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# Wetland Environmental Conditions Associated with the Risk of Avian Cholera Outbreaks and the Abundance of Pasteurella multocida

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Avian cholera is a significant infectious disease affecting waterfowl across North America and occurs worldwide among various avian species. Despite the importance of this disease, little is known about the factors that cause avian cholera outbreaks and what management strategies might be used to reduce disease mortality. Previous studies indicated that wetland water conditions may affect survival and transmission of Pasteurella multocida, the agent that causes avian cholera. These studies hypothesized that water conditions affect the likelihood that avian cholera outbreaks will occur in specific wetlands. To test these predictions, we collected data from avian cholera outbreak and non-outbreak (control) wetlands throughout North America (winter–spring 1995–1996 to 1998–1999) to evaluate whether water conditions were associated with outbreaks. Conditional logistic regression analysis on paired outbreak and non-outbreak wetlands indicated no significant association between water conditions and the risk of avian cholera outbreaks. For wetlands where avian cholera outbreaks occurred, linear regression showed that increased eutrophic nutrient concentrations (Potassium [K], nitrate [NO3 ], phosphorus [P], and phosphate [PO3 ]) were positively related to the abundance of P. multocida recovered from water and sediment samples. Wetland protein concentration and an El Nin˜o event were also associated with P. multocida abundance. Our results indicate that wetland water conditions are not strongly associated with the risk of avian cholera outbreaks; however, some variables may play a role in the abundance of P. multocida bacteria and might be important in reducing the severity of avian cholera outbreaks.

**Keywords**

avian cholera, environmental conditions, epizootiology, Pasteurella multocida, waterfowl, wetlands

**Disciplines**

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*Research Article*

# Wetland Environmental Conditions Associated with the Risk of Avian Cholera Outbreaks and the Abundance of *Pasteurella multocida*

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## Abstract

*Avian cholera is a significant infectious disease affecting waterfowl across North America and occurs worldwide among various avian species. Despite the importance of this disease, little is known about the factors that cause avian cholera outbreaks and what management strategies might be used to reduce disease mortality. Previous studies indicated that wetland water conditions may affect survival and transmission of* Pasteurella multocida*, the agent that causes avian cholera. These studies hypothesized that water conditions affect the likelihood that avian cholera outbreaks will occur in specific wetlands. To test these predictions, we collected data from avian cholera outbreak and non-outbreak (control) wetlands throughout North America (winter–spring 1995–1996 to 1998–1999) to evaluate whether water conditions were associated with outbreaks. Conditional logistic regression analysis on paired outbreak and non-outbreak wetlands indicated no significant association between water conditions and the risk of avian cholera outbreaks. For wetlands where avian cholera outbreaks occurred, linear regression showed that increased eutrophic nutrient concentrations (Potassium [K], nitrate [$NO_3$], phosphorus [P], and phosphate [$PO_3$]) were positively related to the abundance of* P. multocida *recovered from water and sediment samples. Wetland protein concentration and an El Niño event were also associated with* P. multocida *abundance. Our results indicate that wetland water conditions are not strongly associated with the risk of avian cholera outbreaks; however, some variables may play a role in the abundance of* P. multocida *bacteria and might be important in reducing the severity of avian cholera outbreaks.* (JOURNAL OF WILDLIFE MANAGEMENT 70(1):54–60; 2006)

## Key words

*avian cholera, environmental conditions, epizootiology,* Pasteurella multocida*, waterfowl, wetlands.*

Avian cholera is a significant annual cause of mortality in numerous species of North American waterfowl (Stout and Cornwell 1976, Friend 1989). The disease is caused by the gram-negative bacterium *Pasteurella multocida* and is highly infectious. This bacterium has a worldwide distribution and produces septicemic and respiratory disease in more than 180 species of wild birds (Samuel et al. 2006). The disease has now been reported in most areas of the United States and in many portions of Canada. Avian cholera is also suspected to occur in waterfowl wintering areas in Mexico, but surveillance and diagnostic efforts have been limited. Epizootics occur almost every winter in the Pacific flyway and during winter and early spring in the Central and Mississippi flyways. Although avian cholera occurs in many areas in North America, some locations have consistent, annual recurrence of disease (Wobeser et al. 1979, Wobeser 1992). The source of the disease agent and route of transmission likely play important, but not well understood, roles in epizootic dynamics. In addition, little is understood about the role of wetland water conditions in contributing to the initiation, perpetuation, or severity of avian cholera epizootics. A better understanding of these factors could contribute to the development of effective management strategies to prevent outbreaks or reduce disease losses.

