https://doi.org/10.17221/73/2019-VETMED

Septicaemia due to a *Proteus* infection in a Humboldt penguin (*Spheniscus humboldti*)

Su-Min Baek[1], Seoung-Woo Lee[1], A-Rang Lee[1], Jun-Sun Bang[1], Mi-Mi Seo[2], Taeho Oh[2], Seong-Kyoon Choi[4], Sang-Joon Park[5], Il-Hwa Hong[6], Tae-Hwan Kim[1], Kyu-Shik Jeong[1,3], Jin-Kyu Park[1]**

[1]*Department of Veterinary Pathology, College of Veterinary Medicine, Kyungpook National University, Daegu, Republic of Korea*
[2]*Department of Veterinary Internal Medicine, College of Veterinary Medicine, Kyungpook National University, Daegu, Republic of Korea*
[3]*Stem Cell Therapeutic Research Institute, Kyungpook National University, Daegu, Republic of Korea*
[4]*Core Protein Resources Center, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu, Republic of Korea*
[5]*Laboratory of Veterinary Histology, College of Veterinary Medicine, Kyungpook National University, Daegu, Republic of Korea*
[6]*Department of Veterinary Pathology, College of Veterinary Medicine, Gyeongsang National University, Jinju, Republic of Korea*

**Corresponding author: jinkyu820@knu.ac.kr; jeongks@knu.ac.kr*

Citation: Baek SM, Lee SW, Lee AR, Bang JS, Seo MM, Oh T, Choi SK, Park SJ, Hong IH, Kim TH, Jeong KS, Park JK (2019): Septicaemia due to a *Proteus* infection in a Humboldt penguin (*Spheniscus humboldti*). Veterinarni Medicina 64, 558–564.

Abstract: A 2-year-old male Humboldt penguin (*Spheniscus humboldti*) died after a very brief period of illness at a zoo aquarium; the penguin showed sudden depression, anorexia, dyspnoea, and had recurrent melena a day prior to death. The gross examination revealed an extensive bilious effusion in the abdominal cavity due to a gallbladder rupture. Moreover, abscess formation, purulent exudate, severe congestion, and haemorrhages were observed in the trachea and parenchymal organs such as the kidneys and the lungs. A histopathological examination revealed a fibrin deposition with a severe haemorrhage and secondary infiltration of chronic-active inflammatory cells in the parabronchi, atria, and air capillaries and blood vessels of the lungs as well as in most of the parenchymal organs. Moreover, Gram-negative bacilli were found in the lumen of the gastrointestinal tracts including the small and large intestines accompanied by severe epithelial necrosis and the capsule of the liver. Especially, bile pigments were microscopically observed in the whole liver, which indicated a gallbladder rupture. Samples collected from the trachea, lungs, and blood were cultured on a blood agar, and the pure colonies of *Proteus* genus were isolated. *Proteus mirabilis, P. penneri, P. vulgaris,* and *P. cibarius* were identified with polymerase chain reaction (PCR). As a result, the diagnosis was confirmed as *Proteus* septicaemia. To our knowledge, this is the first report of concomitant infection by different *Proteus* species that eventually resulted in septicaemia in a Humboldt penguin, and it will provide valuable information for zoo veterinarians for its diagnosis as well, since Humboldt penguins are the most widely found penguins in zoos and *Proteus* septicaemia in the penguins has, to the best of our knowledge, not been reported as yet.

Keywords: aquatic animals; aves; haemorrhage; parenchymal organs; bilious effusion; sepsis; histopathology

Supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP; Ministry of Science, ICT & Future Planning) (No. 2017R1C1B5015716).

https://doi.org/10.17221/73/2019-VETMED

Proteus, a genus of the family Enterobacteriaceae, is an opportunistic pathogen that causes severe septicaemia in humans and animals with an underlying disease or infection (Sah et al. 1983; Rozalski et al. 1997). The most common infection-causing species are *P. mirabilis, P. vulgaris,* and *P. penneri.* These species cause bloodstream infections (BSI), wound infections, urinary tract infections (UTI), asymptomatic bacteriuria, meningoencephalitis, and respiratory system infections (Kim et al. 2003; Stock 2003; Endimiani et al. 2005). *Proteus cibarius* has recently been reported to have resistance to all β-lactam antibiotics and causing wound infections in humans, but its clinical significance has not been reported in animals (Adesina et al. 2018).

