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Persistence of Pasteurella multocida in Wetlands Following Avian Cholera Outbreaks

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Abstract

Avian cholera, caused by Pasteurella multocida, affects waterbirds across North America and occurs worldwide among various avian species. Once an epizootic begins, contamination of the wetland environment likely facilitates the transmission of P. multocida to susceptible birds. To evaluate the ability of P. multocida serotype-1, the most common serotype associated with avian cholera in waterfowl in western and central North America, to persist in wetlands and to identify environmental factors associated with its persistence, we collected water and sediment samples from 23 wetlands during winters and springs of 1996–99. These samples were collected during avian cholera outbreaks and for up to 13 wk following initial sampling. We recovered P. multocida from six wetlands that were sampled following the initial outbreaks, but no P. multocida was isolated later than 7 wk after the initial outbreak sampling. We found no significant relationship between the probability of recovery of P. multocida during resampling and the abundance of the bacterium recovered during initial sampling, the substrate from which isolates were collected, isolate virulence, or water quality conditions previously suggested to be related to the abundance or survival of P. multocida. Our results indicate that wetlands are unlikely to serve as a long-term reservoir for P. multocida because the bacterium does not persist in wetlands for long time periods following avian cholera outbreaks.

Keywords

Avian cholera, environmental persistence, epizootiology, Pasteurella multocida, wetlands

Comments

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PERSISTENCE OF *PASTEURELLA MULTOCIDA* IN WETLANDS FOLLOWING AVIAN CHOLERA OUTBREAKS

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ABSTRACT: Avian cholera, caused by *Pasteurella multocida*, affects waterbirds across North America and occurs worldwide among various avian species. Once an epizootic begins, contamination of the wetland environment likely facilitates the transmission of *P. multocida* to susceptible birds. To evaluate the ability of *P. multocida* serotype-1, the most common serotype associated with avian cholera in waterfowl in western and central North America, to persist in wetlands and to identify environmental factors associated with its persistence, we collected water and sediment samples from 23 wetlands during winters and springs of 1996–99. These samples were collected during avian cholera outbreaks and for up to 13 wk following initial sampling. We recovered *P. multocida* from six wetlands that were sampled following the initial outbreaks, but no *P. multocida* was isolated later than 7 wk after the initial outbreak sampling. We found no significant relationship between the probability of recovery of *P. multocida* during resampling and the abundance of the bacterium recovered during initial sampling, the substrate from which isolates were collected, isolate virulence, or water quality conditions previously suggested to be related to the abundance or survival of *P. multocida*. Our results indicate that wetlands are unlikely to serve as a long-term reservoir for *P. multocida* because the bacterium does not persist in wetlands for long time periods following avian cholera outbreaks.

Key words: Avian cholera, environmental persistence, epizootiology, *Pasteurella multocida*, wetlands.

INTRODUCTION

Avian cholera is a significant cause of mortality in numerous species of waterbirds, especially of North American waterfowl (Stout and Cornwell, 1976; Friend, 1989; Botzler, 1991). Although most epizootics during the past two decades have been reported from North America, significant mortality in wild birds also has been found in Europe (Christensen et al., 1997, 1998), Africa (Crawford et al., 1992), and Asia (Kwon and Kang, 2003). This highly infectious disease is caused by the gram-negative bacterium *Pasteurella multocida*. This bacterium has a worldwide distribution and produces septicemic and respiratory disease in more than 180 species of wild birds (Samuel et al., 2005). The disease has now been reported in most areas of the United States and in many portions of Canada. Avian cholera also is suspected to occur in waterfowl wintering areas in Mexico, but surveillance and diagnostic efforts have been limited. Epizootics occurred almost every winter in the Pacific Flyway and during winter and early spring in the Central and Mississippi flyways from 1976 to 1999. Although avian cholera occurs in many areas in North America, some locations have consistent recurrence of disease (Wobeser, 1992).