Two reservoirs have been hypothesized as the source for avian cholera: carrier birds and wetlands (Botzler 1991). Several lines of evidence indicate that carrier birds are the most likely reservoirs of *P. multocida* (Botzler 1991). Serological studies of lesser snow geese (*Chen caerulescens caerulescens*) indicated that many geese were able to survive infection with *P. multocida* (Samuel et al. 1999*a*,*b*), and some of these birds (snow and Ross's geese [*Anser rosii*]) have been found to be carriers of pathogenic strains of bacteria (Samuel et al. 2005*a*), whereas others (white-fronted geese [*Anser albifrons frontalis*]) do not appear to be competent carriers (Samuel et al., 2005*b*). Regardless of the source of bacteria, once an epizootic begins, dead birds shed *P. multocida* organisms that contaminate the environment (especially water) and likely facilitate the transmission of disease (Backstrand and Botzler 1986, Bredy and Botzler 1989, Samuel et al. 2003). The disease can be spread through ingestion of contaminated water and wetland sediment, inhalation of water aerosols, or direct bird-to-bird contact (Botzler 1991, Wobeser 1992). Several laboratory and field studies have found associations between various wetland conditions and avian cholera; however, conclusions differ on the role of these wetland conditions on survival and growth of *P. multocida* (Windingstad et al. 1984, Backstrand and Botzler 1986, Price et al. 1992, Lehr et al. 2005).

Outbreaks of avian cholera are the result of complex interactions between the waterfowl host, the bacterial agent, and the environ-

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ment (Wobeser 1992). The risk of exposure and infection is related to the distribution and density of birds and bacteria, the time the host and agent are together, and the ability of the bacteria to survive in the environment (Wobeser 1992). Our study focuses on the role that physical and chemical characteristics of wetlands play in the abundance of *P. multocida* and the occurrence of avian cholera outbreaks. Identification of water conditions associated with outbreaks or abundance of *P. multocida* is important in understanding the dynamics of avian cholera epizootiology and developing appropriate management strategies to minimize disease transmission and reduce impacts on avian populations. We used a paired case-control design to determine whether wetland water conditions were significantly different between wetlands where avian cholera outbreaks occurred and wetlands where outbreaks did not occur. We also identified conditions associated with abundance of *P. multocida* in wetlands experiencing avian cholera outbreaks.

## Methods

### *Field Collection and Laboratory Processing*

During 4 winter–spring periods (1995–1996 to 1998–1999), 77 wetlands were sampled in the western and central United States (Fig. 1). We used a case-control design common to epidemiological studies (Breslow and Day 1980) that followed the design used by Rocke and Samuel (1999). This approach provides improved efficiency for assessing disease events that are uncommon or unpredictable (McDonald 2004). Wetlands where cholera outbreaks occurred were paired with similar wetlands where outbreaks did not occur, and these wetland pairs were our experimental units. Wetlands with recent avian cholera outbreaks ($\geq$100 dead birds reported) were sampled in the same week in which outbreak mortality was occurring and within 2 weeks of the first observation of bird mortality. Control wetlands were in local proximity to outbreak wetlands, were of comparable size, sampled concurrently (usually within 1 d), and had bird use, but little or no avian cholera mortality.

Ten samples were taken from each wetland sampled. Sampling sites were broadly distributed to obtain adequate coverage of the wetland area. At each site, dissolved oxygen (DO), pH, redox potential, specific conductance, and temperature were measured (Table 1) using a 610 DM multiparameter water quality monitor with 600 XL probe (Yellow Springs Instruments, Inc., Yellow Springs, Ohio). Water depth at each site was measured using a metal meter stick. For chemical analyses and *P. multocida* isolation, 100 mL and 10–15 mL water samples, respectively, were collected at each of the 10 sampled sites (Table 1). Water samples for *P. multocida* isolation were collected in sterile polypropylene centrifuge tubes (Corning®, Inc., Corning, New York) at the surface of the water. Water samples for chemical analyses were combined for each site in a 1-L container. In addition, sediment samples (approximately 30 g) were collected in a sterile polypropylene container with a screw-top lid (PGC Scientifics, Gaithersburg, Maryland) from the upper layer (top 10 cm) of sediment using a metal scoop sampler consisting of an open-faced collection bowl attached to an extension rod. All samples were stored in a cooler with ice packs until processed within 24 hours.

