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Ornamental Fish: Diseases and Problems

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ABSTRACT—Seventy-seven bags of ornamental fish imported from Hong Kong, Taiwan, Singapore, and Bangkok were examined for parasites, bacteria, and viruses. Parasites exotic to native North American fishes were not found. Eighteen genera of bacteria were identified. One virus, tentatively classified as a herpesvirus was isolated from a pooled sample of macerated Kuhli loach, Acanthophthalmus *sp., tissue.*

Approximately 600 million tropical fish are imported into the continental United States each year. The objective of this study was to evaluate the potential ecological impact which these fish and/or the water in which they are shipped could have on the health of humans, domestic animals, or native fish species. In this study, 16 shipments of tropical fish originating in Hong Kong, Taiwan, Singapore, and Bangkok were examined for presence of parasites, bacteria, and viruses; 77 bags of fish were examined.

METHODS

Parasitological Examinations

Five fish from each bag of fish were examined following standard methods (Reichenbach-Klinke, 1973) of dissection and examination. Wet mounts of gills, skin and fin scrapings, and internal organs were examined using 5 and 10 magnification objectives.

Bacteriological Examinations

Upon arrival, the fish and their shipping water were systematically processed for the presence of bacterial flora. The bags were opened and a sample of fish removed for parasitic studies. From the remaining fish in each bag, five randomly selected individuals were cultured for detection of possible bacteremia. This was accomplished by killing and surface sterilization of the fish with aseptic dissection to reveal the appropriate organ (kidney) for culture. In small fish a mixture of blood and/or kidney was cultured.

An additional sample of fish was killed, surface sterilized, and homogenized in a blender with sterile phosphate buffered saline at pH 7.2 to achieve a 10 percent weight/volume suspension of fish.

Concurrent with fish processing, aliquots of water from each bag were pooled to achieve approximately a 1,500-ml sample of water representative of the respective shipment. This sample was centrifuged at 5,000 RPM for 20 minutes in an RC-2B[1] centrifuge at 4°C.

The above samples were cultured as follows:

1) Blood and/or kidney were streaked on blood agar and Ordall's agar with subsequent incubation at 35°C and 23°C. Resulting growth was subcultured and identified.

2) A battery of several media was inoculated with fish suspension to assure recovery of a wide spectrum of bacteria. Selective media were used where possible to enhance possible isolations. The media used and justification are: A) Blood agar was used as a general medium for detection of fastidious organisms as well as common organisms; B) Ordall's agar was selected for detection of the presence of myxobacteria; C) Trypticase soy agar was used as a generalized media which would grow practically all organisms: D) Rimler-Shotts agar (Shotts and Rimler, 1973) was used to specifically select members of the *Aeromonas hydrophila* complex; E) Bismuth sulfide agar was used specifically to detect the possible presence of *Salmonella typhosa;* F) Selenite-brilliant green broth and dulcitol selenite broth—brilliant green agar. This battery of media was used for the detection of possible *Salmonella*.

Unless otherwise necessary because of special requirements of the media, all media were incubated at 35°C and 23°C. Resulting growth was subcultured and identified by standard methods (Breed et al., 1957).

3) Water. The sedimented material

[1]Reference to trade names or commerical firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

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resulting from centrifugation was resuspended in approximately 100 ml of the centrifuged supernatant. This constituted the inoculum for the following battery of media: A) Selenite brilliant green broth and dulcitol selenite broth—brilliant green agar. This battery of media was used for detection of possible *Salmonella;* B) MacConkey agar was used to detect gram negative bacteria and also group them for further processing; C) TCBS—was used as a selective medium for *Vibrio cholera* or *Vibrio parahemolyticum* which might be present; D) Trypticase soy agar with 1.5 percent sodium chloride was used for detection of possible halophilic organisms; E) Rimler-Shotts agar was used for selection of members of the *Aeromonas hydrophila* complex; and F) Pseudosel was used as a selective medium for the genus *Pseudomonas*.

These media were incubated at 35°C and 23°C unless otherwise indicated by the selective procedure involved. Resulting growth was subcultured and identified. Aliquots of all samples were processed on special media for the presence of *Mycoplasma* sp. and *Mycobacterium* sp.

Virological Examinations

Samples were processed for virus isolation by making 10 percent suspension of whole fish in phosphate buffered saline. The suspension was filtered through four layers of cheesecloth and centrifuged at 1,000 *g* for 15 minutes. The supernatant was removed and recentrifuged at 4°C at 5,000 *g* and then filtered through a 47-μm pore size membrane filter using positive pressure.

Cell cultures used for virus detection were rainbow trout gonad (RTG-2), fathead minnow (FHM), brown bullhead (BB), VERO (African green monkey kidney), pig kidney, bovine kidney, rabbit kidney, and feline kidney. Medias, cell culture procedures, and microculture techniques have been described previously (Gratzek et al., 1973; Rovozzo and Burke, 1973). All samples were passed three times at 5-day passage intervals. Samples were judged negative if cytopathic effects were not present at the end of the third passage.

