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PREVALENCE OF *BORDETELLA AVIUM* INFECTION IN SELECTED WILD AND DOMESTICATED BIRDS IN THE EASTERN USA

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ABSTRACT: *Bordetella avium* is the etiologic agent of bordetellosis, a highly contagious upper respiratory disease of young poultry. Its prevalence among domesticated turkeys is well-known, but information on prevalence of this bacterium in other birds is limited. A survey of the prevalence of *B. avium* in wild and domesticated birds was conducted from June 1998 to January 2000, using tracheal cultures and serology. Of 237 blood samples from 61 species, 100 individuals from 41 species had antibodies against *B. avium* as determined with a microtiter agglutination test. Nine isolates of *B. avium* were cultured from 128 tracheal samples. Ribotype analysis of seven isolates from mallards (*Anas platyrhynchos*), one from a wild turkey (*Meleagris gallopavo*), and one from a Canada goose (*Branta canadensis*) indicated that they represent three strains, two of which were indistinguishable from clinical isolates from domesticated turkeys. *Bordetella avium* is present in wild bird populations of multiple species. Transmission from free-living avian populations to domesticated poultry populations may be possible and should be examined.

Key words: Birds, *Bordetella avium,* bordetellosis, serologic survey, turkey.

INTRODUCTION

Bordetella avium causes bordetellosis in domesticated turkeys (Skeeles and Arp, 1997) and is an opportunistic pathogen in chickens (Jackwood et al., 1995). Although mortality is typically low, morbidity in young turkeys is high and causes several-million dollars in losses to the U.S. turkey industry every year (Skeeles and Arp, 1997). Current treatments do not effectively manage this disease (Skeeles and Arp, 1997). Knowledge of the epizootiology of *B. avium* may allow better control of bordetellosis.

Bordetella avium has been isolated from domesticated species in Germany, including: muscovy ducks, domesticated geese, a yellow-crested cockatoo (*Kakatoe galeria*), parrot finches (*Erythrura psittacea*), and partridges (*Perdix perdix*) (Hinz and Glunder, 1985). *Bordetella avium* has also been implicated in a respiratory syndrome of cockatiels (*Nymphicus hollandicus*) and ostriches (*Struthio camelus*) (Clubb et al., 1994). Hopkins et al. (1990) reported that 42 of 44 wild turkeys in Arkansas were seropositive. In contrast, little is known about transmission or prevalence of *B. avium* in wild birds other than turkeys or if the bacterium causes disease in wild birds.

We collected blood and tracheal samples from wild birds to investigate the extent of *B. avium* infection and to determine if strains carried by wild birds are similar to those isolated in domesticated turkeys. Serology allows detection of antibodies to a pathogen long after infection, whereas bacterial strains can only be isolated during active infection. When obtained, isolates are valuable because it is possible to compare strains from wild birds with pathogenic strains. The purpose of this study was to determine the prevalence of this bacterium among wild birds and non-commercial domesticated birds using serology and tracheal cultures. Additionally, ribotype analysis was carried out on the isolates to determine similarity to strains known to be pathogenic in domesticated turkeys.

MATERIALS AND METHODS

Serum samples were collected between June 1998 and January 2000 from wild birds at the Raptor Trust (Millington, New Jersey, USA; 39°49′N, 75°44′W), the Florida Keys Wild Bird Rehabilitation Center (Tavernier, Florida, USA; 24°58′N, 80°32′W), Tri-State Bird Rescue and

Research, Inc. (Newark, Delaware, USA; 39°42′N, 75°44′W), and the Wildlife Care Center (Ft. Lauderdale, Florida; 26°04′N, 80°32′W). Additional samples were obtained from birds caught in the Drew University Arboretum (Madison, New Jersey; 40°45′N, 74°25′W). Age was determined by examining the plumage. Birds were handled according to procedures described in Gaunt and Oring (1999). Usually, samples were obtained immediately after the bird was caught. Blood samples were taken from the medial metatarsal vein in waterfowl and the ulnar or brachial vein in other birds. For small birds about to be euthanized, blood was taken directly from the jugular vein. Blood was placed in a sterile plastic tube and permitted to clot. Serum was separated and stored in microfuge tubes at −20 C.

