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Source: Journal of Wildlife Diseases, 40(3) : 377-382

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-40.3.377

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Journal of Wildlife Diseases, 40(3), 2004, pp. 377–382
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ARE WETLANDS THE RESERVOIR FOR AVIAN CHOLERA?

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ABSTRACT: Wetlands have long been suspected to be an important reservoir for *Pasteurella multocida* and therefore the likely source of avian cholera outbreaks. During the fall of 1995–98 we collected sediment and water samples from 44 wetlands where avian cholera epizootics occurred the previous winter or spring. We attempted to isolate *P. multocida* in sediment and surface water samples from 10 locations distributed throughout each wetland. We were not able to isolate *P. multocida* from any of the 440 water and 440 sediment samples collected from these wetlands. In contrast, during other investigations of avian cholera we isolated *P. multocida* from 20 of 44 wetlands, including 7% of the water and 4.5% of the sediment samples collected during or shortly following epizootic events. Our results indicate that wetlands are an unlikely reservoir for the bacteria that causes avian cholera.

Key words: Avian cholera, disease reservoir, *Pasteurella multocida,* wetlands.

INTRODUCTION

Avian cholera kills thousands of waterfowl annually in North American wetlands; however, the reservoir for the bacteria that causes the disease (*Pasteurella multocida*) remains uncertain (Botzler, 1991). Two important reservoirs—a reservoir is defined as a "place where the infective agent can survive on a year-round basis" (Botzler, 1991)—have been suggested as a source of avian cholera in waterfowl populations: carrier birds and epizootic wetland sites. Although neither of these hypotheses has been thoroughly or consistently investigated, several observations have contributed to the idea that soil or water at specific wetland areas may serve as the reservoir for this disease. First, although the disease has occurred in many areas, there has been a consistent pattern of recurrence of winter and spring avian cholera outbreaks in northern California (USA), the Rainwater Basin in Nebraska (USA), in Texas (USA) (Fig. 1), and in the prairies of Canada (Wobeser et al., 1979; Wobeser, 1992). In addition, a number of researchers have isolated *P. multocida* from wetlands where avian cholera outbreaks were occurring (Rosen and Bischoff, 1950; Rosen, 1969; Korschgen et al., 1978; Price and Brand, 1984; Backstrand and Botzler, 1986; Samuel et al., 2003), indicating that wetlands can be contaminated with bacteria during outbreaks. Second, several researchers have observed that *P. multocida* can survive for considerable time periods (e.g., weeks to >1 yr) in the laboratory (Bendheim and Evan-Shoshan, 1975; Awad et al., 1976; Bredy and Botzler, 1989; Price et al., 1992), providing the theoretical potential for long-term survival under favorable environmental conditions. And third, researchers have also found that survival of *P. multocida* in the laboratory can depend on the water or sediment characteristics (Rosen and Bischoff, 1950; Bredy and Botzler, 1989; Price et al., 1992) and that wetland water chemistry may be associated with outbreak areas (Windingstad et al., 1988), providing the possibility that differential survival of bacteria may occur among wetlands.

We investigated the hypothesis that wetlands are the most likely reservoir for *P. multocida* and thus play an important role in the epizootiology of avian cholera. If wetlands serve as reservoirs for the bacterium that causes avian cholera, then *P. multocida* should be present in these wetlands before migratory birds arrive. In addition, the patterns of consistent disease mortality in specific locations and isolation

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FIGURE 1. Distribution of avian cholera epizootics reported in wild waterfowl (1944–97) in the United States and shown in shaded circles, which represent the relative magnitude of mortality events (National Wildlife Health Center, unpubl. data). Fall wetland sample locations 1995–98 shown with stars. All sampled wetlands had avian cholera outbreaks during the previous winter–spring. Each star may represent multiple wetlands and multiple years of sampling (see Table 1 for details).

of *P. multocida* from wetlands with outbreaks imply that wetlands with recent mortality are a potential reservoir for the disease agent. Each fall, from 1995 to 1998, we sampled wetlands throughout the United States where avian cholera outbreaks were reported during the previous winter or spring. We collected sediment and water samples and attempted isolation of *P. multocida* from each wetland. Although we attempted to isolate all *P. multocida* serotypes, we were primarily concerned with serotype 1, which typically causes avian cholera mortality in the Pacific, Central, and Atlantic flyways (Brogden and Rhoades, 1983; Windingstad et al., 1983; Hirsh et al., 1990; Wilson et al., 1995). If wetlands are an important reservoir for avian cholera, we expected to isolate *P. multocida* during the fall, prior to use by migratory waterfowl.

