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NOTE

Mortalities in red claw crayfish *Cherax quadricarinatus* associated with systemic *Vibrio mimicus* infection

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ABSTRACT: Two cases of mortalities in cultured red claw crayfish *Cherax quadricarinatus* associated with systemic *Vibrio mimicus* infections are described. In these cases, *V. mimicus* appears to have been an opportunistic pathogen following stress caused by either overcrowding or mismanagement and poor water quality. Humans consuming raw or improperly cooked infected crayfish could be at risk of contracting gastrointestinal disease.

KEY WORDS: Red claw · *Cherax quadricarinatus* Crayfish · *Vibrio mimicus* · Vibriosis

The red claw crayfish *Cherax quadricarinatus* is an Australian tropical freshwater crayfish belonging to the family Parastacidae, its habitats varying from shallow clear-flowing creeks to deep turbid clay-based water holes (Rouse et al. 1991). In Australia, it is found in watercourses ranging from the Daly River in the Darwin region of Northern Territory through the Gulf of Carpentaria rivers to the westerly flowing rivers of Cape York Peninsula (Morrissy et al. 1990). Red claw are highly suitable for intensive aquaculture in tropical and subtropical zones (Jones 1990). They tolerate low oxygen levels and a wide range of other water quality parameters including ammonia, nitrite, hardness, alkalinity, salinity and pH. In addition, they are not territorial, do not dig burrows, accept food items ranging from hay to formulated rations and are capable of breeding several times a year (Rouse et al. 1991). They have been cultured in Australia since 1985 and are now farmed in Queensland and northern New South Wales. To date, infectious disease has not caused significant mortalities in Australian crayfish. Stock losses have been primarily due to problems associated with site selection, water quality and management (Morrissy et al. 1990). This paper reports 2 cases of mortalities in cultured red claw in Australia associated with a systemic infection of *Vibrio mimicus*.

Materials and methods. Since 1989, sick or dead red claw from aquaculture operations have been submitted to our laboratory for disease diagnosis. Tissues for histological examination were fixed in Davidson's solution (ethanol 31.7%, formalin 22.2%, glacial acetic acid 11.1% in an aqueous solution). Sections (5 µm) were cut and stained with haemotoxylin and eosin. Selected sections were stained with Brown & Brenn's modification of the Gram stain (Lillie & Fuller 1976). Bacteriological culturing was done on Blood Agar (containing 5% sheep blood) and Trypticase Soy Agar plates (Becton Dickinson, Cockeysville, MD, USA) incubated aerobically at 25 °C. Following overnight incubation, single colonies were streaked onto another Blood Agar plate and incubated overnight. The following day, the cultures were checked for purity and an oxidase test done on them using Oxidase Reagent Droppers (Becton Dickinson). The cultures were subsequently inoculated into oxidation/fermentation media (Difco, Detroit, MI, USA) and into either the API 20E or the API 20NE identification system (Bio Merieux, Marcy-l'Etoile, France) for presumptive identification. Later, comprehensive identification of Vibrionaceae was made using a modification of tests described by Cowan (1974) as well as tests described by Furniss et al. (1978), Lee et al. (1979), Lee & Donovan (1985) and West & Colwell (1981). Identification was based on a range of tests selected by Bryant et al. (1986a) for the numerical classification of Vibrionaceae and further developed into a computer matrix for the probabilistic identification of species of Vibrionaceae (Bryant et al. 1986b).

Case histories. ***Case 1:*** In November 1989, 3 dead red claw from a commercial crayfish enterprise in northern New South Wales containing a hatchery and ponds were frozen and submitted for examination. Upon draining the ponds, a large number of the excess

adults and juveniles were recovered and placed in holding tanks. Following mortalities in these congested tanks, overcrowding was reduced by the use of additional tanks. Despite this, further mortalities were observed. The submitted crayfish were necropsied and the haemolymph and hepatopancreas were sampled for bacteriological examination.

Case 2: In July 1990, 3 live red claw with carapace lengths ranging from 6.7 to 7.5 cm were submitted from a commercial crayfish farm in southeast Queensland. The farm had a history of low-level mortalities over a 2 yr period which increased to 20% when stocks were transferred to cement tanks. Heaviest losses occurred during hot weather and were initially observed following the addition of hydrated lime to the ponds to raise the pH. Affected crayfish were unable to walk and appeared to be stuck in the residue on the bottom of the ponds. Many had a white powdery coating over their bodies. Two of the submitted crayfish had blistering on the end of the telson, and one of them also had blisters on the uropod. The third crayfish exhibited erosion at the end of the telson. Subsequently, alterations were made in the management practices. Lime was added to a holding dam from which water was drawn to fill the ponds and the residue was cleared from the bottom of drained ponds. Following these changes, mortalities ceased. Haemolymph from the heart of each submitted crayfish was cultured bacteriologically.