All blood samples yielding 20 μl or more of serum were tested with a microtiter agglutination test (Jackwood and Saif, 1980), using a modification of the original procedure (Arp and Skeeles, 1989). Antigen was prepared by growing 500 ml of *B. avium* strain 197N (Gentry-Weeks et al., 1988), *Bordetella hinzii,* (previously termed *B. avium*-like strain GOBL110, Gentry-Weeks et al., 1988; Vandamme et al., 1995), *Bordetella bronchiseptica* RB50 (Cotter and Miller, 1994), and *B. hinzi*i TR96–1212 (received from Dr. Eric Gonder, Goldsboro Milling, North Carolina, USA) aerobically at 37 C for 48 hr in brain-heart infusion broth. At 48 hr and again at 49 and 50 hr, 2.5 ml of 1% neotetrazolium stain (SIGMA, St. Louis, Missouri, USA) in 50% ethanol was added, followed by 4 hr of further incubation. Next, 0.2 ml of a 0.1% solution of merthiolate (thimerosal, SIGMA) was added followed by overnight incubation. The cells were centrifuged at 10,000 x G for 20 min and the pellet washed three times in phosphate-buffered saline (PBS) with 0.01% merthiolate. The packed cells were then resuspended to a 1:20 dilution by volume, in PBS with 0.01% merthiolate. To break up antigen clumps, the stock antigen was passed through a 22-gauge needle with a syringe and was then stored at −80 C.

To run the test, 80 μl of PBS with 0.01% merthiolate was added to all the wells of row A in a 96-well V-bottom low-binding plate (Corning-Costar, Acton, Massachusetts, USA). To each of the remaining wells 50 μl was added, and 20 μl of each serum sample was placed in a well in row A. Known positive and negative turkey serum samples (donated by Dr. Paul Orndorff, North Carolina State University School of Veterinary Medicine, Raleigh, North Carolina) served as controls. Positive serum was obtained from 3- to 4-wk-old turkeys (British United Turkeys Association, Lewisburg, West Virginia, USA) that had been exposed to *B. avium* strain 197N at 10 days of age. One to two serial dilutions were then made through eight wells. The stock antigen was thawed, mixed well, and diluted 1:128 in PBS with 0.01% merthiolate. Into each well 50 μl was dropped, followed by trituration to mix. The plate was covered with parafilm and incubated overnight at room temperature. Negative wells had a purple pellet and positive wells had no pellet and were cloudy. Two intermediate results were also recognized: partial positive was cloudy with a tiny pellet, and partial negative had a smaller pellet than normal. Serum was judged to contain antibodies to *B. avium* if any dilution was definitely positive or two dilutions were consistently partial positive. Samples testing positive to *B. avium* were tested using *B. bronchiseptica* strain RB50, *B. hinzii* strains GOBL110 and TR96–1212 as antigens (data not shown) to check for cross-reactivity with related *Bordetella* species.

Minitab 12 statistical software (Minitab Inc., State College, Pennsylvania, USA) was used for statistical analyses. Data from the New Jersey and Delaware sites were pooled in order to compare groups in different geographic regions, and the prevalence of infection in birds from this area was compared to pooled data from the Florida sites. Prevalence was compared between recently caught birds and birds that had been captive for more than a week, because 1 wk is long enough for a bird to have become infected from resident birds, but not long enough to have detectable antibodies (Skeeles and Arp, 1997). Prevalence in adult birds was compared to that in immature birds. Two-way contingency tables were used in all comparisons.

