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## Screening of Free-Ranging Waterfowl in Oklahoma for *Salmonella*

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Species of *Salmonella* have long been noted as pathogens in avian species, and many reports describe infections of *Salmonella* in domestic or pen-raised waterfowl (Rettger and Scoville, 1920, J. Inf. Dis. 26: 217-231; Levine and Graham, 1942, J. Am. Vet. Med. Assoc. 100: 240-241; Truscott, 1956, Can. J. Comp. Med. 20: 345-346). Reports from free-ranging waterfowl are, however, less frequent. *Salmonella typhimurium* has been isolated from a wild duck (Keymer, 1958, Vet. Rec. 70: 713-720, 736-740), and a prevalence of 4.2% was reported for *Salmonella* cultured from the feces of wild ducks in England (Mitchell and Ridgwell, 1971, J. Med. Microbiol. 4: 359-361). The results of one survey of wild waterfowl in the United States were negative for serologic activity against *S. typhimurium* and *S. pullorum* (Bradshaw and Trainer, 1966, J. Wildl. Manage. 30: 570-576). The present study was undertaken to determine the prevalence of *Salmonella* in wild waterfowl migrating through and wintering in Oklahoma, using culture of cloacal swabs and serologic reactivity to *Salmonella* group antigens B, C and D.

The capture and sampling of wild waterfowl began in January 1976, and continued through December 1978 at Washita National Wildlife Refuge in Custer County, Oklahoma and Hamm's Lake in Payne County, Oklahoma. Birds were captured by cannon net, rocket net and a

baited swim-in trap. Two cloacal swabs using sterile cotton-tipped applicators were taken from each bird. One swab was inoculated into strontium chloride B enrichment broth (Iveson, 1971, J. Hyg. (Camb.) 69: 323-330), and the other was inoculated into selenite-cysteine enrichment broth (Difco Laboratories, Inc., Detroit, Michigan 48201, USA). Blood samples were taken from the brachial vein with syringe and needle. Since some sampling was done in cold weather (temperatures below 0 C), the samples were maintained and transported from the field in insulated styrofoam containers. At the laboratory, the serum was harvested after the sample had clotted and was frozen at -10 C until testing. The enrichment media were incubated at 42 C for 24 hr. After incubation, material from the strontium chloride B broth was inoculated and streaked on *Salmonella-Shigella* agar (Difco Laboratories, Inc., Detroit, Michigan 48201, USA), and material from the selenite-cysteine broth was inoculated and streaked on Brilliant Green agar (Difco Laboratories, Inc., Detroit, Michigan 48201, USA). The inoculated agar media were incubated at 37 C for 18 hr. Suspected colonies of *Salmonella* were inoculated onto Triple Sugar Iron (Difco Laboratories, Inc., Detroit, Michigan 48201, USA) agar slants and Christensen's urea agar (BBL Division of Becton, Dickinson and Company, Cockeysville, Maryland 21030, USA) and were tested with polyvalent "O" anti-*Salmonella* serum (BBL Division of Becton, Dickinson and Company, Cockeysville, Maryland 21030, USA).

Antibody titers were determined using a microagglutination technique (Williams

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TABLE 1. Serologic reactivity of wild ducks to *Salmonella* group antigens in Oklahoma, 1976–1978.

Species	Sex	No. of samples	<i>Salmonella</i> group B titer (inverse)			<i>Salmonella</i> group D titer (inverse)		
			32	64	128	32	64	128
Mallard	M	117	2			2	1	1
( <i>Anas platyrhynchos</i> )	F	93	1			1	1	
American wigeon	M	11						
( <i>Anas americana</i> )	F	3						
Northern pintail	M	6						
( <i>Anas acuta</i> )	F	3						
Green-winged teal	M	26						
( <i>Anas carolinensis</i> )	F	11				1		
Blue-winged teal	M	20				1	1	
( <i>Anas discors</i> )	F	7						
Ring-necked duck	M	8		1				
( <i>Aythya collaris</i> )	F	4						
Lesser scaup	M	8	1				1	
( <i>Aythya affinis</i> )	F	6						
Redhead	M	5						
( <i>Aythya americana</i> )	F	3						
Total		331	4	1	0	5	4	1

