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SURVEY OF WILD DUCKS AND GEESE FOR *Pasteurella* spp.

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Abstract

Nasal pharyngeal swabs were taken from 400 waterfowl and 37 feral pigeons *(Columba livia)* and cultured for the presence of *Pasteurella multocida,* the causative agent of fowl cholera. Blood samples were also taken from these birds and tested for antibodies for *P. multocida. Pasteurella multocida* was not isolated from any of the birds. However, two of the waterfowl had antibodies for *P. multocida.* The nasal pharynges of 50 Canada geese *(Branta canadensis)* were also cultured for *Pasteurella anatipestifer,* the causative agent of anatipestifer infection. *Pasteurella anatipestifer* was isolated from 4 of these geese. Experimental inoculation of mallards *(Anas platyrhynchos)* demonstrated that they are susceptible to anatipestifer infection.

Introduction

The capability of an apparently healthy wild mallard to harbor and transmit *P. multocida,* the causative agent of fowl cholera, was demonstrated by Quortrup et al.[11] There have been several studies demonstrating that *P. multocida* can be harbored in the nasal area of apparently healthy domestic birds.[10,14]

In this study wild mallards, Canada geese, snow geese *(Chen hyperborea),* blue geese *(Chen caerulescens),* and pigeons were examined to determine if *P. multocida* was harbored in their nasal pharynges. These birds were also tested for antibodies for *P. multocida* to determine if they had been exposed to *P. multocida.*

Pasteurella anatipestifer, the causative agent of anatipestifer infection, has not been reported in wild waterfowl. But Hilbert and Witter[5] did report that more than 1000 of 1500 semi-wild black ducks *(Anas rubripes)* died of anatipestifer infection. Therefore, 50 Canada geese were also examined to determine if *P. anatifestifer* was harbored in their nasal pharynges, and *P. anatipestifer* was inoculated into mallard ducklings to determine if they were susceptible to anatipestifer infection.

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Materials and Methods

Experimental Birds: Waterfowl were live-trapped during the fall and winter of 1967-68 from four areas: (1) Swan Lake National Wildlife Refuge, Chariton County, Missouri, on October 20; (2) Squaw Creek National Wildlife Refuge, Holt County, Missouri, on December 29-31; (3) Union County Wildlife Refuge, Union County, Illinois, on January 17-18; and (4) Horseshoe Lake Wildlife Refuge, Alexander County, Illinois, on January 18. The species, sex, and age of the waterfowl are given in Table 1.

Feral pigeons were live-trapped in the area of Jefferson City, Missouri, between February 13 and May 5, 1968. Eggs laid by semi-wild mallards were the source of the mallard ducklings.

Survey for Pasteurella multocida: To survey for *P. multocida* the nasal pharynx of each wild bird was swabbed with a sterile cotton swab. The swabs were streaked on dextrose starch agar plates (Difco), and the plates were incubated at 37° C for 16-18 hours. After incubation the plates were examined for colonies having the morphological characteristics of *P. multocida* by observing through a stereomicroscope with obliquely transmitted light.[1] Colonies having the morphological characteristics of *P. multocida* were transferred to obtain a pure plate, and biochemical tests were then performed. An isolate was considered to be *P. multocida* if it produced indol in SIM medium (Difco) and if dextrose, sucrose, and mannitol (but not lactose and maltose) were fermented without producing gas.[6]

To survey for antibodies for *P. multocida* blood was drawn from each bird and tested using a serum-plate agglutination test. Blood samples were taken from the brachial or metatarsal vein of waterfowl and by heart puncture from pigeons. After the clot formed the serum was removed and stored in a refrigerator pending testing. Each serum sample was tested with three monovalent antigens prepared using the methods of Heddleston and Watko,[4] except that the concentration of the antigens was adjusted to a density of MacFarland 10. Isolates X-73, P-1059, and M-8954 were used for making the three antigens. Isolates X-73 and P-1059[2] were received from Kenneth L. Heddleston, National Animal Disease Laboratory, Ames, Iowa. Isolate M-8954 was obtained from the Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, Missouri, and was isolated from a domestic turkey flock.

Survey for Pasteurella anatipestifer: The dextrose starch agar plates streaked from the swabs of the 50 Canada geese from Swan Lake were also examined for the presence of *P. anatipestifer*. Colonies having the morphological characteristics of *P. anatipestifer* cited by Price[6] were isolated, and the biochemical tests listed by Price[6] were performed.

The wild bird isolates were also checked serologically. Immune sera to three known isolates of *P. anatipestifer* were produced in rabbits by injection of a suspension of an isolate into the marginal ear vein at 4 day intervals. The density of the suspension equaled the density of a MacFarland 3 nephelometer tube, and the volumes of the five doses injected were as follows: 1.0, 2.0, 3.0, 5.0, and 5.0 ml. Five days after the last injection each rabbit was bled from the heart.

The three *P. anatipestifer* isolates were Pennsylvania and Long Island,[3] which were received from Dr. Jessie I. Price, Duck Research Laboratory, Eastport, New York; and P-1050, which was received from Kenneth L. Heddleston. A wild goose isolate was tested with the three immune sera using a plate agglutination test.

Inoculations: Eight-week-old mallard ducklings were inoculated with 1 of 3 isolates of *P. anatipestifer*. The isolates used were Pennsylvania, Long Island, and M-29 (one of the isolates from a wild Canada goose). An isolate was incubated at 37° C in 150 ml of brain heart infusion (Difco) plus 1.0% bovine serum for 22-24 hours. After incubation 0.5 ml of the undiluted culture was inoculated into the trachea of a duckling with a 4 inch, 14 gauge cannula.

