3. Isolate individual turtles showing early lesions ;
4. Provide sufficient basking and water space for each turtle to reduce the possibility of shell injury ;
5. Curette active and benign lesions and cauterize with tincture of iodine.

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A SURVEY OF PARASITES, BACTERIA AND VIRUSES ASSOCIATED WITH TROPICAL FISH IMPORTED FROM SOUTHEAST ASIA

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Summary

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 originating in Hong Kong, Taiwan, Singapore, and Bangkok were examined for presence of parasites, bacteria, and viruses. A total of 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 scrappings, and internal organs were examined uisng 5 and 10 magnification objectices.

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, 5 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 pH 7.2 to achieve a 10% wt/vol. suspension of fish. Concurrent with fish processing, aliquots of water from each bag were pooled to achieve approximately a 1500 ml sample of water representative of the respective shipment. This sample was centrifuged at 5000 RPM for 20 minutes in a RC - 2B centrifuge at 4°C.

The above samples were cultured as follows :

I. *Bacteremia.* Blood and/or kidney were streaked to blood agar and Ordall's agar with subsequent incubation at 35°C and 23°C. Resulting growth was subcultured and identified.

II. *Fish suspension.* A battery of several media were inoculated to assure recovery of a wide spectra of bacteria. Selective media were used where possible to enhance possible isolations. The media used and justification are listed below :
   1. Blood agar was used as a general medium for detection of fastidious organisms as well as common organisms.
   2. Ordall's agar was selected for detection of the presence of myxobacteria.
   3. Trypticase soy agar was used as a generalized media which would grow practically all organism.
   4. Rimler-Shotts agar (Shotts c.s., 1973) was used to specifically select members of the *Aeromonas hydrophila* complex.
   5. Bismuth Sulfide agar was used specifically to detect the possible presence of *Salmonella typhosa.*
   6. Selenite-Brilliant green broth and ducitol 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 c.s., 1957).

III. *Water* - The sedimented material resulting from centrifugation was resuspended in approximately 100 ml of the centrifuged supernatant. This constituted the inoculum for the following battery of media :
   1. Selenite brilliant green broth and ducitol selenite broth-Brilliant green agar. This battery of media was used for detection of possible Salmonella.
   2. MacConkey agar was used to detect gram negative bacteria and also group them for further processing.
   3. TCBS - was used as a selective medium for *Vibrio cholera* or *Vibrio parahemolyticum* which might be present.

4. Trypticase soy agar with 1.5% sodium chloride was used for detection of possible halophilic organism.
5. Rimler-Shotts agar was used for selection of members of the *Aeromonas hydrophila* complex.
6. 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 sampless were processed on special media for the presence of *Mycoplasma* sp. and *Mycobacterium* sp.

Virological methods :

Samples were processed for virus isolation by making a 10% suspension of whole fish in phosphate buffered saline. The suspension was filtered through four layers of cheezecloth, and centrifuged at 1000 times G for 15 minutes. The supernatant was removed and recentrifuged at 4°C at 5000 G. The supernate was then filtered through a 47 μ 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, Pig Kidney, Bovine Kidney, Rabbit Kidney, and Feline Kidney. Mediums, cell culture procedures and microculture techniques have been described previously (GRATZEK c.s., 1973, ROVOZZO c.s., 1973 and WOLF c.s., 1973). All samples were passaged three times at five day passage intervals. Samples were judged negative if cytopathic effects were not present at the end of the third passage.

Results and discussion

Parasitological results : The results of parasitological examinations of 77 bags of fish suggested that of the 77 bags, 61% contained fish with some type of parasite. In 39% of the bags, no parasites at all were found. These results are best portrayed by the following table which presents the incidence of fish parasitezed.

Gill infestation :		*Skin infestation*		*Intestinal infestation*	
PARASITE	PERCENT BAGS INFESTED	PARASITE	PERCENT BAGS INFESTED	PARASITE	PERCENT BAGS INFESTED
Flukes	21.0	Flukes	3.9	Nematodes (in lumen)	12.5
Ichthyophthirius	2.6	Chilodonella	1.3	Nematode (cysts)	6.2
Flukes + Ichthyophthirius	1.3	Oodinium	1.3	Acanthocephalan nematode (cysts)	3.1
Trichodina + ichthyophth.	1.3	Ichthyophthirius	1.3	Acanthocephalan (cysts)	3.1
		Myxosporidian	1.3	Hexamita	3.1

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 to the fish. Also, it appears that combinations of infections, such as flukes and Ich, or trichodina and Ich do occur. The results of intestinal examination for parasites suggested that 12.5% 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 of 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 infestation 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), for instance, 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 our 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 :

During the course of this study, 77 bags containing 30-50 fish were examined. In genral, 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 organism. 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 noraml inhabitants of water and constitute no disease problem to mammals or fish under normal conditions. The latter organism 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 organism which could be considered of human health importance were recovered during the study. These organism 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. Mycoplasmae were not isolated.

Virological results :

One virus isolate was made from a slurry of Kuhli loachs 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, lack of hemagglutination ability. The virus was shown to not cross react with channel catfish herpesvirus nor was it pathogenic to channel catfish. It does not react with antiserums to pseudorabies virus, infectious bovine rhinotracheitis virus, but it does partially cross neutralize with equine rhinopneumonitis virus. Further serological studies are being conducted.

Conclusions

The results of this survey of imported fishes from Southeast Asia indicate that the parasitic load is less than would be expeted 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 United States watersheds (Kenner c.s., 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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