In humans, several cases of *Proteus* septicaemia are reported annually; therefore, its significance is duly recognised (O'Hara et al. 2000; Chen et al. 2012; Kwiecinska-Pirog et al. 2018). However, this is not the case in animals, although some cases of *Proteus* infections have been reported in the canine and feline species (Lees 1996; Ling et al. 2001); moreover, general information, including symptoms, histopathological findings, prognosis, and preventive measures, still remain ambiguous in veterinary medicine. Particularly, cases of *Proteus* septicaemia are extremely rare with no reports available to us in penguins until date. Therefore, *Proteus* was not a primary pathogen to be commonly diagnosed in avian septicaemia. In that regard, a detailed histopathological analysis on the septicaemia caused by the *Proteus* species in this animal may contribute to diagnosing *Proteus* septicaemia in avian species, especially in penguins including *S. humboldti*. For these reasons, we are reporting this apparently first known case of lethal *Proteus* septicaemia in a Humboldt penguin.

Case Description

A 2-year-old, male Humboldt penguin, which was transferred from a local zoo aquarium, was presented for necropsy. The penguin was in moult (Figure 1A) and expressed an aggressive behaviour towards the other penguins, so it was introduced to a new rearing aquarium. A week after, the penguin showed sudden depression, anorexia, dyspnoea, and had recurrent massive melena the day before death. The penguin otherwise had a clean medical history. A complete necropsy was performed 12 h after death. Overall, no ex-