Results. ***Case 1:*** A mixed culture of *Escherichia coli* and *Enterobacter intermedium* was isolated from the haemolymph, and *Aeromonas hydrophila* and *Citrobacter freundii* from the hepatopancreas of 1 crayfish. The haemolymph and hepatopancreas of the other 2 crayfish yielded pure cultures of an organism initially identified as *Vibrio cholerae* by the API 20NE. The isolates did not agglutinate *V. cholerae*:01 polyvalent antiserum and were presumptively called *V. cholerae* non:01. However, on comprehensive characterisation, these isolates were subsequently identified as *V. mimicus*. Histologically, there was severe cellular disruption due to freezing in the ovary and hepatopancreas. However, the heart of one of the crayfish yielding a pure culture of *V. mimicus* exhibited severe inflammation of the pericardium, and foci of small Gram-negative rods were present in the lesion.

Case 2: The haemolymph of each crayfish yielded a pure culture of a Gram-negative organism identified as *Vibrio mimicus* using API 20E test kits. The isolates were not agglutinated by *V. cholerae*:01 polyvalent antiserum and were confirmed as *V. mimicus* following further comprehensive characterisation. On histology, varying degrees of inflammation, indicative of bacterial septicaemia, in the gills and hearts, and also in the antennal gland of 1 crayfish, were observed. The integument of the tails exhibited epithelial necrosis and cuticular erosion with evidence of bacterial invasion.

Discussion. *Vibrio mimicus* was first described by Davis et al. (1981) as a pathogenic species of the genus comprising sucrose negative variants of biochemically atypical strains of *V. cholerae*. Phenotypic and biochemical properties of the 5 *V. mimicus* isolates described here were identical, conforming with the original species description except for their lipolytic ability as they hydrolysed both Tween 20 and Tween 80 (Table 1). However, this difference could be due to the fact that Davis et al. (1981) used corn oil as their lipid substrate rather than the Tween compounds used in this study. This is supported by the positive lipase reactions reported for *V. mimicus* by Bryant et al. (1986b) and Lupiani et al. (1993) where Tween compounds were used as the lipid substrates. However, the isolates differed from the descriptions of both Bryant et al. (1986b) and Lupiani et al. (1993) in their alginase activity and their inability to use Mannose as a sole carbon substrate (Table 1). The API 20NE system does not have *V. mimicus* included in its data base and thus it identified the isolates in Case 1 as *V. cholerae*. In addition, it does not test for carbohydrate fermentation, negating differentiation of *V. cholerae* and *V. mimicus* by sucrose fermentation.

Vibrio mimicus occurs in seawater and shellfish, and in humans, and has been associated with gastroenteritis following ingestion of seafood and with ear infection after exposure to sea water (Ciufecu et al. 1983, Shandera et al. 1983). It has also been found both in brackish and freshwater environments (Bockemühl et al. 1986, Chowdhury et al. 1989), and was isolated from the gills and from under the carapace of freshwater prawns *Macrobrachium malcolmsonii* by Chowdhury et al. (1986). *V. mimicus* would thus appear to be part of the normal bacterial flora of the aquatic environment in aquaculture ponds. As the crayfish in both cases described in this report were fed a diet of commercial chicken pellets, the water would appear to be the most likely source of the *V. mimicus* causing the disease outbreaks.

The significance of the blisters observed on the telson and uropod of crayfish in the second case reported here remains uncertain. Herbert (1987) reported that blisters on the uropods and telson of *Cherax* spp. most commonly occurred following introduction into vinyl-lined swimming pools, concrete tanks or stainless steel troughs. They considered them to be the result of irritation as no deaths could be solely attributed to their presence.

Bang (1970) considered that the haemolymph of healthy crustaceans was sterile and that the presence of bacteria in the circulatory system was a sign of

Table 1. *Vibrio mimicus*. Phenotypic characteristics of isolates (5 strains, 2 from Case 1 and 3 from Case 2) compared with other published results. +: 86 to 100% of strains positive; v: 16 to 85% of strains positive; –: 0 to 15% of strains positive; nd: not done