At the field laboratory, the 15-mL centrifuge tube containing each water sample was mixed, and 4 mL of water for *P. multocida* isolation was removed and transferred to a cryovial containing 1 mL of 50% dimethylsulfoxide (DMSO). Each sediment sample was thoroughly mixed using a sterile wooden applicator to obtain a homogeneous mixture. A sterile cotton swab was used to remove

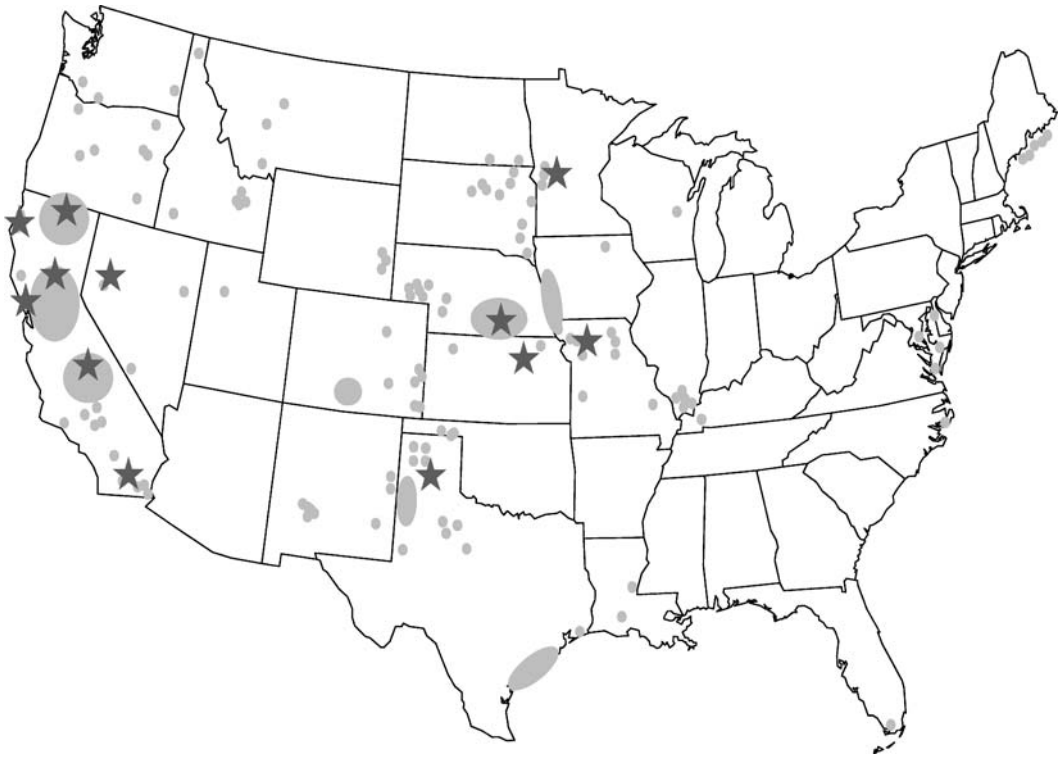

***Figure 1.*** Map of the United States indicating general locations of sampled wetlands (stars) and locations of historical avian cholera outbreaks (shaded).

***Table 1.*** Mean, range, and measurement units of environmental variables measured in control and avian cholera outbreak wetlands in North America.

