (Fig.12) and necrosis and depletion of the haemopoietic tissues of the spleen (Fig.13).

Various sized and types of tubercles were recorded in this study in the kidney (Fig.14&15) and spleen (Fig.16&17) of the experimentally infected Goldfish. Some tubercles were consisted of central area of caseous necrosis surrounded by histocytes, epithelioid cells and a thin fibrous tissue capsule (Soft tubercles), where the other tubercles were consisted of aggregation of epithelioid cells, lymphocytes, melanomacrophages and enclosed in delicate fibrous tissue sheath (Hard tubercles). No calcium deposition was observed in the experimentally infected Goldfish.

**DISCUSSION**

In this study, The clinical examination of randomly collected ornamental fishes indicated that the presence of various clinical signs including excessive mucous secretion, ulcerations detachment of the scales, exophthalmia, body deformity and external hemorrhages. These observations supported those reported by Amlacher [17]. However, these signs could be attributed to other infectious and/or non-infectious causes which are usually present in the aquatic environment, whereas the isolation of the different mycobacterial isolates were negative in some examined naturally infected fishes.

The prevalence of fish mycobacteriosis in different species of ornamental fishes randomly collected during different season revealed that the highest infection rate of mycobacteriosis during summer and the lowest rate was during winter. These findings supported those reported by Ruth and Roy [18] who concluded that the infection rates could vary from 10% to 100% according to the season. Also, They added that much higher prevalence of mycobacteria were recorded in warm water.

Regarding the species susceptibility for fish mycobacteriosis. which indicated that Goldfish was the
most susceptible species to be infected with mycobacteria while the lowest susceptibility rate was recorded in Gambusia. These results are in agreement with Dulin [19] who concluded that any species of fish may be infected with fish mycobacteriosis. Also He added that the disease is most frequently recognized in aquarium fishes, probably because such fishes are maintained under a degree of captivity stress for long periods of time, so allowing the slowly progressive infection to develop.

Bacteriological examination of randomly examined ornamental fishes revealed the isolation of a total number of 30 fish mycobacterium isolates were identified as \textit{M. fortuitum} as described by Ross and Brancato [20] and 8 isolates were \textit{M. marinum} as described by Aronson [21]. However, the 2 other etiological agents of mycobacteriosis were considered to be \textit{M. fortuitum} and \textit{M. marinum} in the present study.

Regarding the relationship between fish age and the isolation rate of the mycobacteria, the results indicated that the highest recovery rate of fish mycobacteria was recorded in aged fish than the younger ones. These results agreed with El-Bouhy [22], who mentioned that the rate of the isolation from mycobacteriosis infected cattfish was higher in adult fish. On other hand, [23] observed mycobacterial lesions in fish as young as three months old and it was attributed to to the transovarian transmission from infected brood stocks.

Results of the experimental inoculation of Goldfish (\textit{Carassius auratus}) with \textit{M. fortuitum} and \textit{M. marinum} revealed different clinical signs mainly ascitis, erected scales, exophthalmia, emaciation, exhaustion, loss of balance and abnormal swimming behavior. These clinical signs described above are more or less similar to those observed by Amlacher [17]. The presence of the ascetic fluid in the abdominal cavity in fish infected with mycobacteriosis were seen also by Prearo et al. [2]. The accumulation of the fluid in the abdominal cavity are thought to be related to the severity of affection of the parenchymatous organs mainly spleen, kidney and liver which revealed sever congestion and degeneration with the subsequent transudation of fluids in the peritoneal cavity. The emaciation and exhaustion were reported by Mahmoud [24] two months post-experimental infection of \textit{M. marinum} in Nile tilapia. Also [25], reported severe emaciation in tilapia species inoculated with a standard strain of \textit{M. fortuitum} at the end of the experiment (3 months post - infection).

The mortality rate was very low during the first month and increased towards the end of the experiment. These results supported those of Plumb [26], who mentioned that fish mycobacteriosis is a chronic problem in the ornamental fishes in home and large public aquaria. These chronicity of the disease results in low morbidity and moderate to high cumulative mortality among the moribund fish.

The gross pathological findings in the experimentally infected Goldfish (\textit{Carassius auratus}) revealed the presence of severe congestion and adhesion of the abdominal viscera and were previously recorded [22, 25].

The current histopathological results supported those of Nigrelli and Vogel [27] who stated that naturally mycobacterial infection in fish resulted in wide spread tubercle formation specially in kidney and spleen. Various sized and types of tubercles were recorded in this study in the kidney and spleen of the experimentally infected Goldfish. Some tubercles were consisted of central area of caseous necrosis surrounded by histocytes, epitheliod cells and a thin fibrous tissue capsule (Soft tubercles), where the other tubercles were consisted of aggregation of epitheliod cells, lymphocytes, melanomacrophages and enclosed in delicate fibrous tissue sheath (Hard tubercles). These results parallel to the histopathological findings recorded by Gauthier \textit{et al.} [28]. No calcium deposition was observed in the experimentally infected Goldfish used in this work however it was recorded by Plumb [26] and Majeed \textit{et al.} [29]. This may be attributed either to the short period post-experimental infection (60 days), the different fish species inoculated and the holding water temperature.

In conclusion, the isolation of mycobacterium organisms from the apparently healthy ornamental fishes proved the public health hazards of the human handling such fishes, therefore the development of rapid and reliable diagnostic techniques of mycobacteria namely PCR in the ornamental fishes in healthy and clinically infected fishes are recommended.

REFERENCES


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