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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Mortality in Common (*Sterna hirundo*) and Sandwich (*Thalasseus sandvicensis*) Terns Associated with Bisgaard Taxon 40 Infection on Marco Island, Florida, USA

Kevin D Niedringhaus[*,†], **Lisa A Shender**[‡], **Adam DiNuovo**[§], **Leanne J Flewelling**[‖], **Grazieli Maboni**[¡,¶], **Susan Sanchez**[¶], **P J Deitschel**[#], **Joanna Fitzgerald**[#] and **Nicole M Nemeth**[*]

[*] *Southeastern Cooperative Wildlife Disease Study, Departments of Pathology and Population Health, University of Georgia, Athens, Georgia,* [†] *Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California-Davis, Davis, California,* [‡] *Florida Fish and Wildlife Conservation Commission, Gainesville,* [§] *Audubon Florida, Naples,* [‖] *Florida Fish and Wildlife Conservation Commission, St. Petersburg, Florida, USA,* [¡] *Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada,* [¶] *Athens Veterinary Diagnostic Laboratory and Department of Infectious Diseases, University of Georgia, Athens, Georgia and* [#] *Conservancy of Southwest Florida, Naples, Florida, USA*

Summary

Widely distributed aquatic species such as terns are highly dependent on, and can serve as indicators of, the global health of marine and other aquatic environments. Documented mass mortality events in terns have been associated with anthropogenic, weather-related and, less commonly, infectious causes. This study describes a multispecies mortality event associated with brevetoxicosis and Bisgaard taxon 40-induced sepsis involving common (*Sterna hirundo*) and sandwich (*Thalasseus sandvicensis*) terns off the southwest coast of Florida, USA, in November and December 2018. During an approximately 6–8-week period, a large number of birds were found dead or displayed weakness, ataxia or other neurological signs. Many were admitted to a wildlife hospital for evaluation, but most died or were euthanized due to poor prognosis. Necropsy of 12 birds revealed minimal or non-specific gross lesions. Initial toxicology screening of tissues for brevetoxins revealed levels that could be consistent with brevetoxicosis. However, histology revealed multiorgan inflammation and necrosis associated with a gram-negative bacillus. A bacterium isolated on aerobic culture of liver and heart tissues was unidentifiable in the MALDI-TOF database. Subsequently, 16 S rRNA gene sequencing revealed that the isolate shared 99.33% homology with Bisgaard taxon 40 from the Pasteurellaceae family. While the source of the bacterium and potential association with brevetoxin exposure are unclear, histopathology suggests that the bacterium was the proximate cause of clinical signs and mortality in all birds examined as well as the scale of the mortality event. This report highlights the need to conduct detailed investigations into wildlife mortality events and expands on the current, limited knowledge of the effects of novel Pasteurellaceae bacteria on avian health.

Keywords: bacteria; Pasteurellaceae; tern; wildlife

Terns are a diverse group of aquatic birds that have a global distribution and rely heavily on marine environments (Jackson *et al*, 2012). There are an

Correspondence to: N M Nemeth (e-mail: nmnemeth@uga.edu).

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estimated 45 species, many of which are considered to be within the order Charadriiformes, family Laridae and subfamily Sterninae (Bridge *et al*, 2005). Terns are colony nesters and often roost and nest in mixed species groups of over 10,000 birds (Pemberton, 1922; Walker, 1991). In the early 1900s, many tern species were heavily persecuted for the ornamental use of their feathers, but the passage of the Migratory Bird Treaty Act between the United States and Canada has helped to support population recoveries (Perkins and Hart, 2020). Nevertheless, due to additional habitat- and environment-related population-level challenges, multiple tern species currently are considered endangered, and a better understanding of the causes of mortality in this group could help shape conservation and management decisions (Croxall *et al*, 2012).

Tern mortality events have been reported globally due to a variety of causes. These have included infectious agents, anthropogenic toxins and chemicals, biotoxins, human-induced traumatic or physical injuries, and natural (eg, weather-related) disasters (Rowan, 1962; Lloyd *et al*, 1976; Sidle *et al*, 1992; Keedwell *et al*, 2002; Shumway *et al*, 2003; Fleischli *et al*, 2004; Drewitt and Langston, 2008; Haney *et al*, 2014; Huang *et al*, 2017). Habitat loss and global climate change also pose continuous threats to many wildlife species, including terns, and their effects may be compounded by additional challenges such as infectious diseases and nutrient deficiencies (Sonne *et al*, 2012; Nisbet *et al*, 2020). Despite documented causes of mortality in terns, little is known about the health threats to these birds, which is an urgent information gap, due to the rapid changes in global environmental conditions (Nisbet *et al*, 2020).

