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Disease outbreak in seafarmed Cobia (*Rachycentron canadum*) associated with *Vibrio* Spp., *Photobacterium damselae* ssp. *piscicida*, monogenean and myxosporean parasites

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Abstract

The first documented outbreak of monogenean and myxosporean parasites associated vibriosis and photobacteriosis in cobia (*Rachycentron canadum*) cultured in the Penghu islands of Taiwan is reported. Monogenean infested fish showed dark skin pigmentation, haemorrhage and severe ulcer on the head. Microbial analysis of these fish revealed the presence of *V. alginolyticus, V. vulnificus* and *V. parahaemolyticu*s. Internally affected fish with no apparent external indications showed paleness of liver and kidney and white tubercles in the spleen. A pure culture of bacteria isolated from these organs was biochemically charecterised as *Photobacterium damselae* ssp. *piscicida* (*Ph. d. piscicida*). Myxosporean parasite was found in the renal tubules.

Introduction

Cobia (*Rachycentron canadum*) has been selected as the target fish for the future cage culture industry in Taiwan and in many other tropical and subtropical countries, because of its excellent meat quality and fast growth rate (Su et al. 1999). Recently farmers are facing problems in cobia culture due to disease outbreak (Chen et al. 2001). In October 2000, an epizootic occurred in the cage-cultured juvenile cobia in Penghu Island, Taiwan. Histopathological, bacteriological and experimental infection studies provided substantial evidence that *Vibrio alginolyticus* was the causal agent of the epizootic (Rajan et al. 2001).

In February 2001, another epizootic occurred in the same place but with different signs of infection. The observations described in this report are based on bacteriological and histopathological studies.

Materials and Methods

Clinical History

Infected cobia exhibited ulcer on the head (Fig. 1), lesion in the oral cavity, erosion in the operculum and gill and haemorrhage in the tail and ventral side of the fish. Behavioural changes such as reduced feeding and frequent surfacing were observed. Macroscopic observation of the infected fish revealed the presence of monogenean ectoparasites (*Neobenedenia* sp.) near the ulcers on the head

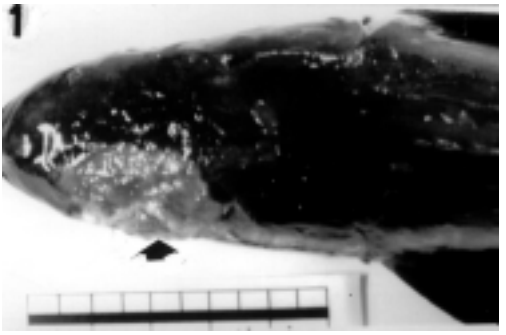

Fig. 1. Photograph of Cobia (*Rachycentron canadum*) showing severe ulcer on the head and eye (arrow).

(Fig.2). Around 15-20 parasites were found on each fish. Ulcers were located on the head but were absent in other parts of the body. In the previous epizootic (October 2000) ulcers were present all over the body except the head and there was no ectoparasite. After the macroscopic observation, samples of all organs were subjected to bacteriological and histological studies. During dissection of fish white focal lesions were observed in the liver, kidney and spleen (Fig. 3). The cumulative mortality of this epizootic was 40%.

Bacteriology

Samples taken from the ulcer, haemorrhage, gill, liver, kidney and spleen of moribund cobia were cultured on tryptic soy agar and brain-heart infusion agar with 1.5% NaCl

Fig. 3. Gross pathology of the infected fish showing whitish tubercles in the spleen (arrow).

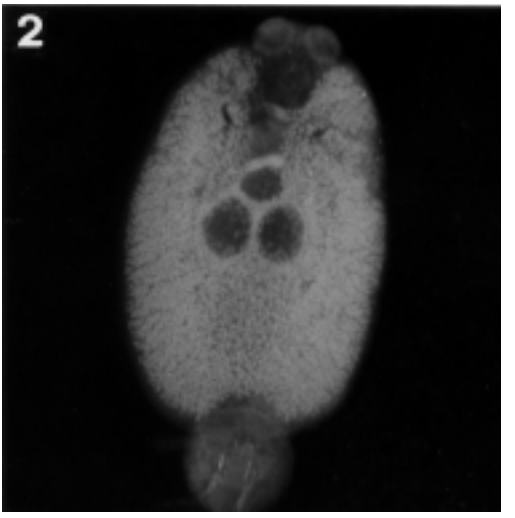

Fig. 2. Photograph showing *Neobenedenia* sp. (monogenean ectoparasite) isolated from cobia (*Rachycentron canadum*).

