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Fatal fibrino-hemorrhagic bronchopneumonia associated with *Morganella morganii* in a bottlenose dolphin: a case report

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ABSTRACT: A 5 yr old, 184 kg, and 262 cm total length female bottlenose dolphin *Tursiops truncatus* was found dead in a display after bloody discharge from the blowhole was observed 3 h prior to death. Pathological examination revealed fibrinous bronchopneumonia with prominent areas of necrosis (sequestra) and numerous Gram-negative bacilli within alveoli and in blood vessels of the lungs and liver and between muscle fibers. The cause of death was attributed to septicemia. Often, cases of fibrinous bronchopneumonia are characterized by bacteremia in the latter stages of infection, resulting in the death of the animal. Septicemia likely accounts for the ecchymoses and petechiae noted on the spleen, pancreas, forestomach, lungs, visceral peritoneum, and small intestine. Additional lesions included hemothorax, stable red frothy fluid in the trachea, and lymphoid depletion in the spleen and lymph nodes. Pure growth of *Morganella morganii* was isolated from the lungs, blood, liver, and blowhole mucosa. Sequencing of 16s rRNA of the isolated bacteria showed more than 99.6% identity with *M. morganii* strain FDAARGOS_172. To our knowledge, this is the first report of fatal fibrinonecrotizing bronchopneumonia associated with *M. morganii* infection in a cetacean.

KEY WORDS: Gram-negative bacilli · 16s RNA · Lungs · Sequestra · Hemothorax

INTRODUCTION

Pulmonary infections are common among wild and captive dolphins and have been attributed to a variety of etiologic agents including *Aspergillus fumigatus* (Delaney et al. 2013), brucellosis (Cassle et al. 2013), salmonellosis (Davison et al. 2010), *Mycobacterium abscessus* (Clayton et al. 2012), and morbillivirus (Kemper et al. 2016) infection.

Morganella morganii is a Gram-negative bacterium that belongs to the tribe *Proteeae* of the family *Morganellaceae*. This species is considered an unusual opportunistic pathogen that primarily causes post-operative wound and urinary tract infections (Liu et al. 2016). Infection with *M. morganii* can result in various clinical presentations including sepsis, abscessation, purple urine bag syndrome, chorioamnionitis, and cellulitis in humans. In animals, *M. morganii* has been associated with abscessation, sepsis, and arthritis in reptiles (Novak & Seigel 1986, Heard et al. 1988, Knipper & Baldwin 1996) and ocular lesions in harbor and elephant seals

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(Thornton et al. 1998). It is also considered a possible cause of swollen head syndrome in broiler chickens in Japan (Tanaka et al. 1995). Respiratory infections associated with this bacterium have been described in piglets (Ono et al. 2001), a jaguar (Choi et al. 2002), a guinea pig (Vandenberge et al. 2013), and a rabbit (Roels et al. 2007).

CLINICAL HISTORY

A 5 yr old juvenile female bottlenose dolphin *Tursiops truncatus* with a body weight of 184 kg and a total length of 262 cm was transported from Japan to a new aquarium in South Korea. The dolphin was transferred by car from Wakayama to the port of Osaka, Japan (6 h journey including waiting time in the port), then ferried to Busan, Korea (19 h journey). Finally, the animal was transported to Ulsan Dolphinarium by car (3 h journey).

Three months later the animal acutely developed signs of anorexia, slight fever, and abnormal vertical movement in the pool. Bloody discharge from the mouth and blowhole as well as bloody feces were observed 6 h prior to death. Fluids (0.9% NaCl 1000 ml, intraveneous [IV]) were administered with antibiotics, including enrofloxacin 900 mg (intramuscular [IM]), gentamicin 720 mg (IM), cefazolin 3500 mg (IV), and cimetidine 1000 mg (IV).

MATERIALS AND METHODS

Necropsy and histopathology

A full necropsy was performed 19 h after death, and representative samples from multiple organs were fixed in 10% neutral formalin and submitted for histopathological examination. An additional set of tissues were sampled and individually labeled for ancillary diagnostic studies. Slides were prepared by conventional histological techniques, and sections were cut to 5 µm and stained with hematoxylin and eosin. Representative sections of the bronchopneumonia-affected tissues were also prepared with Gram stain following the method of Brown & Hopps (1973).

