Isolation of *Vibrio parahaemolyticus* from Salt Springs in Florida

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*Vibrio parahaemolyticus* was isolated from various locations in two salt springs of Florida and appears to be a normal inhabitant of these artesian waters.

The distribution of *Vibrio parahaemolyticus* in the marine environment and on seafoods has been described (1, 4). However, the isolation of this pathogenic halophile has never been reported from an area not directly influenced by the sea. With a number of springs in Florida, some of which are notably salty (2), it seemed possible that these environments could harbor this organism.

The salt contained in these springs probably results from either the flushing of salt entrapped in the underlying rock formation or from salt water that became trapped in Pliocene, or older, rock beds when the sea level in Florida was at a much higher level than at the present time (2, 6) and not from a current encroachment of sea water.

The two springs selected for this study were Salt Springs in Marion County and Warm Mineral Springs in Sarasota County, Fla. Salt Springs (2), located 45 km northeast of Ocala, Fla., represents a calcium bicarbonate water mixed with remnants of sea water or salt in the aquifer. The spring area is approximately 30 m in diameter, and the surrounding area is relatively undeveloped. The spring run flows for about 6 km, entering Lake George in the St. John's River Basin. Both fresh water and salt water fish may be found in or near the spring. Principle usage of the spring is for fishing and swimming. The water temperature is about 24 C, with a sodium chloride concentration of 0.25%.

Warm Mineral Springs (2) is located about 13 km northwest of Murdock, Fla. The spring is about 76 m in diameter, with the flow eventually emptying into the Myakka River. The spring water has a relatively high sulfate content from anhydrite in the water-bearing rock formations. The spring is used as a health spa. The water has a year-round temperature of about 30 C and a sodium chloride concentration of 1.57%.

For comparison, three freshwater springs were sampled. These were Rum, Fannin, and Ichataucknee. These springs had a sodium chloride content of less than 0.04%.

Isolation and identification of the isolates described was by recognized methods (3, 5). Samples of sediment, grass, and water were obtained from areas within the springs as well as from locations at the outfall. Most isolations were qualitative; when quantitated, enumeration was by the most-probable-number technique. Samples from Salt Springs were obtained in 10/72, 12/72, and 8/73; samples from Warm Mineral Springs were obtained in 5/73, and samples from the freshwater springs were obtained in 11/72. A total of 54 isolates was obtained from 47 samples. No isolates were obtained from the freshwater springs. Results of these investigations are given in Table 1.

The question that immediately arises from these data is how these organisms gain entrance to the springs. In the case of Warm Mineral Springs, there would be little opportunity for contaminated species to move up the run from the estuarine areas into the spring. In addition, the sample of well water examined was obtained from a pipe that extended 21 m below the surface of the water into the spring. In the case of Salt Springs, there is ample opportunity for seeding of the spring from external sources. Mullet and blue crab are common inhabitants of the spring, gaining entrance from the estuarine area by way of the St. John's River. However, *V. parahaemolyticus* was isolated directly from the upflowing area of the boil, an extremely forceful current against which to penetrate. It is therefore conceivable that these organisms are inhabitants of the underlying aquifer of the salt springs and are moved to the surface by these waters.

The three freshwater springs, which have as much opportunity for external contamination...
and seeding as the salt springs (because of their location), did not yield any isolates. This would indicate the inability of these springs to support the growth of *V. parahaemolyticus* because of their low salt content or, more likely, the fact that these organisms are absent from these waters.

Although only a limited number of isolates were serologically typed, all of them were subjected to the Kanagawa hemolysin test; a positive reaction is reportedly characteristic of pathogenic strains of these organisms (1). None was found to be positive, which was not unexpected in view of earlier reports (7) concerning the lack of this trait in sea water isolates. Until additional information is obtained concerning the significance of this test, little can be stated regarding the pathogenicity of these isolates.

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