| Variable | Measurement Unit | Control ($n = 34$) | | | Outbreak ($n = 43$) | | |
|---|---|---|---|---|---|---|---|
| | | Mean | Minimum | Maximum | Mean | Minimum | Maximum |
| Aluminum (Al) | ppm[a] | 5.27 | 0.00 | 119.90 | 2.39 | 0.00 | 41.43 |
| Boron (B) | ppm | 0.35 | 0.03 | 1.72 | 0.34 | 0.00 | 2.61 |
| Calcium (Ca) | ppm | 47.06 | 4.77 | 145.80 | 49.74 | 7.37 | 149.30 |
| Chloride (Cl) | ppm | 98.17 | 3.00 | 940.00 | 69.00 | 2.30 | 425.30 |
| Iron (Fe) | ppm | 2.99 | 0.00 | 60.79 | 1.51 | 0.00 | 22.18 |
| Magnesium (Mg) | ppm | 25.94 | 2.81 | 116.40 | 22.39 | 3.73 | 79.00 |
| Manganese (Mn) | ppm | 0.12 | 0.00 | 1.43 | 0.08 | 0.00 | 0.63 |
| Nitrate ($NO_3$) | ppm | 0.32 | 0.00 | 2.60 | 0.31 | 0.00 | 1.84 |
| Phosphate ($PO_3$) | ppm | 0.34 | 0.00 | 3.20 | 0.47 | 0.00 | 4.50 |
| Phosphorous (P) | ppm | 0.39 | 0.00 | 3.58 | 0.55 | 0.00 | 4.91 |
| Potassium (K) | ppm | 11.22 | 1.25 | 44.81 | 11.23 | 1.78 | 29.78 |
| Sodium (Na) | ppm | 90.14 | 5.24 | 537.30 | 79.64 | 5.29 | 455.20 |
| Sulfate ($SO_4$) | ppm | 21.62 | 0.00 | 112.70 | 19.86 | 0.00 | 94.40 |
| Sulfur (S) | ppm | 41.57 | 1.17 | 231.80 | 38.86 | 0.61 | 201.50 |
| Zinc (Zn) | ppm | 0.07 | 0.00 | 0.91 | 0.05 | 0.00 | 0.51 |
| Depth | inches | 14.24 | 4.80 | 40.20 | 15.51 | 4.70 | 31.10 |
| Dissolved Oxygen (DO) | mg/L | 9.34 | −2.60 | 18.63 | 9.93 | 0.80 | 19.52 |
| Dissolved Protein (Protein) | mg/mL | 11.81 | 2.70 | 25.90 | 11.85 | 3.10 | 33.10 |
| pH | pH | 7.95 | 6.60 | 9.73 | 7.88 | 6.34 | 9.80 |
| Redox Potential | mV | 89.54 | −74.60 | 235.20 | 96.68 | −89.20 | 218.30 |
| Specific Conductance | mS/cm | 588.12 | 53.00 | 2,981.00 | 545.63 | 68.00 | 1,964.00 |
| Temperature | C | 9.24 | 0.96 | 16.97 | 9.05 | 1.16 | 18.00 |
| Turbidity | NTU | 1,048.21 | 9.30 | 14,100.00 | 747.55 | 7.60 | 10,590.00 |

[a] ppm, parts per million; mV, millivolts; mS, milliSiemens; C, degrees celsius; NTU, nephelometric turbidity units.

approximately 0.5 g of sediment/water from the sample and immersed and swirled vigorously in a cryovial containing 4 mL of a 10% solution of DMSO. The cryovials containing water and sediment samples for testing were stored frozen (−170 C) in a dry-shipper liquid-nitrogen tank (model SC 4/2v, Minnesota Valley Engineering, Inc., Bloomington, Minnesota).

Turbidity for the wetland was measured within 8 hours of sampling, using the pooled sample of water from each site. Measurements were taken in nephelometric turbidity units, using a Hach® 2100P Turbidimeter (Hach Company, Loveland, Colorado). Very turbid samples were diluted as needed with demineralized water to bring them into range of the equipment.

For the analysis of dissolved proteins, samples taken from the 10 sites within each wetland were pooled (total volume = 1 L), and 50 mL of the sample was filtered through both 0.8-μm (Nalgene™ no. 190-2580, Nalge Company, Rochester, New York) and 0.45-μm (Corning, Inc. no. 21053–25) syringe filters within 8 hours of sampling to remove particulate matter that might interfere with testing. They were then chilled and returned to the National Wildlife Health Center (NWHC; Madison, Wisconsin, USA) where they were frozen at −20C. Total protein concentration (μg/mL) was determined using a bicinchoninic protein assay kit (Sigma Chemical Company, St. Louis, Missouri), using Procedure no. TPRO-562, a modification of Smith et al. (1985).

At each of the 10 sites with sampled wetlands, 100 mL of water was collected using a glass beaker and combined in a 1-L Nalgene bottle. Samples were kept chilled and sent to the NWHC, where a 250-mL subsample was then removed, filtered with a vacuum pump through a Buchner funnel assembly using a 15-cm, 1-μm glass fiber filter (Whatman International, Maidstone, England), and stored chilled in a small Nalgene bottle for analytical chemistry analysis. Chemical analyses of water samples were conducted at the Soils and Plant Analysis Laboratory Soils Department, University of Wisconsin-Madison, USA, using an Applied Research Laboratories 34,000 RTB ICP Optical Emission Spectrometer (Thermo Jarrell Ash Corporation, Franklin, Massachusetts).