Bacteriological and molecular examination

One Touch Transport plastic swabs containing Amis transport media (Yuhan Lab Tech) were prepared from the lung, liver, and blood from the pericardium, then inoculated into tryptic soy agar and blood agar media for routine aerobic bacterial isolation and special culture to screen for *Vibrio, Salmonella, Listeria*, and *Brucella*. After 24 h of incubation at 37°C, to further identify isolated colonies, bacteria were inoculated onto 5% sheep blood agar (Hanil Komed) and tryptic soy agar (Difco).

Polymerase chain reaction (PCR) investigations for *Brucella* spp. and *Morbillivirus* were performed on frozen samples (liver, lungs, lymph nodes, kidney, pancreas, and spleen) using 2 previously published protocols (Herman & De Ridder 1992, Frisk et al. 1999). The 16S rRNA of the isolated bacteria in the culture media was further identified using universal primers 27F (5-AGA GTT TGA TCC TGG CTC AG-3) and 1492R (5-GGT TAC CTT GTT ACG ACT T-3), and the amplicon was sequenced by Macrogen, Inc. (ROK). In addition, to confirm the presence of *Morganella morganii* in the dolphin, a PCR assay was also adopted using primers Mm208F/Mm1017R (Kim et al. 2003). Genomic DNA from the frozen organs was prepared with a DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol. These extracts were submitted for direct PCR amplifications.

RESULTS

The most significant findings at necropsy were localized to the respiratory system and consisted of widespread pulmonary consolidation and congestion of the dorsal regions of the lungs. Multiple round white punctate nodules consistent with bronchiectasis and abscessation were observed in the dorsocranial aspect of the lungs. The lungs were heavy and wet, and a large amount of stable red froth was found on incision of the trachea. Fibrinous attachments between the visceral and parietal pleura were noted (Fig. 1a), and there was moderate hemothorax. Areas of encapsulated caseous necrosis were observed in the lung parenchyma (Fig. 1b). Petechiae and ecchymoses were observed on the surface and randomly throughout the pancreas (Fig. 1c), intestinal mucosa, and spleen. Mucosa of the stomach was slightly swollen and showed ecchymosis (Fig. 1d). The kidneys were congested; the parenchyma was dark red, and a considerable amount of blood oozed from cut surfaces (Fig. 1e). The spleen was friable and slightly enlarged (Fig. 1f).

Histopathology revealed fibrino-hemorrhagic and necrotizing bronchopneumonia. Bronchioles throughout the lungs were distended and occluded by dense mononuclear inflammatory cells, fibrinous exudate,