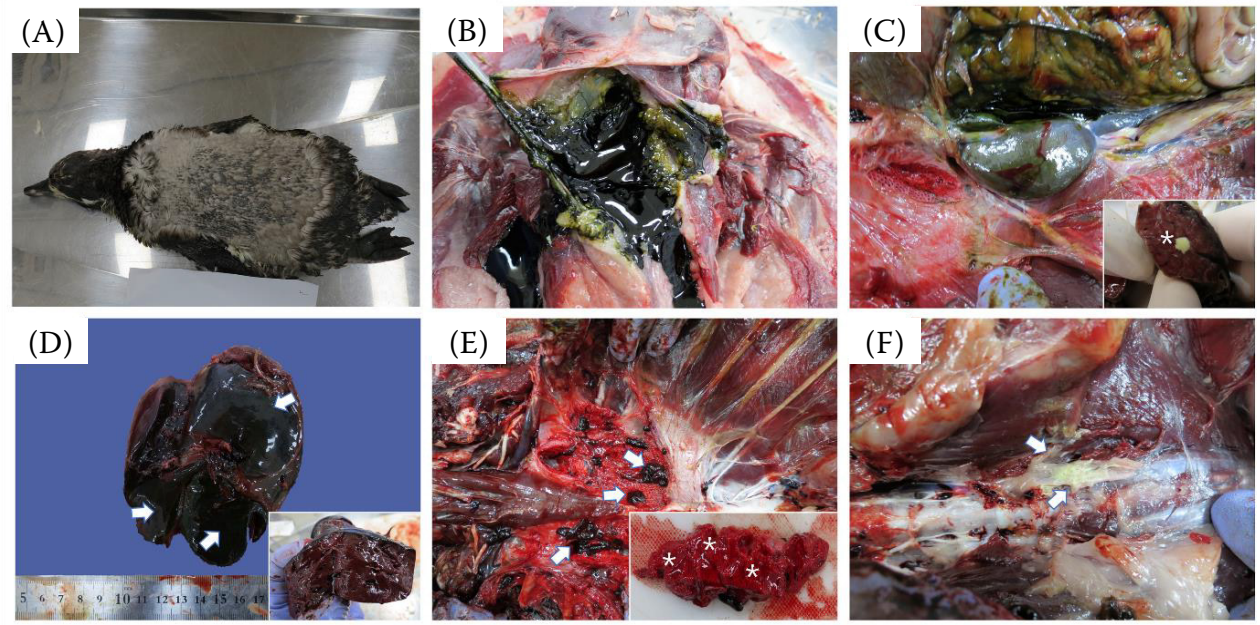

Figure 1. The abdominal cavity, liver, kidneys, and lungs of the 2-year-old Humboldt penguin. **(A)** The appearance of the Humboldt penguin. No specific gross lesion, except for partial baldness due to moulting, was observed. **(B)** A dark greenish bilious effusion in the whole abdominal cavity. The visceral organs were stained with the bilious effusion. **(C)** The cut-surface of the kidney with a green-yellowish abscess in the cortex (asterisk). **(D)** The stained liver (arrows) accompanied by congestion. **(E)** The lungs with severe congestion, haemorrhaging (arrows), and oedema (asterisks). **(F)** The green-yellowish purulent exudate at the tracheal carina (arrows)

https://doi.org/10.17221/73/2019-VETMED

ternal signs of haemorrhages or trauma were evident (Figure 1A). However, the whole abdominal cavity contained large quantities of a bilious dark greenish effusion resulting from a ruptured gallbladder (Figure 1B). The abdominal viscera were stained with the dark greenish effusion (Figure 1C and 1D). The kidneys were severely oedematous and congested with abscesses on the cortex of both the kidneys (Figure 1C). The liver was slightly swollen and severely congested (Figure 1D). The mucosa of the gastrointestinal tracts was also oedematous and hyperaemic. Marked consolidation, thrombi, oedema, haemorrhages, and congestions were diffusely identified in both lungs (Figure 1E). Along with the kidneys, a purulent and haemorrhagic exudate was collected at the caudoventral portions of the lungs and at the tracheal carina (Figure 1F). Bilateral congestion and hyperaemia of the brain were also noted.

For the microscopic examination, the collected tissue samples were fixed in a 10% neutral buffered formalin, routinely processed, paraffin-embedded,

