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Survival of *Pasteurella multocida* in Soil and Water in an Area Where Avian Cholera is Enzootic

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Avian cholera has been reported among wildfowl in California since 1944, (Rosen and Bischoff, 1949, Calif. Fish Game 35: 185–192) and in Humboldt County, California since 1945 (Titche, 1979, Calif. Dept. Fish Game, Wildl. Manage. Branch Adm. Rept. 79-2, Sacramento, California, 49 pp.). Despite some studies on the survival of the causative agent, *Pasteurella multocida*, in soil and water (Dimov, 1964, Nauchn. Tr. Vyssh. Vet.-Med. Inst. Sofia 12: 339–345; Olson and Bond, 1968, Proc. Annu. Meet. Livestock Sanit. Assoc. 72: 244–246; Price and Brand, 1984, J. Wildl. Dis. 20: 90–94), the role of soil and water as year-round reservoirs of these bacteria in wildfowl has not been established.

The objectives of this study were to determine 1) whether *Pasteurella multocida* could be isolated from the natural soil or water of an enzootic avian cholera site, and 2) how long detectable concentrations of these bacteria could survive in inoculated soil and water of this site.

The study was conducted at the Centerville Gun Club, a 100 ha area on the Eel River delta of Humboldt County, California. This land is used to pasture sheep and beef cattle between January and October of each year. The waterfowl hunting rights are then leased by eight to 10 local residents from late October to late January.

The ponds lie within 700 m of the Pacific Ocean, from which they are separated by a strip of low, vegetated coastal dunes and some pasturelands. The predominant soil type is a poorly drained soil classified as a siltic clay loam of the Bayside Soil Series (McLaughlin and Harradine, 1965, Soils of Western Humboldt County, California, Dept. Soils and Plant Nutrition, Univ. California, Davis, 85 pp.). To attract waterfowl onto the area, water is pumped from an on-site well to form two shallow ponds, approximately 5.5 ha and 3.9 ha in size, beginning each September. The sizes of these ponds vary each winter with the amount of rainfall.

These two ponds are the only bodies of fresh water in the immediate area and are attractive to migrating waterfowl. They have also been the site of numerous avian cholera epornitics in past years (Titche, 1979, op. cit.; Hazlewood et al., 1978, J. Wildl. Dis. 14: 229–232; Oddo et al., 1978, J. Wildl. Dis. 14: 317–321). In 1977–1978, the winter prior to this study, 1,113 dead wildfowl were observed during an avian cholera epornitic on the study area. Dur-

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TABLE 1. Survival of *Pasteurella multocida* in soil in culture tubes at the Centerville Gun Club, Humboldt County, California. Samples were inoculated on 7 November 1978 with 10^5 viable *P. multocida* and tested weekly.

Time after inoculation	Sterilized soil[a]	Nonsterilized soil[a]
0	4/4	4/4
6 days	2/4	3/4
13 days	1/4	0/4
20–125 days	0/64	0/64

[a] No. of samples with detectable *P. multocida*/no. samples tested.

ing the current study an avian cholera epornitic began on 25 December 1978, and was completed by 22 January 1979; at least 306 birds were known to have died (Botzler, unpubl. data).

Beginning 7 November 1978, the natural soil and water at the Gun Club were tested weekly for 18 consecutive weeks for the presence of *Pasteurella multocida* (Study A). Two 3-g plugs of surface soil were taken each week with a modified syringe barrel from each of two sites located within 10 m of one of the waterfowl ponds. In addition two 10-ml samples of surface water were collected each week along the shore of the pond. Nine ml of 0.5% sterile saline were added to each 3-g sample of soil. The samples were mixed thoroughly, the particulate matter was allowed to settle, and 0.5 ml of the supernate was injecteed intraperitoneally (i.p.) into 16–23-g white laboratory mice; one mouse was used for each sample. Each undiluted water sample was mixed thoroughly and 0.5 ml was injected into a mouse. All mice were observed for up to 2 wk. An attempt was made to isolate *Pasteurella multocida* from each mouse that died.