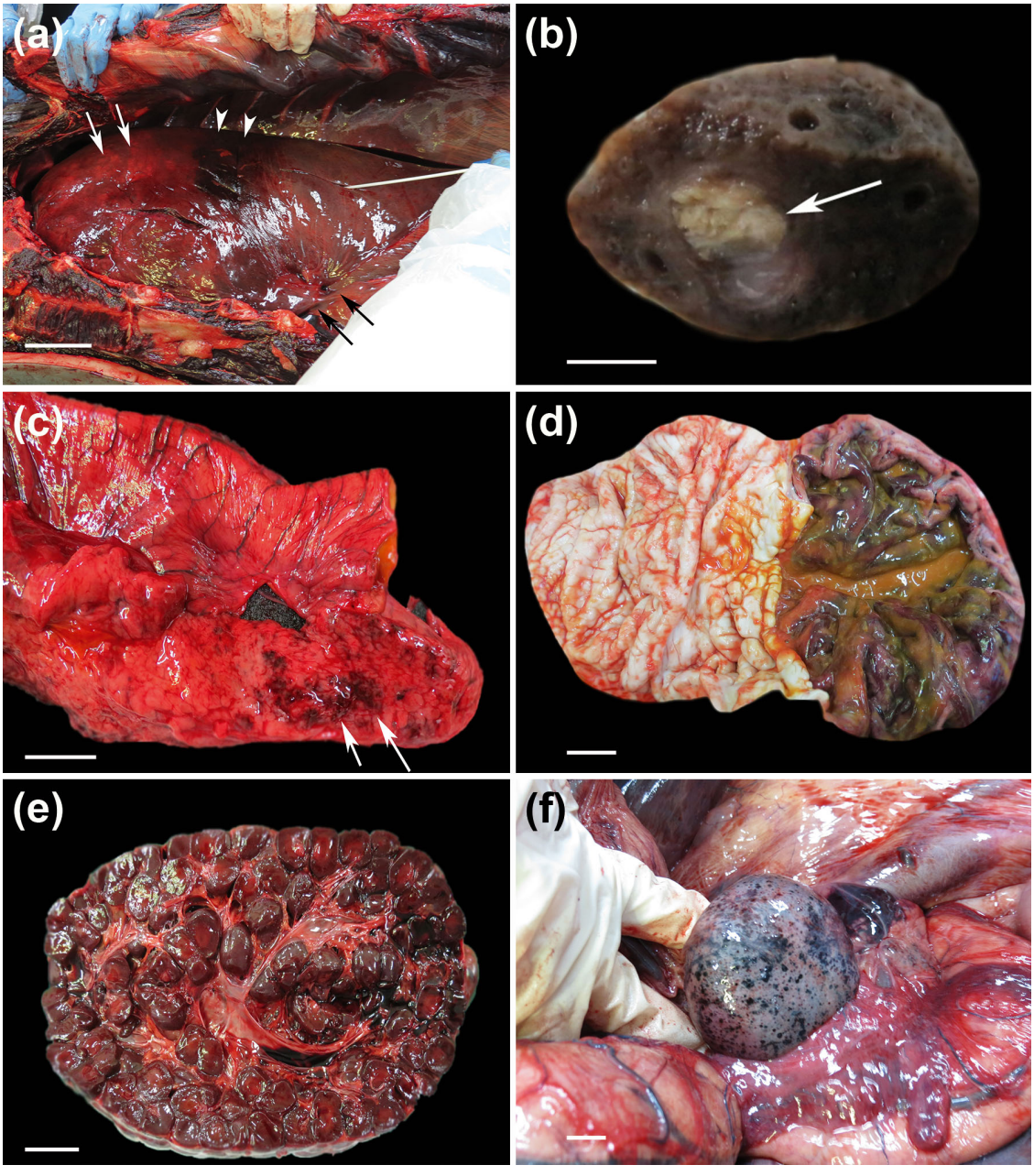

Fig. 1. Gross lesions of a 5 yr old bottlenose dolphin *Tursiops truncatus* that died from fibrino-hemorrhagic bronchopneumonia and septicemia. (a) Multiple white spots (white arrows) most probably consisting of inflamed bronchioles in the cranial aspect of the lung; area of hemorrhagic consolidation (white arrowheads). Fibrinous adhesions were seen between the pleura and the thoracic cavity (black arrows). Scale bar = 70 mm. (b) Cross section through pulmonary parenchyma showing isolated areas in the lungs with a central white area of caseous necrosis (arrow) encapsulated by a fibrous capsule; these areas of necrosis were termed 'sequestra.' Scale bar = 5 mm. (c) Ecchymotic hemorrhages on the surface of the pancreas (arrows). Scale bar = 30 mm. (d) Ecchymotic hemorrhages and congestion in the main stomach with slight swelling of the mucosa, as seen in the right aspect of the image compared to the normal mucosa on the left. Scale bar = 30 mm. (e) Kidneys were congested; the parenchyma was dark red and a considerable amount of blood was released from large vessels in the cut section. Scale bar = 30 mm. (f) The spleen was friable and showed multiple petechial and ecchymotic hemorrhages. Scale bar = 30 mm

and numerous intra- and extracellular bacterial colonies (Fig. 2a,b). Multifocal necrosis of bronchial respiratory epithelia was evident, and alveoli were variably occluded by large numbers of lymphocytes and erythrocytes, abundant fibrin, and edema with numerous bacterial colonies (Fig. 2c). The pleura was

