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ISOLATION OF *Edwardsiella tarda* FROM AQUATIC ANIMAL SPECIES AND SURFACE WATERS IN FLORIDA*

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Abstract: *Edwardsiella tarda* was isolated from lakes and streams in Northcentral Florida. This emerging enteric bacterial pathogen also was isolated from alligators *(Alligator mississippiensis)* and brown pelicans *(Pelecannus occidentalis carolinensis)* on several occasions, and from other species including the ring-billed gull *(Larus delewarensis)*, bald eagle *(Haliaeetus leucocephalus)*, great blue heron *(Ardea herodias)*, sandhill crane *(Grus canadensis)*, common loon *(Gavia immer)*, and largemouth bass *(Micropterus salmoides)*. Hemorrhagic enteritis was found in association with *E. tarda* in some of the species presented.

INTRODUCTION

Ewing *et al*,[5] described a new genus and species, *Edwardsiella tarda*, to be included in the family *Enterobacteriaceae*. Their description was based on 37 cultures sent to them between 1959 and 1965, derived primarily from man.

Sakazaki[11] isolated the same organisms, primarily from snakes in Japan, but referred to these isolants as the "Asakusa group."

Edwardsiella tarda has been associated with a number of infections in man, including wound infections,[8] abscesses,[6,8] meningitis,[10,12] and *Salmonella*-like intestinal infections.[2,3,8,9]

D'empaire[4] investigated the growth requirements of *E. tarda* isolants from France, Tahiti, Tchad, and Vietnam, using two cultures from cattle, three cultures from man, and one culture each from a pig and a panther, in addition to numerous isolants from snakes. Arambulo *et al.*,[1] found *E. tarda* in the bile of 3 of 1000 apparently healthy pigs at slaughter, but not in the bile of 500 apparently normal cattle.

In 1966, the isolation of *E. tarda* from a sea lion *(Zalophus californianus)* and an alligator, both dead after illness, was reported from Florida.[13] The recovery of *E. tarda* from the small intestine of an ostrich *(Struthio camelus)*, which died from an acute intestinal infection, was reported in 1969.[14] Also, in 1969, Jackson *et al.*,[7] found many turtles (nine species) carrying *E. tarda*.

The present report records the isolation of *E. tarda* from surface waters, a sick largemouth bass *(Micropterus salmoides)*, six species of wild birds, and five alligators *(Alligator mississippiensis)*.

MATERIALS AND METHODS

Water specimens

Water specimens were collected from August 1970 through December 1971

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from numerous lakes and streams within a 40 km radius of Gainesville, in northcentral Florida. A total of 108 specimens was collected on an irregular schedule, two or more from each of 20 locations. Water varied in temperature from 12 to 28C, and in pH from 6.5 to 8.5. Specimens of 10 ml were collected from 15 to 25 cm below the water surface and added directly to 10 ml of double strength selenite F or tetrathionate enrichment broth. The cultures were held in an ice chest until returned to the laboratory, usually within 2 to 3 hours, and then were incubated at 37 C. After incubation for 18 to 24 hours, the cultures were plated onto EMB and SS agars. After approximately 24 hours of incubation, colorless colonies appearing similar to *Salmonella* colonies were transferred to TSI agar slants, and were subsequently identified by biochemical tests described as adequate to identify *E. tarda*.[5]

Alligators *(Alligator mississippiensis)*

In May 1971, a die-off of fish, turtles, alligators and some water birds occurred in Lake Apopka, near Orlando, Florida. During an investigation of the die-off, five apparently normal alligators, 1 to 1.2 meters in length, were presented for necropsy. Internal organs and intestinal contents were examined for bacteria, and sections were prepared for histopathologic examination.

In addition, 22 apparently normal alligators were captured in Alachua County, near Gainesville, Florida in order to collect blood for another study. Cloacal specimens also were obtained for enteric bacterial studies.

Brown pelicans *(Pelecannus occidentalis carolinensis)*

During 1971-72, many brown pelicans were found dead on Florida beaches. Others were unable to fly, and apparently sick. Some died shortly after capture. Forty-eight pelicans, most of them received frozen, were examined bacteriologically. Internal organs and intestinal contents were examined for pathogens except for those badly decomposed before freezing, in which case only intestinal contents were examined. Several weak, live pelicans were necropsied and examined bacteriologically and histologically. One similarly affected pelican was examined for evidence of enterotoxemia by mouse inoculation. In the latter tests, different pairs of mice were inoculated intravenously with 0.2 ml and intraperitoneally with 0.3 ml of intestinal contents, both heated and unheated, after filtration through 0.22 μm Millipore filters (Millipore Filter Corp.).

Common loons *(Gavia immer)*

Beginning in December 1971, numerous common loons were found dead on Florida beaches, under circumstances resembling the brown pelican die-off. Eleven loons were examined bacteriologically, most of them received frozen in January 1972. Several typically affected live loons were necropsied. Two were examined for evidence of enterotoxemia.

Sandhill cranes *(Grus canadensis)*

During 1970-71, 32 apparently normal sandhill cranes became available for examination in connection with other management and biological studies. The cranes were on wintering grounds in northcentral or southern Florida.

Bald eagles *(Haliaeetus leucocephalus)*

Two bald eagles were found unable to fly and apparently sick, one in 1970 in Levy County, Florida, and the other near Lake Apopka during the previously mentioned wildlife die-off in 1971. Since attempts were to be made by the U.S. Fish and Wildlife Service to save the lives of the eagles, only cloacal swab enteric cultures could be made.