Infectious agents, particularly bacteria, have rarely been associated with mass mortality in terns (Fenton *et al*, 2018). Rather, assessments of bacterial infections have focused on the anthropogenic effects of human-associated food and water contamination as related to subclinical shedding of zoonotic bacteria (Rivera *et al*, 2012; Contreras-Rodríguez *et al*, 2019). Thus, the present report describes a rarely documented occurrence of sizeable mortality in a large colony of terns with both evidence of brevetoxicosis and disseminated infection with the Bisgaard taxon 40 bacterium, which has been rarely described in aquatic, avian species (Knowles *et al*, 2019). Epidemiological, clinical and pathological findings in two affected tern species are described, with discussion of comparative pathology and epidemiology in other wildlife species infected with this bacterium.

Hurricane Michael swept through Marco Island, Florida, on October 10, 2018 and resulted in an influx of approximately 10,000 terns along a 0.8 km stretch of shoreline, a historically popular roosting area. Subsequently, beginning in early November of 2018, common (*Sterna hirundo*) and sandwich (*Thalasseus sandvicensis*) terns were commonly reported moribund or dead on the beach (Figs. 1A and B). Moribund birds had ataxia, convulsions, pronounced head wobbles and an inability to fly or right themselves (Supplemental Video 1) and were easily captured with handheld nets. Clinical signs were consistent among the affected tern species. Tern surveys and monitoring of mortality and abnormal behaviour are regularly performed at this site twice weekly by an experienced biologist, and visits were increased to three times daily during the morbidity and mortality event. The event was initially presumed to be due to brevetoxicosis as it coincided with a severe red tide (*Karenia brevis*) that impacted southwest Florida, including Marco Island, for much of 2018 (Weisberg *et al*, 2019).

Approximately 120 sick terns were captured for transport to wildlife rehabilitation facilities in November and December 2018. Some birds deteriorated clinically during transport, but others arrived at the rehabilitation facilities seemingly normal only to decline the following day. On admission, most terns displayed non-specific signs consistent with central nervous system disease (Fauquier *et al*, 2013). The birds initially were provided with supportive care via supplemental heat (ie, placed within heated incubation chambers), oxygen and parenteral fluid and vitamin B complex therapy. Most affected birds died within 24 h of arrival, and the mortality rate was estimated to be approximately 97% in sandwich and common terns and 70% in royal terns (*Thalasseus maximus*).

The carcasses of seven common and five sandwich terns that had died within 24 h of hospital admission at various times during the event were frozen at

Table 1
Brevetoxin concentrations (ng/g) in tissues of five terns during a mass mortality event on Marco Island, Florida in November and December 2018

Species, Case no.	*Liver*	*Gallbladder*	*Kidney*	*Ventriculus*	*Intestines*
Sandwich tern CC18-647A	54.1	181.2	<LD	38.1	<LD
Common tern CC18−647B	31.7	27.8	<LD	<LD	14.0
Common tern CC18−647C	31.9	48.7	<LD	<LD	15.8
Sandwich tern CC18-647D	30.4	53.5	<LD	10.1	9.5
Common tern CC18-647E	45.4	99.2	14.8	<LD	33.2

<LD, below limit of detection.

−20°C for 1 week and submitted to the Southeastern Cooperative Wildlife Disease Study at the University of Georgia, Athens, Georgia, USA, for diagnostic evaluation. After thawing, necropsies of the 12 carcasses revealed only little autolysis and inconsistent non-specific gross lesions, which included poor nutritional condition in 3/12 (25%), white or green urate staining of the feathers around the vent in 4/12 (33.3%), empty ventriculus in 5/12 (41.7%), pale viscera in 1/12 (8.3%) and focal renal haemorrhage in 1/12 (8.3%).

