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Health Surveillance of Penguins in the Barton Peninsula on King George Island, Antarctica

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ABSTRACT: Samples from 29 adult Gentoo (*Pygoscelis papua*), Chinstrap (*Pygoscelis antarcticus*), and Adélie Penguins (*Pygoscelis adeliae*) at the King Sejong Station on Narębski Point, King George Island, Antarctica, were investigated to detect antibodies to avian influenza, Newcastle disease virus, infectious bursal disease virus, infectious bronchitis virus, *Mycoplasma*, and *Salmonella*. Antibodies were identified from one Gentoo Penguin and one Chinstrap Penguin against infectious bronchitis virus; from one Gentoo Penguin against Newcastle disease virus; from one Gentoo Penguin against *Mycoplasma synoviae*; and from two Chinstrap Penguins against *Salmonella pullorum*. Thirty-three dead penguin chicks were collected from the breeding colony for necropsy, histopathological examination, and polymerase chain reaction. Pulmonary hemorrhage and congestion were the main necropsy findings.

Key words: Antarctica, health surveillance, histopathology, penguin, pulmonary hemorrhage.

Along with the rapid climate change in the Antarctic ecosystem, introduced infectious diseases with increased human activities are a long-recognized threat to Antarctic wildlife (Gauthier-Clerc et al. 2002; Grimaldi et al. 2015). Penguins are one of top predators in the Antarctic marine ecosystem threatened by both risks (Grimaldi et al. 2015), and therefore, knowledge about their health conditions is important to understand the changes in Antarctic wildlife and ecosystem. The Republic of Korea (hereafter, Korea) manages the King Sejong station near the Antarctic Specially Protected Area (ASPA no. 171; Narębski Point), located at the tip of Barton Peninsula on King George Island in the South Shetland Islands, Antarctica. This region hosts around 3,000 breeding pairs of Chinstrap Penguins (*Pygoscelis antarcticus*) and 2,300 pairs of Gentoo Penguins (*Pygoscelis papua*) annually (Kim et al. 2016). After the Narębski Point Penguin Village of Antarctic King Sejong Station was designated as a protected zone in 2009, three types of monitoring on penguins were conducted: distribution of breeding birds, reproduction rate (egg production rate, hatching rate), and fluctuation of the breeding bird community. However, analysis of external environmental factors, including diseases that may influence the penguins, has not been performed. Because the ultimate purpose of the ASPA is the protection of ecosystems, strategies for species conservation in the protected areas should be implemented (Grimaldi et al. 2015). This health surveillance study aims to investigate the presence of some common infectious avian diseases and to understand the cause of mortality of penguin chicks in order to increase the knowledge of any possible limiting factors of the population.

From 10 January 2013 to 20 January 2013, carcasses of penguin chicks were collected from penguin rookeries at Narębski Point (ASPA no. 171; 62°14′14″S, 58°46′27″W) on King George Island in the South Shetland Islands, Antarctica. We also collected samples at nearby Ardley Island (ASPA no. 150; 62°12′48″S, 58°55′10″W) to capture Adélie Penguins (*Pygoscelis adeliae*), which do not breed at Narębski Point on King George Island in the South Shetland Islands. Due to the short sampling period, limited numbers of blood samples from adult penguins and