Table 1.—Gill infestation.

Parasite	Bags infested %
Flukes	21.0
Ichthyophithirius	2.6
Flukes + *Ichthyophthirius*	1.3
Trichodina + *Ichthyophthirius*	1.3

Table 2.—Skin infestation.

Parasite	Bags infested %
Flukes	3.9
Chilodonella	1.3
Oodinium	1.3
Ichthyophthirius	1.3
Myxosporida	1.3

Table 3.—Intestinal infestation.

Parasite	Bags infested %
Nematodes (in lumen)	12.5
Nematode (cysts)	6.2
Acanthocephalan nematode (cysts)	3.1
Acanthocephalan (cysts)	3.1
Hexamita	3.1

RESULTS AND DISCUSSION

Parasitological Results

The results of parasitological examinations of 77 bags of fish suggested that 61 percent contained fish with some type of parasite. In 39 percent of the bags, no parasites at all were found. The results are best seen in Tables 1, 2, and 3, which present the incidence of fish parasitized.

Gill flukes were the commonest parasites observed on these fish. However, it is also important to note that only in one case was the fluke infestation high enough to create immediate problems for the fish. Also, it appears that combinations of infections, such as flukes and *Ichthyophthirius,* or *Trichodina* and *Ichthyophthirius* do occur.

The results of intestinal examination for parasites suggested that 12.5 percent of the 77 bags contained fish that had nematodes in the interior of the intestine. The significance of cysts of nematodes or acanthocephalans found imbedded in tissues outside the intestine either alone or in combination is that these fish are most probably intermediate hosts for these parasites where the adult stages of the worms exist in birds or larger fishes. Heavy infestations of these intermediate forms are harmful to these fish. It is significant to note that similar families of parasites are found in fish native to this country. It has been stated (Meyer, 1954) that under conditions in nature there is rarely a single individual fish among all the numerous species from the smallest minnows to game fishes which does not harbor at least one or more species of parasites somewhere in its body. Often the parasites are confined to the internal organs and hence are usually not noticed when the fish is cleaned or dissected. It would appear then that the incoming fish are possibly parasitized certainly no more than native fish species.

We were surprised to note that these fish did not harbor more intermediate forms of digenetic flukes or tapeworms. A possible explanation is that most of the fishes imported from the Far East are raised in aquaria or in small ponds where there is less chance for infestation of these fish by free swimming intermediate forms of these parasites. Preliminary studies from South American fishes suggest that the opposite is true.

Bacteriological Results

In this study, 77 bags containing 30-50 fish each were examined. In general, the bacteria isolated were primarily rod shaped, gram negative staining types which, in most instances, can be associated with the fish's natural environment. Bacteremias were noted in fish in 51 bags and represented 11 genera of bacteria. This high incidence of bacteremia suggests that fish are under severe stress during shipment. Further detailed bacteriological cultures of each lot of fish resulted in the isolation of 18 genera of bacteria. Examination of the water in which the fish were shipped resulted in the isolation of 14 genera of organisms. More prevalent among the

bacteria isolated were those of the genera *Pseudomonas, Aeromonas, Proteus, Citrobacter, Enterobacter,* and *Escherichia*. The first two, while they may be potential fish pathogens, are considered normal inhabitants of water and constitute no disease problem to mammals or fish under normal conditions. The latter organisms are usually indicative of human or other animal association and while occasionally associated with human or animal disease are not considered of public health importance under normal circumstances.

Only two organisms which could be considered of human health importance were recovered during the study. These organisms were *Salmonella arizona,* which in high numbers may cause human diarrhea, and *Mycobacterium* sp., which are universally found in water. The former was recovered from fish and water in one instance each. The latter was recovered from the water on three occasions. *Mycoplasma* sp. were not isolated.

Virological Results

One virus isolate was made from a slurry of Kuhli loachs, *Acanthophthalmus* sp., and from the water in which these fish were transported. The virus was isolated on rabbit kidney cell cultures. The virus was characterized as a herpesvirus based on size and morphology, DNA content, ether susceptibility, and lack of hemagglutination ability. The virus was shown to not cross-react with channel catfish herpesvirus nor was it pathogenic to channel catfish, *Ictalurus punctatus*. It does not react with antiserums to pseudorabies virus or infectious bovine rhinotracheitis virus, but it does partially cross-neutralize with equine rhinopneumonitis virus. Further serological studies are being conducted.

SUMMARY AND CONCLUSIONS

The results of this survey of imported fishes from Southeast Asia indicate that the parasitic load is less than would be expected in native fish (Hunter, 1942). The presence of bacteria of definite public health importance is also minimal based on reported studies on *Salmonella* distribution in continental U.S. watersheds (Kenner and Clark, 1974). The isolation of a herpesvirus, so far not completely characterized, supports the observation that viruses, like bacteria, are found in water from various sources.

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