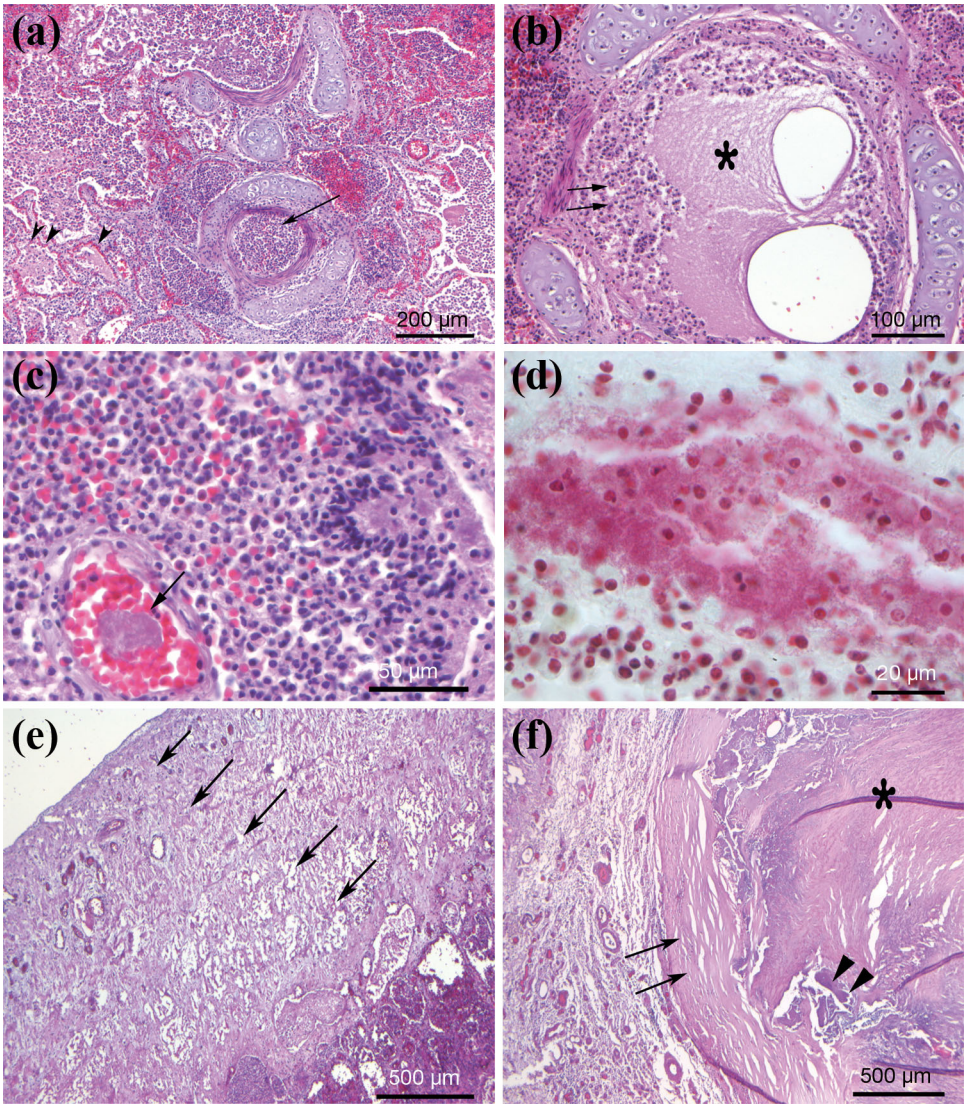

Fig. 2. Histological lesions of a 5 yr old bottlenose dolphin *Tursiops truncatus* that died from fibrino-hemorrhagic bronchopneumonia and septicemia. (a) Mix of inflammatory cell infiltrate consisting primarily of mononuclear cells was seen in the bronchi (black arrow). Fibrinous exudate was observed in the alveoli (black arrowheads). Hemorrhage was observed in the lung parenchyma (H&E stain). (b) Higher magnification of a bronchus filled with fibrin threads and a mix of mononuclear inflammatory cells. Fibrin threads (*); mononuclear inflammatory cells (arrows). (c) Presence of a bacterial embolus (arrow) lodged inside a blood vessel and surrounded by a population of mononuclear inflammatory cells. (d) Gram stain demonstration of large numbers of gram negative bacilli in lung tissue. (e) Thickening of the visceral pleura by fibrinous exudate (arrows; H&E stain). (f) Sequestration in lung tissue with a central area of necrosis (black asterisk), dystrophic calcification (arrowheads), and fibrous encapsulation (black arrows) (H&E stain)

