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# Analysis of Archival Specimens Confirms White-nose Syndrome in Little Brown Bats (*Myotis lucifugus*) from New York, USA, in Spring 2007

**Saskia Keller,[1,2] Jeffrey M. Lorch,[2,3] Brenda M. Berlowski-Zier,[2] Anne Ballman,[2] and David S. Blehert[2]** [1]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, 2015 Linden Drive, Madison, Wisconsin 53706, USA; [2]US Geological Survey National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, USA; [3]Corresponding author (email: jlorch@usgs.gov)

ABSTRACT: White-nose syndrome (WNS), an emerging fungal disease of North American bats, was first diagnosed in January 2008, although mortality and photodocumentation suggest the disease might have been present earlier. Using archived samples, we describe a definitive case of WNS in little brown bats (*Myotis lucifugus*) from New York, US, in spring 2007.

White-nose syndrome (WNS) is an emerging fungal disease of hibernating bats of North America, named for the distinctive white growth often apparent on muzzle, ears, and wing membrane of infected animals (Blehert et al. 2009; Meteyer et al. 2009). Since the introduction of *Pseudogymnoascus destructans* (Pd), the causative agent of WNS, to North America, the fungus has become widespread and is now found in 39 states and seven Canadian provinces (Lorch et al. 2011; White-nose Syndrome Response Team 2018). Contrary to insignificant impacts on bat species in Eurasia, where the fungus is thought to have established a host-pathogen equilibrium, Pd causes massive mortality events in at least four cave-hibernating North American bat species (*Myotis lucifugus*, *Myotis sodalis*, *Myotis septentrionalis*, and *Perimyotis subflavus*), leading to major declines in their populations (Turner et al. 2011; Zukal et al. 2016).

Unusually large mortality events of hibernating bats along with clinical signs suggestive of WNS were first noted in January 2007 west of Albany, New York, US (Turner and Reeder 2009). The disease spread into adjacent states the following winter, and carcasses were collected between January and April 2008 for an extensive disease investigation. This investigation resulted in the discovery of Pd and description of WNS (Blehert et al. 2009), and led to clear criteria for diagnosing the disease (Meteyer et al. 2009). Thus, although WNS was strongly suspected to be present in North America since the winter of 2006–07, diagnostic confirmation of WNS prior to 2008 was lacking.

We examined archived carcasses of six little brown bats (*Myotis lucifugus*) that were found dead and collected by the New York Department of Environmental Conservation in March and April of 2007 at Hailes Cave near Albany, to investigate a mortality event involving approximately 14,000 hibernating *M. lucifugus*. In the field, bats were reported with a white powdery substance around their noses, which disappeared after handling. In April 2007, 14 *M. lucifugus* carcasses collected within (or outside the entrance to) the cave were submitted to the US Geological Survey–National Wildlife Health Center for diagnostic evaluation, and three carcasses in fair postmortem condition were necropsied. The only significant gross finding at necropsy was visual depletion of subcutaneous, visceral, and pericardial fat reserves. Histopathological examination of internal organs (brain, heart, lung, liver, spleen, and kidney) was impaired by autolysis, although no evidence of an inflammatory process or other significant changes were observed. Bacterial and viral tests failed to detect any pathogenic organisms (Table 1). Due to the novel nature of the mortality event, and lack of gross skin lesions observed at necropsy, skin samples were not collected for histopathologic or culture analyses. Rabies virus was not detected via direct fluorescent antibody assay on brain tissue in four additional carcasses in poor postmortem

TABLE 1. Results of tests performed on 14 carcasses of little brown bats (*Myotis lucifugus*) originating from a large-scale mortality event near Albany, New York, USA, in 2007 for suspected white-nose syndrome (WNS).

