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CHARACTERISTICS OF *PASTEURELLA MULTOCIDA* ISOLATED FROM WATERFOWL AND ASSOCIATED AVIAN SPECIES IN CALIFORNIA

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ABSTRACT: Characteristics of *Pasteurella multocida* isolated from tissues of dead waterfowl and associated avian species found at 23 sites located in northern and central California, from January 1986 through January 1988 are reported. Two hundred ninety five isolates of *P. multocida* were obtained from 23 avian species. Most of the isolates belonged to the subspecies *P. multocida multocida* (63%), followed by *P. multocida gallicida* (37%), and by *P. multocida septica* (<1%). There appeared to be a higher prevalence of *P. multocida multocida* in Ross' geese (*Chen rossi*) and Snow geese (*Chen coeruleus*). All of the isolates belonged to somatic serotype 1, possessed the A capsule type and were susceptible to the 8 antimicrobial agents tested. None contained plasmid DNA.

Key words: *Pasteurella multocida*, avian cholera, waterfowl, plasmids, antimicrobial agents, characterization, subspecies, isolation, survey.

INTRODUCTION

Avian cholera, caused by the gram negative bacterium, *Pasteurella multocida*, is a significant cause of morbidity and mortality of migrating waterfowl (Stout and Cornwell, 1976). In addition, this disease is one of the most significant causes of mortality and economic loss in poultry, especially turkeys (Rhoades and Rimler, 1984). There is concern among poultrymen that infected waterfowl may transmit the microorganism to poultry. Over 95% of California meat turkeys are grown in the Central Valley region, a major staging and wintering area for waterfowl in the Pacific Flyway (Snipes et al., 1987).

Characterization of the *P. multocida* organism affecting waterfowl is important in determining the epidemiology of avian cholera in waterfowl, and in assessing the risk migrating waterfowl pose for domestic turkeys. Past surveys reported data gathered from isolates obtained over many years and from geographically diverse regions, and are valuable for the elucidation of the epidemiology of *P. multocida* as a whole (Brogden and Rhoades, 1983). However, these data may not provide sufficient detail to characterize the epidemiology of this disease in a specific locality. We obtained isolates of *P. multocida* from waterfowl that had died from avian cholera in California during 1986 to 1988. These isolates were characterized according to subspecies, serotype, susceptibility to antimicrobial agents and plasmid content. The objectives of this study were to determine whether these characteristics were of value in determining the epidemiology of avian cholera among migrating waterfowl in California, and whether migrating waterfowl are a source of *P. multocida* for turkeys raised on or near areas of waterfowl concentrations in California.

MATERIALS AND METHODS

Study area

The study area consisted of 23 sites in northern and central California (USA). The location, together with the number of samples collected at each site, are shown in Figure 1. One hundred sixty four of the 295 isolates of *P. multocida* were obtained from birds that died on National Wildlife Refuges, 20 that died on State wildlife areas, 31 that died on private waterfowl hunting areas, 55 that died on privately owned agricultural land, and 25 that died on sewer ponds or other artificial bodies of water. Samples were

obtained from January 1986 through January 1988.

Bacteria

Isolates of *P. multocida* (n = 295) were obtained from heart blood of dead waterfowl collected during epizootics. All birds sampled were believed to have died from avian cholera based on gross pathology, the observation of large numbers of rod shaped bacteria in stained smears of blood, and the isolation of *P. multocida*. Portions of heart blood were placed onto blood agar plates (containing 5% bovine erythrocytes). The plates were incubated at 37 C in air. Isolates were identified to genus and species according to established criteria (Carter, 1984).

Determination of subspecies

The subspecies of each isolate was determined by assessing the utilization of various carbohydrates as previously described (Mutters et al., 1985). The carbohydrates used in this determination were trehalose, maltose, xylose, arabinose, sorbitol and dulcitol.

Determination of serotype

The somatic serotype was determined by the agar gel immunodiffusion method (Heddleston et al., 1972). The capsular type was determined by assessing lability of the capsule to hyaluronidase (Carter and Rundell, 1975). Possession of capsule was determined by examining colonies under indirect light after growth on dextrose starch agar (Bond et al., 1970).

Determination of susceptibility to antimicrobial agents

Isolates were tested for susceptibility to chloramphenicol, gentamicin, kanamycin, penicillin G, streptomycin, sulfonamides, tetracycline and trimethoprim–sulfonamide (ratio of 1:20) using an agar dilution assay (Washington, 1985). Mueller-Hinton agar was used for testing all of the antimicrobial agent except for sulfonamides and trimethoprim–sulfonamide; Mueller-Hinton agar supplemented with laked horse blood (5%) was used for the latter antimicrobial agents.

Preparation and demonstration of plasmid DNA

A colony lysis technique was used to isolate plasmid DNA (Kado and Liu, 1981). Plasmid DNA was demonstrated by agarose gel electrophoresis (Meyers et al., 1976).

