Antibodies Against Enteric Bacteria in Brown Bullhead Catfish (Ictalurus nebulosus, Le Sueur) Inhabiting Contaminated Waters

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Brown bullhead catfish were collected from sewage- and acid mine waste-polluted waters in an attempt to detect antibodies against human enteric bacteria in their sera and to investigate the association of antibody response with environmental conditions. Agglutination antigens prepared from isolates obtained from water collected at the same locations as the fish habitats were used to demonstrate such antibodies. The results showed large percentages of reactive sera for common isolates such as Escherichia coli and Enterobacter cloacae as well as lesser incidences of antibodies to other, less common isolates. In general, fish with the highest titers were collected from habitats with higher coliform counts. Acid mine drainage reduced the total coliform counts, but did not appear to affect the titers of sera from fish collected from water so affected. It was concluded that the bottom-feeding catfish might be a better subject for the study of fish as an ecological indicator of fecal pollution in acid-polluted waters.

Fish inhabiting waters contaminated by fecal coliform bacteria can easily come in intimate contact with these microorganisms. Studies have shown that enteric bacteria are not part of the normal flora in the intestinal tract of fish and their presence is considered a direct result of the association of fish with sewage-polluted waters (3, 4, 5, 12). These bacteria can also survive and multiply in the fish intestine with residency lasting from a few days to a few weeks (3, 4, 5, 6, 11). Possible consequences of this association may be either infection of fish or fish acting as vectors of human disease. Although there are substantial data that cold-blooded vertebrates such as reptiles may act as reservoirs of human enteric bacteria (11), few reports are available concerning the role that fish may play in this regard. Japanese investigators have been able to isolate antibiotic resistant strains of certain Enterobacteriaceae from cultured marine fish (1). Since the eating of raw fish is common in their country, the public health significance of either infection or transfer of R (resistance) factor to normal human flora is great. A number of significant human enteric pathogens have been isolated from bottom-feeding fish in Korea (9).

Brown et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, M92, p. 81) were able to isolate several enteric viruses including reoviruses, adenoviruses, coxsackie viruses, and all three types of poliovirus from fish inhabiting a polluted river in a high density human population area. They were also able to show that cell cultures obtained from fresh fish tissues supported the multiplication of certain of these same viruses, suggesting that the fish could become infected under natural conditions with agents of human significance. This situation as a health hazard to man and in relation to management of sports fisheries needs to receive further study.

An approach to further study of this situation is through the immune response of fish. It might be assumed that antibody production to a particular human pathogen would indicate that the fish had been infected and could act as carriers. In an unconfirmed study, Janssen and Meyers (7) showed that antibody production against human pathogens occurred in white perch, Roccus americanus (Gmelin), taken from waters adjacent to areas of heavy human populations. Their results showed a low incidence of precipitin-positive fish sera to antigens prepared from stock cultures.

The purpose of this research was to determine if brown bullheads collected from water with fecal bacteria contained circulating antibodies.

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against the species of enteric bacteria isolated from the same environment.

**MATERIALS AND METHODS**

**Fish.** Brown bullhead catfish were collected at each station from July to August. The fish were caught using a Nichols Special Frame net. The size of fish ranged from 208 to 290 mm and weights ranged from 104 to 303 g. The fish were transported to the laboratory where they were bled within 24 h of capture for 1 to 2 ml by intracardiac puncture. The recovered serum samples were stored at −20 C until serologically examined.

**Water samples.** Water samples were collected from four separate stations during May through August. The stations included two areas receiving sewage effluent from outfalls on the acid-mine polluted Monongahela River, a lake receiving septic tank drainage, and an unpolluted pond. On the river, samples were taken at the outfall and at midstream. All stations' water samples were collected from the surface and bottom depths. Total coliform counts were obtained using the membrane filter technique and Endo medium (Difco, Detroit, Mich.)-saturated pads as substrate. A Beckman Expandomatic model line operated meter was used to obtain pH determinations.

**Antigens.** Antigens for the serological tests were prepared from bacterial cultures isolated from water samples taken at each station. Five isolates were prepared in 0.6% formal-saline according to conventional procedures (2). Selected bacteria were harvested from Roux bottles containing nutrient agar inoculated with 24-h broth cultures of each isolate. Antigens included an untypable *Escherichia coli*, *Enterobacter cloacae*, two *Klebsiella* sp. isolates which differed in morphology (type A and type B), and an unidentified *Mima* species. Selection of bacterial antigens was based on numbers and distribution of species found among the stations.

**Serological examination.** All sera were first screened at 1/2 dilution of serum by the tube agglutination technique with an incubation period of 4 to 5 h at 45 C followed by overnight in the cold. All positive specimens were titrated by the microtiter technique.

**RESULTS**

Table 1 presents the results of screening of all of the sera against the study antigens. The high incidence of serological reactions with *E. coli* and *E. cloacae* reflects the intimate association of the fish with the most common isolates from the collecting stations. Neither the *Klebsiella* nor the *Mima* isolates were very common, which was also reflected by the low serological reactions to these organisms. Fish from river habitats exhibited higher incidences of antibodies than did fish from the contaminated lake.