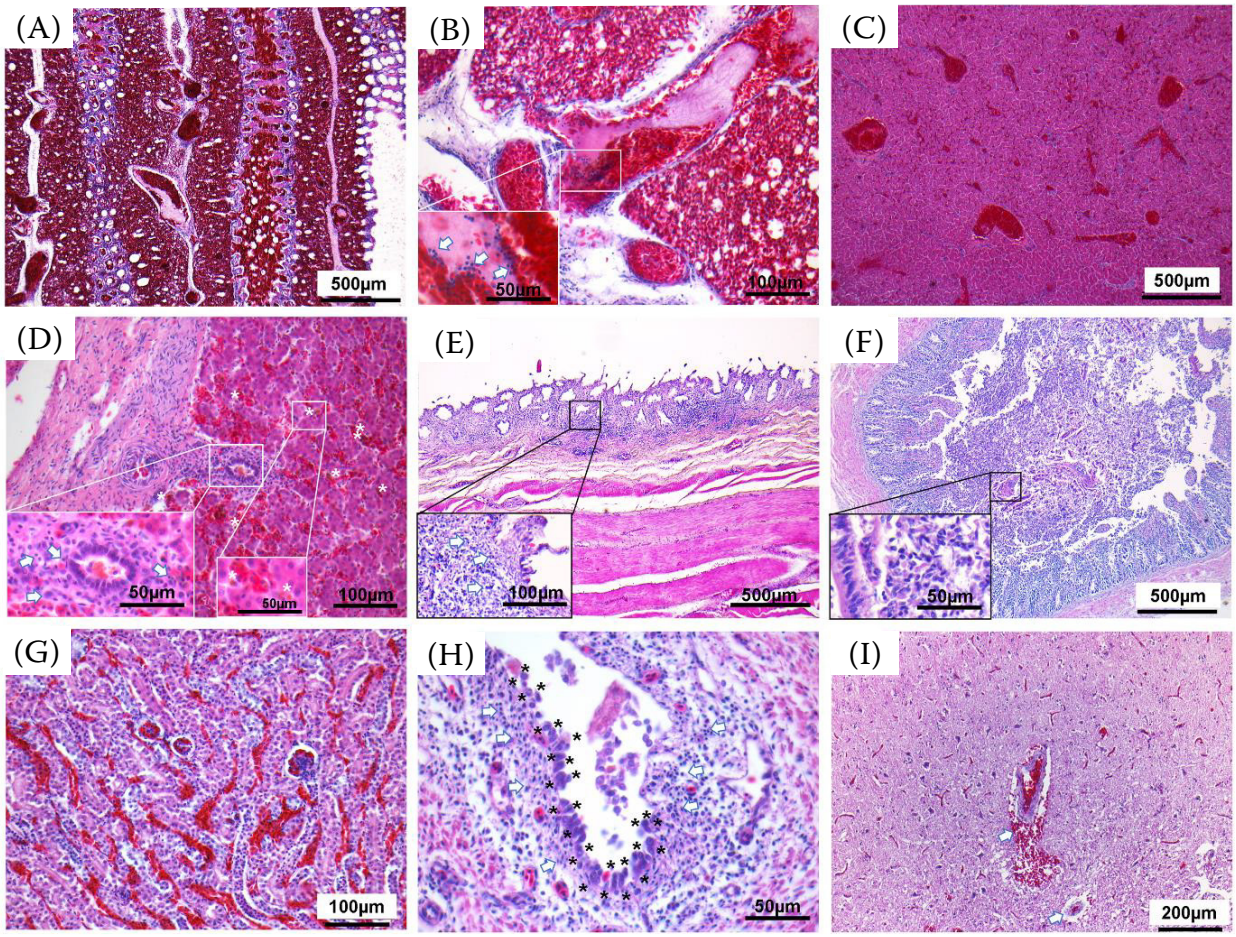

Figure 2. The representative photomicrographs of the lungs (**A** and **B**), liver (**C** and **D**), stomach (**E**), small intestine (**F**), kidneys (**G**), ureter (**H**), and brain (**I**). **(A)** The lung with severe haemorrhaging and excessive fibrin deposition. H&E stain. Bar = 500 μm. **(B)** A microvascular thrombus accompanied by infiltration of the inflammatory cells (arrows) in the lungs. H&E stain. Bar = 100 μm. Inset, Bar = 50 μm. **(C)** The parenchymal congestion of the liver. Bar = 500 μm. H&E stain. **(D)** The liver showing infiltration of the inflammatory cells (arrows) around the bile duct and extensive deposition of the bile pigments (asterisks). H&E stain. Bar = 100 μm. Inset, Bar = 50 μm. **(E)** Necrosis of the villi and infiltration of the chronic active inflammatory cells (arrows) in the stomach. H&E stain. Bar = 500 μm. Inset, Bar = 100 μm. (**F**) Necrosis and fusion of the villi accompanied by infiltration of the chronic active inflammatory cells, and colonies of gram-negative bacteria intermingled with the detached villi in the lumen of the small intestine. H&E stain. Bar = 500 μm. Inset, Bar = 50 μm **(G).** The congestion and detachment of the tubular epithelial cells of the cortex in the kidney. H&E stain. Bar = 100 μm. **(H)** The detachment of the transitional epithelial cells in the ureter (asterisks) and infiltration of the inflammatory cells (arrows) in the submucosal layer. H&E stain. Bar = 50 μm. **(I)** A perivascular oedema and haemorrhaging (arrows) of the brain. H&E stain. Bar = 200 μm