Avian cholera is likely transmitted through inhalation of water aerosols, direct bird-to-bird contact, or ingestion of contaminated water and sediment (Botzler, 1991; Wobeser, 1992). Once an epizootic begins, contamination of the environment, especially water, likely facilitates transmission of *P. multocida* (Price and Brand, 1984; Backstrand and Botzler, 1986; Bredy and Botzler, 1989; Samuel et al., 2003b). Several laboratory and field studies have reported associations be-

tween various environmental conditions and avian cholera (Windingstad et al., 1984; Backstrand and Botzler, 1986; Bredy and Botzler, 1989; Price et al., 1992; Lehr et al., 2005; Blanchong et al., 2006); however, conclusions differ on the role these environmental conditions play in the survival of *P. multocida* (Bredy and Botzler, 1989; Price et al., 1992).

Evaluation of the ability of *P. multocida* to persist in wetlands and identification of factors that promote the persistence of *P. multocida* are important in understanding the dynamics of avian cholera epizootiology, determining the reservoir for the bacterium (Samuel et al., 2004), and developing appropriate management strategies to minimize disease transmission and reduce impacts on avian populations. Our objectives were to determine how long *P. multocida* persists in wetlands following avian cholera outbreaks and to identify environmental factors associated with bacterial persistence.

MATERIALS AND METHODS

Field collection and laboratory processing

We sampled wetlands in the western and central United States that experienced avian cholera outbreaks (≥100 dead birds reported and avian cholera diagnosed as the primary cause of mortality) during winters and springs of 1996–97, 1997–98, and 1998–99. Initial sampling typically took place during avian cholera outbreaks and within 2 wk of the first observation of waterfowl mortality (Blanchong et al., 2006). We collected samples from 10 sites broadly distributed within each wetland to obtain adequate coverage of the wetland area. At each of the 10 sites, water and sediment samples were collected for chemical analyses and *P. multocida* isolation, following standardized methods (Samuel et al., 2003b). Chemical analyses of water samples were conducted at the University of Wisconsin Soils and Plant Analysis Laboratory, Soils Department, University of Wisconsin–Madison, USA, using an Applied Research Laboratories 34000 RTB ICP Optical Emission Spectrometer (Thermo Jarrell Ash Corporation, Franklin, Massachusetts, USA). Water and sediment samples were processed for isolation of both encapsulated and nonencapsulated colonies of *P. multocida* at the National Wildlife Health Center (Madison, Wisconsin, USA) following the procedure described by Samuel et al. (2003b). All *P. multocida* isolates were serotyped using the agarose gel precipitin test (Heddleston et al., 1972).

Isolate virulence

We conducted challenge studies using Pekin ducks to evaluate the virulence of *P. multocida* serotype-1 isolates (the serotype commonly associated with avian cholera in waterfowl in western and central North America) as described in detail in Samuel et al. (2003a). When more than one *P. multocida* isolate was obtained from a wetland, isolates were selected arbitrarily for virulence testing in ducks. When isolates were recovered from both water and sediment samples, we tested at least one isolate from each substrate. Virulence of isolates recovered from a wetland was quantified as the number of ducks (out of four challenged) that died, or the average number of ducks that died from wetlands where isolates were obtained from both water and sediment.

Persistence of *P. multocida*

Wetlands where avian cholera outbreaks occurred were resampled one to three additional times to evaluate the ability of *P. multocida* to persist in wetlands. Resampling of wetlands after the initial sampling occurred at roughly 2- to 4-wk intervals for up to 13 wk following the beginning of the outbreak. The water and sediment sampling and testing methods described above were followed during resampling. It should be noted that, on detection of an avian cholera outbreak, wetlands were systematically searched, and dead birds were collected for disease control.

We used separate logistic regression analyses to evaluate associations between the probability of recovering *P. multocida* during resampling periods (persistence) and several independent variables including the number of sample sites out of 10 (abundance) from which the bacterium was obtained within a wetland at initial sampling (outbreak), whether *P. multocida* was isolated from a single substrate (water or sediment) or both substrates, the virulence of isolates obtained during initial sampling, and initial values of environmental conditions previously reported to be related to the abundance or survival of *P. multocida*. Wetlands in which *P. multocida* was recovered during any resampling event were assigned a value of "1" to indicate

TABLE 1. Mean and range of environmental variables observed in the 23 wetland sites included in the evaluation of persistence of *P. multocida* following avian cholera outbreaks.