RESULTS

The distribution of subspecies of *P. multocida* among waterfowl and associated avian species was as follows: 63% belonged to *P. multocida multocida*, 37% belonged to *P. multocida gallicida*, and <1% belonged to *P. multocida septica* (Table 1). There was no obvious preference of subspecies for species of waterfowl except for isolates from geese (*Chen* spp.). There were 2.1 times and 2.5 times more isolates of *P. multocida multocida*, compared with isolates of *P. multocida gallicida* from Ross' geese (*Chen rossi*) and Snow geese (*Chen caeruleus*), respectively. Although certain subspecies of *P. multocida* seemed more common in other species of waterfowl (*P. multocida gallicida* was isolated almost twice as often from pintail ducks (*Anas acuta*) as *P. multocida multocida*), the number of isolates was too small to draw any conclusions. However, there were 2.3 and 5.4 times as many isolates belonging to *P. multocida gallicida* and *P. multocida multocida*, respectively, obtained from all species of geese as compared to waterfowl categorized as dabbling ducks (*Anas platyrhynchos, A. crecca, A. acuta, A. clypeata*

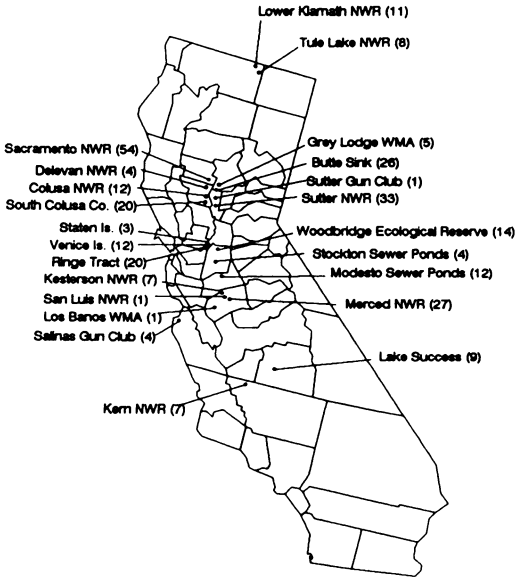

FIGURE 1. Collection sites of waterfowl and associated avian species dying from avian cholera from which *Pasteurella multocida* was isolated. The number in parenthesis represents the number of isolates obtained at each location (NWR, National Wildlife Refuge; WMA, Waterfowl Management Area).

TABLE 1. Subspecies of *Pasteurella multocida* isolated from waterfowl and associated avian species in California, 1986 to 1988.

Species	n	No. of isolates (%)		
		P. multocida gallicida	*P. multocida multocida*	*P. multocida septica*
Black-necked stilt (*Himantopus mexicanus*)	1	0	1	0
Canada goose (*Branta canadensis*)	39	16 (41)*	23 (59)	0
(*B. moffitti*, honker)	9	5	4	0
(*B. minima*, cackling)	14	7	7	0
(*B. leucopareia*, Aleutian)	16	4	12	0
Canvasback (*Aythya valisineria*)	1	0	1	0
American Coot (*Fulica americana*)	21	9 (43)	12 (57)	0
Grebe (species unknown)	4	1	3	0
Green-winged teal (*Anas crecca*)	1	0	1	0
Gull (*Larus* spp)	2	1	1	0
Lesser Scaup (*Aythya affinis*)	2	0	2	0
Mallard (*Anas platyrhynchos*)	13	6 (46)	6 (46)	1 (8)
Peregrine falcon (*Falco peregrinus*)	1	1	0	0
Pintail (*Anas acuta*)	13	8 (62)	5 (39)	0
Ring-billed gull (*Larus delawarensis*)	4	1	3	0
Ring-necked duck (*Aythya collaris*)	2	0	2	0
Ross' goose (*Chen rossi*)	46	15 (33)	31 (67)	0
Ruddy Duck (*Oxyura jamaicensis*)	18	9 (50)	9 (50)	0
Sandhill crane (*Grus canadensis*)	2	1	1	0
Shoveler (*Anas clypeata*)	5	3	2	0
Snow goose (*Chen caeruleus*)	87	25 (29)	62 (71)	0
Snowy egret (*Egretta thula*)	1	1	0	0
Swan (*Cygnus* sp)	1	0	0	1
Whistling swan (*Cygnus columbianus*)	13	4 (31)	9 (69)	0
White-fronted goose (*Anser albifrons*)	3	0	3	0
Wigeon (*Anas americana*)	15	7 (47)	8 (53)	0
Total	295	108 (37)	185 (63)	2 (<1)

* Percent infected.

and *A. americana*; $\chi^2 = 5.6$, df = 1, $P = 0.018$). Comparisons between other groups of waterfowl were unremarkable due to the small number of samples.

The distribution of subspecies on the various sites generally reflected the distribution of species of waterfowl sampled at that location; for example, if samples from Snow or Ross' geese predominated, then isolates from that location were predominantly *P. multocida multocida*.