https://doi.org/10.17221/73/2019-VETMED

sectioned, and subjected to haematoxylin and eosin (H&E) and Gram staining. Microscopically, the lungs were characterised by severe haemorrhages filled in the parabronchi, atria, air capillaries, and interparabronchial septa with a microvascular thrombosis indicating disseminated intravascular coagulation (DIC) (Figure 2A). Numerous lymphocytes, macrophages, and septic heterophils were also diffusely infiltrated in the parabronchi, atria, air capillaries, and interparabronchial septa and intermingled with fibrin clots in the blood vessels (Figure 2B). The liver was characterised by dilated sinusoids with overall sinusoidal congestion (Figure 2C). Inflammatory cells, such as macrophages and lymphocytes, infiltrated around the bile ducts, and extensive deposition of the bile pigments were noted in the liver (Figure 2D). In addition, rod-shaped Gram-negative bacterial colonies on the capsule surface of the liver, along with inflammatory cells such as heterophils, macrophages, and lymphocytes, were identified on both the H&E and Gram staining (Figure 3A). The gastrointestinal tracts indicated severe gastroenteritis. Villi necrosis and infiltration of the chronic-active inflammatory cells such as heterophils, macrophages, and lymphocytes in the mucosal and submucosal layers were observed in the stomach (Figure 2E). The small intestines consisted of necrotic and detached villi intermingled with colonies of rod-shaped Gram-negative bacteria and septic heterophils, macrophages, and lymphocytes in the lumen (Figure 2F, Figure 3B). The large intestines showed similar microscopic findings as seen in the small intestine (Figure 3C). Both kidneys showed diffuse congestion, haemorrhages and necrosis of the tubular epithelial cells in the cortex (Figure 2G). The ureters also showed desquamation of the necrotic transitional epithelial cells and infiltration of the inflammatory cells in the submucosal layer (Figure 2H). Myositis accompanied by haemorrhaging and fatty atrophy were observed in the skeletal muscles such as the *pectoralis* muscle and hindlimb muscle. The brain was characterised by a perivascular oedema and haemorrhage (Figure 2I). To determine the aetiologic agent, sterile Transport Swabs containing Amies transport media (COPAN Diagnostics Inc., Murrieta, CA, USA) were prepared from the abdominal cavity, the blood was collected from the jugular vein, trachea, lung, and the kidney to collect the bile, blood, abscess contents, and purulent exudate. These samples were inoculated onto a blood agar media (KisanBio, Seoul, Republic of Korea) for routine aerobic bacterial isolation. Moreover, an AnaeroBag kit (KisanBio, Seoul, Republic of Korea) was used for the anaerobe and microaerophile culture. All

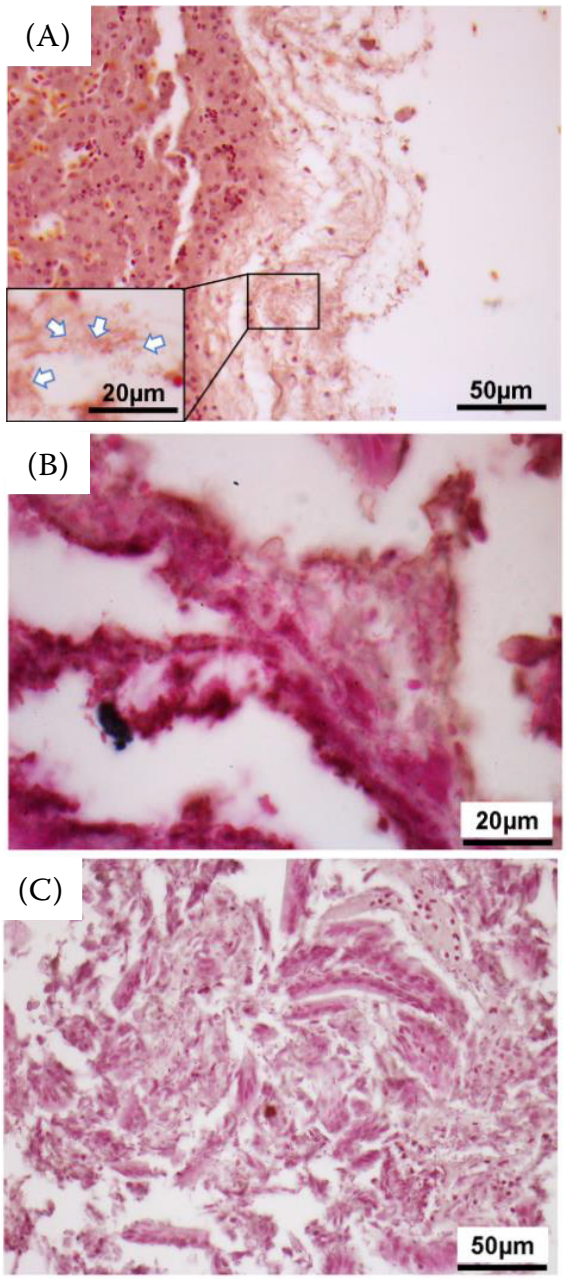

Figure 3. The representative photomicrographs of the liver **(A),** small intestine **(B),** and large intestine **(C). (A)** The gram-negative bacterial colonies in the capsule surface of the liver (arrows). Gram stain. Bar = 50 μm. **(B)** A large quantity of rod-shaped gram-negative bacilli in the villi of the small intestine. Gram stain. Bar = 20 μm. **(C)** The colonies of the gram-negative bacilli intermingled with the necrotic microvilli in the lumen of the large intestine. Gram stain. Bar = 50 μm