Environmental variable	Mean	Range
Potassium (K)[a]	13.19	1.78–51.12
Nitrate (NO_3)	0.28	UD[b]–1.84
Phosphorous (P)	1.93	UD–4.70
Phosphate (PO_3)	0.4	0.1–1.9
Calcium (Ca)	35.94	UD–149.3
Magnesium (Mg)	15.53	0.65–63.09
Temperature (°C)	8.20	2.94–18.00
pH	7.73	6.34–8.82
Protein (mg/ml)	12.8	3.8–29.7

[a] All chemical ions measured in parts per million (ppm).

[b] UD: undetectable.

"persistence," and wetlands where we failed to recover *P. multocida* during resampling were assigned a value of "0."

In a previous study of wetlands with avian cholera outbreaks (Blanchong et al., 2006), increased eutrophic nutrient concentration (K, NO_3, P, and PO_3) and wetland protein concentration were positively related to the abundance of *P. multocida* recovered from water and sediment during outbreaks (Table 1; see Blanchong et al., 2006 for a summary of environmental conditions measured in sampled wetlands across North America). We also evaluated calcium (Ca) and magnesium (Mg) concentration as well as temperature and pH, water quality variables previously demonstrated in laboratory studies to be related to survival of *P. multocida* (Bredy and Botzler, 1989; Price et al., 1992) (Table 1). Because wetlands differed in the number of resampling events, we also tested for a relationship between recovery of *P. multocida* during resampling and the number of times a wetland was resampled. Statistical analyses were carried out using the program R (R Development Core Team, 2004).

RESULTS

We studied persistence of *P. multocida* in 23 wetlands from the western and central United States that experienced avian cholera outbreaks during the winters and springs of 1996–99. Wetlands were resampled one to three times (for up to 13 wk) following initial sampling during the outbreak. *Pasteurella multocida* was recovered from 11 of 23 wetlands during the initial outbreak sampling (Table 2). We recovered *P. multocida* serotype-1 from six wetlands during resampling (Table 2). In five of the six wetlands in which we recovered *P. multocida* during resampling, the outbreak was still ongoing. No additional *P. multocida* were recovered from these wetlands after the outbreak ended.

We found no significant relationship between the persistence of (probability of recovering) *P. multocida* during resampling periods and the number of times the wetland was resampled ($Z=1.338$, $P=0.18$), abundance of *P. multocida* at initial sampling ($Z=-1.144$, $P=0.25$), or wetland conditions during initial sampling. Specifically, eutrophic nutrients ($Z=1.313$, $P=0.19$), protein ($Z=-0.983$, $P=0.33$), Ca ($Z=-1.128$, $P=0.26$) and Mg ($Z=-0.731$, $P=0.47$) concentrations, water temperature ($Z=-0.389$, $P=0.70$) and pH ($Z=-1.116$, $P=0.27$) during outbreaks were not related to persistence of *P. multocida*. There was no significant relationship between persistence of *P. multocida* and where it was recovered (water and sediment versus only a single substrate) during the outbreak ($Z=-0.544$, $P=0.59$) or the virulence of *P. multocida* isolates recovered during initial sampling ($Z=-0.517$, $P=0.61$).

DISCUSSION

There have been very few studies evaluating the long-term persistence of *P. multocida* in wetlands following avian cholera outbreaks. Our results provide evidence that *P. multocida* does not persist in wetlands for long periods, especially once an avian cholera outbreak has ended. We recovered *Pasteurella* from only six of our 23 study wetlands during resampling events, and five of these recoveries occurred while the outbreak was ongoing (Table 1). We found no relationship between the persistence of *P. multocida* and its abundance, the substrate from

TABLE 2. Location, year, number of sampling events, number of *P. multocida* isolates, and day of reisolation from wetlands in the western and central United States experiencing avian cholera outbreaks during winters and springs of 1996–99.