thickened by abundant fibrin and focal aggregates of degenerated and necrotic neutrophils (Fig. 2e). Lymphoid follicles in the spleen and lymph nodes were depleted and comprised predominantly plasma cells and large lymphocytes (Fig. 3b). Aggregates of extracellular Gram-negative bacilli colonies were inside blood vessels of the lungs (Fig. 2d), pancreas, and liver, consistent with bacteremia. In the kidneys, multifocal hemorrhage, acute tubular necrosis, and protein casts were observed in renal tubules (Fig. 3a). Routine microbial culture isolated heavy growth of *Morganella morganii* from lung, liver, blood of the blowhole, and pericardial fluid (Table 1). There was no growth of *Vibrio* spp., *Salmonella* spp., or *Brucella* spp. The isolated colonies were oxidase-negative, catalase-positive, and β-hemolytic colonies of Gram-negative straight rods on 5% sheep blood agar. Using the universal primers (27F/1492R),

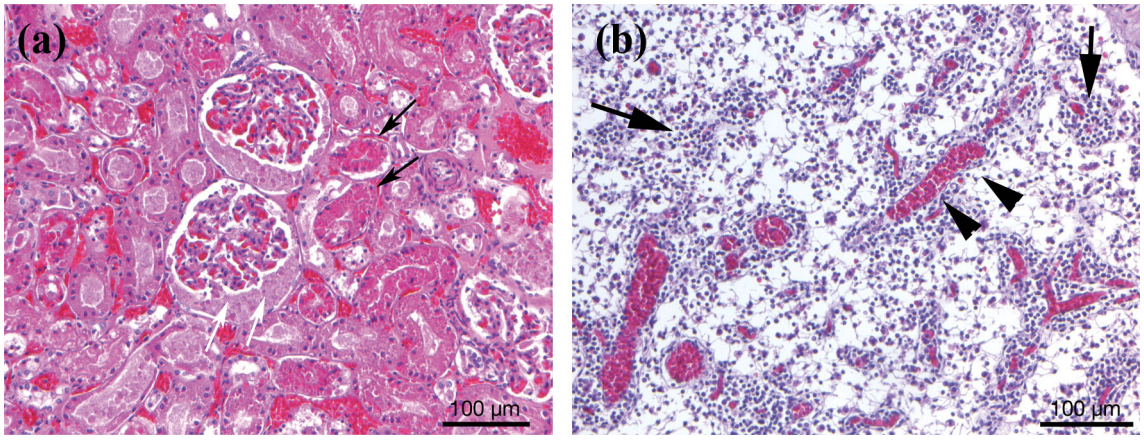

Fig. 3. Histological lesions of a 5 yr old bottlenose dolphin *Tursiops truncatus* that died from fibrino-hemorrhagic bronchopneumonia and septicemia. (a) Acute tubular necrosis with degraded and necrotic tubular epithelia (black arrows), intervening congestion, and acute hemorrhage (H&E stain). (b) Lymphoid depletion (arrows) and congestion (arrowheads) were observed in the lymph nodes (H&E stain)

Table 1. Bacterial species isolated from a 5 yr old bottlenose dolphin *Tursiops truncatus* that died from fibrino-hemorrhagic bronchopneumonia and septicemia. *M.*: *Morganella*; α: α-hemolytic isolate; β: β-hemolytic isolate; γ: γ-hemolytic isolate

No.	Lung	Liver	Blood discharge of the blowhole	Pericardial fluid
1	*M. morganii* (β)	*M. morganii* (β)	*M. morganii* (β)	*M. morganii* (β)
2			*Enterococcus* spp. (α)	
3			*Salinivibrio* spp. (γ)	
4			*Escherichia* spp. (γ)	

the amplified 16S rRNA sequences from these cultures were compared with the NCBI GenBank database using a BLAST search (www.ncbi.nlm.nih.gov/BLAST). All of these isolates showed more than 99.6% match with *M. morganii* strain FDAARGOS_172 (GenBank accession no. CP014026) and strain KT (GenBank accession no. CP004345) which were isolated from human clinical samples. One of the *M. morganii* isolates (*M. morganii* strain KC-Tt-01, isolated from the pericardial fluid) was deposited in GenBank under accession number MF033453. Cultures of the blood discharge from the blowhole yielded mixed bacterial growth including *Enterococcus* spp., *Salinivibrio* spp., and *Escherichia* spp., which may represent normal commensals or environmental contaminants (Table 1), with *M. morganii* which was 100% identical to MF033453.