https://doi.org/10.17221/73/2019-VETMED

Table 1. The PCR-based bacterial identification of the samples

No.	Abdominal cavity	Blood	Trachea	Lung	Kidney
1	*Proteus cibarius*	*Proteus cibarius*	*Proteus cibarius*	*Proteus cibarius*	*Escherichia fergusonii*
2	*Proteus mirabilis*	*Proteus mirabilis*	*Proteus vulgaris*	*Proteus vulgaris*	*Escherichia fergusonii*
3	*Proteus vulgaris*	–	–	–	–

The PCR-based bacterial identification of the samples collected from the abdominal cavity, kidneys, trachea, lungs, and blood. The *Proteus* species and *E. fergusonii* were detected

the media under aerobic, anaerobic, and microaerophilic culture conditions were incubated at 37 °C for 24 hours. After that, the isolated colonies were once again inoculated onto a 5% sheep blood agar (Mbcell, Seoul, Republic of Korea), incubated at 37 °C for 24 h for further identification, and polymerase chain reaction (PCR) investigations were performed. The 16S rRNA of these isolated bacterial colonies in the culture media were identified by using the universal primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'). As a result, the *Proteus* species was identified in the samples, including the blood sample (Table 1).

DISCUSSION AND CONCLUSIONS

Septicaemia is a life-threatening and complicated form of bacteraemia characterised by fever, toxaemia, organ dysfunction, and final shock (Zachary and McGavin 2011; Tizard 2012). It is indicated that one of the most common aetiologies for septicaemia is a biliary tract infection, which is most commonly caused by the family *Enterobacteriaceae* ascending from the gastrointestinal tract (Melzer et al. 2007). The bacteria while ascending invades the biliary tract from the duodenum (Sung et al. 1992). The increased intraductal pressure leads to biliary content reflux and bacteraemia, which, in turn, causes septic shock and, finally, sudden death (Hanau and Steigbigel 2000).

In the present case, the penguin showed several lesions representing a systemic bacterial infection on both the H&E and Gram staining. For instance, several parenchymal organs showed infiltration of the chronic active inflammatory cells, detachment of the epithelial cells, haemorrhages, and congestion. Particularly, the bile ducts were infiltrated by the inflammatory cells with haemorrhaging; microscopically, bile pigments were also observed in the whole liver section, indicating a gallbladder rupture. Moreover, rod-shaped Gram-negative bacteria were found in the capsule surface of the liver. Additionally, the intestines were characterised by fusion and necrosis of the villi, with numerous Gram-negative bacilli in the lumen. The lungs also showed severe haemorrhages in the parabronchi, atria, air capillaries, and interparabronchial septa and diffuse microvascular coagulation intermingled with the inflammatory cells without an indication of a primary underlying vasculopathy disease. These findings strongly suggest that this bird succumbed to death due to septicaemia occurring after the gallbladder rupture.

P. mirabilis, P. vulgaris, P. cibarius, and *P. penneri* were identified in all the samples, except for the pus collected from the kidneys. However, their pathogenicity in animals has not been reported.

Escherichia fergusonii, a Gram-negative opportunistic pathogen causing UTIs was identified in the kidneys (Lai et al. 2011; Glover et al. 2017). However, this screening PCR test ruled out the most common organisms known to cause biliary tract infections such as *E. coli* and *Klebsiella pneumoniae*. Although *Proteus* species are saprophytes and saprogenic bacteria reside in soil, sewage, and carcass, it was confirmed that the infection occurred when the bird was alive, and death resulted from a *Proteus* infection as the penguin's body was maintained at 4 °C during the transportation and the necropsy was performed within 12 h of death. Moreover, the presence of the bacterial colonies in various organs including the parenchymal organs such as the liver, the DIC in the blood vessels, and the secondary inflammatory responses, such as infiltration of the chronic active inflammatory cells in various organs, were observed microscopically.

