

Pathology Caused by the Bacterium *Edwardsiella tarda* in *Anabas testudineus* (Bloch)

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Abstract

A virulent strain of *Edwardsiella tarda* isolated from an outbreak in an organized *Clarias batrachus* hatchery was used to study the infectivity pattern and pathology in *Anabas testudineus*. The 50% lethal dose for *A. testudineus* injected intraperitoneally with *E. tarda* was $10^{7.8}$ cells. The organism produced acute disease pattern with characteristic histopathological lesions viz., hepatic necrosis and intravascular thrombus, myocardopathy and enteritis along with mild necrosis in the spleen and kidney. *E. tarda* specific antigen was detected in the liver, kidney and spleen of dead fish through dot-ELISA.

Introduction

Edwardsiellosis caused by the bacterium *Edwardsiella tarda*, a gram-negative motile rod-shaped flagellated pathogen occurs in a wide range of fishes, reptiles, birds and mammals including humans (Bullock and Herman 1985). *E. tarda* infections with economically important losses have been reported from a variety of cultured fishes in Asia, especially Japan, India and channel catfish in U.S.A. (Herman and Bullock 1986). The organism causes mass mortality in many fish species. The pathological changes caused by this organism has widely been studied in many other fish species (Thune et al. 1993; Sahoo et al. 1998) except *Anabas* sp. This is the first description of experimental *E. tarda* infection of *Anabas testudineus* (common name: koi; Family: *Anabantidae*). *E. tarda* has the ability to live outside the host in pond water and mud (Thune et al. 1993). Since *C. batrachus* and *A. testudineus* prefer to live in similar environmental conditions and there has been a report of serious *E. tarda* infection outbreak in *Clarias* species (Sahoo and Sahoo 1997), the possible susceptibility of *A. testudineus* to this infection was explored.

Materials and Methods

Bacteria and media

The pathogenic isolate of *E. tarda* used for infecting *A. testudineus* was obtained from dermal lesions of catfish fry (length: 12 to 15 mm; 12-day old) during one outbreak in an organized hatchery. The organism isolated from catfish fry was also proven to be pathogenic in the same species following Koch's postulate and produced ulcerative lesions only on the dermis (Sahoo and Sahoo 1997). Infectivity studies were conducted with the original bacterial isolate grown on brain heart infusion (BHI) broth (Hi Media, Bombay, India) for 48 hours at 30°C. The bacteria were washed in phosphate buffer saline (PBS, pH 7.2) and the number of viable bacterial cells in PBS was determined following MacFarland's standard and subsequently confirmed through plate count.

Fish

The fish used for the experimental infectivity were obtained from a commercial farm (mean weight, 21 g). The fish were free from bacterial infections, through kidney culture of randomly sampled fish and were zero-negative for *E. tarda* antibodies. The fish were held in 1000 l aquaria supplied with tap water at 20 to 22°C simulating the water temperature of ponds during the season when outbreak of edwardsiellosis mostly occur (Sahoo et al. 1998). They were acclimatized for a period of two weeks before the start of the experiment.

Experimental design

Duplicate groups of 40 fish were used in the infectivity studies. Virulence of the bacteria was tested through intraperitoneal (i.p.) injection, that will help to know the exact number of bacteria required to produce pathology. Fish were injected with serial 10-fold dilution of original bacterial suspension (0.1 ml-fish) with five fish for each dilution. The final concentrations of the bacteria injected to each dose group of fish were 10^6 , 10^7 , 10^8 and 10^9 cells-ml of PBS. Control fish were injected intraperitoneally with 0.1 ml of PBS.

Fish were observed for a period of seven days and the moribund or freshly dead fish were preserved for histological examination in 10% neutral buffered formalin. For detection of bacteria in exposed animals, kidney tissue of dead fish and of survivors was cultured on BHI agar and gram-stained. *E. tarda* were identified by colony and cell morphology, motility, negative oxidase reaction, positive indole test, and reactions in triple sugar iron agar slants. The LD₅₀ value of the bacterium in *Anabas* sp. was calculated following Reed and Muench (1938).

Serology

The fish which survived after 21 days were bled and the serum samples were checked for antibodies through agglutination test (Klesius et al. 1991).

Similarly, the tissues of liver, spleen and kidney collected from dead and moribund fish were tested for presence of bacterial antigen through dot-enzyme linked immunosorbent assay (Dot-ELISA) (Ahmad et al. 1992).

Results

E. tarda isolated from *C. batrachus* were pathogenic for *A. testudineus* when injected i.p. The 50% lethal dose value was $10^{7.8}$ cells·ml of PBS. All fish exposed to 10^9 cells·ml died within three days of injection. Ninety per cent of total mortality occurred within the third day of injection. Pure cultures of *E. tarda* were reisolated from kidneys of fish in all treatments except in the control. The bacteria were gram-negative motile rods, cytochrome oxidase-negative, acid and gas positive, and hydrogen sulfide – positive in triple sugar iron agar. These characteristics provided a presumptive identification of *E. tarda* that was confirmed by a positive agglutination reaction with rabbit anti *E. tarda* serum. The moribund fish became anorexic, lethargic, laid down at the bottom, revealed yellowish to reddish distended abdomen and pale gills before death. Freshly dead fish showed extensive hemorrhages and hyperaemia on the ventral body surface and at the base of fin. The scales were roughened in the highest dose group of fish.

