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Authors: Mensik, J. Gregory, and Botzler, Richard G.

Source: Journal of Wildlife Diseases, 25(2) : 240-245

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-25.2.240>

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## EPIZOOTIOLOGICAL FEATURES OF AVIAN CHOLERA ON THE NORTH COAST OF CALIFORNIA

J. Gregory Mensik<sup>1,2</sup> and Richard G. Botzler<sup>1</sup>

<sup>1</sup> Department of Wildlife, Humboldt State University, Arcata, California 95521, USA

<sup>2</sup> Present address: Sacramento National Wildlife Refuge, Route 1, Box 311, Willows, California 95988, USA

**ABSTRACT:** An avian cholera (*Pasteurella multocida*) epizootic was observed among wildfowl at the Centerville Gun Club, Humboldt County, California (USA) in January 1978. Compared to their live populations and use of the area, coots (*Fulica americana*) died in proportionately greater numbers than any other species. Coots collected by gunshot were evaluated for sex and age composition, and morphometry from November 1977 through mid-January 1978 at this site. There was no substantial difference in the sex, age or morphometry between birds dying of avian cholera and from those dying from gunshot. Assuming coots dying of gunshot are representative of the general population, it appears there was little selection among coots by *P. multocida*. There was evidence for a sequential mortality similar to that reported previously at this site: coots were the first birds to die, followed by American wigeon (*Anas americana*) and northern pintails (*A. acuta acuta*); northern shovelers (*A. clypeata*) and mallards (*A. platyrhynchos*) died late in the epizootic.

**Key words:** Avian cholera, disproportionate mortality, *Fulica americana*, morphometry, sequential mortality, *Pasteurella multocida*, epizootic, field study.

### INTRODUCTION

Avian cholera, caused by the bacterium *Pasteurella multocida*, is a serious disease among wildfowl throughout North America (Rosen, 1971). There is little information on the dynamics of the disease in susceptible wildfowl populations during an epizootic. For example, it is uncertain whether all individuals in the population of a particular species are equally susceptible to avian cholera. Among common eiders (*Somateria mollissima*) in Maine (USA), Korschgen et al. (1978) found that >90% of the total avian cholera mortality occurred in females. In contrast, McLandress (1983) found significantly greater mortality from avian cholera in male Ross' geese (*Chen rossii*) and male lesser snow geese (*C. caerulescens caerulescens*) in California (USA), when compared to the sex ratio among hunter-killed birds. We are not aware of any reports of variations in susceptibility to avian cholera based on age or other physical features in wild populations. Coots (*Fulica americana*) are often the major species dying from avian cholera on the California north coast during an epizootic (Hazlewood et al., 1978; Oddo et al. 1978). If intraspecies variation

to disease susceptibility does occur, managers should know which population subsets are most susceptible.

In a past study at the Centerville Gun Club (Humboldt County, California, USA) there was evidence for a distinct sequence of mortality of the affected wildfowl population: coots and American wigeon (*Anas americana*) died early in the epizootic, followed by gadwalls (*A. strepera*) and northern pintails (*A. acuta acuta*); northern shovelers (*A. clypeata*) and mallards (*A. platyrhynchos*) died late in the epizootic (Oddo et al., 1978). There have been no comparable reports of sequential mortality at other sites.

The objectives of this study were to determine (1) whether there are significant differences in the sex, age or morphometry of coots dying from avian cholera, compared to those dying of gunshot wounds, (2) whether the sequential mortality observed previously at Centerville is a consistent feature of this site, and (3) the mortality rate among related species on the Centerville site.

### MATERIALS AND METHODS

The study was conducted at the Centerville Gun Club (Humboldt County, California, USA;

40°30'N, 124°10'W). This area has been described previously (Hazlewood et al., 1978; Backstrand and Botzler, 1986; Mensik, 1986).

A population survey of all species of waterfowl was conducted on the club once each week from 1 November 1977 to 19 January 1978. The number of birds of each species present was multiplied by seven and summed to yield an estimated total of bird use days for that week [one bird use day (BUD) = one bird observed on club lands on a given day]. Weekly totals were then added to yield a grand total, on which a percent use by each species was computed.