Proteus, a proteobacterium belonging to the family Enterobacteriaceae, is a well-known opportunistic pathogen mainly reserved in the intestines of humans as well as domestic and wild

https://doi.org/10.17221/73/2019-VETMED

animals (Rozalski et al. 1997; Drzewiecka 2016). These bacteria can cause severe pathologic conditions in humans and animals with an immunocompromised status with an environmental stress, a co-morbid disease, or a concurrent infection (Sah et al. 1983; Rozalski et al. 1997; Olinda et al. 2012). *Proteus* septicaemia in avian species has been clinically characterised by rapid depression, coma, and sudden death and pathologically by severe congestion of the parenchymal organs such as the liver, lungs, and kidneys (Sah et al. 1983). Similarly, in the present case, the penguin exhibited an abrupt and rapid depression and coma before death. Moreover, most of the parenchymal organs, such as the liver, kidneys, and lungs showed severe congestion and haemorrhaging. However, the most interesting gross lesion in this present case was the gallbladder rupture accompanied by a secondary bilious effusion in the whole abdominal cavity. In addition, a purulent exudate was observed in the other organs such as the tracheal carina, lungs, and kidneys.

Humboldt penguins live in colonies (Simeone et al. 2004). However, in the present case, the penguin was isolated and introduced to a new rearing aquarium during the season of moulting because it ruthlessly attacked other penguins in the zoo flock. A biliary infection, which shows high morbidity and mortality, is associated with immunosuppressed patients exposed to stress, a primary underlying disease, a co-morbid disease, and a co-infection by other bacterial pathogens (Melzer et al. 2007). Therefore, in this case, the possibility of stress associated with the environmental changes cannot be excluded as a contributing factor along with the *Proteus* infection, and it also explains the concurrent infection with another Gram-negative opportunistic pathogen, *E. fergusonii.* Thus, *Proteus* residing in the intestines retrograded via the small intestine. Sequentially, the ascending infection toward the gallbladder may have resulted in cholecystitis, which, in turn, led to a blood flow obstruction of the gallbladder, leading to necrosis and the gallbladder rupture. Bile ascites that leaked from the gallbladder probably caused bile peritonitis, resulting in the systemic inflammatory response syndrome (SIRS). For these reasons, it is highly suspected that the penguin died due to septic shock.

A case of septicaemia caused by *Proteus* species in a bird was reported (Sah et al. 1983). Although *Proteus* is known as a potential pathogen in birds (Olinda et al. 2012), it is an opportunistic pathogen that does not cause diseases in healthy animals. For this reason, neither septicaemia due to *Proteus* infections nor the pathological examination in penguins have been reported, although few cases of septicaemia caused by other pathogens, such as *P. aeruginosa* and *Erysipelothrix rhusiopathiae*, in penguins were reported (Boerner et al. 2004; Widmer et al. 2016). In addition, *S. humboldti* has been categorised as a threatened species, therefore, we can mostly see these penguins in zoos, but pathological information of septicaemia in this species has been rarely reported; thus, the detailed histopathological description of septicaemia caused by the bacterium may provide a clue for the diagnosis of septicaemia in penguins caused by *Proteus* and other bacterial species later on. For this reason, we hereby report this apparently first known case of septicaemia due to *Proteus* infection in a Humboldt penguin.

REFERENCES

Adesina T, Nwinyi O, De N, Omonigbehin E (2018): First detection of Proteus cibarius sp. of clinical significance. IOP Conference Series: Earth and Environmental Science, 210, 012001. doi: 10.1088/1755-1315/210/1/012001.

Boerner L, Nevis KR, Hinckley LS, Weber ES, Frasca Jr S (2004): Erysipelothrix septicemia in a little blue penguin (Eudyptula minor). Journal of Veterinary Diagnostic Investigation 16, 145–149.

Chen CY, Chen YH, Lu PL, Lin WR, Chen TC, Lin CY (2012): Proteus mirabilis urinary tract infection and bacteremia: risk factors, clinical presentation, and outcomes. Journal of Microbiology, Immunology and Infection 45, 228–236.

Drzewiecka D (2016): Significance and roles of Proteus spp. Bacteria in natural environments. Microbial Ecology 72, 741–758.