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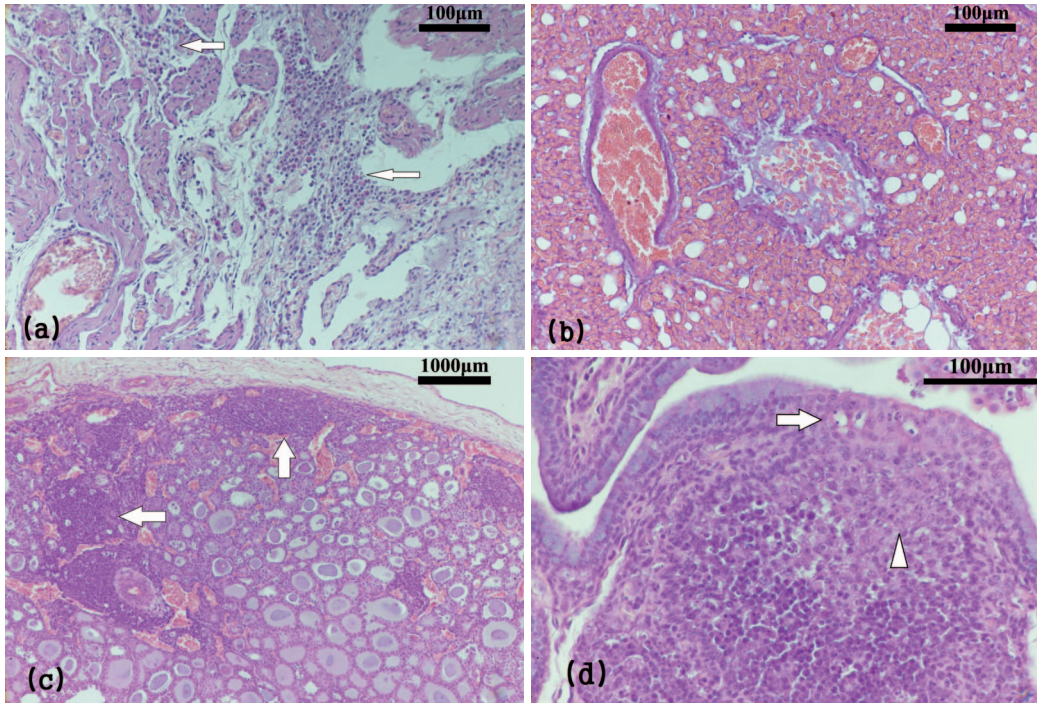


FIGURE 1. Representative histology from a dead Chinstrap Penguin (*Pygoscelis antarcticus*) chick found at Narębski Point, King George Island, Antarctica, in January 2013. (a) Myocarditis. Heterophilic migration and lymphocytic infiltration in the myocardium (arrows). (b) Pulmonary hemorrhage. (c) Inflammatory cell infiltration (arrows) in the thyroid gland. (d) Single-cell necrosis of epithelia (arrow) and depletion of lymphocytes (arrowhead) of bursa of Fabricius. H&E stain.

carcasses of chick penguins were collected. Healthy-looking penguins were randomly captured with a dip net on their way to their nests (ASPA nos. 171 and 150). Blood samples were collected from the brachial vein of 13 adult Gentoo Penguins, 15 Chinstrap Penguins, and one Adélie Penguin. Blood was kept in an icebox before separating sera and then frozen at –20 C. The presence of antibodies to avian influenza virus and infectious bursal disease virus was tested for with the agar gel precipitation method. If the precipitation line from the reaction of tested sera and antigen was similar to precipitation from positive sera, the tested sera evaluated as positive to the antigens. To identify infectious bronchitis virus and Newcastle disease virus, sera were treated with kaolin and detected with hemagglutination inhibition with 10% chicken red blood cells at room temperature for 10 min. Titers greater than 8 were evaluated as positive results. *Mycoplasma* and *Salmonella* were tested with plate agglutination. If the agglutination occurred with 2 min, the tested sera evaluated as positive to antigens.

Due to the high predatory and scavenging activities by birds, only carcasses of chicks guarded by parents were available. Dead adults could not be collected because they were immediately scavenged. Thirty-three penguin chick carcasses were collected (one Adélie, six Gentoo, and 26 Chinstrap Penguins; 0.12–1.02 kg) and were necropsied in a laboratory in the King Sejong Station (Fig. 1a). Organs were fixed in 10% neutral formalin, and the tissues were later processed for histological examination in Seoul, Korea.