The stations receiving the greatest volumes of sewage exhibited the highest bacterial counts, which in turn were influenced by the pH at the time, a product of acid mine waste load of the collecting body of water (Table 2). Also there was the great difference in coliform counts between the lake receiving septic tank effluents

|                        | Total coliforms (per 100 ml) | pH |
|------------------------|-------------------------------|----|
| *River stations*       |                               |    |
| Midstream              | 7 July                        | 1,500 | 4.3 |
|                        | 2 August                      | 32,000 | 6.8 |
| *Near sewage outfall*  | 19 July                       | 340  | 3.9 |
|                        | 16 August                     | 2,200 | 5.3 |
| *Pond stations*        | 12 July                       | 340,000 | 7.4 |
|                        | 28 July                       | 44,000 | 7.5 |
| *Doe Pond*             | 9 August                      | 400  | 7.2 |

**Table 1. Percentage of bullhead catfish reacting with selected antigens at screening dilution 1/2**

| Antigens | Monongahela River | Suncrest Lake* | Doe Pond* |
|----------|-------------------|----------------|-----------|
|          | Midstream         | Sewage outfall |           |
|          | Sera tested       | Sera tested    | Sera tested | Sera tested |
|          | % Positive        | % Positive     | % Positive | % Positive |
| *E. coli* | 177               | 35.5            | 359        | 48.7       | 132        | 9.1       | 27        | 7.4       |
| *Enterobacter cloacae* | 187           | 12.8            | 372        | 14.2       | 154        | 0.6       | 27        | 0.0       |
| *Mima sp.* | 173             | 2.3             | 375        | 2.7        | 139        | 2.2       | 27        | 0.0       |
| *Klebsiella A* | 154             | 0.0             | 327        | 0.0        | 117        | 0.0       | 26        | 0.0       |
| *Klebsiella B* | 188             | 0.0             | 376        | 1.6        | 168        | 0.6       | 28        | 0.0       |

*Control source known to receive septic tank effluent pollution.

*Control source not known to receive any external source of pollution.
Table 3. Serum titer distributions of catfish sera showing positive agglutination reactions

| Antigen   | Station or habitat | No. of sera tested | Titer       |
|-----------|-------------------|--------------------|-------------|
|           |                   |                    | %  | %  | %  | %  | %  |
| E. coli   | Midstream river   | 63                 | 7.9*| 36.5| 38.0| 17.5| 0  |
|           | Sewage outfall    | 175                | 2.3 | 29.7| 38.3| 21.1| 8.0 |
| E. cloaca | Midstream river   | 24                 | 16.7| 29.0| 32.5| 4.0 | 12.5|
|           | Sewage outfall    | 33                 | 11.3| 49.1| 22.6| 5.7 | 11.3|

*Results are expressed as the percentage of sera showing a titer at this end point.

(Suncrest Lake) and the relatively uncontaminated lake (Doe Pond).

Table 3 presents the titers of all positive sera, and the data are interpreted as to the degree of association between the fish and the bacteria, i.e., the higher the titer, presumably the more intimate the contact. A comparison of the ranges of titer distribution among the fish collected at the two river stations and for the major bacteria involved indicates that there were no statistically significant differences in serum titers in relation to the quality of the habitat from which the fish were obtained.

**DISCUSSION**

The present studies confirm and extend the significant findings of Janssen and Meyers (7), who demonstrated low incidences of antibodies in white perch by the precipitin technique, using bacterial extracts of stock cultures as antigens. The incidence of antibodies reported here was much higher, possibly due to the facts that in the present study (i) the more sensitive agglutination technique was used to demonstrate antibodies, (ii) that antigens were prepared from strains indigenous to the same habitat from which the fish were taken, and (iii) that the feeding habits of catfish differ appreciably from the white perch used by Janssen and Meyers (7). Brown bullhead catfish are primarily bottom feeders which would ingest large numbers of bacteria that settle out from flocculation deposits caused by the iron and acid mine pollution found in certain Appalachian bodies of water. By contrast, white perch feed on crustacea and small fish. Another way in which catfish might become immunized is by high counts of bacteria, either suspended in the ambient water or stirred up from the bottom sediment during the feeding process, coming in intimate contact with the gill structures. This route might serve as an excellent route of immunization. The development of rather high titers suggests that the degree of immunization results from a rather intimate contact with the antigen.

The incidence and amount of antibody present in the serum of a given fish appeared to be related to the kind and amount of bacteria present in the habitat from which the fish was taken. An exception to this thesis was the finding of a low incidence of antibody in the fish taken from the contaminated lake.

In comparing the two river stations, there was no correlation between antibody titer and bacterial counts. Although acid mine drainage does influence the bacterial counts of the collecting body of water (8), the numbers of bacteria are still high enough to remain a significant antigenic stimulant to vertebrates living in such a habitat.

High microbial pollution may affect the health of fish, something that must be studied further by those interested in the management of sport fisheries. Whether the bullheads in the present study were infected was not determined, but the high antibody titers in some of the fish certainly suggest this may have happened. An organism of some human significance, *Edwardsiella tarda*, has not only been isolated from fish, but has been incriminated in disease outbreaks of another species of catfish (10). The total impression from these various data sources leads one to the conclusion that fish as reservoirs and hosts of human infectious agents also deserve more microbiological study.

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