

An Outbreak of *Aeromonas hydrophila* Infection in Turtles (*Pseudemis scripta*)

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An outbreak of *Aeromonas hydrophila* infection with a high rate of mortality (95%) in turtles (*Pseudemis scripta*) in Italy is reported. Pure cultures of the pathogen were isolated from liver, lung, kidney, and heart specimens of the turtles. The *A. hydrophila* isolate was resistant to amoxicillin, ampicillin, cephalothin, and trimethoprim-sulfamethoxazole but was sensitive to a number of other antibiotics tested. The study indicates that pet turtles can act as reservoirs of this pathogen and may play an important role in the etiology of *Aeromonas*-associated human infections.

Aeromonas species have long been known to cause different types of infections in poikilothermic animals such as fishes, reptiles, and amphibians (2, 5, 13). These pathogens have also been associated with several categories of human infections such as gastroenteritis, peritonitis, endocarditis, meningitis, septicemia, urinary tract infections, and wound infections (6, 12, 15), while epizootic forms of *Aeromonas hydrophila* infection have been reported to occur in fishes as well as in frogs (9, 13). A case of septicemia in a Canadian beaver caused by *A. hydrophila* has also been reported (14). In this report, we describe an outbreak of *A. hydrophila* infection in turtles (*Pseudemis scripta*) from southern Italy.

In July 1993, a severe outbreak of infection occurred in turtles (illegally imported to Italy from an unknown country) in a pet shop in Naples, Italy. In 10 days, 95 out of 100 animals died of acute infection. During the infection, the animals were apathetic, moved very slowly, and refused food.

Gross pathological findings at the time of autopsy of the 21 turtles which were examined were minimal and limited to general congestion of the internal organs. Tissue samples from four organs, namely, the liver, lungs, kidneys, and heart, of each of the 21 turtles were taken aseptically with sterile instruments. The tissue samples were separately homogenized under aseptic conditions and later inoculated into tubes containing sterile brain heart infusion (BHI) broth (Difco, Detroit, Mich.). These BHI culture tubes were incubated overnight at 37°C. The material from the BHI broth cultures was streaked with an inoculating loop onto blood agar (5% sheep erythrocytes in BHI agar) and incubated overnight at 37°C. Among the bacteria from the tissue samples, only a single pure culture of beta-hemolytic colonies was present on blood agar plates (8). For further examination, three to five colonies were randomly selected from each blood agar plate. The bacteria in each of these colonies were further identified as gram-negative rods which were motile, oxidase positive, glucose fermenting, and O/129 resistant and were thereby considered presumptive aeromonads (8, 11).

Further identification of presumptive aeromonads (84 isolates, i.e., one from each organ sample from the 21 turtles) was performed by the API ID 32 GN assay (API System, Marcy l'Étoile, France). They showed identical biochemical profiles in

TABLE 1. Biochemical profiles^a and other properties of *A. hydrophila* isolated from turtles (*P. scripta*)

Substrate or property	Reaction
Rhamnose	-
D-Melibiose	-
Propionate	-
Citrate	-
5-Ketogluconate	-
3-Hydroxybenzoate	-
3-Hydroxybutyrate	-
L-Serine	+
Salicin	+
D-Ribose	+
D-Saccharose	-
Itaconate	-
Malonate	-
DL-Lactate	+
Mannitol	+
D-Sorbitol	-
L-Arabinose	+
Caprate	+
Histidine	+
Glycogen	+
2-Ketogluconate	-
4-Hydroxybenzoate	-
L-Proline	+
N-Acetylglucosamine	+
Inositol	-
Maltose	+
Suberate	-
Acetate	+
L-Alanine	+
D-Glucose	+
Oxidase	+
Motility	+
O/129 resistance	+
Beta-hemolysins	+

^a As determined by the API ID 32 GN assay.

