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An Outbreak of *Aeromonas salmonicida* Infection of Ayu *(Plecoglossus altivelis)* in Taiwan

W.-S. WANG, D.-H. WANG and J.-S. LEE

Department of Veterinary Medicine
National Chung Hsing University
Taichung, Taiwan 403

Abstract - We report possibly the first case of *Aeromonas salmonicida* infection of ayu (*Plecoglossus altivelis*) in Taiwan. The biological characteristics of the isolate indicate that it was 'atypical' *A. salmonicida*. Antimicrobial sensitivities indicate that the isolate is sensitive to most antimicrobial agents, except sulfasoxazole and nitrofurantoin.

In April 1994, a severe outbreak of infection occurred in a cultured ayu (*Plecoglossus altivelis*) farm in central Taiwan. In 4 d, 8,000 of 10,000 fish died. The infected fish showed darkening, lethargy and loss of appetite. Gross patho-logical findings during the autopsy of 20 fish showed petechiation at the fin bases, and sometimes gross swelling (furuncles) (Fig. 1). In some cases, ulcers
at the fin bases developed to release necrotic tissue debris and bacteria. Hemorrhage around the eyeball was found on some infected fish (Fig. 2). The gills of infected fish were pale. Internally, blood vessels surrounding the lower intestines were conjested. The liver was swollen and cherry-red (Fig. 3), while the spleen was gray. Petechiae were common on all serosal surfaces. In some cases, the swim bladder was swollen and cloudy. The kidney was convex and appeared to be liquefied. The peritoneum and pericardium commonly had accumulations of bloody fluid.

Fig. 1. Petechiation at fin bases and sometimes gross swelling.

Tissue samples taken aseptically from four locations including surface lesions, heart, kidney and spleen, in each of 20 infected fish were cultured on tryptone soya agar (TSA) and blood agar (5% sheep erythrocytes in brain heart infusion agar). All agar plates were incubated at 28°C for 2 d. Among bacteria from the tissue samples, only a single pure culture of β-hemolytic colonies

Fig. 2. Hemorrhage around the eye ball.

was present on blood agar plates. Three to five colonies were randomly selected from each blood agar plate for further examination. The bacteria in each colony were identified as non-motile gram-negative rods that failed to grow at 37°C. They were positive for catalase, glucose fermentation, acid production from sucrose and xylose, degradation of gelatin, starch and arginine dihydrolase. The organism was negative for urea, gluconate oxidation, ornithine decarboxylase production. The bacteria were resistant to 0/129. Although the bacteria isolated from TSA were positive for oxidase, they did not produce

Fig. 3. The cherry-red, swollen liver.

brown pigment at 28°C (Table 1). According to *Bergey's Manual of Systematic Bacteriology* (Krieg and Holt 1984), the organism was consistent with the characteristics of 'atypical' *Aeromonas salmonicida.*

When the isolated bacterium was inoculated intraperitoneally into healthy ayus by 10^5 ml^{-1} (McCarthy 1983), these fish showed the same signs as the natural outbreak after 3-4 d. The same bacterium was isolated from the experimentally infected fish.

The sensitivity of *A. salmonicida* to several antibacterial agents was tested according to the disk diffusion method. The results revealed that most agents such as chloramphenicol, tetracycline and nalidixic acid inhibited growth of the isolate; however, the isolate was resistant to nitrofurantoin, sulfasoxazole and SXT-25 (Table 2).

A. salmonicida have long been known to cause furunculosis in all species of salmonids (Austin and Austin 1987; Inglis et al. 1993). The disease has not

Table 1. The biological characteristics of the isolate.

Biology characteristics tests	The isolate	*Aeromonas salmonicida*[1]
Gram stain	-	-
Morphology	rod	rod
Growth at 37°C	-	-
Growth at 28°C	+	-
Growth at 25°C	+	+
Motile	-	-
Pigment production	-	+
Indole	+	-
Cytochrome oxidase	+	+
ONPG	+	-
Catalase	+	+
Oxidation/fermentation glucose	O/F	O/F
Sucrose	+	-
VP	+	-
Gelatinase	+	+
Urea	-	-
Arginine	+	+
Ornithine	-	-
Gluconate oxidase	-	-
0/129	resistant	resistant

[1]adapted from *Bergey's Manual of Systematic Bacteriology* (Krieg and Holt 1984)
- negative reaction; + positive reaction

Table 2. Sensitivities of the isolate to several antibacterial agents.

Antibacterial agents	ug·disc^{-1}	Diameter of inhibition zone (mm)	Sensitivity
Chloramphenicol	30	22	++
Tetracycline	30	25	++
Novobiocin	30	23	+
Nalidixic acid	30	23	++
SXT-25	Sulfa (23.75)+ Trime (1.25)	-[a]	-[b]
Sulfsoxazole	300	-[a]	-[b]
Nitrofurantoin	30	-[a]	-[b]
0/129	150	8	-[b]

++ very sensitive;
+ sensitive
-[a] no inhibition zone
-[b] resistant

been reported in Taiwan. Although the clinical signs of infected ayu in Taiwan were distinct from those in Japan or other countries (Nakatsugawa 1994), this disease is worthy of attention in coldwater fish species, and even warmwater species, in Taiwan because trout, ayu and salmon are popular in high mountain areas in Taiwan. The case might be the first report of furunculosis caused by *A. salmonicida of* ayu in Taiwan.

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