Tracheal swab samples were obtained by inserting an ultrafine applicator swab (Fischer, Pittsburgh, Pennsylvania) into the trachea of birds and immediately inoculating agar plates. All samples were cultured at 35 C on MacConkey's agar plates (Difco, Detroit, Michigan, USA), on which *B. avium* has a distinctive clear, mucoid colony morphology after 48 hr (Arp and Skeeles, 1989). All colonies with this morphology were subcultured onto Bordet-Gengou agar plates (Difco) supplemented with 12% sheep's blood.

Colonies that were white with no hemolysis on the blood agar were subjected to five biochemical tests and a hemagglutination test, and the results of these tests were compared to known results for *B. avium* (Pickett, 1980; Kersters et al., 1984). *Bordetella avium* is negative for nitrate reduction, tested by incubating the bacteria aerobically in nitrate broth at 37 C for 48 hr followed by addition of alpha-naph-

TABLE 1. Presence of serum microtiter agglutination antibodies against *Bordetella avium* in 62 avian species.

Order[a]	Prevalence[b] (% positive)	Common name	Species name	Prevalence[b] (% positive)
Gaviformes	1/1	common loon	*Gavia immer*	1/1[b]
Pelecaniformes	7/9	anhinga	*Anhinga anhinga*	2/3
		brown pelican	*Pelecanus occidentalis*	1/1
		double-crested cormorant	*Phalacrocorax auritus*	1/1
		northern gannet	*Morus bassanus*	3/4
Ciconiiformes	17/22 (77)	American bittern	*Botaurus lentiginosus*	1/1
		black-crowned night heron	*Nycticorax nycticorax*	1/1
		cattle egret	*Bubulcus ibis*	1/2
		glossy ibis	*Plegadis falcinellus*	1/1
		great blue heron	*Ardea herodias*	8/10 (80)
		great egret	*Ardea alba*	2/2
		green heron	*Butorides virescens*	0/1
		snowy egret	*Egretta thula*	0/1
		tricolored heron	*Egretta tricolor*	1/1
		turkey vulture	*Cathartes aura*	2/2
Anseriformes	31/46 (67)	Canada goose	*Branta canadensis*	19/23 (83)
		mallard duck	*Anas platyrhynchos*	2/9
		muscovy duck	*Cairina moschata*	9/13 (69)
		wood duck	*Aix sponsa*	1/1
Falconiformes	2/10 (20)	broad-winged hawk	*Buteo platypterus*	0/1
		Cooper's hawk	*Accipiter cooperii*	0/2
		osprey	*Pandion haliaetus*	2/3
		red-tailed hawk	*Buteo jamaicensis*	0/2
		sharp-shinned hawk	*Accipiter striatus*	0/1
		short-tailed hawk	*Buteo brachyurus*	0/1
Galliformes	7/12 (58)	ring-necked pheasant	*Phasianus colchicus*	1/1
		wild turkey	*Meleagris gallopavo*	2/4
		domesticated chicken		4/7
Gruiformes	3/6	American coot	*Fulica americana*	1/1
		common moorhen	*Gallinula chloropus*	0/3
		limpkin	*Aramus guarauna*	1/1
		sora rail	*Porzana carolina*	1/1
Charadriiformes	9/14 (64)	great black-backed gull	*Larus marinus*	1/1
		herring gull	*Larus argentatus*	1/1
		laughing gull	*Larus atricilla*	1/2
		ring-billed gull	*Larus delawarensis*	4/6
		royal tern	*Sterna maxima*	2/4
Columbiformes	1/47 (2)	mourning dove	*Zenaida macroura*	0/9
		ringed turtle-dove	*Streptopelia risoria*	0/6
		rock dove	*Columbia livia*	1/30
		white-winged dove	*Zenaida asiatica*	0/2
Psittaciformes	1/3	monk parakeet	*Myiopsitta monachus*	0/2
		blue and yellow macaw	*Ara ararauna*	1/1
Cuculiformes	0/3	yellow-billed cuckoo	*Coccyzus americanus*	0/3
Strigiformes	2/3	eastern screech owl	*Otus asio*	1/2
		great horned owl	*Bubo virginianus*	1/1
Coraciiformes	1/1	belted kingfisher	*Ceryle alcyon*	1/1
Piciformes	0/2	downy woodpecker	*Picoides pubescens*	0/1
		northern flicker	*Colaptes auratus*	0/1
Passeriformes	18/58 (31)	American crow	*Corvus brachyrhynchos*	2/16 (13)
		American robin	*Turdus migratorius*	0/5
		blue jay	*Cyanocitta cristata*	3/11 (27)
		boat-tailed grackle	*Quiscalus major*	4/5
		common grackle	*Quiscalus quiscula*	3/4
		eastern kingbird	*Tyrannus tyrannus*	1/2