Between 8 November 1977 and 19 January 1978, two to 10 coots were shot on the wing each week and frozen at  $-4\text{ C}$  until they were examined. Sex was determined at necropsy. The age of each bird was based on leg color as described by Gullion (1952). The following weights (in g) and measurements (in mm) were defined and recorded for each coot: (1) total length as the length from tip of bill to tip of tail; (2) lower leg length as the length of leg from tibiotarsus/femur joint to tip of longest claw; (3) wing length as the length from tip of primaries to the humerus/coracoid joint, along anterior edge of the wing; (4) total weight as the weight of entire bird; (5) viscera weight as the weight of all body cavity organs extending from proximal end of trachea to distal end of cloaca with specific weights recorded for the empty heart, empty gizzard, liver, spleen, and lungs; (6) external extremities as the weight of feathers, skin, tail, wings, lower legs, and head with the head detached from the neck at the base of the skull, the lower legs detached at the tibiotarsus/femur joint, the wings detached at the humerus/coracoid joint, and the tail detached at the ilium/caudal vertebral joint; (7) carcass weight as the weight of remaining bone and muscle after viscera and external extremities were removed; (8) neck weight as the weight of cervical vertebrae with attached flesh; (9) rib weight as the weight of rib to anterior end of ilium (synsacrum), not including the coracoid and sternum; (10) pelvis/leg weight as the weight of whole legs from femur/illium joint to toe claws, and pelvic girdle from anterior end of the ilium to the posterior end/caudal vertebral joint; (11) cardiac fat as the width of the cardiac fat measured at its widest point; (12) gizzard fat index (GFI) as the gizzard removed at the poies, opened and cleaned of all grit and vegetation, all fat scraped from the outside surface, and the weight of the gizzard fat divided by the weight of the stripped and cleaned gizzard, multiplied by 100, being defined as the gizzard fat index; (13) muscle to bone ratio as the combined weight of the pectoralis major and supracoracoideus muscles di-

vided by the combined weight of the sternum and coracoid; and (14) depth of lower back fat as the deepest point of the fat at the base of the tail. Mean values for all physical parameters were calculated weekly for the coots killed by gunshot to determine any significant changes in the Centerville population during the 1977–1978 winter season. Heart blood was not evaluated for *P. multocida* among birds collected by gunshot.

An avian cholera epizootic occurred on the Centerville Gun Club from 5 to 31 January 1978. Carcass collections of affected birds were made throughout the epizootic. Additional air and ground searches of surrounding lands were conducted on 9 and 10 January 1978.

Forty-four intact coots and two intact carcasses of most other species collected were frozen and later necropsied and tested for the presence of *P. multocida*. Heart blood was streaked onto Brain Heart Infusion Agar (Difco Lab., Inc., Detroit, Michigan 48232, USA). Identification of suspect isolates was based on the procedures of Rosen (1971) and Heddleston (1975). Each coot was then measured as described above for those that were killed by gunshot.

Ten additional coots were sent to the California Department of Fish and Game Wildlife Investigational Laboratory (Rancho Cordova, California 95670, USA). Cultures isolated from these birds were serotyped and biochemically confirmed as *P. multocida* at the National Animal Disease Laboratory (Ames, Iowa 50010, USA).

Steel and Torrie (1960) were used as a basis for evaluating significant variations with the Chi-square test, binomial confidence limits, and the *t*-test. The G-test for heterogeneity was used as described by Sokal and Rohlf (1969). The significance level for all tests was set at  $\alpha = 0.05$ .

## RESULTS

Coots were the most common bird on the club, with an estimated 21,812 (56%) of the total 39,158 BUD calculated during the January 1978 avian cholera epizootic (Table 1). The most commonly observed duck species were northern pintails, American wigeon, American green-winged teal (*Anas crecca carolinensis*), northern shovellers and ruddy ducks (*Oxyura jamaicensis rubida*). The only other species with a >1% prevalence of the observed waterfowl use was the tundra swan (*Cygnus columbianus*) (Table 1).

One thousand one hundred four dead

TABLE 1. Estimated waterfowl use and avian cholera mortality during January 1978, Centerville Gun Club, Humboldt County, California.

Species	Estimated bird use days (BUD) <sup>a</sup>		Avian cholera mortality <sup>b</sup>	
	n	%	n	%
American coot ( <i>Fulica americana</i> )	21,812	56	901	82
Northern pintail ( <i>Anas acuta acuta</i> )	8,351	21	55	5
American wigeon ( <i>Anas americana</i> )	4,004	10	67	6
Tundra swan ( <i>Cygnus columbianus</i> )	2,506	6	7	<1
Ruddy duck ( <i>Oxyura jamaicensis rubida</i> )	1,316	3	10	<1
Northern shoveler ( <i>Anas clypeata</i> )	371	<1	35	3
American green-winged teal ( <i>Anas crecca carolinensis</i> )	315	<1	9	<1
Mallard ( <i>Anas platyrhynchos</i> )	203	<1	8	<1
Lesser scaup ( <i>Aythya affinis</i> )	147	<1	4	<1
Others	133 <sup>c</sup>	<1	8 <sup>d</sup>	<1
Total	39,158	100.0	1,104	100.0

<sup>a</sup> Monitored from 5 to 19 January 1978.