| | | | | | WNS diagnostic testing[c] | | | | Ancillary testing | |
|---|---|---|---|---|---|---|---|---|---|---|
| Case | Collected | Examined | BCS[a] | PM state[b] | UV light[d] | Histology[e] | qPCR[f] | Fungal culture[g] | Micro-biology[h] | Virology[i] |
| 001 | 7 April 2007 | 13 April 2007 | Ema | Fair | NP | NP | NP | NP | no growth | VI neg (Lu, BrS, SaG) |
| 002 | 7 April 2007 | 19 February 2020 | U | Poor | + | + | + | + | NP | NP |
| 003 | 7 April 2007 | 19 February 2020 | U | Poor | + | + | + | − | NP | NP |
| 004 | 7 April 2007 | 19 February 2020 | U | Poor | + | + | + | + | NP | NP |
| 005 | 6 April 2007 | 13 April 2007 | Ema | Fair | NP | NP | NP | NP | no growth | VI neg (Lu, BrS) |
| 006 | 15 March 2007 | 13 April 2007 | Ema | Fair | NP | NP | NP | NP | no growth | VI neg (Lu, BrS) |
| 007 | 15 March 2007 | NP | U | U | NP | NP | NP | NP | NP | Rabies: neg |
| 008 | 10 April 2007 | 13 April 2007 | U | Poor | NP | NP | NP | NP | NP | NP |
| 009 | 10 April 2007 | NP | U | U | NP | NP | NP | NP | NP | Rabies: neg |
| 010 | 10 April 2007 | NP | U | U | NP | NP | NP | NP | NP | Rabies: neg |
| 011 | 10 April 2007 | NP | U | U | NP | NP | NP | NP | NP | Rabies: neg |
| 012 | 10 April 2007 | 19 February 2020 | Ema | Poor | − | + | + | + | NP | NP |
| 013 | 10 April 2007 | 19 February 2020 | Poor | Poor | − | + | + | + | NP | NP |
| 014 | 13 April 2007 | 19 February 2020 | U | Poor | + | + | + | − | NP | NP |

[a] BCS = body condition score; ema = emaciated; U = unknown.

[b] PM = postmortem state at time of examination; U = unknown.

[c] NP = not performed; + = positive detection; − = negative detection.

[d] Ultraviolet (UV)-light screening for detection of orange fluorescence indicative of WNS (Turner et al. 2014).

[e] Histologic examination of patagium, pinnae, nose, and/or muzzle (Meteyer et al. 2009).

[f] Quantitative (q) real-time PCR specific for *Pseudogymnoascus destructans* (Muller et al. 2013).

[g] Isolation of *P. destructans* by fungal culture (Lorch et al. 2010).

[h] Routine bacterial culture of liver tissue using tryptic soy agar with 5% sheep blood and eosin methylene blue agar.

[i] Virus isolation (VI) using Vero cells (ATCC-CCL-81; Burleson et al. 1992) on lung (Lu), brain swabs (BrS), and/or salivary gland (SaG); rabies testing by rabies virus inclusion body detection via direct fluorescent antibody assay on brain tissue (neg = negative).

condition that were sent to an external laboratory (Wisconsin State Laboratory of Hygiene, Madison, Wisconsin, USA) for testing. Based on these findings, the mortality event was attributed to emaciation. The six remaining carcasses were saved frozen at –20 C.

In 2020, the six frozen carcasses were retrieved from the National Wildlife Health Center archive. All carcasses were in poor postmortem condition and unsuitable for internal examinations. Unfurred skin was examined under ultraviolet (UV) light, and skin samples of wing, pinnae, and muzzle were taken for histopathological analyses. Four carcasses, desiccated at the time of collection (Fig. 1A), exhibited pinpoint orange fluorescence typical of WNS on their patagia under UV light (Turner et al. 2014). The patagia from the remaining two carcasses were in poor condition (sloughing from the body; Fig. 1B) and exhibited no obvious UV fluorescence. Despite severe autolysis, histopathology revealed distinctive epidermal cupping lesions with intralesional fungal hyphae and superficial curved conidia, consistent with WNS (Meteyer et al. 2009) in all six animals (Fig. 1C). Presence of Pd was confirmed by real-time PCR (Muller et al. 2013) in all six archived carcasses. Additionally, viable Pd was recovered in culture (Lorch et al. 2010) from four of the six carcasses. In accordance with defined criteria (combination of histopathological lesions characteristic of WNS and detection of Pd), all six examined animals were confirmed as having WNS (Meteyer et

