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ISOLATION OF *Aeromonas hydrophila* FROM THE AMERICAN ALLIGATOR, *Alligator mississippiensis* [1]

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Abstract: Aeromonas hydrophila was isolated from the internal organs of nine adult alligators, *Alligator mississippiensis*, which died without apparent cause, suggesting the bacterium may have been a factor. One hundred and twenty-three alligators ranging in age from six months to over 10 years were captured from five locations in the southeastern United States and sampled for *A. hydrophila*. The bacterium was isolated from the oral cavity of 85% of the animals, on the external jaw area from over 50% and from 70% of the internal tissue samples. *A. hydrophila* is ubiquitous with alligators in their natural habitats, but apparently does not cause clinical disease. However, stress factors such as trapping, handling, and warm water temperatures may be conducive to the rapid proliferation of the bacteria, thereby facilitating disease.

INTRODUCTION

Aeromonas hydrophila is a pathogen for a wide range of freshwater fish, amphibians and reptiles.[1,4,8] It has been directly linked with massive fish and alligator kills in Lake Apopka, Florida,[9] and with epizootics of red-sore disease among a variety of fish species in North and South Carolina.[5]

Studies in Par Pond, a 1092-ha reservoir located on the Savannah River Plant near Aiken, South Carolina, indicate that *A. hydrophila* and red-sore disease are related to temperature-induced stress within the largemouth bass *(Micropterus salmoides)* population.[5]

During the course of routine bacteriologic studies conducted in the spring and summer of 1975, nine Par Pond alligators died suddenly and without apparent cause.

At necropsy, *A. hydrophila* was isolated from the liver, lungs, and kidneys (Hazen *et al.*, unpubl.). The presence of *A. hydrophila* within the internal organs of these alligators, as well as the relatively high prevalence of red-sore disease within the Par Pond bass population, suggested that mortality of the alligators was related to *A. hydrophila* or was induced by it. Subsequent studies on the histopathology study of the livers, lungs and kidneys of these alligators, revealed damage similar to that induced by lytic toxins produced by *A. hydrophila* (Huizinga, pers. commun.). The sudden and unexpected mortality among the alligators together with the isolation of *A. hydrophila*, suggested that the

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bacterium was present in low densities but with a high degree of frequency within the internal organs of the alligators. Since *A. hydrophila* may require stress conditions to initiate an epizootic we suggest that the bacterium is present in or on the alligators and conditions of stress favor a rapid increase in *A. hydrophila*, contributing to host mortality. Such an hypothesis is in complete agreement with Dubos[3] who stated, "there are many situations in which the microbe is a constant and ubiquitous component of the environment but causes diseases only when some weakening of the patient by another factor allows infection to proceed unrestrained, at least for a while." The present report surveys external surfaces, oral cavity, and internal organs of alligators from widely scattered populations in the southeastern United States to determine whether, indeed, *A. hydrophila* were commonly present.

MATERIALS AND METHODS

Numerous alligators (123) ranging in age from six months to greater than 10 years were sampled for *A. hydrophila* using aseptic techniques. Samples were taken, using sterile cotton applicators, on the abdomen and/or jaws of the animal, along the oral cavity and inside the mouth. Tissue samples, including lung, heart, liver, kidney, and intestine, were aseptically excised from recently killed animals and placed in sterile containers until processed. Internal organs normally were sampled immediately after death; however, several Par Pond animals were not sampled for 8 h. Tissue samples were weighed, suspended in 25 ml sterile 0.1M phosphate buffered saline (pH 7.2) and homogenized in a Vitro Tissue Homogenizer.[5] Aliquots of the suspension were filtered through sterile 0.45 μm Millipore filters.[6] Both swabs and filters were streaked or placed on Rimler-Shotts (RS) medium[10] and incubated aerobically at 35 C for 18-24 h. Presumptive isolates of *A. hydrophila* on RS medium were confirmed using API-20[7] identification system.

Alligators were obtained from Rockefeller Refuge, Louisiana; South Carolina coastal ponds; Okefenokee Swamp, Georgia; Orange Lake, Florida; and Par Pond, a reactor cooling reservoir in South Carolina. All animals were captured under federal government permits.