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TABLE 2. Susceptibility of *A. hydrophila* to various antimicrobial agents

Antibiotic	MIC ($\mu\text{g/ml}$)	Result ^a
Amikacin	<16	S
Amoxicillin-clavulanic acid	>32	R
Ampicillin	>32	R
Ampicillin-sulbactam	>16	R
Aztreonam	<8	S
Cefamandole	<8	S
Cefotaxime	<8	S
Cefotetan	<16	S
Cefoxitin	<8	S
Ceftazidime	<8	S
Ceftizoxime	<8	S
Ceftriaxone	<8	S
Cephalothin	>32	R
Ciprofloxacin	<1	S
Chloramphenicol	<8	S
Gentamicin	<4	S
Imipenem	8	I
Netilmicin	>4	S
Ofloxacin	<2	S
Pefloxacin	<2	S
Piperacillin	<16	S
Tetracycline	<4	S
Tobramycin	<4	S
Trimethoprim-sulfamethoxazole	>0.5	R

^a R, resistant; S, susceptible; I, intermediate.

this system and were confirmed as *A. hydrophila* (Table 1) (8, 11). The susceptibility of the isolates to different antibiotics was tested by the Sceptor system (Becton Dickinson), and the different isolates yielded identical results (Table 2).

Microbiological identification of bacteria isolated from the internal organs of the turtles thus showed the presence of single pure cultures of beta-hemolytic *A. hydrophila*. All 84 *A. hydrophila* isolates from organs of different turtles were resistant to amoxicillin, ampicillin, cephalothin, and trimethoprim-sulfamethoxazole but susceptible to the rest of the antibiotics used, except for imipenem, against which the isolates yielded intermediate sensitivity results (Table 2).

A. hydrophila has been shown to cause a variety of infections in humans and other mammals as well as reptiles and fishes. We recently reported a case of human septicemia attributed to *A. hydrophila* infection (7). On the other hand, it has been shown that the immediate cause of death in the majority of cases of *A. hydrophila* infection in frogs is septicemia (4). Similarly, motile *Aeromonas* septicemia in fishes and its synonymous diseases hemorrhagic septicemia, infectious dropsy, and red sore disease have frequently been reported (2). In this report, we describe an outbreak of *A. hydrophila* septicemia in turtles in which 95 out of 100 animals died during a period of 10 days.

It is well known that there are two forms of *Aeromonas* infections in fishes and frogs: (i) the acute form, with a very high mortality rate, and (ii) the chronic form, with a low mortality rate and variable clinical signs of disease depending on the form of infection. It seems that in the outbreak reported here, the turtles suffered from the acute form of infection, since it resulted in a mortality rate of 95%. The ubiquitous nature of *A. hydrophila* in the environment, especially the aquatic environment, suggests that the disease was derived from an exogenous source. Unfortunately, no microbiological examination of water samples from the aquarium in which the turtles were living was performed.

The results of the bacteriological analysis of the internal organs showed pure cultures of beta-hemolytic *A. hydrophila*; some investigators consider beta-hemolysis to be a virulence factor of this species (1). In recent years, some of the motile *Aeromonas* species have been recognized as primary human pathogens (10), with the most common infections caused by these species being gastrointestinal, extraintestinal, and wound infections (6, 15). In particular, young children, elderly persons, and immunocompromised patients are vulnerable to such infections (6, 12, 15). It is in such cases that *Aeromonas* infections may lead to very high rates of mortality (6).

Since *A. hydrophila* has now been recognized worldwide as a human pathogen, and since turtles (*P. scripta*) are becoming more popular as household pets (3), it is highly probable that children who are often in contact with pet turtles run a risk of infection with *A. hydrophila* if the turtles are infected by this pathogen. This report indicates that turtles infected with *A. hydrophila*, while acting as reservoirs for these pathogens, may well play an important role in the etiology of *Aeromonas*-associated human infections that is similar to their role in cases of pet turtle-associated *Salmonella* infections in children (16).

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