MATERIALS AND METHODS

During 1995–98, wetlands where avian cholera mortality, confirmed by diagnostic pathology and culture, occurred were selected for sampling the next fall to determine whether *P. multocida* could be isolated from water or sediment. We selected wetlands where estimated mortality was at least 100 waterbirds because we believed that these losses increased the likelihood that *P. multocida* was present in the wetland during the outbreak. We sampled wetlands during September to November, depending on geographic location, prior to major influxes of migratory waterfowl, which could also be a reservoir for the disease (Botzler, 1991; Samuel et al., 1999a, b).

We collected water and sediment samples for isolation of *P. multocida* using the methods described in Samuel et al. (2003). We used the cryopreservation method and quality assurance procedures described by Samuel et al. (2003) to preserve water and sediment samples prior to attempting isolation of *P. multocida.* In addition to water and sediment samples, we collected water samples for chemical, turbidity, and dissolved protein analyses and we measured other water quality characteristics (temperature, pH, redox potential, conductivity, and dissolved oxygen) using a Yellow Springs Instruments 610 DM water quality meter and 600 XL probe (Yellow Springs, Ohio, USA).

Following field collection, samples were transported to the US Geological Survey National Wildlife Health Center (NWHC), Madison, Wisconsin (USA), in liquid nitrogen vapor shippers. Cryovials containing the water and sediment samples were transferred to liquid nitrogen tanks for storage until they could be conveniently processed in the laboratory. Attempted isolation of *P. multocida* followed the methods described in Moore et al. (1998) and Samuel et al. (2003). Suspect bacterial colonies were identified by methods described in Samuel et al. (1997).

We calculated the proportion of water and sediment samples and the proportion of sampled wetlands that had detectable *P. multocida.* We also calculated the exact 95% confidence intervals on these prevalence data (Zar, 1984).

RESULTS

During September–November of 1995–98, we sampled 44 wetlands in six different states in the United States (Table 1). Many of the sampled wetlands were distributed throughout enzootic avian cholera areas in the Klamath Basin, Central Valley, and San Joaquin Valley of California and the Rainwater Basin in Nebraska (Fig. 1). We also sampled wetlands in areas with less frequent occurrences of avian cholera, including Swan Lake National Wildlife Refuge (NWR) in Missouri, Lac Qui Parle Wildlife Management Area (WMA) in

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Minnesota, the eastern San Francisco Bay in California, Stillwater NWR in Nevada, and the panhandle of Texas (all sites in the United States). Reported avian cholera mortalities from our sampled wetlands varied from approximately 90 to >8,000 during the previous winter–spring period (Table 1).

We were unable to isolate *P. multocida* from any of the water or sediment samples collected during our fall sampling. Thus, estimated prevalence was 0.0% for water, sediment, and wetlands. Assuming that each of the water or sediment samples we collected had independent probabilities of containing *P. multocida*, then the 95% confidence interval on the proportion of water or sediment samples with detectable concentrations of *P. multocida* was between 0.0% and 0.63%. Alternatively, the 95% confidence interval on the proportion of wetlands with detectable *P. multocida* was 0.0% to 6.2%.

DISCUSSION

Whether wetlands or waterbirds serve as a reservoir for avian cholera has been a controversial issue with important implications for understanding the epizootiology of this disease. In part, debate about the reservoir for the disease has persisted because of lack of consistent research investigating the proposed hypotheses. We were unable to recover *P. multocida* from any of the 440 water and 440 sediment samples we collected from the wetlands we sampled during the fall, prior to the return of migratory waterfowl populations.