In a second study (Study B), surface soil and water were collected at the Gun Club on 7 November 1978. Three-gram samples of soil were dispensed into each of 160 screw-cap culture tubes; half of the samples were sterilized by steam at 121 C for 20 min. Nine ml of pond water were added to each of 80 screw-cap culture tubes; half of these were similarly sterilized.

Each soil and water sample was inoculated with 10^5 viable cells of *P. multocida* isolated from a northern pintail (*Anas acuta*) that died on the Gun Club in January 1978. The LD_{50} for this isolate was $10^{1.55}$ bacteria in white laboratory mice, over a 7-day period.

The inoculated samples were returned to the site on the day of collection. Culture tubes containing inoculated water were suspended in the ponds from a wood-styrofoam float such that the water level in the tubes approximated the water level of the pond. This was done to maintain the samples at ambient water temperatures. Culture tubes with inoculated soil similarly were placed in the soil such that the top surface of the soil in the tubes approximated the level in the surrounding soil. The caps on all samples were loosened to allow gaseous exchange. Ambient air temperatures ranged from a mean (±SD) weekly low of −1.5 (±3.9) C to a mean weekly high of 13.3 (±2.0) C; water temperatures were not monitored during this period.

Each week for 18 wk, beginning 7 November 1978, one sample of sterilized water, one sample of nonsterilized water, two samples of sterilized soil and two samples of nonsterilized soil were collected randomly and tested for the presence of *P. multocida* as described above.

Following the onset of an avian cholera epornitic on 25 December 1978, an additional study (Study C) on the survival of *Pasteurella multocida* in soil was begun. Soil was exposed at two sites of one of the waterfowl ponds by removing the upper layer of vegetation; each site was within 10 m of the pond. A grid was established on each site by means of a 20 × 20-cm wooden frame, with string tied from side to side at 4-cm intervals. For both grids,

each of the 25, 4 × 4-cm compartments was inoculated on 8 January 1979 with 10^5 viable *P. multocida*. Two 3-g plugs of soil were collected randomly from each grid for 8 consecutive wk, using a modified syringe barrel. Each sample was transferred to a sterile screw-cap culture tube and tested for the presence of *P. multocida* by mouse inoculation.

Pasteurella multocida was not isolated from any of the natural soil samples adjacent to the waterfowl ponds before, during or after the avian cholera epornitic in Study A. However, *P. multocida* was isolated from the natural water of the ponds on the third and 10th days after the epornitic began on 25 December 1978.

For both the sterilized and nonsterilized water samples in Study B, *Pasteurella multocida* was recovered from all four samples tested on the day of inoculation. No pasteurellae were recovered thereafter.

Detectable concentrations of *Pasteurella multocida* were present for less than 20 days in inoculated soil samples contained in culture tubes (Table 1). There was little difference in survival of *P. multocida* between previously sterilized, and nonsterilized samples.

On the grids of exposed soil in Study C inoculated after the avian cholera epornitic began, *P. multocida* was isolated from three of four samples taken on the day of inoculation, but was not recovered after that time. We speculate that exposures to the winter rains may have washed away the bacteria in the exposed soil grid, and thus accounted for their reduced presence, compared to the pasteurellae in culture tubes with soil; rainfall of 65 mm was recorded at the study site in the week following inoculation of the exposed soil grids. Another consideration is that the bacteria inoculated were still in a logarithmic growth phase; survival may have been increased if a late stationary growth phase had been used. It is also possible that *P. multocida* may have survived as injured cells, which may require more specialized techniques for recovery.

In summary, detectable concentrations of *Pasteurella multocida* declined rapidly in both soil and water, with and without competing organisms. This poor survival occurred shortly before and during an avian cholera epornitic. The isolation of *P. multocida* from the natural pond water on the third and 10th days after the onset of the epornitic may have been due to the presence of waterfowl carcasses which shed the bacteria, as suggested by the findings of Price and Brand (1984, op. cit.). Thus, while water may be capable of transmitting the pasteurellae between susceptible wildfowl, there is little evidence from this study that soil or water can serve as year-round reservoirs of *Pasteurella multocida*.

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