Samples of brain, heart, lungs, liver, spleen, intestines and kidney were collected in 10% neutral buffered formalin for histopathological examination,

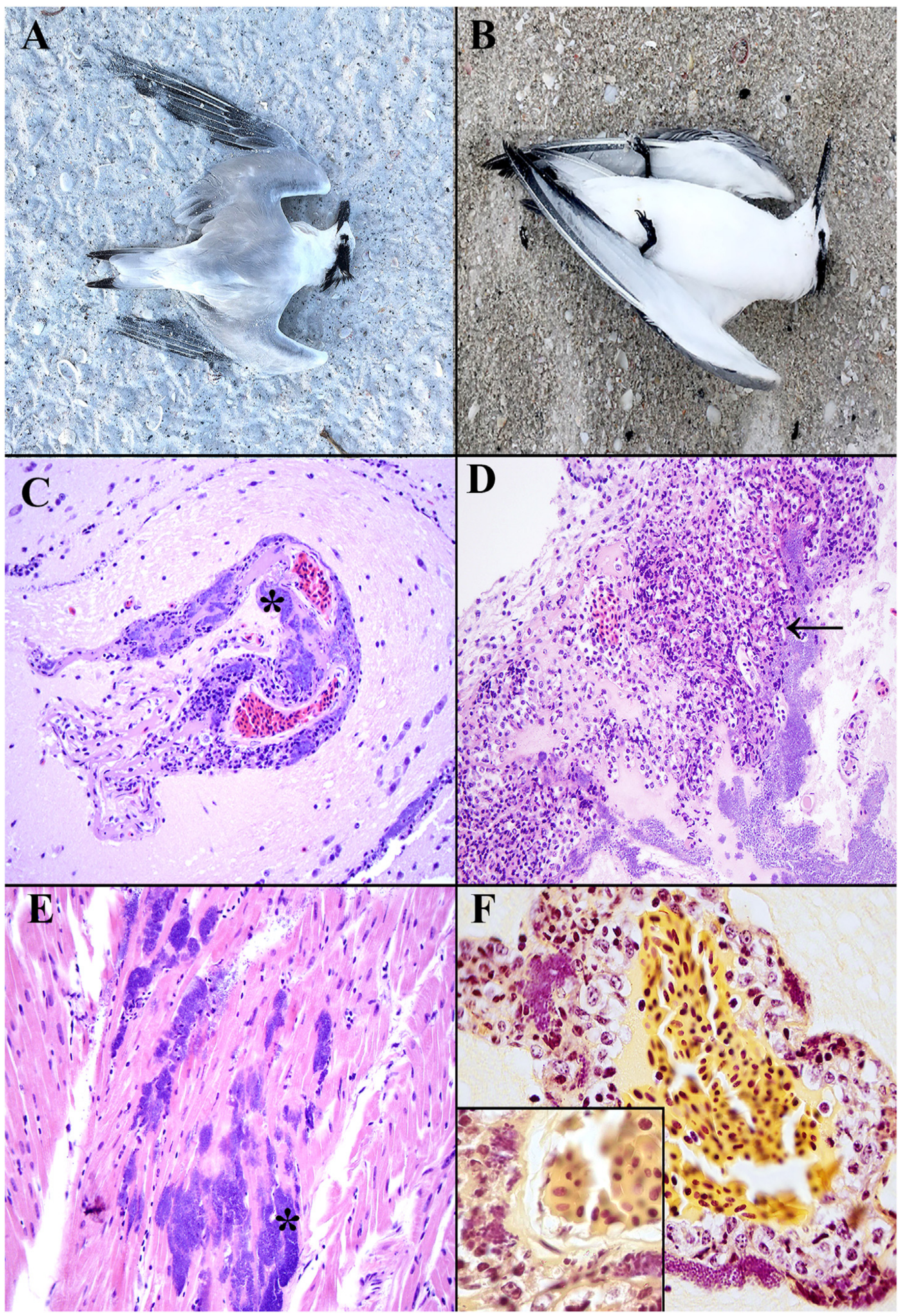

Fig. 1. (A) Tern found dead in ventral recumbency. (B) Tern found with marked ataxia and inability to right itself. (C and D) Colonies of bacilli (asterisk) surround blood vessels in the brain (C) and meninges (D) and are associated with heterophilic infiltration (arrow) and fibrinoid necrosis. HE. (E) Coccobacilli (asterisk) multifocally within sarcoplasm of myocardiocytes. HE. (F) Gram-negative coccobacilli within perivascular cuffs of inflammatory cells consisting of heterophils, lymphocytes and plasma cells. Lillie–Twort Gram stain. Inset: higher magnification (oil immersion) of bacteria. Lillie–Twort Gram stain.

which revealed multiorgan necrosis and lymphoplasmacytic and heterophilic inflammation that was associated with small bacterial rods in 9/12 terns (75%). The most commonly affected tissues included the liver (6/12; 50%), brain and meninges (6/12; 50%; Figs. 1C and D), lung (4/12; 33.3%), spleen (3/12; 25%), skeletal muscle (quadriceps femoris; 3/12; 25%), heart (2/12; 16.7%; Fig. 1E) and adrenal gland (1/12; 8.3%). Lillie–Twort Gram staining of numerous tissues revealed the bacteria to be gram-negative bacilli (Fig. 1F). Additional microscopic findings included hepatic lipidosis in 5/12 terns (41.7%) and hepatic hemosiderosis and oesophageal nematodiasis in one bird each (8.3%).