The most striking histological lesions in the fish were in the liver, heart, anterior kidney and intestine and were dose-related. The heart showed vacuolative degeneration to necrosis of cardiac muscle, leucocytic infiltration and wavy appearance of the muscle bundles (Fig. 1) along with intramuscular hemorrhages. There were increased activities of fixed phagocytes of the atrium, particularly in the endothelial lining, with excessive engorgement of blood sinusoids in the muscular trabeculae (Fig. 2). The liver had perivascular fatty degeneration and necrosis, intravascular thrombus formation along



Fig. 1. Myocardium showing necrosis (arrow) and wavy appearance of cardiac muscle along with leucocytic infiltration and intramuscular hemorrhages. H and E X 400 (Bar = 30 μ m).



Fig. 2. Increased activities of fixed phagocytes in the atrial endothelial lining (arrow head) along with engorged blood vessels (arrow). H and E x 1000 (Bar = 30 μ m).

with the presence of macrophages and serous exudate in the lumen, periportal accumulation of serous exudate (Fig. 3), massive congestion and engorged sinusoids. The spleen showed dilated sinusoids with necrotic debris lying in the white pulp area. The posterior kidney also revealed necrotic changes in the tubular epithelia and interstitium. The

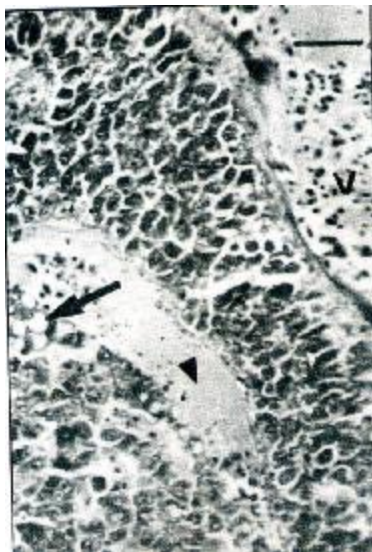


Fig. 3. Liver revealing periportal fatty degeneration (arrow) to necrosis and serous exudate accumulation (arrow head), intravascular macrophages with serous exudate (V) and intact exocrine pancreas. H and E x 625 (Bar = 30 μ m).



Fig. 4. Anterior kidney revealing engorged blood vessels (bv), focal necrosis (arrow head) along with acute interstitial nephritis (arrow). H and E x 625 (Bar = 30 μ m).

anterior kidney revealed engorged blood vessels, focal necrosis with acute interstitial nephritis, mostly infiltrated with neutrophilic granulocytes (Fig. 4). The presence of microabscesses were also evident in the anterior kidney. Mucosal congestion, submucosal hemorrhages with leucocytic infiltration were evident in the intestine. The gills and stomach did not reveal any appreciable change. The fish that received the pathogen at low doses survived and did not show presence of antibodies in the sera when subjected to plate agglutination test. However *E. tarda* specific antigen was detected in the liver, kidney and spleen of experimentally infected and moribund fish through Dot-ELISA.

Discussion

E. tarda has not been previously reported to be pathogenic for *A. testudineus*. Using an *E. tarda* isolate from channel catfish, Meyer and Bullock (1973) reported that no deaths occurred in brown trout held in 13°C water after i.p. injection with 8×10^7 bacterial cells. However, in this study, *A. testudineus* showed mortality with *E. tarda* isolate from *Clarias batrachus* when injected intraperitoneally with a similar dose of bacterial cells. This isolate was also pathogenic to *Labeo rohita* (Sahoo 2000).

Miyazaki and Egusa (1976a, 1976b), who described two forms of diseases in eels *Anguilla japonica* due to *E. tarda*; i.e. suppurative interstitial nephritis and suppurative hepatitis-reported nephritis to be more common. They considered the initial lesions in both forms to be bacte-

ria-laden phagocytic cells in the sinusoids. However, these lesions were poorly noticed in infected *A. testudineus*. This might have been due to the small size of the fish along with the acute course of the disease in experimental infections. The lesions in the internal organs of *A. testudineus* were hepatic necrosis, intravascular thrombus, myocardopathy and enteritis that were also reported in *Clarias batrachus* (Sahoo et al. 1998). The ulcers at the injected site observed in tilapia and *Clarias* sp. (Kaige et al. 1986; Sahoo et al. 1998) were not seen here. However, the congestion and scale erosion observed around the injection site at the ventral part before death along with LD₅₀ value of 10^{7.8} indicated the probable virulent nature of the organism. Kaige et al. (1986) observed similar types of cardiac lesions as seen in our case. Thrombosis in the hepatic veins along with other associated lesions proved the septicaemic nature of the organism. The changes observed here might be due to the release of enterotoxin, cytotoxin, hemolysin and dermatotoxins by the bacterium as discussed by Thune et al. (1993).

The presence of *E. tarda* specific antigen in sensitive organs like liver, kidney and spleen as detected through Dot-ELISA along with the marked histopathological changes suggest the susceptibility of this species to *E. tarda* infection. Therefore, care must be taken prior to composite culture of *A. testudineus* along with other susceptible carrier fish like *Clarias* sp.

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