We determined the sex of dead penguin chicks by post-mortem and histological examination on genital organs. However, for live adults, we used a molecular technique to

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TABLE 1. Primer sequences and amplification conditions for reverse transcriptase (RT)-PCR or PCR used to detect the presence of avian influenza virus, infectious bursal disease virus, Newcastle disease virus, and fowl adenovirus 29 adult Gentoo (*Pygoscelis papua*), Chinstrap (*Pygoscelis antarcticus*), and Adélie Penguins (*Pygoscelis adeliae*) at the Narębski Point, King George Island, Antarctica, in January 2013. RT-PCR was used to detect avian influenza virus (AI), infectious bursal disease virus (IBD), and Newcastle disease virus (ND), and PCR was used for fowl adenovirus (FA).

Virus	Target gene[a]	Primer name	Sequence	PCR conditions	Target size (base pair)	Reference
AI	NP	MMU43	CATCCCAGTGCTGGGAARGAYCCTAAGAA	94 C 30 sec–58 C 30 sec, 72 C 30 sec; 35 cycles	218	Munch et al. 2001
		MMU42	AGAGCTCTTGTTCTCTGATAGGTG			
IBD	VP2	MK114	AGC CTT CTG ATG CCA ACA AC	94 C 1 min–50 C 1 min–72 C 2 min; 35 cycles	365	Caterina et al. 2004
		MK115	ATC TGT CAG TTC ACT CAG GC			
ND	F	NDCom-F	ATA CAC CTC RTC YCA GAC AG	94 C 20 sec–50 C 30 sec–72 C 30 sec; 40 cycles	379	Lee et al. 2009
		NDCom-R	TGC CAC TGM TAG TTG YGA TA			
		NDPt-F	TGG AGG CAT ACA ACA GGA CA	94 C 20 sec–50 C 30 sec–72 C 30 sec; 40 cycles	204	Lee et al. 2009
		NDPt-R	TGC CAC TGM TAG TTG YGA TA			
FA	Hexon	HexonA	CAA RTT CAG RCA GAC GGT	94 C 2 min–54 C 1 min–72 C 90 sec; 35 cycles	897	Meulemans et al. 2001
		HexonB	TAG TGA TGM CGS GAC ATC AT			

[a] NP = nucleoprotein; F = fusion.

determine the sex of sampled individuals using their blood samples and three primers: P0, P2, and P8 (Han et al. 2009). An emulsion of lung, spleen, bursa of Fabricius, and liver from 26 fresh penguin chick carcasses (three Gentoo and 23 Chinstrap Penguins) kept at –20 C over 7 mo was used for PCR. Reverse transcriptase-PCR was used to identify avian influenza virus, infectious bursal disease virus, and Newcastle disease virus. We used PCR to detect fowl adenovirus (Table 1). The nucleic acids were extracted using the IQeasy Plus Viral DNA/RNA Extraction Kit (iNtRON Biotechnology, Gyeonggi-do, Korea). Finally, 30 μL of elution buffer was used to obtain nucleic acid and centrifuged at 18,214 × G for 1 min. Antibody testing and PCR procedures were carried out in an isolated room of the Animal and Plant Quarantine Agency, Anyang, Korea.

Serology results for adult penguins are shown in Table 2. Antibodies were identified from the following: one Gentoo Penguin and one Chinstrap Penguin against infectious bronchitis virus; one Gentoo Penguin against Newcastle disease virus; one Gentoo Penguin against *Mycoplasma synoviae*; and two Chinstrap Penguins against *Salmonella pullorum*.

At necropsy, food was found in the stomachs of all dead penguin chicks. The subcutaneous fat was within normal range. Atrophy or degeneration of the internal organs was not found at gross necropsy from these carcasses.