Samples of liver, kidney, ventriculus and intestines from five terns were submitted to Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute for brevetoxin testing (Table 1). Tissues (2 g) were extracted using 80% methanol and assayed for the presence of brevetoxins and brevetoxin metabolites using a competitive ELISA (Naar *et al*, 2002) with modifications as described by Flewelling (2008).

Bacterial culture was performed on brain, liver, heart and spleen tissues from five birds at the Athens Veterinary Diagnostic Laboratory (AVDL), University of Georgia, Athens, Georgia, USA, an American Association of Veterinary Laboratory Diagnosticians (AAVLD)-accredited laboratory. Specimens were cultured on 5% sheep blood agar and MacConkey agar for aerobic incubation and on Brucella agar for anaerobic incubation (Remel, San Diego, California, USA). After 24 h of aerobic incubation, a pure bacterial growth with identical isolated colonies was obtained from the heart and liver samples of three birds on blood agar. No bacterial growth was obtained on MacConkey or Brucella agar. The isolates formed smooth, shiny, circular, whitish–pale tan, beta-haemolytic colonies. All isolates investigated were small, gram-negative rods similar to those observed on histological examination. No bacterial identification was obtained from the matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry analysis (VITEK MS; bioMérieux, Marcy-l'Étoile, France). GEN III Microbial ID analysis (Biolog, Hayward, California, USA) revealed mixed isolates with a low percentage of probability, including *Pasteurella canis/stomatis* (51.6%), *Histophilus somni* (15.5%), *Nicoletella semolina* (9%) and *Pasteurella pneumotropica* (9%).

Conventional eubacteria polymerase chain reaction (PCR) was performed on isolated colonies obtained on blood agar at the AVDL. Bacterial DNA was extracted using a commercial kit (QIAmp cador Pathogen Mini Kit; Qiagen, Hilden, Germany) according to the manufacturer's instructions. Using published primers, the 16 S rRNA gene was targeted in the PCR assay (Yang *et al*, 2002). DNA from a *Salmonella* strain (ATCC14028) was used as a PCR-positive control. The PCR product was purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced by Sanger method at the AVDL (SeqStudio Genetic Analyser; Thermo Scientific, Waltham, Massachusetts, USA). Basic Local Alignment Search Tool Analysis (http://www.ncbi.nlm.nih.gov/BLAST) was used to identify related bacterial species.

The sequence obtained in this study was 99.3% homologous to the Bisgaard taxon 40 sequence available in Genbank (AY172732.1) and between 93 and 94% homologous to sequences from *Pasteurella*, *Haemophilus*, *Gallibacterium* and *Avibacterium* genera. The DNA sequence was deposited in Genbank under accession number MT158225.

Additional ancillary tests performed at a variety of diagnostic laboratories on samples from a subset of terns included *Salmonella* culture and PCR for herpesviruses and influenza viruses at AVDL, paramyxoviruses according to standard protocols (Kim *et al*, 2008; van Boheemen *et al*, 2012), *Clostridium botulinum* toxin bioassay and liquid chromatography/mass spectrometry toxicology screening for toxic compounds, including pesticides, environmental contaminants, drugs and other natural products at the AAVLD-accredited California Animal Health and Food Safety Laboratories, and domoic acid at the Florida Fish and Wildlife Conservation Commission Harmful Algal Bloom Biotoxin Laboratory. All of these yielded negative or non-significant results.

This large-scale mortality event involved thousands of birds of multiple tern species on Marco Island in Collier County, Florida, USA. It was estimated that when the mortality event started, there were approximately 4,000 birds on the beach, and by mid-December only approximately 400 birds remained at the site. It is unclear whether the majority of the birds died or relocated to other areas, but high mortality is suspected on the basis of the extent of morbidity and mortality observed in a local area over a 6-week period.

Bacteriological and molecular test results suggest the aetiological bacteria to be members of the Bisgaard taxon 40 group within the Pasteurellacea family. Pasteurellaceae are commonly isolated in healthy hosts but many are considered opportunistic, secondary invaders. Bacteria identified as Bisgaard taxon 40 were first isolated from the respiratory tract of a healthy gull (Christensen *et al*, 2003) and more recently from a mass mortality event involving the rhinoceros auklet (*Cerorhinca monocerata* (Knowles *et al*, 2019).