At the NWHC, the cryovials containing the water and sediment samples were processed for *P. multocida* isolation following the procedure described by Moore et al. (1998). After 20–24 hours of incubation, suspect *P. multocida* colonies were selected (Samuel et al. 2003). After re-isolation, suspect colonies were Gram-stained, and the API® 20E or API NE identification system (bio-Merieux, Inc., Hazelwood, Missouri) was used to identify *P. multocida* isolates. All *P. multocida* isolates were serotyped using the agarose gel precipitation test (Heddleston et al. 1972).

### Statistical Analyses

Several variables were measured from the pooled water samples from each wetland (aluminum [Al], boron [B], calcium [Ca], chlorine [Cl], dissolved protein, iron [Fe], potassium [K], magnesium [Mg], manganese [Mn], sodium [Na], nitrate [$NO_3$], phosphate [$PO_3$], sulfur [S], sulfate [$SO_4$], turbidity, and zinc [Zn]). Measurements for the other water quality variables were obtained directly at each of the 10 sites within a wetland (depth, DO, pH, redox potential, specific conductance, and temperature). We considered each wetland to be our experimental unit of interest for the following analyses; however, to assess the effect of averaging the measurements taken at each of the 10 sites within a wetland, we conducted a nested analysis of variance (ANOVA) to compare within and among wetland variation for variables measured at each of the 10 sampled sites.

We standardized redox potential for pH and temperature (Smoot and Pierson 1979, Rocke and Samuel 1999) to compare redox potential over the range of conditions observed in our study wetlands. In addition, several variables (Ca, Cl, K, Mg, S, $SO_4$,

depth, dissolved protein, specific conductance, and turbidity) had skewed distributions and were natural-log (ln) transformed for normality. Other variables could not be easily transformed to normality (Al, B, Fe, Mn, $NO_3$, P, $PO_3$, and Zn). We used principal component analysis (SAS version 8.1, Cary, North Carolina) to produce multivariate, linear combinations of correlated variables and to reduce the number of covariates in our analyses. Principal components with eigen values >1.0 were selected for varimax rotation to produce factors that maximized the contribution of each variable to a single factor and enhanced biological interpretation. Temperature and dissolved protein, previously reported to be related to isolation of *P. multocida* or its survival (Windingstad et al. 1988, Bredy and Botzler 1989), were retained as individual factors. The resulting factors were used for subsequent analyses of wetland water conditions associated with avian cholera outbreaks or the abundance of *P. multocida*.

We used McNemar's test (StatXact version 4, Statistical Solutions, Cambridge, Massachusetts) to evaluate the null hypothesis that *P. multocida* was more likely to be recovered from outbreak wetlands than from control wetlands. Because of small sample sizes, the association between wetlands and isolation of *P. multocida* was evaluated using an exact test. Exact test statistics tend to be overly conservative, especially in the case of small sample sizes (Lancaster 1961). Rather than using the exact $P$ value to assess statistical significance, we report the 1-tailed mid-$P$ value, which adjusts for the overly conservative results given by exact tests (Lancaster 1961, Agresti 2001, Lydersen and Laake 2003).

We used paired $t$-tests to determine whether wetland water factors consistently differed between paired avian cholera outbreak and control wetlands. We also used conditional logistic regression (program R version 2.0.0, R Development Core Team 2005) in a case-control design to evaluate the association between measured water factors and the relative probability (risk) that a wetland experienced an avian cholera outbreak.

For wetlands with significant avian cholera mortality (outbreak wetlands), we used multiple linear regression (R version 2.0.0, R Development Core Team 2005) to evaluate potential associations between wetland water conditions and abundance (number of sites) of *P. multocida* serotype-1 isolated from the wetland. In addition to water conditions, other covariates in our analysis included duration of the outbreak (weeks), size of the wetland (hectares), and whether sampling took place during an El Niño event (winter–spring 1997–1998). Regression models were evaluated using backward stepwise regression, Akaike information criterion (AIC) values, and model averaging (Burnham and Anderson 2002).

## Results

We sampled 34 pairs of outbreak and control wetlands and 9 additional outbreak wetlands that were not paired with a control, across western and central North America during 4 winter–spring periods (1995–1996 to 1998–1999) (Fig. 1). We recovered 78 *P. multocida* serotype-1 isolates from 71 (9%) of 770 sampled sites in 31 (40%) of these 77 wetlands. Isolates were obtained from 20 (47%) of 43 outbreak wetlands and 11 (32%) of 34 control wetlands. We recovered *P. multocida* from wetlands in each year of sampling. *P. multocida* was only isolated from wetlands located in California and Nebraska (Pacific and Central flyways, respectively).