Endimiani A, Luzzaro F, Brigante G, Perilli M, Lombardi G, Amicosante G, Rossolini GM, Toniolo A (2005): Proteus mirabilis bloodstream infections: Risk factors and treatment outcome related to the expression of extended-spectrum β-lactamases. Antimicrobial Agents and Chemotherapy 49, 2598–2605.

Glover B, Wentzel J, Jenkins A, Van Vuuren M (2017): The first report of Escherichia fergusonii isolated from non-human primates, in Africa. One Health 3, 70–75.

Hanau LH, Steigbigel NH (2000): Acute (ascending) cholangitis. Infectious Disease Clinics of North America 14, 521–546.

https://doi.org/10.17221/73/2019-VETMED

Kim BN, Kim NJ, Kim MN, Kim YS, Woo JH, Ryu J (2003): Bacteraemia due to tribe Proteeae: A review of 132 cases during a decade (1991–2000). Scandinavian Journal of Infectious Diseases 35, 98–103.

Kwiecinska-Pirog J, Skowron K, Gospodarek-Komkowska E (2018): Primary and secondary bacteremia caused by Proteus spp.: epidemiology, strains susceptibility and biofilm formation. Polish Journal of Microbiology 67, 471–478.

Lai CC, Cheng A, Huang YT, Chung KP, Lee MR, Liao CH, Hsueh PR (2011): Escherichia fergusonii bacteremia in a diabetic patient with pancreatic cancer. Journal of Clinical Microbiology 49, 4001–4002.

Lees GE (1996): Bacterial urinary tract infections. Veterinary Clinics: Small Animal Practice 26, 297–304.

Ling GV, Norris CR, Franti CE, Eisele PH, Johnson DL, Ruby AL, Jang SS (2001): Interrelations of organism prevalence, specimen collection method, and host age, sex, and breed among 8,354 canine urinary tract infections (1969–1995). Journal of Veterinary Internal Medicine 15, 341–347.

Melzer M, Toner R, Lacey S, Bettany E, Rait G (2007): Biliary tract infection and bacteraemia: Presentation, structural abnormalities, causative organisms and clinical outcomes. Postgraduate Medical Journal 83, 773–776.

O'Hara CM, Brenner FW, Miller JM (2000):Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. Clinical Microbiology Reviews 13, 534–546.

Olinda RG, Souza MCA, Figueiredo JN, Silva JMC, Alves ND, Bezerra FSB, Feijo FMC (2012): Diagnosis of Proteus spp. in wild birds raised under captivity in Rio Grande do Norte, Brazil. Arquivos do Instituto Biológico 79, 301–303.

Rozalski A, Sidorczyk Z, Kotelko K (1997): Potential virulence factors of Proteus bacilli. Microbiology and Molecular Biology Review 61, 65–89.

Sah RL, Mall MP, Mohanty GC (1983): Septicemic Proteus infection in Japanese quail chicks (Coturnix japonica). Avian Diseases 27, 296–300.

Simeone A, Luna-Jorquera G, Wilson RP (2004): Seasonal variations in the behavioural thermoregulation of roosting Humboldt penguins (Spheniscus humboldti) in north-central Chile. Journal of Ornithology 145, 35–40.

Stock I (2003): Natural antibiotic susceptibility of Proteus spp., with special reference to P. mirabilis and P. penneri strains. Journal of Chemotherapy 15, 12–26.

Sung JY, Costerton JW, Shaffer EA (1992): Defense system in the biliary tract against bacterial infection. Digestive Diseases and Sciences 37, 689–696.

Tizard IR (2012): Innate immunity:proinflammatory and antimicrobial mediators. In: Tizard IR (ed.): Veterinary Immunology. 9th edn. Elsevier, St. Louis, Missouri. 21–29 pp.

Widmer D, Ziemssen E, Schade B, Kappe E, Schmitt F, Kempf H, Wibbelt G (2016): Pseudomonas aeruginosa infection in a group of captive Humboldt penguins (Spheniscus humboldti). Journal of Avian Medicine and Surgery 30, 187–195.

Zachary JF, McGavin MD (2011): Inflammation and healing. In: McGavin MD (ed.): Pathologic Basis of Veterinary Disease. Elsevier, St. Louis, Missouri. 89–146 pp.

Received: June 18, 2019
Accepted after corrections: October 26, 2019