State	Location	Wetland	Initial sampling	No. resamplings[a]	Last sampling	Initial abundance[b]	Day reisolate[c]	Resampling abundance[d]
California	Delevan NWR[e]	T11	17 January 1997	3	11 March 1997	0	—	—
California	Sutter NWR	T15	12 March 1997	2	2 April 1997	0	—	—
California	Sutter NWR	T19-2	28 January 1997	3	13 March 1997	0	—	—
California	Llano Seco NWR	T10-1	13 January 1998	3	25 March 1998	3	—	—
California	Los Banos WMA[f]	Gadwall-1	6 January 1998	3	17 March 1998	0	28	5
California	Los Banos WMA	Gadwall-2	7 January 1998	3	17 March 1998	6	—	—
California	Mud Slough	Unit 5	2 February 1998	2	18 March 1998	4	—	—
Nebraska	Rainwater Basin	Eckhardt	3 March 1998	3	20 May 1998	1	—	—
Nebraska	Rainwater Basin	Funk	2 April 1998	2	19 May 1998	2	—	—
Nebraska	Rainwater Basin	Johnson	3 April 1998	2	19 May 1998	2	—	—
Nebraska	Rainwater Basin	Massie	5 March 1998	3	20 May 1998	1	—	—
Nebraska	Rainwater Basin	Smith	4 April 1998	2	18 May 1998	0	—	—
California	Sutter NWR	T20-2	6 January 1998	2	1 April 1998	0	—	—
Missouri	Swan Lake	Swan Lake	9 February 1998	2	30 March 1998	0	—	—
California	Colusa NWR	T13A	15 January 1999	3	26 March 1999	1	20	2
California	Delevan NWR	T2-1	14 January 1999	2	23 February 1999	0	—	—
California	Hayward Park	2B	6 January 1999	3	20 March 1999	2	42	2
California	Kesterson NWR	West Teal	30 January 1999	1	19 February 1999	3	—	—
California	Merced NWR	Mariposa	11 January 1999	2	18 February 1999	0	38	2
California	Sacramento NWR	T10-3	14 January 1999	3	25 March 1999	0	40	3
California	San Luis NWR	Page Lake	8 January 1999	2	20 February 1999	0	—	—
California	West Bear Creek	Pintail Marsh	7 January 1999	2	19 February 1999	0	21, 43	1, 6
California	Yolo Bypass	Unit F	7 January 1999	2	24 March 1999	2	—	—

[a] No. resamplings: Number of times a wetland was resampled after initial sampling.

[b] Initial abundance: Number of wetland sites (out of 10) where *P. multocida* was isolated during initial sampling.

[c] Day reisolate: Day after original sampling on which *P. multocida* was isolated.

[d] Resampling abundance: Number of wetland sites (out of 10) where *P. multocida* was isolated during resampling.

[e] NWR: National Wildlife Refuge.

[f] WMA: Wetland Management Area.

which isolates were collected, the virulence of the isolates, pH, temperature, eutrophic nutrient (K, NO_3, P, and PO_3), or protein, Ca, or Mg concentrations during initial sampling. Our results are in general agreement with those of Backstrand and Botzler (1986), who sampled a waterfowl pond in California for *P. multocida* for 18 consecutive weeks before, during, and following an avian cholera outbreak. They recovered *P. multocida* only from water samples taken shortly after the onset of the outbreak (days 3 and 10), well before the outbreak ended (outbreak duration = 29 days) (Backstrand and Botzler, 1986).