Histopathology revealed that 55% (18/33) of dead penguin chicks had focal lymphocytic infiltration of the pericardium and focal necrosis of the myocardium (Fig. 1a). Further, 67% (22/33) of dead chicks had pulmonary hemorrhage, and 45% (15/33) dead penguin

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TABLE 2. Results of serological antibody tests of 29 adult Gentoo (*Pygoscelis papua*), Chinstrap (*Pygoscelis antarcticus*), and Adélie Penguins (*Pygoscelis adeliae*) from Narebski Point, King George Island, Antarctica. Antibodies were identified from the following: one Gentoo Penguin and one Chinstrap Penguin against infectious bronchitis virus; one Gentoo Penguin against Newcastle disease virus; one Gentoo Penguin against *Mycoplasma synoviae*; and two Chinstrap Penguins against *Salmonella pullorum*.

Agent	Test[a]	% Positive	*N* positive/ *n* tested	Titer
Avian influenza virus	AGP	0	0/29	NA[b]
Newcastle disease virus	HI	3	1/29	8[c]
Infectious bursal disease virus	AGP	0	0/29	NA
Infectious bronchitis virus	HI	7	2/29	8,[c] 16[d]
Mycoplasma gallisepticum	PA	0	0/29	NA
Mycoplasma synoviae	PA	3	1/29	2[d]
Salmonella gallinarum	PA	0	0/29	NA
Salmonella pullorum	PA	7	2/29	NA[d]

[a] AGP = agar gel precipitation; HI = hemagglutination inhibition; PA = plate agglutination.

[b] NA = not applicable.

[c] Gentoo Penguin.

[d] Chinstrap Penguin.

chicks showed pulmonary hemorrhage and congestion. Bronchopneumonia was detected in 24% (8/33) by the presence of lymphocytes in the bronchial lumen (Fig. 1b). In addition, lymphocytic aggregation of the thyroid (10 cases), single-cell necrosis of epithelia and focal lympholysis of the bursa of Fabricius (six cases), and inflammatory cellular infiltrates around arteries and peripheral nerves (one case) were observed (Fig. 1c, d). The other histological findings included pigmented crystals in the renal tubules and focal hepatic necrosis of the liver (two cases). All penguin chicks were negative by PCR for avian influenza virus, Newcastle disease virus, infectious bursal disease virus, and fowl adenovirus.

The overall health status of Gentoo and Chinstrap Penguins at Narębski Point has not been studied previously, although Newcastle-positive penguins have been found at this location (Thomazelli et al. 2010). Positive serology results do not imply the presence of disease as none of the serologically positive penguins had clinical signs of disease. When serological tests are used to diagnose a disease, the predictive value of positive results mainly depends on the prevalence of the disease. If the disease prevalence is only 10%, the predictive value of positive results would be only 68.5%, with a serological test of 95% specificity. Therefore, examination of adult penguin tissue samples is required to obtain an exact diagnosis for the prevalence of diseases in a penguin colony. Although tissues from adult penguins are required to confirm these findings, feces or blood or choanal swabs can be used for PCR.

In our study, one adult female Gentoo Penguin was positive for Newcastle disease virus (Table 2). Paramyxovirus, which includes Newcastle disease virus, is widespread in Adélie Penguin colonies around the Australian Casey Station of the Australia Antarctica Division on Bailey Peninsula (Morgan and Westbury 1981). Adélie and Royal Penguins (*Eudyptes schlegeli*) have been previously reported positive for avulaviruses and Newcastle disease virus (Morgan and Westbury 1981; Wille et al. 2019). One hundred penguins of three different species (Gentoo, Adélie, and Chinstrap Penguins) were assessed for Newcastle disease virus infection at King George Island, and two from Narębski Point were positive (Thomazelli et al. 2010). Similarly, in our study, one adult female Gentoo Penguin was positive for Newcastle disease virus (Table 2). Given these findings and previous reports, it appears that the Newcastle disease virus is relatively widespread among penguins that live in Antarctica and sub-Antarctic regions, but these are likely to be lentogenic strains as no disease outbreaks have been reported. In our study, a positive reaction to *Salmonella pullorum* was also confirmed in the serum from one male and one female adult Chinstrap Penguin (Table 2). Five serotypes of *Salmonella* were identified from Adélie Penguins on Ross Island in Antarctica; however, positive results did not correlate to disease (Oelke and Steiniger 1973). Further, we identified infectious bronchitis virus in the serum from one Chinstrap and one Gentoo adult female