added. Pure cultures of the isolated colonies were subjected to taxonomic studies using standard morphological, physiological and biochemical plates following the procedures of Fouz et al. (1990) and West and Colwell (1984). For rapid diagnosis the commercial miniaturized API 20E system was used in parallel. Bacteria isolated from the surface of skin, ulcer, gill and lesion of the infected fish were found to grow on the TCBS plate supplemented with 1% NaCl in yellow and green colour. Colonies were circular with an entire margin and convex after 24h incubation at 23°C. Gram's stain revealed it to be Gram-negative rod-shaped bacteria. It was halophilic and grew at 37°C. Biochemical analysis of the bacteria using API 20E showed the yellow colony bacteria in TCBS isolated from the surface of skin, ulcer, gill and lesion to be *Vibrio alginolyticus* and the green colonies in TCBS isolated from the lesion to be *V. vulnificus* and *V. parahaemolyticus* (Table 1). A pure culture of bacteria isolated from the liver,

Test	Bacterial isolates			
	a	b	c	d
Gram	-	-	-	-
motility	-	-	-	-
swarming on TSA	-	-	-	-
growth on TCBS	Y	G	G	-
Sensitivity to 0/129(150um)	+	+	+	+
growth in 3% NaCl	+	+	+	+
growth in 6% NaCl	-	+	+	-
growth in 8% NaCl	-	-	-	-
growth at 25°C	+	+	+	+
growth at 37°C	+	+	+	-
oxidase	+	+	+	+
catalase	-	-	-	-
nitrate reductase	+	+	+	-
ß-galactosidase	-	+	-	-
Arginine dihydrolase	-	-	-	+
Lysine decarboxylase	+	+	+	-
ornithine decarboxylase	-	+	+	-
citrate	-	-	-	-
production of H_2S	-	-	-	-
urease	-	-	-	-
tryptophan deaminase	-	-	-	-
indol	+	+	+	-
Voges-Proskauer	-	+	-	+
gelatinase	+	+	+	-
glucose	+	+	+	+
mannitol	+	-	+	-
inositol	-	-	-	-
sorbitol	-	-	-	-
rhamnose	-	-	-	-
sucrose	+	-	-	-
melibiose	-	-	-	-
amygdalin	+	+	+	-
arabinose	-	-	+	-

Table 1. Biochemical and morphological characterization of the bacterial isolates from infected cobia *V. alginolyticus* (a), *V. vulnificus* (b), *V. parahaemolyticus* (c) & *Ph. d. piscicida* (d). TSA: tryptic soy agar, TCBS: thiosulfate citrate bile salt sucrose agar; 0/129: 0-nitrophenyl-b-D-galactopyranoside. Symbols: Y yellow colour colonies, G green colour colonies, + positive reaction, - negative strain

kidney and spleen was shiny, raised, entire, translucent, slightly viscid and grew in TSA and BHI agar. No growth was observed in TCBS plates. Based these criteria and biochemical characterization it was identified as *Ph. d. piscicida* (Table 1).

Drug resistance patterns of *V. alginolyticus*, *V. vulnificus*, *V. parahaemolyticus* and *Ph. d. piscicida* isolated from cobia and the control strains, *V. anguillarum* (CCRC 12908) and *Ph. d. piscicida* (CCRC 17065) were tested for different antibiotics by disc diffusion by Muller-Hinton agar (bioMerieux) (Table. 2). For treatment of this outbreak, oxytetracycline (75mg/kg fish/day) and nalidixic acid (40mg/kg fish/day) were mixed with dry pellet feed as a dose for 7 days. The fish were also dipped in fresh water for 2 minutes to detach the monogeneans. Mortality was controlled after these treatments.

Histopathology

Tissues were fixed in 10% seawater formalin and paraffin sections were stained by haematoxylin-eosin (H&E). The histological study showed the presence of bacteria in gill, liver, spleen and kidney. *Ph. d. piscicida*- infected fish showed chronic lesions in the spleen (Fig. 4) and kidney. Sporogonic stages of a protozoan parasite, *Sphaerospora*-like myxosporidean were detected in the lumen of the renal tubules of kidney (Fig. 5). In few cases, intracellular stages of myxosporidian were also observed within the tubule epithelium. The intensity of this parasite was low and the damage was also not obvious in the kidney tissue.