We did not detect any relationships between the probability of recovering *P. multocida* during resampling and wetland water conditions during outbreaks. A laboratory study of the relationship between chemical ions and bacterial survival showed that addition of Ca and Mg to thawed wetland water increased the survival of *P. multocida* (Price et al., 1992). It should be noted, however, that the concentrations of Ca and Mg added (600 and 180 mg/l, respectively) far exceeded values we observed in wetlands during outbreaks (8.7–149.3 and 4.8–63.1 mg/l, respectively), and that the duration of their observations was a maximum of 9 days. Another laboratory study found significant differences in the survival of *P. multocida* over 12 wk as a function of temperature (2 C vs. 18 C) (Bredy and Botzler, 1989). They also found a short-term effect (1–3 days) of pH (6.3 vs. 7.3) on bacterial survival. Bredy and Botzler (1989), however, evaluated survival of *P. multocida* at concentrations (1.83×10^5–3.19×10^5 bacteria/ml) far higher than levels detected in wetlands in our study (minimum detection probabilities of 2–18 organisms/ml, Moore et al., 1998). Given the low concentration at which *P. multocida* was detected from wetlands experiencing avian cholera outbreaks in our study and the heterogeneity among wetlands in both temperature (2.9 C–18 C) and pH (6.3–8.8), it is unclear whether either factor plays an important role in the long-term survival of *Pasteurella* at concentrations found in wetlands under natural conditions.

Although our study on persistence of *P. multocida* in wetlands is one of the most comprehensive field studies to be undertaken thus far, the number of wetlands from which *P. multocida* was recovered during resampling limited our ability to determine conclusively if a relationship exists between water quality variables and persistence of *P. multocida*. In addition, there are likely some limitations in our ability to detect *P. multocida*, especially at low concentrations (minimum consistent isolation 2–18 organisms/ml; Moore et al., 1998). However, we consistently used the same methods throughout the study to maintain comparable rates of *P. multocida* isolation. We cannot determine whether the few times we recovered *P. multocida* following outbreaks represent true persistence of the bacteria or reintroduction to the wetlands by infected birds. In some wetlands, we cannot determine if *P. multocida* was present during the outbreak and we failed to recover it, or if the organism was introduced to the wetland after initiation of the outbreak. Despite these apparent limitations, our inability to recover *P. multocida* from most of the wetlands during resampling, especially after outbreaks ended (end of bird mortality), strongly supports the conclusion that *P. multocida* abundance declines after an outbreak and does not likely persist in wetlands for long enough time periods to serve as long-term reservoirs of avian cholera. However, we did not evaluate the possibility that *P. multocida* may occur in other parts of the wetland ecosystem (e.g., Rosen and Morse, 1959).

The short duration of *P. multocida* survival in wetlands following avian cholera outbreaks contrasts with patterns found for avian botulism, another common disease affecting waterbirds worldwide. *Clostridium botulinum* Type C, the caus-

ative agent of botulism, is widely distributed in wetland sediments (Smith and Sugiyama, 1988) and forms a spore state that can remain viable for decades (Hofer and Davis, 1972) until favorable environmental conditions precipitate an outbreak (Rocke et al., 1999; Rocke and Samuel, 1999). Thus wetlands likely serve as an important reservoir for avian botulism epizootics that occur when the toxin produced by *C. botulinum* is transmitted to birds that consume maggots or wetland invertebrates with botulinum toxin (Rocke and Friend, 1999). Studies indicate that transmission of *P. multocida* by ingestion of infected invertebrates or arthropods during an avian cholera epizootic may be possible but is highly unlikely (Botzler, 1991; Miller and Botzler, 1995).

Our findings support the increasing evidence that wetlands are not reservoirs of avian cholera in the absence of birds (Samuel et al., 2004) and that avian cholera is not strongly influenced by environmental conditions (Blanchong et al., 2006). Instead, *P. multocida* likely is introduced to wetlands when they are used by carrier or clinically ill birds. Once an outbreak begins, infected carcasses may lead to a short-term accumulation of *P. multocida* in wetlands and serve as an important source of infection for susceptible birds (Botzler, 1991). Upon detection of an avian cholera outbreak, biologists systematically search the wetland, collecting dead birds in an effort to reduce the severity of outbreaks and help prevent spread of the disease. Removal of carcasses, a major source of *P. multocida* (Price and Brand, 1984), may have reduced accumulation of the bacteria in wetlands we studied and decreased our ability to isolate *P. multocida* over time. As such, a management strategy of removing carcasses to reduce the accumulation of *P. multocida* may be a successful method for reducing the persistence of *P. multocida* and controlling avian cholera outbreaks.

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