RESULTS AND DISCUSSION

The results of the study are given in Table 1. *A. hydrophila* was isolated from the lungs, hearts, livers, kidneys, and intestines on 16 of 23 (70%) of the alligators. *A. hydrophila* was isolated from the internal organs of 53% of the healthy alligators collected near Rockefeller Refuge. Additional samples were taken from the external surface of 39 live animals. Of the alligators captured in the wild, *A. hydrophila* was isolated from swabs taken outside of the oral cavity in 50% (35/70) of the animals, while 92% (12/13) of the animals hatched and raised in growth chambers also had the bacterium externally. *A. hydrophila* was isolated from the oral cavity of 85% (40/47) of the animals sampled in the wild. No data are available for growth chamber animals.

These data indicate that healthy alligators held and raised in captivity, or captured in the field, possess *A. hydrophila* on the mouth, in the oral cavity and in internal organs. Further, the high percentage of alligators positive for *A. hydrophila*, strongly suggests that

[5] Vernitron Medical Products, Inc., Empire Blvd. & Terminal La., Carlstadt, New Jersey 07072, USA.

[6] Millipore Corp., Asby Rd., Bedford, Massachusetts 01730, USA.

[7] Analytab Products, 200 Express St., Plainview, New York 11803, USA.

TABLE 1. Isolation of *A. hydrophila* from alligators captured in various locations in the Southeastern United States.

Location	Date	Age	Alligators positive for *A. hydrophila*/number sampled			Comments
			External	Oral Cavity	Tissue	
Par Pond	Spring/76	Adult[a]	8/8	ND[b]	8/8	Necropsy - intestines and lungs were sampled
Rockefeller[c]	9/23/76	Adult	ND	ND	8/15	Harvest,[d] lung, heart, liver, kidney tissue
Rockefeller[c]	9/24/76	Adult	2/16	ND	ND	Live, wild
Coastal, SC	10/14/76	Juv.[e]	18/22	ND	ND	Live, wild
Rockefeller	2/4/77	Juv., 3 yr	6/7	ND	ND	Live, growth chamber[f]
Rockefeller	2/4/77	Juv., 2 yr	3/3	ND	ND	Live, growth chamber
Rockefeller	2/4/77	Juv., 1 yr	3/3	ND	ND	Live, growth chamber
Coastal, SC	4/12/77	Juv.	2/9	9/9	ND	Live, wild
Coastal, SC	6/21/77	Juv.	3/9	10/11	ND	Live, wild
Okefenokee	7/10/77	Adult	2/6	2/4	ND	Live, wild
Coastal, SC	8/9/77	Juv.	ND	6/6	ND	Live, wild
Orange Lake	8/14/77	Juv., 1 yr	ND	13/17	ND	Live, clutch mates
		Totals	47/83	40/47	16/23	

[a]Adult = adult alligator (>6 feet long, 6 years or older).

[b]ND = Not Determined.

[c]Inadequate incubation facilities for accurate bacterial analyses probably resulted in a conservative number of positive samples.

[d]Harvest = animals killed in federally controlled alligator harvest.

[e]Juv. = juvenile alligator (<6 feet long, less than 6 year of age).

[f]Growth chamber = hatched and raised in growth chambers under controlled temperature conditions.

the bacterium may be ubiquitous among the sampled alligator populations.

While *A. hydrophila* may not cause disease under normal conditions, during periods of stress the bacterium could be a problem. Recent studies of *A. hydrophila* in other poikilothermal organisms indicate that a number of factors, i.e. stress and warm water temperatures, may have contributed to the mortality of Par Pond alligators in the spring and summer of 1975. Groberg *et al.*[6] demonstrated a positive correlation between water temperature and the probability of mortality in steelhead trout *(Salmo gairdneri)* inoculated with low numbers of *A. hydrophila*. They concluded that higher temperatures (20.5 C) favored rapid proliferation of the bacteria and subsequent host mortality. In this regard, mortality of Par Pond alligators occurred when water temperatures were in excess of 20 C, and when rapid reproduction of *A. hydrophila* was favored. However, mortality occurred only among those alligators subjected to handling and not among those which were not trapped or otherwise manipulated, suggesting that stress of handling, in addition to temperature, contributed to their death.

Based on these observations, we suggest that low densities of *A. hydrophila* in internal organs, coupled with high water temperatures and stress induced by extreme trapping procedures and handling, are sufficient to create physiologic conditions conducive to rapid proliferation of the bacteria. We also suggest that the increases in *A. hydrophila* within the internal organs result in the production of sufficient quantities of lytic toxins to induce histopathology characteristic of red-sore disease,[7] (Huizinga, pers. commun.) and subsequent mortality.

If *A. hydrophila*, in combination with stress, does indeed cause death, trapping, handling and manipulation of alligators should be eliminated when water temperatures are warm or when red-sore disease is known to be present among other species.

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