The 16S rRNA sequence of strain KC-Tt-01 was further compared with those of closely related enterobacterial species in GenBank by multiple sequence alignments using CLUSTAL X (version 2.1) (Thompson et al. 1997). Using primers Mm208F/Mm1017R, positive PCR amplicons were obtained from isolates recovered from the liver, lungs, lymph nodes, kidney, pancreas, and spleen samples, with sequences corresponding to strain KC-Tt-01.

Clinical chemistry of blood sampled at the time of death showed elevated creatinine (>24.0 mg dl^{-1}) and blood urea nitrogen (117.7 mg dl^{-1}), indicative of renal impairment, which may have further exacerbated the *M. morganii* infection. Petechiae and ecchymoses in multiple organs, bloody effusion in the chest cavity, multisystem intravascular bacterial emboli, lymphoid depletion in lymph nodes and the spleen, and acute renal tubular necrosis were attributed to septicemia and in this case, would have been sufficiently severe to account for the death of this animal. There were no apparent pre-existing lesions in the examined tissues which may have predisposed this animal to bacterial pneumonia.

DISCUSSION

Morganella morganii is an unusual opportunistic pathogen that primarily causes post-operative wound and urinary tract infections (Liu et al. 2016). Based on histopathology and extensive diagnostic tests, no

underlying primary causative agents or disease process could be determined. One of the most common etiologies for fibrinous bronchopneumonia in veterinary medicine is *Pasteurella* spp., which are responsible for shipping fever in cattle and septicemia in a variety of other animals. Several samples from different organs were cultured in sheep blood agar which is a differential medium of several hemolytic bacteria, including *Pasteurella* spp.; however, no isolates were recovered. Ante-mortem antibiotic administration may have impeded recovery of other pathogens. Moreover, there was no evidence for growth of *Vibrio* spp., *Salmonella* spp., *Listeria* spp., or *Brucella* spp. in selective or enriched media, and PCR was negative for *Brucella* spp. and *Morbillivirus*. Our primary screening tests ruled out most of the common organisms that are known to cause disease in dolphins. *M. morganii* was the only species that was isolated in large numbers by routine culture of sampled organs. One possible entry route of *M. morganii* into the body is retrograde, via the urethra and urinary bladder. Although renal tubular necrosis and elevated creatinine and blood urea nitrogen levels suggest primary renal disease that may have resulted from a lower urinary tract infection, the possibility of bacteremia and localization to the kidney from a remote site of infection cannot be entirely discounted. Petechial and ecchymotic hemorrhages in mucosal and serosal membranes of a wide range of organs without indication of an underlying vasculopathy, and presence of bacterial emboli in blood vessels in different organs strongly suggest death of the animal due to septicemia. This animal succumbed 3 mo after transport and introduction to a new facility, and the possibly of stress associated with these events cannot be excluded as a contributing factor along with infection.

A case of pleuropneumonia due to *M. morganii* in a piglet was reported by Ono et al. (2001) with isolation of large numbers of bacteria from the lung. In this case, *M. morganii* were demonstrated in the necrotic areas of the lungs by immunohistochemistry. Interestingly, the distribution of bacterial antigens was closely correlated to the histological lesion. Ono et al. (2001) also indicated that no other bacteria were isolated from lung tissue. In this case, considered porcine reproductive and respiratory syndrome virus (PRRSV), infection and transportation could be the underlying primary cause of the lung infection. Vandenberge et al. (2013) isolated *M. morganii* from the lungs in a case of bronchointerstitial pneumonia in a guinea pig. However, the presence of another bacterial or viral primary infection could not be excluded. Also, *M. morganii* was isolated from a domestic rabbit with bronchopneumonia and the possibility that the rabbit was immunosuppressed could not be excluded (Roels et al. 2007). Many reports of *M. morganii* infection show the potential importance of this bacterium as a respiratory pathogen. Therefore, the evolution and the virulence factors of this bacterium should be further investigated.

In conclusion, we believe this is the first case report of *M. morganii* infection in a dolphin. Septicemia was diagnosed as the cause of death, and transport stress and introduction to a new facility could not be excluded as contributing factors.

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