We believe our research provides the strongest evidence to date that wetlands are not the primary reservoir for the *P. multocida* serotypes that cause avian cholera. However, we acknowledge some limitations of our sampling methods and the difficulty of proving the hypothesis that wetlands never serve as a reservoir for the disease. Nevertheless, we believe several lines of evidence support our conclusion. In previous laboratory and field studies (Moore et al., 1998) we found the preservation and culturing methods used in our investigations to be highly sensitive for detecting *P. multocida* at concentrations of two to 18 organisms per milliliter in wetland water samples. This method was superior to the standard mouse inoculation method used for previous work (Moore et al., 1998). Concurrent to this study, we attempted to isolate *P. multocida* from wetlands where avian cholera outbreaks occurred, using the same sampling and isolation procedures described in this study. During the winters of 1996–99, we recovered 51 *P. multocida* isolates (49 serotype 1) from 20 (46%) of the 44 wetlands with outbreaks. *Pasteurella multocida* was isolated from 31 (7%) of 440 water samples and from 20 (4.5%) of 440 sediment samples collected during or shortly following epizootic events. Representative wetland isolates were tested for virulence in Pekin ducks (four ducks per isolate), with most of the isolates being pathogenic (Samuel et al., 2003). In addition, 17 of the wetlands we sampled during the fall were also sampled the previous spring during avian cholera outbreaks. During spring we recovered *P. multocida* serotype 1 from 41% (seven) of these wetlands, and from 10% (17) of the water and 2.4% (four) of the sediment samples, but none of the samples collected in the fall. For these samples collected in the fall, 95% confidence intervals indicated that <1% of the water and sediment samples and <7% of the epizootic wetlands we sample contained detectable bacteria. Thus, although we cannot prove that wetlands we sampled were completely free of *P. multocida*, our data do not support the hypothesis that wetlands are an important reservoir for avian cholera because the bacteria were not present in sufficient amounts or in a sufficient number of wetlands to ensure annual infection of migrating waterbirds. Based on serologic studies conducted on lesser snow geese (*Chen caerulescens caerulescens*), we have also found that many geese were infected with the *P. multocida* serotype 1 bacteria, but survived the infection (Samuel et al.,

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TABLE 1. Time period of sample collection, geographic location of wetland, and number of waterbirds reported dead during previous avian cholera outbreaks at wetlands sampled during the subsequent fall.

State	Watershed location	Wetland[a]	Latitude	Longitude	Sampling period	Mortality[b]
California	Central Valley	Delevan NWR, T11	39°19′N	122°07′W	October 1997	125
California	Central Valley	Delevan NWR, T28	39°19′N	122°06′W	September 1996	100
California	Central Valley	Sutter NWR, T2	39°41′N	121°45′W	September 1996	250
California	Central Valley	Sutter NWR, T15	39°03′N	121°44′W	October 1997	100
California	Central Valley	Sutter NWR, T19-2	39°03′N	121°44′W	October 1997	254
California	Central Valley	Sutter NWR, T20-2	39°04′N	121°44′W	October 1997	93
California	Klamath Basin	Tule Lake NWR, Sump 1B	41°51′N	121°27′W	September 1996	950
California	Klamath Basin	Upper Klamath Lake	42°28′N	121°56′W	September 1997	300
California	San Joaquin Valley	Los Banos WMA, Gadwall-1	37°03′N	120°47′W	November 1998	1,200
California	San Joaquin Valley	Los Banos WMA, Gadwall-2	37°03′N	120°47′W	November 1998	800
California	San Joaquin Valley	Merced NWR, Deadman Marsh	37°11′N	120°38′W	October 1997	200–300
California	San Joaquin Valley	Merced NWR, EGB	37°11′N	120°38′W	October 1996	80–100
California	San Joaquin Valley	Merced NWR, EGC	37°11′N	120°38′W	October 1996	80–100
California	San Joaquin Valley	Merced NWR, EGF	37°10′N	120°37′W	October 1997	300
California	San Joaquin Valley	Merced NWR, EGG	37°10′N	120°37′W	October 1997	300
California	San Joaquin Valley	Merced NWR, Honker Lake	37°12′N	120°37′W	October 1997	100
California	San Joaquin Valley	Mud Slough, Unit 5	37°04′N	120°46′W	November 1998	750
California	San Joaquin Valley	Arena Plains, North Lake	37°17′N	120°44′W	November 1998	180
California	San Joaquin Valley	San Luis NWR, Page Lake	37°40′N	121°12′W	October 1996	120
					November 1998	150
California	San Francisco Bay	Hayward Regional Park, 2A	37°38′N	122°08′W	September 1997	100–150
California	San Francisco Bay	Hayward Regional Park, 2B	37°38′N	122°08′W	September 1997	100–200
Minnesota	Upper Mississippi	Lac Qui Parle WMA	45°04′N	95°54′W	September 1997	150
Missouri	Lower Missouri River	Fountain Grove	39°42′N	93°18′W	November 1995	90
Missouri	Lower Missouri River	Swan Lake NWR	39°37′N	93°14′W	November 1995	80–100
					October 1997	160
Nebraska	Rainwater Basin	Funk	40°31′N	99°13′W	November 1995	100
					September 1996	270
					September 1997	590
					September 1998	8,840
Nebraska	Rainwater Basin	Greenshack	40°29′N	97°42′W	September 1996	200
Nebraska	Rainwater Basin	Hansen	40°27′N	97°51′W	November 1995	250
					September 1998	215
Nebraska	Rainwater Basin	Harvard	40°37′N	98°11′W	November 1995	700–800

