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An Outbreak of Avian Cholera in Wild Waterfowl in Ordos Wetland, Inner Mongolia, China

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ABSTRACT: Eleven species of wild waterfowl (Anseriformes and Charadriiformes) were found dead in the Hongjian Nur Lake in the Ordos wetland, Inner Mongolia of northern China, in 2007. *Pasteurella multocida* was isolated from tissue samples of dead and sick birds and identified as *P. multocida* subsp. *multocida*, serotype A1, using serologic and molecular techniques. Eight bird species in this outbreak had never been previously reported with *P. m. multocida* infection. This was also the first report of fowl cholera in wild waterfowl in China.

Key words: Fowl cholera, *Pasteurella multocida*, wild waterfowl.

Fowl cholera is an acute, fatal, septicemic disease of various domestic and wild bird species. Its causative agent, *Pasteurella multocida*, is a gram negative, oxidase positive, nonmotile, nonspore-forming, facultative aerobic, rod-shaped or coccoid bacterium. The species is divided into three subspecies: *P. multocida* subsp. *multocida*, *P. multocida* subsp. *gallicida*, and *P. multocida* subsp. *septica*, mainly on the basis of the ability to ferment dulcitol and sorbitol (Mutters et al., 1985). In addition, using serologic techniques, *P. multocida* is classified into five capsular serotypes (A, B, D, E, and F) and 16 somatic serotypes (1–16; Rimler and Rhoades, 1989). *Pasteurella multocida* serotypes, causing severe pasteurellosis in animals, display certain host predilections. The majority of isolates from wild birds belong to the *P. m. multocida* subsp., followed by *P. m. gallicida*, whereas the *P. m. septica* subsp. constitutes only a minor fraction of the isolates (Hirsh et al., 1990; Snipes et al., 1990).

Hongjian Nur Lake (39°05'N, 109°54'E), one of the larger lakes in the Ordos wetland adjacent to Yellow River, is located to the south of Inner Mongolia in northern China. On 24 August 2007, waterfowl were found dead during routine surveillance. In further surveillance, over 3,300 wild waterfowl were found dead during 11 days from 24 August to 5 September (Table 1). The Ordos wetland is an important aggregation and breeding site for many waterfowl species. According to the annual survey data of the Ordos National Natural Conservation, the species mentioned above are visitors to the area from April to September annually.

Following necropsy, fresh tissues (heart, lung, liver, pericardial sac, and air sacs; dead birds) and serum samples (sick birds) were immediately submitted to the National Research Center for Wildlife Borne Diseases at the Institute of Zoology (IOZ), Chinese Academy of Sciences (CAS) for bacteriology or virology. Primary cultures from tissue samples were grown on blood agar (blood agar base supplemented with 5% calf blood) and incubated overnight, aerobically, at 37 C. Water samples (Table 1) contaminated by feces or decomposed birds were streaked on blood agar plates. In addition, serologic testing was performed using an indirect enzyme-linked immunosorbent assay (ELISA) method as previously described (Lu et al., 2008).

Identifications were based on Gram's stain, morphologic characteristics, and biochemical tests (Fegan et al., 1995; Koneman et al., 1999). *Pasteurella multocida*

TABLE 1. Bird and water samples collected for *Pasteurella multocida* isolation and serology from Hongjian Nur Lake, Ordos wetland of Inner Mongolia, China in August, 2007.

Source of samples	Number of dead birds	Number of moribund birds	Tested (tissues/serum samples)	Positive ^a (tissues/serum samples)
Ruddy Shelduck (<i>Tadorna ferruginea</i>) ^b	2,145	126	422/126	417/116
Green-winged Teal (<i>Anas crecca</i>)	660	82	253/82	248/78
Gadwall (<i>Anas strepera</i>)	59	20	59/20	56/19
Black-winged Stilt (<i>Himantopus himantopus</i>) ^b	200	26	200/26	196/25
Pied Avocet (<i>Recurvirostra avosetta</i>) ^b	100	21	100/21	98/21
Common Greenshank (<i>ringa nebularia</i>) ^b	78	19	78/19	77/17
Northern Lapwing (<i>Vanellus vanellus</i>)	20	13	20/13	20/13
Common Sandpiper (<i>Actitis hypoleucos</i>) ^b	18	19	18/19	17/19
Kentish Plover (<i>Charadrius alexandrinus</i>) ^b	9	15	9/15	9/15
Little Ringed Plover (<i>Charadrius dubius</i>) ^b	6	12	6/12	6/12
Relict Gull (<i>Larus relictus</i>) ^b	5	10	5/10	4/10
Water samples			145	86/NA

^a *Pasteurella multocida* was isolated from tissue samples by bacterial culturing; antibodies were detected by an ELISA.