Great blue herons *(Ardea herodias)*

Two herons, both frozen, were examined. One had been weak and unable to fly when it was found, the other was found freshly dead.

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Ring-billed gull *(Larus delewarensis)*

One ring-billed gull was found unable to fly at the Lake Apopka die-off, and was examined at necropsy.

Largemouth bass
(Micropterus salmoides)

A bass (34 cm long) was found floating in obvious distress in Lake Santa Fe in Alachua County, Florida in July 1971. It was necropsied immediately after death, bacteriologic cultures were made, and tissues were taken for histopathologic examination. A number of other bass in the lake were reported ill and dying at the same time.

RESULTS

Edwardsiella tarda was isolated from 13 of 108 water specimens (12%). It was isolated two different times at one of the 20 sites. *Salmonella hartford* was isolated from one water specimen during the study.

Except for an old wound lesion in the tail of one alligator, no gross or histopathologic evidence of disease was found in the alligators from Lake Apopka. No bacteria were isolated from the tissues of the internal organs, however *E. tarda* was isolated from the contents of the large intestine of three of the five reptiles. *Edwardsiella tarda* also was recovered from cloacal swabs of two of the 22 examined in the Alachua County survey. In addition, three different *Salmonella* serotypes were isolated in the latter survey. They were *S. miami, S. java,* and *S. hartford.*

The lesion found regularly in brown pelicans received alive or frozen was hemorrhagic enteritis. The intestinal contents in these birds were dark and tarry. *Edwardsiella tarda* was isolated from the lungs and liver of one pelican received frozen and in poor condition for examination. *Edwardsiella tarda* was isolated from the intestinal contents of three of the pelicans with hemorrhagic enteritis. *Salmonella litchfield* was isolated from the intestinal contents of one of the three. Mice inoculated either intravenously or intraperitoneally with Millipore filtered intestinal contents remained alive and unaffected. Histopathologic examinations of the small intestine of several birds with hemorrhagic enteritis revealed ova and segments of flukes firmly embedded in the lamina propria and a heavy infiltration with lymphocytes. Heavy deposits of hemosiderin were found in the sinusoids of the liver and lymphocytic infiltration was noted in the portal triads.

Hemorrhagic enteritis was also found in common loons examined at necropsy. Dark and tarry intestinal contents were present, resembling those seen in brown pelicans. *Edwardsiella tarda* was isolated from the intestinal tract of one loon received frozen and in poor condition. From one loon presented alive, a *Vibrio* species was isolated from the mucosa of the small intestine. Enterotoxins were not demonstrated by mouse inoculations. Histopathologic lesions in the small intestine of four loons with hemorrhagic enteritis consisted of hemorrhage, congestion, and ulceration of the mucosa. In another similarly affected loon, large cystic areas in the kidney contained numerous fluke ova, and there was interstitial lymphocytic infiltration. The liver contained heavy deposits of hemosiderin in the sinusoids and lymphocytic infiltration in the area of the portal triad.

No significant lesions were observed in the apparently normal sandhill cranes. Bacteriologic cultures of internal organs revealed no pathogens. *Edwardsiella tarda* was isolated from the large intestinal contents of one crane from southern Florida. Two cranes were carrying *Salmonella* in their intestinal tracts, one *S. hartford,* and the other *S. java.*

Edwardsiella tarda was isolated from a cloacal swab culture of one bald eagle found sick at Lake Apopka.

No significant lesions were noted in the two frozen great blue herons. *Edwardsiella tarda* was recovered from the large intestine of one.

Although unable to fly, no lesions were found at necropsy in the otherwise

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normal ring-billed gull. *Edwardsiella tarda* was recovered from the large intestine. The gull's inability to fly may have been due to badly worn plumage which had not been replaced on schedule.

Internal organs and gills of the largemouth bass were pale and anemic at necropsy, and the blood was watery. Hemorrhagic nodules were observed on the stomach wall. There were hemorrhages in the serosa of the intestine. Histologic examination showed bile stasis in the liver, and unidentified parasite larvae, hemorrhage, and hemosiderin in the submucosa of the stomach. The spleen contained hemosiderin-like pigment, with no discrete splenic corpuscles. *Edwardsiella tarda* was isolated from the liver and spleen.

DISCUSSION

There are but few reports of the isolation of *E. tarda* from domestic animals, only several instances from cattle[4,5] and pigs.[1,4] There are no previous reports of *E. tarda* from birds other than from an ostrich in a zoological park,[14] and no reports from wild mammals, except for the isolant from a panther.[4]

Although it might be expected that *E. tarda* would be present in water as contamination from reptiles, it has not been previously reported in surface waters.

It is possible that the relatively recent appearance of *E. tarda* in the environment may be related to increased water pollution, thus providing metabolites needed for growth of the organism. There must be some reason other than increased awareness for the increasing isolations of *E. tarda* from aquatic sources.

At the same time, *E. tarda* has been increasingly isolated and described as the causative agent of infections of man, from both intestinal and extraintestinal sources, as previously mentioned.[2,3,6,8,9,10,12]

Edwardsiella tarda may be compared to *Salmonella* in regard to its affinity for the intestinal tract, its ability to produce extraintestinal infections in man, and its pathologic characteristics. However, there are striking differences in host relationships, since *E. tarda* has been isolated only on rare occasions from either wild or domestic mammals.

In the present study, *E. tarda* was found in association with enteric disease in aquatic species of birds in several instances, and in one case of a hemorrhagic disease in a fish. Further observations are needed in order to determine the significance of *E. tarda* in the aquatic environment.

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