DOI: 10.7589/2012-02-063 *Journal of Wildlife Diseases*, 50(1), 2014, pp. 136–139

Acute Pasteurellosis in Wild Big Brown Bats (*Eptesicus fuscus*)

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ABSTRACT: We report acute fatal pasteurellosis in wild big brown bats (*Eptesicus fuscus*) in Wisconsin, USA. Mortality of approximately 100 bats was documented over 4 wk, with no evidence for predatory injuries. *Pasteurella multocida* serotype 1 was isolated from multiple internal organs from four of five bats examined postmortem.

Emergence of white-nose syndrome (WNS) among hibernating bats of the northeastern USA in 2007 (Blehert et al. 2009) and the recent discovery that bats harbor several viral zoonoses (Amman et al. 2011) have fostered heightened interest in investigation of diseases in bats. Over the 28 yr preceding the emergence of WNS (January 1978–December 2006), the US Geological Survey–National Wildlife Health Center (USGS-NWHC) investigated 47 incidents of morbidity and mortality involving 79 bats. In the 6 yr after the emergence of WNS (January 2007–December 2012), NWHC investigated 310 cases involving 979 bats. For the latter period, 53% of cases ($n=215$) investigated during months associated with hibernation (October–May) were attributed to WNS. Additional pathogens (bacteria, viruses, parasites, and other fungi) were implicated in 8% of those cases; others were diagnosed as emaciation (5%) or remain undetermined (22%). Causes of morbidity and mortality for cases ($n=95$) involving nonhibernal bats were primarily attributed to pathogens (bacteria, viruses, and parasites; 12%), predation (9%), other trauma (13%), emaciation (16%), or remain undetermined (38%). With the exception of WNS, epizootics among bats were rare. We report a mortality event among big brown bats (*Eptesicus fuscus*) at a private residence in Winnebago County, Wisconsin, USA.

On 16 June 2008, the property owner discovered four dead bats. Ongoing mortality was observed for approximately the next 4 wk. On 8 July 2008, eight dead big brown bats were collected and transported to NWHC. Total event mortality was approximately 100 bats.

Five big brown bats were selected for postmortem examination. Histologic examinations using hematoxylin and eosin–stained tissue sections of major organs (lung [$n=3$], liver [$n=4$], heart [$n=3$], kidney [$n=3$], and spleen [$n=2$]) were completed for the four bats least autolysed. To identify bacteremia, Giemsa and Brown and Hopps gram stains were applied to lung ($n=2$), liver ($n=3$), heart ($n=3$), and kidney ($n=3$) from three of the bats examined histologically. Wing skin was collected from three bats as previously described (Meteyer et al. 2009), and sections were stained with Periodic acid–Schiff to rule out lesions diagnostic for WNS.

Routine aerobic bacterial isolation was performed on the indicated tissues (Table 1) using blood agar plates (BD Diagnostic Systems, Sparks, Maryland, USA) incubated overnight at 37 C, and bacterial isolates were identified using biochemical tests (API strips, BioMerieux, Marcy l'Etoile, France). Standard techniques for isolation of *Salmonella* were completed on sections of colon from three bats. Somatic serotype for all *Pasteurella multocida* isolates was determined using gel diffusion precipitin (Heddleston et al. 1972) with antisera representing each of the 16 somatic serotypes of *P. multocida* (National Veterinary Services

TABLE 1. Diagnostic summary for five big brown bats (*Eptesicus fuscus*) found dead on a farm in Wisconsin, USA, July 2008.

Sex	Age	Body condition	Postmortem condition	Organ	Gross observations	Bacterial isolates	Final diagnoses
F	Adult	Fair	Fair	Lung	Multifocal pneumonia	*P. multocida* serotype 1 (pure growth)	Pasteurellosis
				Liver	No visible lesions	*P. multocida* serotype 1 (pure growth)	
M	Juvenile	Emaciated	Fair	Spleen	Mild splenomegaly	*P. multocida* serotype 1	Pasteurellosis
				Liver	No visible lesions	*P. multocida* serotype 1	
F	Adult	Fair	Poor	Liver	No visible lesions	*Enterococcus* sp.	Undetermined
						Klebsiella oxytoca	
F	Juvenile	Emaciated	Poor	Lung	Multifocal pneumonia	*P. multocida* serotype 1 (pure growth)	Pasteurellosis
				Liver	No visible lesions	*P. multocida* serotype 1 (pure growth)	
F	Adult	Emaciated	Poor	Lung	No visible lesions	*P. multocida* serotype 1	Pasteurellosis
				Spleen	Mild splenomegaly	*P. multocida* serotype 1	
				Liver	No visible lesions	*P. multocida* serotype 1 (pure growth)	
				Heart	No visible lesions	*P. multocida* serotype 1	
				Mammary gland	Focal hemorrhagic mastitis	*P. multocida* serotype 1	