TABLE 1. Continued.

Order[a]	Prevalence[b] (% positive)	Common name	Species name	Prevalence[b] (% positive)
		European starling	*Sturnus vulgarus*	2/4
		house sparrow	*Passer domesticus*	0/1
		northern mockingbird	*Mimus polyglottos*	0/1
		song sparrow	*Melospiza melodia*	0/1
		tufted titmouse	*Baeolophus bicolor*	0/1
		wood thrush	*Hylocichla mustelina*	3/7

[a] As classified on the American Ornithologists' Union Checklist (American Ornithologists' Union, 1999).
[b] Number positive/number tested.

thylamine and sulfanilic acid (Difco) (Kersters et al., 1984). It is also negative for urease production, tested by incubating the bacteria aerobically in urease broth at 37 C overnight with a phenol red indicator (Difco)(Kersters et al., 1984). In the other three tests, phenol red indicator was added to a Greenwood's low-peptone agar slant containing one of the three amides (Pickett, 1980). Bacteria were streaked and incubated on the medium at 35 C. *Bordetella avium* is positive for acetamide and formamide alkalinization but negative for malonamide alkalinization (Kersters et al., 1984). In all these biochemical tests, a positive result was indicated by a color change in the medium from yellow to red. *Bordetella avium* is hemagglutination positive, whereas *B. hinzii* is hemagglutination negative (Rimler and Simmons, 1983; Kersters et al., 1984).

A commercial bacterial identification system (Biolog, Hayward, California, USA) was used for definitive identification of bacterial isolates based on use of a carbon source and a color change, and subsequent matching with known Gram negative bacterial strains. A similarity index of > 0.5 is considered highly indicative of species identification. This system distinguishes unequivocally between *B. hinzii* and *B. avium* (data not shown).

Chromosomal DNA was purified using a commercially available kit (Wizard® Plus Minipreps DNA Purification System, Promega, Madison, Wisconsin, USA). Ribotype analysis was based on hybridization of digestion fragments with a probe derived from the *Escherichia coli* rRNA operon as reported previously (Register et al., 1997).

RESULTS

Both serology and tracheal cultures detected *B. avium* exposure and infection, respectively, among birds tested. Antibodies against *B. avium* were detected in 100 (42%) of 237 tested, and 41 (67%) of 61 species tested (Table 1). None of the sera tested against *B. bronchiseptica* and *B. hinzii* had detectable titers. There was no significant difference in prevalence between birds at different locations ($\chi^2 = 0.027$, df = 1, $P = 0.870$) or between birds held captive for less than 1 wk and those held for $>$ 1 wk ($\chi^2 = 0.086$, df = 1, $P = 0.769$) (Table 2). Overall, however, adult birds had a higher prevalence of antibodies than immature birds ($\chi^2 = 7.69$, df = 1, $P = 0.006$) (Table 2).