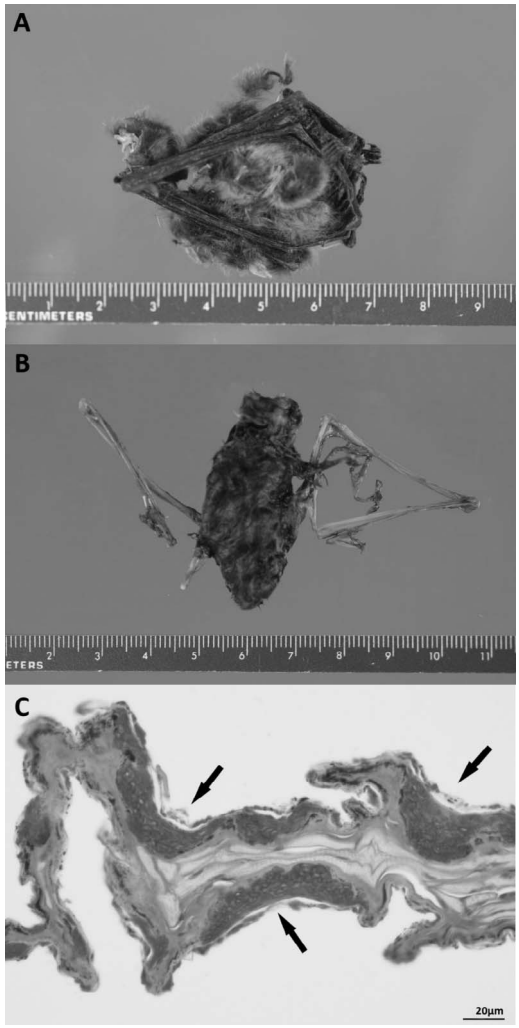


FIGURE 1. Gross and microscopic photographs of little brown bats (*Myotis lucifugus*) originating from a large-scale mortality event near Albany, New York, USA, in early 2007. (A) Mummified carcass with severe anatomical distortion and desiccation of the integumentary and skeletal system. (B) Carcass with wet decay of the body and complete decomposition of the patagium. (C) Skin stained with Periodic-acid-Schiff, showing cupping ulcers filled with dense agglomerates of Periodic-acid-Schiff–positive fungal hyphae (arrows) typical of white-nose syndrome. Bar=20 μm.

al. 2009; White-nose Syndrome Response Team 2019).

The diagnosis of WNS in these bats predates previously confirmed cases by 1 yr and affirms that the initial bat mortality events observed near Albany were indeed due to WNS. A photograph of a hibernating bat with clinical signs consistent with WNS taken in a cave near Albany in February 2006 (Blehert et al. 2009) represents the only known instance of possible WNS in North America prior to the 2007 mortality event described herein. Because no samples were collected in 2006 following the February observation, presence of the disease in early 2006 remains speculative.

Identifying the cause of emerging diseases in wildlife often takes considerable time. Due to the seasonality of WNS and the novelty of a psychrophilic fungus causing mass mortality in healthy mammals, it took approximately a year following reports of initial mortality events to identify an association between Pd and the disease (Blehert et al. 2009). An additional year was needed to develop a sensitive molecular assay to detect Pd (Lorch et al. 2010), and another year to confirm that Pd was the causative agent of WNS (Lorch et al. 2011). Thus, it is perhaps not surprising that the initial examination of bats in April 2007 overlooked the fungal skin infection. Furthermore, the initial cause of death based on the April 2007 necropsies was attributed to emaciation. Consistent with this conclusion, hibernating bats affected by WNS are often emaciated, because epidermal invasion by Pd leads to physiological and behavior perturbations that accelerate depletion of fat reserves (Reeder et al. 2012; Verant et al. 2014).

Considering the increased incidence of emerging diseases, wildlife biobanks can hold valuable information about pathogens' host ranges, epidemiology, and genetics (Astrin and Betsou 2016; Lajaunie and Ho 2018). For example, sampling of museum specimens helped to demonstrate that Pd has been present in Europe since at least 1918 (Campana et al. 2017). In our case, the presence of a biobank allowed us to confirm that WNS was present in North America earlier than previously documented. Despite poor sample quality, multiple techniques, including histopathology and PCR, yielded a clear diagnosis and further insight into the disease.

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history of this case. The use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

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