TABLE 1. Continued

State	Watershed location	Wetland[a]	Latitude	Longitude	Sampling period	Mortality[b]
					September 1996	1,000
Nebraska	Rainwater Basin	Johnson	40°34′N	99°20′W	September 1997	280
					September 1998	1,420
Nebraska	Rainwater Basin	Mallard Haven	40°27′N	97°45′W	November 1995	400
					September 1996	530
		Meat and Animal Research Center	40°28′N	98°08′W	September 1996	1,200
Nebraska	Rainwater Basin				September 1997	185
Nebraska	Rainwater Basin	Springer	40°51′N	98°08′W	September 1996	200
Nevada	Interior Basin	Stillwater NWR	39°41′N	118°23′W	September 1996	495
Texas	Red River Drainage	Rita Blanca Lake	36°02′N	102°30′W	October 1997	270

[a] NWR = national wildlife refuge; WMA = wildlife management area; EG = east grasslands.
[b] Estimated number of deaths of waterbirds in previous winter or spring.

1999a, b). Further research has also confirmed that birds, especially snow geese, are carriers of pathogenic strains of *P. multocida* and that enzootic transmission occurs year round in these waterfowl (Samuel et al., unpubl. data).

A persistent issue in the epizootiology of avian cholera has been the identification of a reservoir for the disease agent. Ambiguity about whether birds or wetlands are the primary source of *P. multocida* has inhibited our understanding about factors such as transmission, carrier birds, and persistence of disease in waterfowl populations. Our study provides evidence that wetlands are not a likely reservoir for the bacteria, although wetlands likely play an important role in disease transmission. Alternatively, other studies give support to the hypothesis that carrier birds are an important reservoir for the disease. Future bird research should focus on their role in maintaining disease throughout the year and the role of environmental conditions and other stressors on the initiation of disease outbreaks. Research on wetlands should evaluate the role of wetlands in disease transmission and determine which factors influence the growth of *P. multocida.* Strategies for prevention and control of avian cholera outbreaks should consider that carrier birds are the most likely source of disease outbreaks and disease spread. Management actions that decrease potential disease transmission by separating carrier species from other species, reducing stress factors that may precipitate epizootic events, and reducing densities of waterfowl offer potential strategies to minimize the impact of avian cholera on waterfowl and other bird populations.

ACKNOWLEDGMENTS

We are grateful to M. McCollum and S. Smith for assistance with fieldwork. Many state and federal wildlife biologists provided considerable logistic support and assistance to our study; in particular we thank J. Beckstrand, G. Mensik, G. Mack, G. Gerstenberg, and K. Sandie for their help. D. Johnson and M. Moore provided laboratory assistance. R. G. Botzler,

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G. Wobeser, C. J. Brand, and R. G. McLean provided many helpful suggestions for improvement of the manuscript.

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Received for publication 8 August 2003.

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