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penguin. Infectious bronchitis virus has not been previously reported in this region. Our study also confirmed a positive reaction to *Mycoplasma synoviae* from the serum of an adult female Chinstrap Penguin. Dewar et al. (2013) detected Mycoplasmataceae in fecal samples of King Penguins (*Aptenodytes patagonicus*) with 16S rRNA pyrosequencing.

Although many viruses including avian influenza viruses H11N2 were previously confirmed from Adélie Penguins on King George Island (Morgan and Westbury 1981; Grimaldi et al. 2015; Hurt et al. 2016; Wille et al. 2019), all penguin chicks were negative with PCR for avian influenza virus, Newcastle disease virus, infectious bursal disease virus, and fowl adenovirus. This may be due to the 7 mo storage time before testing samples due to the difficulty of performing PCR test for samples in situ at time of collection. We attempted to keep the samples in appropriate conditions at the field station and during transport to our lab in Seoul, but there may have been fluctuating temperatures during the storage and transportation. The PCR results for viruses were all negative; however, it is impossible to exclude the tested agents as a cause of death. Methods of preservation and transportation of the samples must be reviewed and improved in the future. In addition, improvements are needed to obtain fresh samples from the chick and adult penguins.

Because good nutrition is key to maintaining a functioning immune system as well as the survival of penguins (Kogut and Klasing 2009; Grimaldi et al. 2015), starvation because of decreasing food resources like krill (*Euphausia* spp.) due to changing climate and sea conditions may also become an evolving threat to penguin chicks (Grimaldi et al. 2015). However, gross and histopathological findings of penguin chicks suggested that death of penguin chicks may be due to cold stress and hypothermia causing pulmonary hemorrhage rather than starvation (Kretschmer et al. 2018; Hillman and Lam 2019). The gastric contents and fat reserves in the penguin chicks indicated that their death was not related to starvation. Most penguin chicks had pulmonary hemorrhage, pericarditis, and myocarditis. Factors associated with increased risk of severe pulmonary hemorrhage include hypothermia (Kretschmer et al. 2018). The average of atmosphere temperature when we collected the carcasses of penguin chicks was lower than those of 2011 and 2112. This suggests that cold stress from snowstorms that occurred at this time following weakening in the polar vortex may have made the penguins susceptible to hypothermia and pulmonary hemorrhage (Hillman and Lam 2019). However, this hypothesis needs further investigation. Histologically, atrophy and epithelial necrosis were found in the bursa of Fabricius (six cases), and focal lympholysis was found in the liver (two cases). The bursa of Fabricius is well known as a primary lymphoid organ in birds, and infectious agents stimulate the immune system through this organ. Penguins lose resistance to infection when the bursa of Fabricius is infected. Therefore, additional diagnostic methods, such as immunohistochemistry and electron microscopy techniques, are needed to possibly identify the specific pathogens involved in these lesions. Infectious bursal disease has been reported in King Penguins (Gauthier et al. 2002). Pathogens observed in penguin breeding groups may result in diseases of penguin chicks related to the stress of climate change and other ecological changes. It is not clear whether collection of 33 dead fledged penguins in 5 d from one breeding site represents an adequate sample of the normal population as there are currently no data for the survival rate of chick penguins in the area. Ongoing investigations to identify pathogens that infect penguin groups are needed to formulate a plan for climate change or potential sudden mass mortality. It is also necessary to systematically investigate the effects of diseases related to climate stress by accumulating data related to disease. Future research of the health of penguins and other wildlife near stations with increasing human activities should be conducted to prepare for changes in the Antarctic ecosystem.

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