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TABLE 1. *A serological survey of wild waterfowl for antibodies for* Pasteurella multocida *winter, 1967-68.*

		Serological results*					
		HY**		AHY***			
Source of Birds	Species of Birds Sampled	Males	Females	Males	Females	Sex Unknown	Totals
Swan Lake Oct. 20	Canada Geese	0/0	0/0	0/0	0/0	0/50	0/50
Squaw Creek	Canada geese	0/6	0/4	0/1	0/4	0/0	0/15
Dec. 29-31	Blue geese	0/8	0/3	0/14	0/14	0/0	0/39
	Snow geese	0/17	0/12	1/38	0/22	0/0	1/89
Union County Jan. 17-18	Mallards	0/0	0/0	1/76	0/0	0/0	1/76
Horseshoe Lake	Mallards	0/0	0/0	0/66	0/0	0/0	0/66
Jan. 18	Canada geese	0/0	0/0	0/0	0/0	0/65	0/65
							2/400

*The number of positive sera is listed over the total number tested.
**HY indicates that the birds were less than one year old.
***AHY indicates that the birds were over one year old.

Results

No *P. multocida* was detected from the nasal pharynx of any of the 400 waterfowl or 37 pigeons. The serological survey revealed no positive reactors among the 37 pigeons, but 2 of the waterfowl had antibodies for *P. multocida* (Table 1). The serum from an after-hatching-year, male snow goose from Squaw Creek agglutinated with the X-73 antigen. And the serum from an adult male mallard from Union County reacted with the P-1959 antigen.

Organisms having the morphological and biochemical characteristics of *P. anatipestifer* were isolated from 4 of the 50 Canada geese from Swan Lake. One of the 4 isolates agglutinated with the P-1050 and Long Island antisera. The other three isolates were negative, indicating that they were serologically different.

One of the ducklings died within 72 hours after being infected with the Pennsylvania isolate of *P. anatipestifer* (Table 2). The other ducklings survived, but at autopsy they exhibited some of the lesions of anatipestifer infection.

Discussion

Fowl cholera is an important disease among both domestic and wild birds. The source of infection is usually unknown in wild birds and often unknown in domestic birds, but wild birds have often been accused of serving as a reservoir for fowl cholera. In this study 2 of the waterfowl had antibodies for *P. multocida,* indicating that they were potential carriers of the organism. But no organisms were isolated from any of the waterfowl.

It is very probable that domestic birds, especially turkeys, act as a source of infection for wild waterfowl. In Missouri in 1968 there were over 120 known outbreaks of fowl cholera involving over 1,000,000 turkeys.[13] Most of these turkeys were ranged on areas where they could come into contact with waterfowl and other wild birds.

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TABLE 2. *Experimental infection of mallard ducklings with* Pasteurella anatipestifer

Duckling	Isolate*	Results
200	Penn.	Killed after 31 days; mottled spleen and cloudy air sacs; no *P. anatipestifer.***
164	Penn.	Killed after 31 days; mottled spleen and air sacs slightly clouded; no *P. anatipestifer.*
128	Penn.	Died on day 3; spleen greatly enlarged and mottled, air sacs cloudy with some cheese-like material, lungs reddened and firm; *P. anatipestifer* isolated from spleen and air sacs.
127	Long Is.	Killed after 31 days; mottled spleen and cloudy air sacs; no *P. anatipestifer.*
119	Long Is.	Killed after 31 days; mottled spleen and cloudy air sacs; no *P. anatipestifer.*
118	Long Is.	Killed after 31 days; mottled spleen and cloudy air sacs; no *P. anatipestifer.*
101	Goose 29	Killed after 26 days; enlarged, mottled spleen; cloudy air sacs; no *P. anatipestifer.*
102	Goose 29	Killed after 26 days; spleen normal, air sacs cloudy; no *P. anatipestifer.*
103	Goose 29	Killed after 26 days; spleen mottled and slightly enlarged; air sacs cloudy; no *P. anatipestifer.*

*One-half ml. of a twenty-four-hour old broth culture was inoculated into the trachea.

**An attempt was made to isolate *P. anatipestifera* from the spleen and air sacs of each bird.

Even if waterfowl don't come into direct contact with infected turkeys, these turkeys could still serve as an indirect source of fowl cholera. Other wild birds, such as pigeons and sparrows, that commonly feed around domestic turkeys could transmit the disease to waterfowl. None of the pigeons surveyed in this study had antibodies for *P. multocida* or the organisms in its nasal area. But Heddleston and Watko[8] reported that *P. multocida* could be isolated from the nasal cleft of a pigeon 6 days after inoculation with *P. multocida.* Wild waterfowl could also pick up the organisms by feeding or drinking on areas once inhabited by diseased turkeys. Rosen and Bishoff[11] reported that a mouse inoculated with pond water succumbed in 4 hours to *P. multocida* infection. And Olson and Bond[7] demonstrated that the organisms could survive for up to 21 days in soil. Dead turkeys left on a range could be another source of infection.

Pasteurella anatipestifer was isolated from 4 of the 50 Canada geese. But there are no published reports of mortality in wild waterfowl due to anatipestifer infection. In this study one duckling succumbed to anatipestifer infection, and the other 8 exhibited some of the lesions associated with the disease, indicating that wild mallards might be susceptible to anatipestifer infection. Because *P. anatipestifer* is difficult to isolate from infected birds,[8,9] mortality from anatipestifer infection in wild birds might go undetected.

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