We found considerable variation in physical and chemical measures among wetlands (Table 1). Nested ANOVAs showed that the magnitude of variation among wetlands was substantially larger than variation within wetlands ($F$ ratios ranged from 10.3 to 610.4) for water condition variables measured at each of the 10 sampled sites (water depth, DO, pH, redox potential, specific conductance, and temperature).

Six principal components accounted for 78.6% of the variation in the 23 original variables. The varimax rotation produced 6 factors with relatively clear biological interpretation (Table 2). Factor 1 contained variables associated with increased conductivity (Dunson et al. 1997) and explained the largest portion of the variation found in water quality measurements. Factor 2 accounted for higher trace metals and increased turbidity and accounted for the second highest portion of variation. The variables aggregated in Factor 3 were associated with eutrophic nutrients (K, $NO_3$, P, and $PO_3$; Berner and Berner 1996). Factor 4 consisted of pH and redox potential, Factor 5 contained Mn and a negative effect of DO, and Factor 6 was represented by water depth and a negative association with turbidity. These 6 factors, along with temperature and dissolved protein, were used in subsequent analyses.

We found a marginally significant increase in the probability of isolating *P. multocida* from outbreak wetlands (odds ratio = 2.25, $\chi_1^2 = 1.23$, 1-tailed mid-$P = 0.09$). Paired $t$-tests, however, indicated no significant differences between outbreak and control wetlands for any factor (all df = 33, all $P > 0.17$). Conditional logistic regression analysis on paired outbreak and control wetlands indicated no significant association between wetland water conditions and the probability that a wetland had an avian cholera outbreak (likelihood ratio test $\chi_9^2 = 7.62$, $P = 0.57$).

For wetlands where avian cholera outbreaks occurred ($n = 43$), visual assessment of the relationship between wetland water conditions and the abundance of *P. multocida* showed potential nonlinear patterns for Factor 4, Factor 6, temperature, and dissolved protein. We used a quadratic model to assess these relationships. The distribution of wetland *P. multocida* abundance fit a natural-log (ln) distribution far better (AIC = 4.66) than a Poisson distribution (AIC = 14.97; program R version 2.0.0, R Development Core Team 2005). Therefore, abundance of *P. multocida* was natural-log (ln) transformed for subsequent analyses. Multiple linear regression indicated that 4 models had similar ability in explaining *P. multocida* abundance (Table 3). Factor 1 (conductivity), Factor 3 (nutrients), dissolved protein, sampling during an El Niño season, duration of outbreak, and wetland size were the covariates included in these models. We used model averaging from these 4 models to determine average parameter coefficients, standard errors, and the relative importance of each factor (Table 4). Ninety-five percent confidence limits on model-averaged coefficients indicated that Factor 3 (eutrophic nutrients), dissolved protein, and the El Niño event of 1997–1998 had the highest relative importance and were significantly related (95% confidence limits did not overlap 0.0) to the abundance of *P. multocida* in outbreak wetlands (Table 4).

## Discussion

We were marginally more likely to recover *P. multocida* from outbreak wetlands than from non-outbreak wetlands sampled

***Table 2.*** Six principal components obtained after varimax rotation of environmental variables measured in wetlands throughout North America in 1995–1999. Standardized regression coefficients indicate sign and relative strength of environmental variables influencing each factor. Cumulative explained variation indicates the proportion of total variation explained by addition of each factor.

| Factor | Environmental variable[a] | Coefficient | Cumulative explained variation |
|---|---|---|---|
| 1 (Conductivity) | Specific conductance | 0.92744 | 0.3072 |
| | Na | 0.91767 | |
| | S | 0.91606 | |
| | $SO_4$ | 0.86452 | |
| | Mg | 0.86318 | |
| | Cl | 0.81037 | |
| | Ca | 0.78070 | |
| | B | 0.67175 | |
| 2 (Metals/turbidity) | Al | 0.96862 | 0.4866 |
| | Fe | 0.95953 | |
| | Zn | 0.87592 | |
| | Turbidity | 0.50270 | |
| 3 (Nutrients) | P | 0.91424 | 0.5859 |
| | $PO_3$ | 0.90783 | |
| | $NO_3$ | 0.66704 | |
| | K | 0.60564 | |
| 4 (Redox/pH) | Redox potential | 0.78514 | 0.6743 |
| | pH | 0.75458 | |
| 5 (Mn/DO) | Mn | 0.76582 | 0.7346 |
| | DO | −0.70615 | |
| 6 (Water depth) | Water depth | 0.88441 | 0.7857 |
| | Turbidity | −0.63404 | |

