

OCCURRENCE OF MYCOBACTERIA IN VARIOUS ORGANS OF CLINICALLY AFFECTED AND APPARENTLY HEALTHY GUPPIES (*POECILIA RETICULATA*)

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Introduction

Ornamental fish aquaculture is a rapidly growing profitable industry in most part of the world including Sri Lanka. Economic losses due to diseases, in terms of morbidity mortality and the cost associated with prevention and control is one of the major constraints faced by the farmers. Health certification of imported and exported stock is a mandatory requirement by many countries and one of the diseases considered as important in this trans-boundary transmission is fish mycobacteriosis. Fish mycobacteriosis is a sub-acute to chronic disease characterized by emaciation, chronic ulcers and death over a period of months to year (Nigrelli and Vogel, 1963). It has been reported in many countries and Sri Lanka imports ornamental fish brood stocks from such countries. Despite this risk, no systematic study has been carried out in Sri Lanka to investigate this problem. The aim of this study was to determine the occurrence of mycobacteria in different organs/systems of clinically affected and apparently healthy fancy guppies: *Poecillia reticulata*.

Materials and Methods

Guppies showing lesions similar to mycobacteriosis were collected from

three different farms located in Kandy, Kurunegala and Kaluthara districts and apparently healthy guppies (n=23) were also collected from five farms situated in Kandy, Kurunegala, Polonnaruwa and Kaluthara districts. After external examination, all fish were humanely euthanized and subjected to post-mortem examinations. Internal lesions (if any) were recorded and liver, spleen, intestine and muscle tissues were sampled aseptically for microbiological examinations.

Squash preparations of the samples were stained with Ziehl-Neelsen (ZN) stain and at least 50 fields of the smear were examined to detect the presence of acid-fast organisms. Samples for culture were decontaminated by incubating for 30 minutes with an equal volume of 1M NaOH (w/v) and washing with distilled water. Decontaminated samples were cultured on Ogawa egg medium in duplicates and incubated aerobically at 25° C and 37° C for two months. All samples were examined daily for seven days and then twice a week for the rest of the period. Colonies were subjected to ZN stain and the acid alcohol fast colonies were subcultured on Ogawa egg medium.

Data were analysed statistically by chi square test using Minitab version 12.

Results and Discussion

The clinically affected guppies showed skeletal abnormalities, emaciation and protrusion of scales and two of the nine clinically affected guppies also had granuloma in the internal organs. Acid fast bacilli were detected microscopically in squash preparations of 33 organ samples. Mycobacteria were isolated from 43 (43 %) of a total of 99 samples (liver, intestine, musculature and gonads) collected from 32 guppies and most often the isolates were from either intestines or liver (Table 1). The organisms were less frequently isolated from musculature or gonads. The prevalence of mycobacteriosis in sick fish was high (8/9 = 88.9 %) when compared with healthy fish (16/23= 69.6 %) but this difference is not statistically significant (P > 0.05). This discrepancy may be related to the imbalance in the sample size (23 vs 9).

Both slow-growing (colonies appear within seven days) and fast-growing

egg medium. The rapidly growing mycobacteria took four to five days to grow while slow growers took 15-20 days. The prevalence of slow-growing mycobacteria was significantly high (P < 0.001 chi square test) in clinically affected fish (8/9=88.9 %) compared with healthy fish (4/23=17.4 %) and the prevalence of fast growing mycobacteria was significantly high (P < 0.05) in healthy fish (14/23= 60.9 %) when compared with clinically affected fish (2/9=22.2 %). The occurrence of mycobacteria (both slow growing and fast growing) in the liver of clinically affected fish was significantly higher (7/9= 77.8 %) than that of healthy fish (6/23=26.1 %) (P < 0.01). Although the occurrence of mycobacteria (both slow-growing and fast-growing) was high in the intestines of healthy fish (15/23=65.2) compared with clinically affected fish (5/9=55.6%), the difference was found to be not significant.

Isolation of mycobacteria from fish tissues needs proper decontamination. When the samples were decontaminated with NaOH-HCl, the

Table 1. Occurrence of slow and fast growing mycobacteria in different organs/systems of clinically affected and apparently healthy guppies

Condition of fish	Intestine		Liver		Musculature		Gonads	
	SG*	FG**	SG	FG	SG	FG	SG	FG
Clinically affected	5	2	7	0	3	0	3	0
Apparently healthy	1	14	3	3	0	0	0	2

*SG-Slow growing mycobacterium species

**FG- Fast growing mycobacterium species

mycobacterium species were isolated from the samples cultured on Ogawa

isolation of mycobacteria was found to be adversely affected (results not

shown). Washing the samples with distilled water after incubating with 1M NaOH for 30 minutes reduced the contamination without affecting the isolation of mycobacteria.

Fast growing mycobacteria were isolated more frequently from intestines of apparently healthy fish and it is important to examine the histological sections to determine whether the tissues were merely colonised with mycobacteria present in aquatic environment or in the process of producing early or latent phase of infection.

It is noteworthy to record the high occurrence of mycobacteria, particularly the slow growers in liver tissues of clinically affected fish compared with healthy fish. However, the sample size used in this study was not enough for meaningful statistical analysis and as this is an on going

study more samples will be examined in the future.

Conclusion

Both slow-growing and fast-growing mycobacteria were present in internal organs of fancy guppies and slow-growing mycobacteria were more often isolated from the guppies with clinical infection.

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References

- Nigrelli, R.F. and Vogel, H., (1963). Spontaneous tuberculosis in fishes and other cold blooded vertebrates with special reference to *Mycobacterium fortuitum* cruz from fish and human lesions. *Zologica*, 48:1-144.