Antibiotic	μg/disc	Mean zone diameter (mm)					
		a	b	c	d	e	f
Amoxycillin	25	0	0	0	0	12	34
Ampicillin	10	9	0	0	0	22	34
Enrofloxacin	5	27	18	34	30	34	36
Flumequine	5	24	24	22	34	40	40
Furazolidone	50	22	17	16	22	17	14
Kanamycin	30	9	10	9	11	10	0
Nalidixic acid	30	21	22	21	26	32	34
Oxolinic acid	30	20	18	17	20	32	30
Oxytetracycline	30	27	25	24	0	24	34
Trimethoprim/Sulphamethoxazole	25	20	22	22	24	25	32

Table 2. Antibiotic susceptibility profile of the isolates *V. alginolyticus* (a), V. *vulnificus* (b), *V. parahaemolyticus* (c) and *Ph. d. piscicida* (d) from cobia and the control strains {*V. anguillarum*, CCRC 12908 (e) & *Ph. d. piscicida*, CCRC 17065 (f)}.

Discussion

In recent years, myxosporean parasites have been reported as pathogens for cultured marine fish (Alvarez-Pellitero & Sitjà-Bobadilla, 1993; Branson et al. 1999; Chen et al. 2001; Diamant et al. 1994; Lom & Dyková, 1995). Chen et al. (2001) reported cobia mortality in Penghu Island associated with *Sphaerospora*-

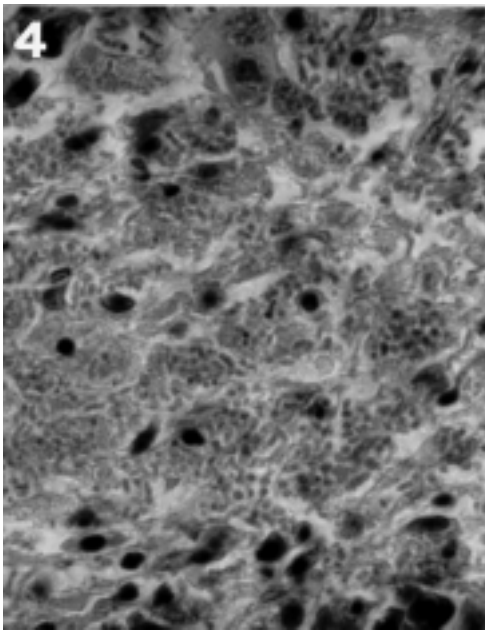

Fig. 4. Spleen of cobia showing acute multifocal necrosis with large masses of bacteria (1000X).

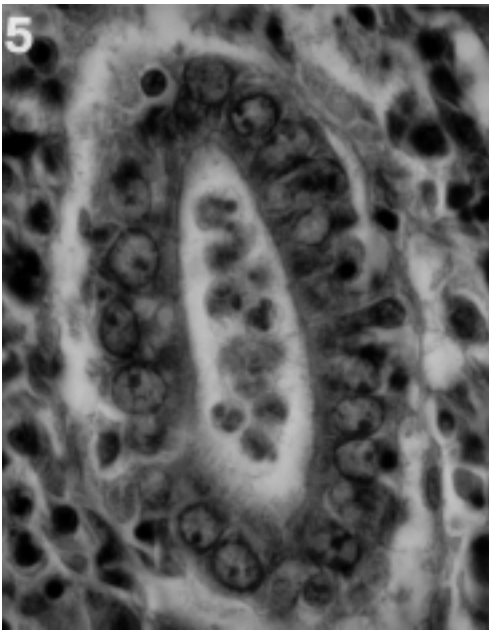

Fig. 5. Kidney of cobia showing *Sphaerospora*-like myxosporidean in the renal tubule (1000X).

like myxosporidean but they did not isolate any bacteria from the infected fish. In the contrary, in our study *V. alginolyticus*, *V. vulnificus*, *V. parahaemolyticus* and *Ph. d. piscicida* were isolated. *Vibrio alginolyticus* and *V. vulnificus* isolated in this epizootic were found to be different biochemically and in the antibiotic profile from the previous epizootic reported by Rajan et al. (2001). The intensity of the protozoan parasite was low and the tissue damage in the kidney was less when compared with the previous report. In this epizootic the bacteria could be the causative agent for the mortality. Paperna and Overstreet (1981) reported the secondary bacterial infection associated with ectoparasite as the cause of fish mortality. Ho (2001) reported the monogenean ectoparasite, *Neobenedenia* sp. infection in cobia from Taiwan. In this epizootic we found *Neobenedenia* sp. on the head near the ulcers. The ectoparasite could have caused skin irritation and damage in the fish. Bacteria could have entered into the fish through the damaged skin and caused ulcers in the skin. However, the factors responsible for photobacteriosis in cobia and its mode of transmission are unknown. Pathology of various organs was consistent with photobacteriosis (pasteurellosis) and vibriosis described in other fish species (Bakopoulos et al., 1997; Jones & Cox, 1999; Fouz et al., 2000; Toranzo et al., 1991). Experimental infection studies are required to prove the Koch's postulate.

Acknowledgement

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