^b *Pasteurella multocida* has not been previously reported from these species.

cida was isolated on the blood agar plate from tissue samples and water samples. Morphologic and biochemical characteristics were typical of *P. m. multocida*. The capsular serotype of the *P. multocida* strain was determined with multiplex PCR assay (Townsend et al., 2001) reagents (BD Biosciences, San Jose, California, USA). Heat-stable antigens were serotyped by immunodiffusion following the method of Heddlestone et al. (1972) using antisera from the China Institute of Veterinary Drug Control. Based on the results of morphologic, biochemical, and serotyping characteristics, the isolate was identified as serotype A1. In order to detect other possible pathogens, the tissue homogenates were inoculated in 9-day-old embryonated eggs via an allantoic route. After 4 days, the eggs were chilled overnight. Under aseptic conditions, the allantoic fluid was collected to test for avian influenza virus (AIV), infectious bursal disease virus (IBDV), and New-

castle disease virus (NDV) as previously described (Spackman et al., 2002; Wise et al., 2004; Wang et al., 2008). All samples tested negative for AIV, IBDV, and NDV.

To test the pathogenicity of the *P. multocida* isolate, 60 specific-pathogen-free muscovy ducks (12 days old) were purchased from the Centre of Beijing Laboratory Animals. Forty ducks were challenged periorally with 1×10^8 colony forming units of *P. multocida* in 1 ml phosphate-buffered saline (PBS). Another 20 ducks served as an uninoculated control group that was inoculated periorally with PBS. All experimental ducks were individually housed in stainless steel cages that were placed in isolated rooms with a barrier regime and independent ventilation. They were fed ad libitum and had free access to water.

Culture and serologic results from wild birds are included in Table 1. Of the 60 *P. multocida*-challenged birds, 40 died within 2 to 9 days after inoculation, with acute

clinical signs. *Pasteurella multocida* was isolated from the lungs and livers of the dead muscovy ducks.

Results suggest that *P. m. multocida* (serotype A1) was the agent that caused mortality in the wild birds; the isolate was pathogenic in experimentally infected muscovy ducks. Although similar events have been reported in other countries (Samuel et al., 2007), to our knowledge, this is the first report of an outbreak of avian cholera in wild waterfowl in China. *Pasteurella multocida* infections have been reported from over 190 species of wild birds (Samuel et al., 2007), but infections in the eight bird species involved in this outbreak have not been previously reported (Table 1). Thus, it is suggested that the host range may be expanded, and pasteurellosis may become epidemic among wild birds in China. The origin of *P. multocida*, as related to this outbreak, is unclear, but it may have originated from domestic birds or other wild birds. In the latter case, it could have been introduced during migration of wild waterfowl (Christensen et al., 1999).

According to the data of Ordos National Natural Conservation, species affected by this outbreak (Table 1) are migratory. Beginning in September, these birds migrate southwards to Poyang Lake in Jiangxi Province, Taiwan, and even to Vietnam, and return to northern China around April. Therefore, our findings indicate that *P. multocida* may disseminate through migratory birds between northern and southeastern China and even outside the country. In addition, other migratory avian species, as well as domestic poultry and possibly mammals, may also be vulnerable to infection.

In the light of our findings, *P. multocida* may become a threat to wild waterfowl and poultry in Southeast Asia which, in connection with highly pathogenic H5N1 influenza, may become a huge catastrophe for migratory birds. Because avian cholera affects many waterfowl species, further research is needed to focus on creating

strategies for the prevention and control of avian cholera, according to the implications of our research that wild birds are a likely source of disease outbreaks and play a key role in spreading this disease.

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