The histological findings in the terns presented here, and in the auklets reported by Knowles *et al* (2019), are similar in terms of the pattern of inflammation, necrosis and presence of bacteria in multiple tissues, which is suggestive of septicaemia. However, a key difference in the auklet study was the finding of severe pleuropneumonia as the most common lesion and a higher percentage of auklets were emaciated or in poor post-mortem condition.

In the auklet mortality event, the authors acknowledged that the role of Bisgaard taxon 40 as a primary or opportunistic pathogen in wild birds is unknown (Knowles *et al*, 2019). While Knowles *et al* (2019) speculated that poor nutritional condition may have compromised the health status of auklets with Bisgaard taxon 40 infection, the terns in this case may have had increased susceptibility to disease, following bacterial infection, due to the presence of brevetoxins or other unknown stressors, including hospitalization in a few birds. Avian and other wildlife species, dependent on the health of marine or other aquatic ecosystems, are at continued risk of underlying physiological stress from substandard or deficient dietary or other habitat-related resources, adverse weather events and the effects of low-grade natural and synthetic environmental contaminants (Newman *et al*, 2007; Mallory *et al*, 2010).

While brevetoxicosis can result in a variety of neurological signs in shorebirds (Kreuder *et al*, 2002), the observation of myriad bacteria surrounding many blood vessels in the brain, with corresponding inflammation and necrosis in the meninges of many of these birds, suggests that the bacterial infection may have been the ultimate cause of severe morbidity and mortality and may have caused or contributed to the neurological signs. Furthermore, the presence of bacteria in multiple organs is consistent with sepsis, although the method and site of initial bacterial entry into the host are unclear. Histological examination and bacteriological culture yielded considerable variation in the detection of bacteria within tissues. The inconsistent culture results were, in part, likely due to variable sample quality as well as the duration and conditions of storage. The brevetoxin concentrations in the five tested birds were cautiously interpreted to be moderately elevated. Some reports of brevetoxicosis in similar species describe much higher tissue levels, but in other cases the range of brevetoxins measured in suspected or presumed cases of avian brevetoxicosis has varied greatly. Validated reference intervals that correspond to exposure and clinical disease have not been established for many species, including terns (Atwood, 2008; van Deventer *et al*, 2012; Fauquier *et al*, 2013).

We speculate that brevetoxin exposure may have caused immunosuppression and initial illness and debilitation, facilitating bacterial proliferation in the host, but this could not be confirmed histologically due to the presumed acute progression of disease. Ancillary tests were performed for many other potential causes of the severe illness, including central nervous system disease, and excluded herpesvirus, paramyxovirus, influenza virus and *Salmonella* infections, *C. botulinum* intoxication, domoic acid toxicosis and a variety of other toxic compounds, including pesticides and environmental contaminants. While thiamine deficiency has been proposed as a cause of central nervous system disease and mortality in marine birds in Europe, the clinical signs and acute nature of this event are inconsistent with those reports (Balk *et al*, 2009; Sonne *et al*, 2012).

Following the diagnoses of bacteraemia and sepsis in the evaluated terns, the rehabilitation clinic began to administer broad-spectrum antibiotics (enrofloxacin at 30 mg/kg/day) to the remaining live terns, which were royal terns. Although treated royal terns appeared to have greater survival than untreated birds, the efficacy of antibiotic treatment was unclear. Compared with sandwich and common terns, the royal terns were treated during the later stages of the outbreak when the diagnosis was known. They also had less severe clinical signs on admission.

The challenges of investigating mortality events in migratory, aquatic, avian species are similar to those posed in all wildlife mortality investigations. They often include the logistical difficulty in prompt retrieval of fresh carcasses and samples of high diagnostic quality, and challenges in monitoring and forming rapid responses to outbreaks. Estimating the extent of mortality in these birds emphasizes the important roles that volunteers and wildlife biologists play in monitoring shorebird populations. Additionally, this substantial mortality event, associated with a rarely documented bacterial infection in conjunction with ongoing brevetoxin exposure, highlights the need for ongoing and thorough investigations into wildlife mortality events including long-term population monitoring of threatened and endangered species. These birds serve as environmental indicators and depend on the global health of oceans, which demands urgent attention.

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Supplementary data

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Conflict of Interest Statement

The authors declare that they have no potential conflicts of interest with respect to the research, authorship or publication of this article.

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