Laboratory, Ames, Iowa, USA). Brains from four bats were tested for rabies virus by fluorescent antibody analysis at the State Laboratory of Hygiene (Madison, Wisconsin, USA). Spleen ($n=4$) and kidney ($n=5$) were tested for West Nile virus by PCR (Lanciotti et al. 2000), and routine virus isolation using Vero cells was completed for lung ($n=2$) and intestine ($n=5$) samples. Wing skin from each bat ($n=5$) was placed on Sabouraud dextrose agar containing gentamycin and chloramphenicol (BD Diagnostic Systems) and incubated at 7 C for up to 30 days, as previously described (Lorch et al. 2010), in an attempt to isolate *Pseudogymnoascus destructans*. Fungal isolates were identified by PCR amplification followed by DNA sequence analysis of the ribosomal RNA gene internal transcribed spacer region using primers ITS4 and ITS5 (White et al. 1990).

External examinations of carcasses were unremarkable. On internal examination, lungs of two bats had gross evidence suggesting multifocal pneumonia, exhibiting dark red coalescing foci in partially consolidated lobes. Two other bats had mild splenomegaly, and one of those also had focal hemorrhagic mastitis (Table 1). Gastrointestinal tracts of all bats examined ($n=5$) showed diffuse serosal congestion or hemorrhage. Microscopically, numerous small gram-variable coccobacilli were seen in vascular lumens and within endothelial cells of lung (2 of 3 bats), liver (4 of 4), and spleen (1 of 2). Alveolar capillaries had intravascular thrombosis, necrosis of endothelial cells, and phagocytosed bacteria within endothelial cells; alveolar septae were infiltrated by neutrophils and other leukocytes. The final diagnosis was septicemia resulting in peracute, diffuse, moderate coccobacillary interstitial pneumonia.

Pasteurella multocida serotype 1 was isolated from lung, liver, spleen, heart, or mammary gland tissues from four of five bats; tissues from three of four bats from which *P. multocida* was isolated yielded pure cultures (Table 1). Other bacterial

pathogens such as salmonellae were not isolated. *Klebsiella oxytoca* and *Enterococcus* sp. were isolated in mixed culture from the liver of one of five bats (Table 1), however tissues from this animal showed advanced autolysis, and we could not conclude whether these bacteria contributed to the death of this bat or colonized tissues postmortem. Assay results for rabies ($n=4$) and West Nile virus ($n=5$) were negative, and no other viruses were cultured from lungs ($n=2$) or intestines ($n=5$) of the bats. Histopathology of wing skin ($n=3$) did not indicate lesions diagnostic for WNS, nor was *P. destructans* cultured from wing skin ($n=5$). Other fungi, however, were isolated, including *Helicostylum* sp., *Mucor heimalis*, *Mucor* sp., and *Rhizomucor variabilis*. Because there was no histologic evidence of related disease processes, these fungi were likely nonpathogenic inhabitants of the skin surface.

Pasteurella multocida and other *Pasteurella* species have been documented to cause fatal infections in bats; however, previous cases involved captive bats (Helmick et al. 2004) or individual wild bats often associated with traumatic injuries suggestive of cat predation (Simpson 2000; Mühldorfer et al. 2011a, b). This report is unique in that it documents microbiologic and pathologic changes consistent with an outbreak of acute pasteurellosis in wild bats without associated traumatic injuries. Follow-up epidemiologic studies of *P. multocida* isolates from this and additional bat mortality events, as they are identified, could provide insights into potential relationships among bacterial isolates, outbreak severity, and patterns of disease spread. Ongoing investigations of unusual mortality among insectivorous bats will likely continue to expand our understanding of the role of infectious diseases in the ecology of these uniquely adapted and highly diverse mammals.

We acknowledge a Society for Applied Microbiology fellowship to R.P.M. We thank K. Egstad and R. Long for conducting virus PCR and culture analyses and V. Shearn-Bochsler for helpful discussions. Use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

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Submitted for publication 27 February 2012.
Accepted 15 July 2013.