<sup>b</sup> Carcasses collected 5 to 31 January 1978.

<sup>c</sup> Includes canvasback (*Aythya valisineria*) and snow goose (*Chen caerulescens caerulescens*).

<sup>d</sup> Includes two canvasbacks, and one each of bufflehead (*Bucephala albeola*), white-winged scoter (*Melanitta fusca*), western gull (*Larus occidentalis*), long-billed dowitcher (*Limnodromus scolopaceus*), eared grebe (*Podiceps nigricollis*), and great blue heron (*Ardea herodias*).

birds, consisting of 16 species, were collected during the avian cholera epizootic: coots, wigeon, pintails and shovelers were the species most frequently affected (Table 1). *Pasteurella multocida* was isolated from coots, wigeon, pintails, shovelers, tundra swans and a long-billed dowitcher (*Limnodromus scolopaceus*). This is the first known report of avian cholera from the latter species.

*Pasteurella multocida* was isolated from all of the 44 intact coots collected during the epizootic. All isolates were Gram-negative, bipolar staining rods. All were positive for catalase, oxidase, and indol; further, all isolates were negative for methyl red, acetylmethylcarbinol, urease, and growth on citrate. Serotype 1 *P. multocida* was isolated from all 10 additional coots sent to the California Department of Fish and Game Wildlife Investigational Labo-

ratory. In addition to the aforementioned biochemical characteristics, the isolates from these latter birds also were nonmotile, and negative for gelatinase, and growth on MacConkey's Agar; they fermented dextrose, sucrose, mannose, mannitol, sorbitol, galactose, glycerol, and levulose, but did not ferment lactose, maltose, trehalose, arabinose, dulcitol, xylose, raffinose, rhamnose, dextrin, inulin, salicin or inositol.

Based on binomial confidence limits for this sample size, it is 99% certain that at least 90% of the coots dying during the epizootic died from avian cholera. Thus, mortality due to factors other than avian cholera in this epizootic was unlikely. Avian cholera mortality was not observed at other sites in the Eel River delta, or reported elsewhere on the California north coast during this epizootic.

There was evidence for a disproportion-

TABLE 2. Physical characteristics of coots dying from avian cholera and gunshot at Centerville Gun Club, Humboldt County, California. Mean values followed by standard deviations in parentheses.

	Gunshot <sup>c</sup>					
	Total (n = 91)		January only (n = 28)		Avian cholera <sup>a</sup> (n = 44)	
	$\bar{x}$	(SD)	$\bar{x}$	(SD)	$\bar{x}$	(SD)
Length (in mm)						
Total	388	(18)	389	(17)	383	(24)
Leg	138	(8)	141	(8)	141	(8)
Wing	318	(14)	322	(14)	319	(14)
Weight (in grams)						
Total	551	(81)	611 <sup>b</sup>	(86)	568	(66)
Viscera	156	(27)	181 <sup>b</sup>	(30)	159	(21)
Heart	5	(1)	5	(1)	7 <sup>d,e</sup>	(2)
Gizzard	53	(10)	59	(10)	54	(8)
Gizzard fat	4	(2)	5	(2)	5	(1)
Liver	13	(3)	16 <sup>c</sup>	(3)	18 <sup>d</sup>	(3)
Spleen	1	(<1)	<1	(2)	1 <sup>d,e</sup>	(<1)
Lungs	6	(2)	6	(2)	7	(1)
External extremities	167	(30)	179	(31)	168	(23)
Remaining carcass	147	(24)	161	(24)	155	(20)
Neck	18	(3)	19	(3)	19	(3)
Ribs	31	(6)	31	(5)	30	(5)
Legs	90	(15)	96	(15)	95	(12)
Miscellaneous						
Heart band fat (mm)	3	(1)	4	(<1)	3	(<1)
Depth of back fat (mm)	1	(<1)	1	(1)	2	(<1)
Muscle to bone ratio	10	(2)	12	(2)	11	(1)

<sup>c</sup> Coots dying from gunshot collected 8 November 1977 to 19 January 1978.

<sup>a</sup> Coots dying from avian cholera collected 6 to 10 January 1978.

<sup>b</sup> Based on a two-tailed *t*-test, significantly ( $P < 0.05$ ) different from coots collected by gunshot in November and December 1977.

<sup>d</sup> Based on a two-tailed *t*-test, significantly ( $P < 0.05$ ) different from total sample of coots collected by gunshot.

<sup>e</sup> Based on a two-tailed *t*-test, significantly ( $P < 0.05$ ) different from coots collected by shooting in January 1978.

ate mortality among the species affected. Eighty-two percent of all recorded avian cholera losses were coots, but this species consisted of only 56% of the population during the avian cholera epizootic (Table 1). In contrast, lower frequencies of most other species were observed dead than would be expected, based on their observed use of the area.