[a] N, sodium; S, sulfur; $SO_4$, sulfate; Mg, magnesium; Cl, chlorine; Ca, calcium; B, boron; Al, aluminum; Fe, iron; Zn, zinc; P, phosphorus; $PO_3$, phosphate; $NO_3$, nitrate; K, potassium; Mn, manganese; DO, dissolved oxygen.

throughout North America during 1995–1999. However, we found no significant differences in water conditions between paired avian cholera outbreak and control wetlands. The absence of an association between wetland water conditions and relative risk of avian cholera is in contrast to the strong association between water conditions and risk of avian botulism (Rocke and Samuel 1999, Rocke et al. 1999). The differing influence of water conditions on the risk of botulism versus avian cholera might be explained by differences between the causative agents. Unlike *P. multocida*, which is relatively short-lived in wetlands (Samuel et al., 2004; Blanchong et al., in press), *Clostridium botulinum* Type C, the causative 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), waiting for favorable conditions that facilitate bacterial replication and precipitate an outbreak.

Reported relationships between wetland water conditions and avian cholera outbreaks have differed among studies. Most studies investigating associations between avian cholera and water conditions were carried out in a laboratory setting often under ecological conditions quite different from those found in wetland ecosystems (Hutyra et al. 1949, Dimov 1964, Olson and Bond 1968, Awad et al. 1976, Bredy and Botzler 1989). Previous field studies were limited to a single geographic location or a short period of time (Windingstad et al. 1984, Lehr et al. 2005). Our study represents the most comprehensive spatial and temporal investigation of the relationship between wetland conditions and avian cholera outbreaks to date; however, we were unable to document an association between wetland water conditions and the risk of avian cholera outbreaks.

We found a significant relationship between wetland chemical and physical characteristics and abundance of *P. multocida* recovered from outbreak wetlands. Water conditions associated with increased eutrophic nutrients (K, $NO_3$, P, and $PO_3$) were positively related to *P. multocida* abundance. Potential sources of increased nutrients in wetlands include urbanization, sewage treatment, and agricultural systems. Runoff from sewage and agricultural areas is usually high in dissolved N, P, nitrates, and phosphates (Dorioz and Ferhi 1994), and avian cholera outbreaks have been associated with wetlands receiving poor irrigation drain water and treatment water (Smith et al. 1989). Based on the association found in our study, research to investigate the relationship between eutrophic nutrients and the abundance of *P. multocida* is warranted. In addition, increased scrutiny of nutrient conditions associated with wetland water sources and artificial wetlands may be appropriate, especially in areas with a regular history of avian cholera outbreaks.

Previous studies of the relationship between avian cholera outbreaks and water conditions detected a positive relationship between protein concentration and avian cholera outbreaks (Rosen and Bischoff 1950, Titche 1979). These studies hypothesized that increased protein concentrations were related to the presence and decay of bird carcasses in wetlands and that increased protein helped bacteria to survive and facilitated transmission of the bacteria among birds (Rosen and Bischoff 1950, Titche 1979, Botzler 1991). We found a nonlinear relationship between concentrations of dissolved protein and abundance of *P. multocida*. Specifically, there was a positive relationship between the abundance of bacterium and dissolved protein up to approximately 12 μg/L, but the relationship was negative at higher protein concentrations. We did not explicitly test the mechanism by which protein concentration was related to abundance of *P. multocida*. In a laboratory study, Bredy and Botzler (1989) found that soluble

***Table 3.*** Akaike information criterion (AIC) values and parameters included in the best-fitting models explaining variation in the number of sampling sites within avian cholera outbreak wetlands from which *Pasteurella multocida* was isolated.

| Parameters[a] | AIC value | ΔAIC[b] |
|---|---|---|
| Factor 3 (nutrients), protein$^2$, El Niño season | 68.27 | 0 |
| Factor 3, protein$^2$, El Niño season, wetland size | 68.85 | 0.58 |
| Factor 3, protein$^2$, El Niño season, wetland size, outbreak duration | 69.37 | 1.10 |
| Factor 1 (conductivity), F3, protein$^2$, El Niño season, wetland size, outbreak duration | 69.72 | 1.45 |

[a] Refer to Table 2 for environmental variables in Factors 1 (F1) and 3 (F3); protein$^2$, dissolved protein concentration squared.
[b] ΔAIC, difference in AIC value of a model relative to the best fitting model.