Of 128 swab samples from 24 wild bird species, nine isolates were identified with 98% to 100% probability as *B. avium* by the Biolog system (Table 3), as well as other biochemical tests. Seven of these isolates were cultured from mallards (*Anas platyrhynchos*) (five of which were ducklings from a single brood), one isolate was from a Canada goose (*Branta canadensis*), and another isolate was from a wild turkey (*Meleagris gallopavo*), all collected at the Raptor Trust. All isolates came from apparently healthy birds. These isolates were further characterized by ribotype analysis following digestion of chromosomal DNA with the enzyme *Pvu*II (Table 3). Three patterns were identified. Two patterns were indistinguishable from those commonly found in domesticated turkeys (unpubl. data), and have previously been designated ribotypes 1 and 3, respectively (Fig. 1). One pattern was novel and was designated ribotype 7.

DISCUSSION

Based on serology, *B. avium* is widespread in many species of wild birds, with

TABLE 2. Proportion of birds captured in New Jersey, Delaware, and Florida with antibodies to *Bordetella avium* in relation to location, duration of captivity, and age.

Facility (State)	All birds	Captive less than 1 wk[c]	Captive more than 1 wk[c]	Immature[c]	Adult[c]
Raptor Trust (New Jersey)	31/75[a] (41[b])	10/27 (45)	10/25 (36)	2/5 (40)	8/10 (80)
Florida Keys (Florida)	2/4	1/3	1/1	0/0	2/3
Tri-State Bird Rescue (Delaware)	6/13 (46)	3/3	3/10 (30)	0/6	6/6
Drew University (New Jersey)	5/13 (38)	5/13 (38)	0/0	0/2	5/11 (45)
Wildlife Care Center (Florida)	56/132 (42)	47/111 (42)	4/12 (33)	16/47 (34)	39/84 (64)
Total	100/237 (42)	66/152 (43)	22/48 (46)	18/59 (31)	60/114 (53)

[a] Number positive/number tested.
[b] Percent positive.
[c] Data not recorded for all birds.

high prevalence in some species. A high proportion of Canada geese tested positive, showing that this species is commonly infected (Table 1). In contrast, pigeons and doves (Order Columbiformes) had relatively low prevalence, with only one of 47 individuals giving a positive result. It is unknown whether this bacterium causes disease in any of these species or whether it is part of the birds' normal flora. However, a wide range of bird species, including cockatiels, ostriches, turkeys, and chickens, develop disease due to *B. avium,* so other species may be affected as well.

It seems unlikely that horizontal transmission within the holding facilities could account for the high prevalence reported by this study. Most of the samples were taken within a week of capture (Table 2), too soon to develop detectable IgM titers from an infection obtained in captivity (Suresh et al., 1994). In addition, there was no significant difference in prevalence between recently caught birds and birds held in captivity longer than 1 wk.

The results of ribotype analysis establish that some strains isolated from wild birds are indistinguishable from those associated

TABLE 3. Isolates of bacteria from birds at the Raptor Trust, New Jersey, identified as *Bordetella avium.*

Strain	Host	Date sampled	Hemagglutination	Ribotype	Similarity index (Biolog)[a]	Biochemical tests				
						U[b]	A[c]	M[d]	F[e]	N[f]
197N[g]	domesticated turkey	unknown	+	1	0.752	−	+	−	+	−
D4	mallard	7/5/98	+	1	0.763	−	+	−	+	−
D10	mallard	7/5/98	+	3	0.769	−	+	−	+	−
D25[h]	mallard	7/26/98	+	3	0.871	−	+	−	+	−
G24	Canada goose	7/26/99	+	7	0.921	−	+	−	+	−
T4	wild turkey	6/23/98	+	1	0.725	−	−	−	+	−

[a] Similarity to known strains.
[b] Urease test results.
[c] Acetamide test results.
[d] Malonamide test results.
[e] Formamide test results.
[f] Nitrate test results.
[g] Control strain of *Bordella avium.*
[h] This sample is representative of isolates from five ducklings in a single brood.