There were some significant variations in the physical parameters among the 91 apparently healthy coots collected between 8 November 1977 and 19 January 1978 (Table 2). The means for total weight, viscera weight and liver weight among coots that were shot were significantly ( $P < 0.05$ ) greater from birds shot in January

than from birds shot in November and December. However, length measures showed little variation throughout the study period. Likewise, fat deposits and muscle to bone ratio changed very little over the course of the study (Table 2). The January sample of coots that were shot had a higher ratio of males:females (19 males:9 females), compared to November (21 males:19 females) and December (12 males:11 females); but this difference was not significant.

The mean weight of the heart, liver and spleen of birds dying from avian cholera was significantly ( $P < 0.05$ ) greater than those dying from gunshot (Table 2). Because these organs often become hyper-

TABLE 3. Estimated age structure of coots dying from gunshot and avian cholera, Centerville Gun Club, Humboldt County, California, January 1978.

Age (yr)	Gunshot		Avian cholera	
	n	%	n	%
0-1	21	24	6	18
1-2	5	6	3	9
2+	62	70	24	73
Total	88	100	33	100

emic at death and are the sites of severe inflammatory exudation by leukocytes, the observed differences may have been due to sequelae of the disease rather than genuine differences between the birds. There were no significant differences in the age structure of birds found dead during the epizootic, compared to the shot coots sampled (Table 3). Of the 44 intact coots found dead during the epizootic, 26 were males and 18 were females. This was not significantly different from that observed among the January sample, or the total sample of coots that were shot.

There was evidence for a sequential mortality among the affected species (Table 4). A significantly ( $P < 0.05$ ) greater proportion of coots (70%) died before 8 January 1978, compared to wigeon (28%), ruddy ducks (20%), pintail (20%), teal (11%), shovelers (3%), scaup (0%), and mallards (0%) (Table 4). Likewise, significantly fewer mallards, scaup and shovelers died before 8 January compared to coots, tundra swans and wigeon (Table 4).

## DISCUSSION

Hepp et al. (1986) reported that mallards with a smaller body weight/wing length ratio at the time of being banded had a greater probability of being recovered during the hunting season. Similarly, Reinecke and Shaiffer (1988) found that hunter-shot mallards had a smaller body weight/wing length ratio than birds captured by rocket nets. Despite the potential biases of collecting birds by gunshot, it was assumed that coots collected by gunshot were representative of the healthy coot population on Centerville at that time.

The pattern of disproportionate mortality of affected species in this epizootic (Table 1) was similar to that observed during the January 1977 epizootic (Oddo et al., 1978). Coots died first at Centerville in both years. On the first day of the January 1978 epizootic, 171 of 172 dead birds were coots. Mortality among wigeon and pintails followed soon thereafter, while shoveler and mallard mortality occurred late in the epizootics (Table 4). The relative position of tundra swans varied in these two epizootics. We are not aware of comparable reports of sequential mortality at any other avian cholera site.

Avian cholera mortality did not appear to vary by age among the Centerville coots (Table 3). In contrast to the findings of Korschgen et al. (1978) for common eiders, and of McLandress (1983) for Ross' and lesser snow geese, there was no evidence of differential mortality by sex among

TABLE 4. Proportion of total avian cholera mortality for each wildfowl species occurring prior to 8 January 1978, Centerville Gun Club, Humboldt County, California.

7 Jan American coot (n = 901)	7 Jan Tundra Swan (n = 7)	10 Jan American wigeon (n = 67)	10 Jan Ruddy duck (n = 10)	12 Jan Northern pintail (n = 65)	≤31 Jan Green-winged teal (n = 9)	12 Jan Northern shoveler (n = 35)	12 Jan Mallard (n = 8)
0.70	0.57	0.28	0.20	0.20	0.11	0.03	0.00

<sup>a</sup> Median day of death for species.

<sup>b</sup> Lines join those numbers not significantly ( $P > 0.05$ ) different with a G-test for heterogeneity.

coots. Taken as a whole, these findings do not support the idea that there is much variation among the coots in susceptibility to *P. multocida*; all sex, age and morphometric groups had a similar susceptibility.

The sequential mortality at the Centerville Gun Club suggests that avian cholera starts in the coot population, and then spreads to other species. Preventing the disease at this site appears to depend on preventing its occurrence in coots. Because no one subset of the coot population appears to be at greater risk from avian cholera than any other, successful preventive measures must consider the entire coot population.

#### ACKNOWLEDGMENTS

We appreciated the assistance of A. Titcher and K. A. Brogden.

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*Received for publication 18 August 1988.*