***Table 4.*** Coefficients, standard errors (SE), 95% confidence intervals (CI), and relative importance of environmental conditions explaining variation in the number of sites within avian cholera outbreak wetlands from which *Pasteurella multocida* was isolated, as determined by model averaging.

| Parameter[a] | Coefficient | SE | 95 % Lower CI | 95% Upper CI | Relative importance |
|---|---|---|---|---|---|
| Intercept | 0.3184 | 0.1563 | 0.0059 | 0.6310 | 1 |
| Protein$^2$ | −0.2049 | 0.0439 | −0.2926 | −0.1172 | 0.9718 |
| F3 (nutrients) | 0.1024 | 0.0384 | 0.0256 | 0.1792 | 0.9380 |
| El Niño season | 0.3768 | 0.1521 | 0.0727 | 0.6809 | 0.8695 |
| F1 (conductivity) | 0.0010 | 0.0007 | −0.0005 | 0.0024 | 0.4745 |
| Wetland size | −0.0009 | 0.0010 | −0.0029 | 0.0011 | 0.4667 |
| Outbreak duration | −0.0010 | 0.0007 | −0.0024 | 0.0004 | 0.4059 |

[a] Refer to Table 2 for environmental variables included in Factors 1 (F1) and (F3); protein$^2$, dissolved protein concentration squared.

protein concentrations increased survival of *P. multocida*. However, the concentration of soluble protein used in their study (175 μg/mL) far exceeded levels that we observed in natural wetland ecosystems (mean = 11.8 μg/mL, range = 2.7–33.1 μg/mL).

*P. multocida* was isolated from significantly more sites during 1997–1998, one of the strongest El Niño events on record. We suspect this pattern may be explained by warmer temperatures and increased rainfall, resulting in more open water and a wider distribution of birds and bacteria across wetlands. Further research is necessary to explicitly test the relationship between wetland conditions, avian cholera outbreaks, and bacterial abundance.

One limitation of our study was our inability to document the abundance, behavior, or activity of species of birds using our study wetlands. It is becoming increasingly clear that the distribution and behavior of birds on wetlands is likely important for the transmission of avian cholera. The species composition and use patterns of birds may help explain why we isolated *P. multocida* at several control wetlands where outbreaks did not occur. Some control wetlands suffered low levels of bird mortality; however, it is possible that some birds may not die of avian cholera at the same wetlands where they become infected. In addition, carcasses were typically not removed from the control wetlands by managers, unlike outbreak wetlands where high levels of mortality occurred. The long-term presence of a small number of carcasses may have resulted in the accumulation of *P. multocida* in the wetland, but the presence of bacteria alone at control sites was not sufficient to produce an avian cholera outbreak.

## Management Implications

Despite the importance of avian cholera as a cause of mortality in birds using wetlands ecosystems, our knowledge of the epizootiology of this disease remains limited. As a result, management strategies have largely been reactive, focusing on collection and disposal of dead birds as a means to reduce the severity of local outbreaks and to help prevent spread of the disease. Alternative management strategies that help prevent outbreaks or reduce losses would be beneficial and potentially more cost-effective. Previous researchers proposed that water conditions were associated with the risk of outbreaks occurring in wetlands, and thus suggested that wetlands could be manipulated to reduce disease risk. In contrast to previous hypotheses, we were unable to detect an association between wetland water conditions and avian cholera outbreaks. Thus, we were unable to identify wetland water conditions that could be manipulated by managers to reduce the risk of avian cholera outbreaks. Instead, our findings provide additional support for the increasing evidence that wetland water conditions are not the primary factors determining disease outbreaks (Samuel et al. 2004). Other research indicates that wetlands are not the primary long-term reservoirs for avian cholera and that *P. multocida* is likely introduced to wetlands each year by carrier birds (Samuel et al. 2005*a*). Together, these results indicate that strategies for avian cholera management should focus on manipulation of bird species abundance and use patterns to separate species that serve as the reservoir and carriers for *P. multocida* from more susceptible species. We also detected associations between wetland water conditions and abundance of *P. multocida* isolated from outbreak wetlands, which represents a significant new finding. Although we cannot explain the mechanisms underlying these associations, we suspect these factors may play a role in the survival of bacteria in wetlands and potential transmission to susceptible birds. In addition, the association between bacterial abundance and nutrient levels is of particular concern because of increased eutrophic conditions in wetlands and the spread of avian cholera outbreaks throughout North America. We recommend that future research studies focus on the role of carrier and susceptible birds in disease transmission and spread, the role of weather and other stressors in initiating outbreaks, and the potential importance of wetland nutrient levels (and other factors) in survival and growth of the bacterial disease agent.

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