Samples were collected over three time periods: February to May 1986 (period 1), November 1986 to April 1987 (period 2) and January 1988 (period 3). All 87 samples obtained in period 1 were from Snow geese (representing 77% of the samples obtained during this time period), Ross' geese (21%), and Canada geese (*Branta* spp) (3%). On two sites, the Ringe Tract and the Sutter National Wildlife Refuge, samples (20 isolates from each location) were obtained from only Snow geese and Ross' geese in order to determine if the same subspecies of *P. multocida* was present in the same avian species from different locations; there were no statistically significant differences ($\chi^2 = 2.0$, df = 1, $P = 0.16$) in the number of isolates belonging to either subspecies when comparisons were made between isolates obtained from these two locations. Overall, 79% of the isolates belonged to *P. multocida multocida* and 21% to *P. multocida gallicida*.

During period 2, 185 samples were collected from 20 species at 17 geographic

sites. A distribution of subspecies similar to that observed during period 1 was observed (57% *P. multocida multocida* and 43% *P. multocida gallicida*). This probably reflects the smaller number of samples from Snow and Ross' geese (14% and 11% of the samples in this time period, respectively).

Two sites (Sacramento National Wildlife Refuge and the Sutter National Wildlife Refuge) were sampled in periods 1 and 2. Comparison of subspecies isolated on these sites showed approximately the same distribution of biotypes among Snow geese and Ross' geese during period 1 (76% *P. multocida multocida*, 24% *P. multocida gallicida*) as in period 2 (62% *P. multocida multocida*, 38% *P. multocida gallicida*. The distribution of subspecies of isolates from other species was found to be 69% *P. multocida multocida*, and 31% *P. multocida gallicida*. The number of samples was too small to allow statistical evaluation. Whether the smaller number of isolates of *P. multocida multocida* during period 2 was due to samples being obtained in a different year, different locations being sampled, or that there were proportionately fewer Snow geese and Ross' geese sampled at this time period (14% of the samples came from Snow geese, 11% from Ross' geese) cannot be determined. The difference in distribution of subspecies also may be due to samples being collected during a different avian cholera season.

Isolates ($n = 23$) obtained during period 3 at four sites were found to be mainly *P. multocida multocida* (81%), with those of *P. multocida gallicida* accounting for 19%. During this time period, samples from 10 species were collected, but none from Snow geese or Ross' geese. The small number of isolates obtained during this time period precluded statistical analysis.

All of the isolates belonged to somatic serotype 1, possessed the A capsule type, and were susceptible to the antimicrobial agents tested (Table 2). We were unable to demonstrate plasmid DNA in any of the isolates.

DISCUSSION

We have shown that isolates of *P. multocida* from waterfowl and associated avian species possessed the A capsule type, were of the somatic serotype 1, and were predominately the subspecies *P. multocida multocida*. The isolates were very susceptible to antimicrobial agents. None contained plasmid DNA.

These data support what has been shown previously, that most, if not all, isolates from waterfowl in the Pacific Flyway affected with avian cholera belong to somatic serotype 1 (Zinkl et al., 1977; Brogden and Rhoades, 1983; Windingstad et al., 1983; Price and Brand, 1984). In California, epidemics of avian cholera on turkey farms are rarely caused by *P. multocida* belonging to this somatic serotype. Of the 55 episodes of this disease occurring during the same time frame that the waterfowl isolates were obtained, only one was caused by a *P. multocida* of somatic serotype 1 (D.C. Hirsh, unpublished observation). However, the isolate obtained from this particular incident was of the A capsule type, possessed no plasmid DNA, and was susceptible to all antimicrobial agents tested. All these traits are in common with the isolates from waterfowl. Overall, these data support the claim that *P. multocida* causing avian cholera in waterfowl very rarely produce disease in domestic turkeys in California.

We expected that the isolates would be susceptible to antimicrobial agents since *P. multocida* from turkeys and wild birds and mammals trapped in the vicinity of turkey farms are fairly susceptible to these drugs despite being exposed to antimicrobial agents in feed or other medications (Hirsh et al., 1989; D. C. Hirsh, unpubl. obs.).

To our knowledge isolates of *P. multocida* obtained from migratory waterfowl have not been subspeciated previously. We have no explanation as to why two species of geese (Ross' goose and Snow goose) harbored isolates that were predominately *P. multocida multocida* as compared to other

TABLE 2. Susceptibility to antimicrobic agents of *Pasteurella multocida* isolated from waterfowl and other avian species dying of avian cholera in California.

Antimicrobic	Cumulative percent susceptible to μg/ml							
	<0.25	0.5	1	2	4	8	16	32
Chloramphenicol	20	100						
Gentamicin	7	7	86	100				
Kanamycin	0	0	0	1	98	100		
Penicillin G	100							
Streptomycin	0	0	0	0	19	96	100	
Tetracycline	23	91	100					
Sulfonamides	0	0	0	0	15	60	91	98
Trimethoprim-sulfonamides[a]	100							

[a] 1:20, trimethoprim fraction shown.

waterfowl. This association seemed to occur regardless of the geographic location from which the isolate was obtained.

The homogeneity of the characteristics we have measured makes it difficult to derive any conclusions regarding the epidemiology of avian cholera among waterfowl. However, the data do suggest that different strains (clones) of pasteurellae affect ducks than affect geese. This is in contrast to the heterogeneity of characteristics of isolates of *P. multocida* obtained from affected turkeys in California (Snipes et al., 1987, 1988, 1989).

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