and Whittemore, 1971, Appl. Microbiol. 21: 394–399) that is comparable to the agglutination test utilized under the National Poultry and Turkey Improvement Plans (Anonymous, 1975, Publ. ARS-NE-32-1, Ag. Res. Serv. USDA, Beltsville, Maryland, 37 pp.) for the serologic diagnosis of *Salmonella* infection in chickens and turkeys. This technique utilized a group specific, tetrazolium-stained *Salmonella* antigen which was provided by Dr. J. E. Williams of the Southeast Poultry Research Laboratory, Athens, Georgia 30601, USA. *Salmonella* groups B, C and D were the only antigens available at that time, and titers of 1:32 or greater were considered positive.

*Salmonella* was not cultured from any of the birds tested. Of 331 serum samples representing eight species of waterfowl, five (1.5%) were reactive to group B antigens, none was reactive to group C antigens, and 10 (3.0%) were reactive to group D antigens (Table 1). No single bird was reactive to more than one antigen.

There was no significant difference ( $P > 0.05$ ) of reactor prevalence between any species or among combined males or females of all species.

These results indicate that *Salmonella* infections are not common in wild waterfowl in Oklahoma. This study was, however, conducted under the same constraints that are a problem in plaque diagnosis of *Salmonella* infections in domestic poultry. The lack of seroconversion by some infected individuals and the inability to culture the organisms due to intermittent shedding by some infected birds are two of these constraints. *Salmonella typhimurium*, *S. gallinarum* and *S. pullorum* constitute the majority of infections in domestic poultry. The antigens used in this study were designed to detect serologic reactivity to these organisms, but since there are many more antigens in the genus *Salmonella*, some serologic reactors to other *Salmonella* groups could have gone undetected. The possibility of serologic cross-reactions with non-*Salmonella*

antigens also exists, but that has not been a significant problem with the use of this test in domestic poultry.

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### **An Infection by *Vibrio alginolyticus* in an Atlantic Bottlenose Dolphin Housed in an Open Ocean Pen**

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An adult male Atlantic bottlenose dolphin (*Tursiops truncatus*) had a history of recurring skin problems. The dolphin weighed 159 kg and was kept in Kaneohe Bay, Hawaii, in an open ocean floating pen, 6 m square, 3.5 m deep, enclosed by 16-cm-square wire mesh. The lesions were first noted in 1975 and were always similar in size, shape and location: 2.5- to 5.0-cm-diameter oval ulcers at the anterior insertion of the pectoral fins. In 1983, the lesions appeared the most severe since 1975. The dolphin's skin was ulcerated to the musculature in the cranial insertion area of both pectoral fins and, in addition, to the subcutaneous tissue on the tip of the rostrum and on the leading edge and lateral aspects of the dorsal fin. The dermis in the region of the pectoral lesions contained a mild neutrophil infiltrate with some neutrophil migration into the overlying stratum spinosum; such characteristics are consistent with chronic, active dermatitis.

Although the dolphin had been treated under several different therapeutic plans previously, with varying degrees of success, lesions always recurred within 4 to 6 mo.

In May 1983, during a 26-day period of no treatment, the lesions became progressively larger and more inflamed than previously seen. Before cultures were taken, the lesion and surrounding area were aseptically prepared using Povidine Iodine surgical scrub, rinsed with alcohol, and allowed to dry for 60 sec. Single swab culturettes (Precision Culture C.A.T.S.® with Modified Amies Medium, Precision Dynamics Corporation, 3031 Thornton Avenue, Burbank, California 91504, USA) were used to take cultures from the lesions. Samples were transported to the laboratory on ice and were plated within 1 hr of collection.

Each sample was plated on *Salmonella-Shigella* agar, blood agar, M-Endo agar LES, Membrane Filtration Agar for recovery of *Vibrio parahaemolyticus* (Watkins et al., 1976, *Appl. Environ. Microbiol.* 32: 679-684), mannitol salt agar and

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