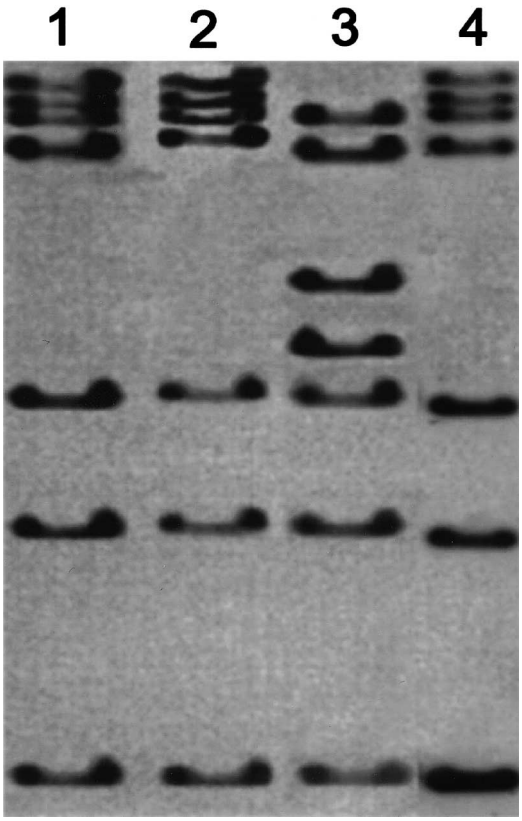

FIGURE 1. Southern blot of three ribotype patterns from *B. avium* cultured from wild birds and domesticated turkeys. Lane 1, ribotype 1 (isolate D4); lane 2, ribotype 3 (isolate D25); lane 3, ribotype 7 (isolate G24); lane 4, ribotype 1 (lab strain 197N). The blot contains genomic DNA digested with *Pvu* II and hybridized with a probe derived from the *E. coli* rRNA operon *rrnB*.

with disease in domesticated turkeys based on data obtained using the restriction enzyme *Pvu*II, shown to be highly discriminating for several *Bordetella* species (Register et al., 1997; Sacco et al., 2000). In a preliminary experiment, 24 (86%) of 28 turkey poults inoculated with strain D4 from a mallard developed clinical signs of coryza 10 days after infection. Our data and those of others (Hopkins et al., 1990) suggest a potential for transmission and spread of virulent *B. avium* between wild birds and commercial turkey flocks. Transmission may result from direct contact or from exposure to a common source. Alternatively, a common source could explain the presence of apparently identical strains in wild and domesticated birds.

Environmental transmission may be important to the spread of the disease. *Bordetella avium* is known to be transmitted by water or litter contamination, and can remain virulent in litter for 1 to 6 mo (Skeeles and Arp, 1997). The prevalence of *B. avium* in solitary species like the wood thrush (*Hylocichla mustelina*) suggests that an environmental reservoir for *B. avium* may be as important as direct contact between birds in the wild. *Bordetella avium* also seems to be long-lived in water as reported in *B. bronchiseptica* (Porter and Wardlaw, 1993). Preliminary work in our laboratory has shown that a population of *B. avium* held in dilute buffer at 4 C remained 90% viable for over 3 wk and 50% viable for 7 wk. Anecdotal evidence (Dr. Eric Gonder, pers. comm.) indicated that chlorination of poultry house water supplies lowers the incidence of this disease in commercially grown turkeys. In addition, birds commonly found in and around fresh water, such as those in the orders Ciconiiformes, Anseriformes, Pelecaniformes, Charadiformes, and Gruiformes, have a relatively high prevalence of *B. avium* exposure. Some of these birds, like the great blue heron (*Ardea herodias*), are solitary hunters, so transmission through water seems more likely than physical contact between birds. Taken together, these observations suggest that water may serve as an environmental reservoir for *B. avium.*

The difference in prevalence between adult and immature birds may be a result of the long persistence of detectable antibodies after infection. Adult birds could have been infected at a young age and sustained a detectable level of antibodies in response to that infection, whereas immature birds have had less time to become infected and develop antibodies. However, since we do not have data on the actual age of these birds, this hypothesis may require further testing.

Work is ongoing to determine if all

strains isolated from wild birds can cause disease in domesticated turkeys.

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