Characteristic Test	This study	Bryant et al. (1986b)	Davis et al. (1981)	Kämpfer et al. (1987)	Lupiani et al. (1993)
Motility	+	+	+	nd	+
Growth					
0% NaCl	+	+	+	nd	+
6% NaCl	–	nd	v	nd	+
Decarboxylation					
Arginine	–	–	–	–	–
Lysine	+	+	+	+	–
Ornithine	+	+	+	+	v
Nitrate reduction	+	+	+	nd	+
Oxidase	+	+	+	+	+
Glucose fermentation / (gas production)	+/(–)	+/(–)	+/(–)	+/(nd)	+/(–)
Indole	+	+	+	+	+
Ortho-nitrophenyl-β-D-galactopyranoside	+	+	+	+	nd
Vosges-Proskauer	–	v	–	v	–
Resistance					
O/129; 10 µg, 150 µg	–[a]	–[a]	–[b]	nd[a]	nd[a]
Ampicillin, 10 µg	–	–	–	nd	+
Polymyxin B, 50 IU	–	–	–	nd	nd
Hydrolysis					
Alginate	+	–	nd	nd	–
Gelatin	+	+	+	nd	+
Lecithin	+	+	nd	nd	nd
Starch	–	–	nd	nd	+
Tween 20, Tween 80[c]	+[a]	+[a]	–[d]	nd[a]	+[a]
Urea	–	–	–	nd	–
Haemolysis					
Sheep Blood Agar	+	+	nd	nd	+
Fermentation					
Sucrose	–	–	–	nd	–
Arabinose	–	–	–	nd	–
Salicin	–	–	–	nd	nd
Inositol, sorbitol	–[a]	–[a]	–[a]	–[a]	–[a]
Mannitol, trehalose, mannose	+[a]	+[a]	+[a]	nd[a]	+[a]
Carbon substrate utilisation					
L-arabinose	–	–	nd	v	nd
Cellobiose	–	v	nd	v	nd
Fructose	–	+	nd	v	nd
Glucose	+	+	nd	v	nd
Mannose	–	+	nd	+	nd
Maltose	+	+	nd	+	nd
Sucrose	–	v	nd	v	nd
Trehalose	+	+	nd	+	nd
Xylose	–	–	nd	–	nd
Ethanol	–	–	nd	nd	nd
Glycerol	+	+	nd	nd	nd
Inositol	–	–	nd	–	nd
D-mannitol	+	nd	nd	v	nd
Sorbitol	–	v	nd	–	nd
D-galacturonate	–	–	nd	–	nd
Gluconate	+	+	nd	+	nd
D-glucuronate	+	+	nd	nd	nd
L-hydroxyproline	–	–	nd	–	nd
D-glucosamine	+	+	nd	nd	nd
DL-3-hydroxybutyrate	–	–	nd	–	nd
Malonate	–	–	nd	nd	nd
Pyruvate	+	nd	nd	+	nd
Putrescein	–	nd	nd	–	nd

[a] Represents the result for each substrate in the group
[b] The concentration of O/129 tested was not determined
[c] Lipolytic activity was determined from the hydrolysis of Tween 20 and Tween 80
[d] Davis et al. (1981) determined lipolytic activity using corn oil as a substrate

disease. Scott & Thune (1986) cultured an array of bacteria of the genera *Acinetobacter, Aeromonas, Arthrobacter, Bacillus, Corynebacterium, Flavobacterium, Pseudomonas* and *Vibrio* from the haemolymph of apparently healthy red swamp crawfish *Procambarus clarkii* exposed to temperatures exceeding 24 °C. Below this temperature the prevalence of bacteria was low, but at temperatures above 28 °C the prevalence and numbers of bacteria increased significantly. They felt that the stress associated with low dissolved oxygen and extremes of temperature affected the crawfish's ability to control bacterial growth in the haemolymph. Subsequently, Thune et al. (1991) reported a bacterial septicaemia in the red swamp crawfish associated with infection with a single bacterial species identified as either *Vibrio mimicus* or *V. cholerae*. Crawfish from 13 of the 15 cases investigated had been held at temperatures of 25 °C or higher, with low dissolved oxygen concentrations recorded in most cases. In the cases reported here, *V. mimicus* appeared to be an opportunistic pathogen causing a systemic infection following the stress of overcrowding or mismanagement and poor water quality. The isolation of *V. mimicus* in pure culture from crayfish haemolymph in a number of unrelated disease outbreaks, and from different countries, highlights the pathogenicity of this bacterium for freshwater crayfish.

Chowdhury et al. (1986) found that most isolates of *Vibrio mimicus* from freshwater prawns in Bangladesh were enterotoxigenic, and thus a potential health hazard, particularly for individuals eating uncooked prawns. Although there are as yet no reported cases of gastrointestinal disease in humans following crayfish consumption, it would appear possible that improper cooking of affected crayfish could present a risk for humans.

None of the isolates was serotypable when tested with a range of antisera to 30 *Vibrio cholerae* non:01 isolates of human origin by Dr P. M. Desmarchalier (Tropical Health Program, University of Queensland). However, Dr Desmarchalier and colleagues were able to demonstrate that 1 isolate from each disease outbreak exhibited the same ribotype pattern when the total DNA was digested with the enzyme *Bgl* I and hybridised with a DNA probe complimentary to 16s and 23s rRNA of *Escherichia coli*.

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