



Evaluation of the air sac volume of penguins with respiratory diseases using computed tomography

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ABSTRACT. Captive penguins with respiratory diseases exhibit advanced pathological conditions upon the appearance of clinical signs. Therefore, the successful treatment of respiratory diseases remains difficult after the onset of clinical signs, leading to high mortality rates. In this study, we measured air sac volume using computed tomography (CT) to evaluate the respiratory condition of penguins. In a regular quarterly health checkup, blood samples were collected from 45 penguins housed at an aquarium in Hokkaido, Japan. A total of 12 penguins with abnormal blood parameters underwent CT. The air sac volumes were calculated in three-dimensional CT, and the scatter plots of the air sac volumes and body weights were analyzed. No correlation was found between the air sac volume and body weight in both the gentoo and king penguins. Two gentoo penguins with infiltration and one king penguin with multiple nodules on CT were tentatively diagnosed with aspergillosis and treated with oral administration of itraconazole. Follow-up CT examination was performed until the outcome: healed or died. The mean air sac volumes of the two gentoo penguins, which recovered after treatment, increased from 273.9 and 329.0 cm³ before healing to 449.0 and 424.6 cm³ after healing, respectively. Meanwhile, the air sac volume of the king penguin, which subsequently died, decreased from 1,556.9 to 920.6 cm³ despite treatment. Changes of the air sac volume in the same individual could be useful for evaluating the respiratory condition of penguins.

KEY WORDS: air sac volume, aspergillosis, computed tomography, penguin

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Captive penguins are often affected by respiratory diseases, and those with respiratory diseases exhibit advanced pathological conditions upon the appearance of clinical signs such as inappetence, open mouth breathing, and exercise intolerance. Therefore, the successful treatment of respiratory diseases remains difficult after the onset of clinical signs, leading to high mortality rates.

The most common respiratory disease is pulmonary aspergillosis [2, 10]. Its poor prognosis has always been a concern in zoos and aquariums [3, 4, 11, 13]. We previously reported the computed tomography (CT) image findings of infiltration in the pulmonary parenchyma as findings specific to aspergillosis [10, 16]. In these studies, we revealed that the volume of the air sacs of the affected penguins was lower than that of the air sacs of healthy penguins. Assessing air sac volume might be an objective method for evaluating respiratory conditions. Thus, we measured the air sac volumes using CT for evaluating the prognosis of the respiratory disease of penguins.

MATERIALS AND METHODS

Animals

A total of 45 penguins (King penguin, *Aptenodytes patagonicus*, n=8, Gentoo penguin, *Pygoscelis papua*, n=21, and Cape penguin, *Spheniscus demersus*, n=16) housed at an aquarium in Hokkaido, Japan, were investigated. The body weight of the penguins was measured once a month, and their blood samples were collected during regular quarterly health checkups. The health check and all diagnostic and treatment procedures were conducted in accordance with practice guidelines of the veterinary department of the aquarium.

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Blood examination

Blood was collected from the medial metatarsal vein, and aspergillosis parameters were evaluated. The levels of aspartate aminotransferase (AST), creatine kinase (CK), albumin-to-globulin ratio (A/G), *Aspergillus* antigens, and *Aspergillus* antibodies were measured. Based on previous reports, penguins with any of the following criteria were selected for this study: AST ≥ 400 IU, CK ≥ 900 IU [8], A/G ratio ≤ 0.8 [12, 15], *Aspergillus* antigen (galactomannan index) ≥ 0.5 [6], or *Aspergillus* antibody titer determined by a complement fixation test (the highest dilution of serum) $\geq 1:16$ in penguins suspected to have aspergillosis [9]. Penguins with abnormal blood parameters underwent CT.

CT examination

Anesthesia was induced by a well-trained veterinarian. The nose and beak of the penguin were covered with a custom-made mask and induced with 5% isoflurane and maintained 2% isoflurane with spontaneous breathing. The penguins were placed in a supine position on the CT table. CT images were obtained, including from the cranial edge to the caudal edge of the air sac, using a four-row multidetector CT scanner (Asteion Super 4, Cannon, Ohtawara, Japan) with 80 kV, 150 mA. One point zero-mm slice thickness in both the scan and reconstruction, and 1.375 pitch factor. Images were reconstructed with lung algorithm and then observed in lung window. DICOM data were sent to a viewer (OsiriX-N, Newton-Graphics, Sapporo, Japan) to read the images. The areas of the air sac in each penguin were measured by using the viewer with the operator-defined region of interests of all air sacs on each two-dimensional CT image, and the volumes were then calculated using the viewer-fixed three-dimensional images. The relationship between the air sac volumes on CT and body weight were compared using scatter plot (Microsoft Excel, Office 2019 for Mac, Microsoft, Redmond, WA, USA).

The penguins detected with infiltrative lesions in the pulmonary parenchyma or multiple nodules in the air sac on CT were tentatively diagnosed with aspergillosis and treated with the oral administration of itraconazole (4–10 mg/kg once a day). The total number of follow-up CT examination was 4–5 times until the outcome: healed or died. The interval of follow-up CT examination was 2–6 months. After observing no abnormality, the interval of follow-up CT examination was 2 months–2 years. The air sac volume was compared between abnormal CT findings and normal CT findings in each case.

RESULTS

A total of 12 penguins (King penguin, n=6 and Gentoo penguin, n=6) with abnormal blood parameters underwent CT (Table 1). All these penguins, except for case 9, did not show clinical sign such as inappetence, open mouth breathing, and exercise intolerance. Infiltrative lesions in the pulmonary parenchyma were detected in cases 1, 2, and 9, whereas multiple nodules were detected in cases 7 and 9. These signs were considered specific for aspergillosis [10]. Case 9 died 19 days after CT; hence, follow-up CT was not performed. Defects in the pulmonary parenchyma were detected in cases 4, 5, and 6. The defects were not considered clinically significant. No abnormality was detected in cases 3, 8, 10, 11, and 12. Follow-up CT examinations were performed in cases 1, 2, and 7.

The scatter plots of the air sac volumes and body weight on the gentoo and king penguins are shown in Figs. 1 and 2,

Table 1. Description of penguins evaluated for aspergillosis diagnostic screening using blood tests and computed tomography

	Species	Sex	Age (years old)	Body weight (kg)	Blood test	CT findings
Case 1	Gentoo	Female	4	5.69	<i>Aspergillus</i> antibody 1:32	Infiltrative lesion in the pulmonary parenchyma
Case 2	Gentoo	Female	3	5.88	<i>Aspergillus</i> antibody 1:16	Infiltrative lesion in the pulmonary parenchyma
Case 3	Gentoo	Female	6	6.06	<i>Aspergillus</i> antigen 0.5	No abnormality
Case 4	Gentoo	Female	6	6.40	<i>Aspergillus</i> antibody 1:16	Defects in the pulmonary parenchyma
Case 5	Gentoo	Male	11	6.62	<i>Aspergillus</i> antibody 1:32	Defect in the pulmonary parenchyma
Case 6	Gentoo	Female	3	5.84	<i>Aspergillus</i> antibody 1:16	Defects in the pulmonary parenchyma
Case 7	King	Female	24	11.23	A/G: 0.75	Multiple nodules in the air sac
Case 8	King	Male	11	14.83	<i>Aspergillus</i> antigen 0.5	No abnormality
Case 9	King	Female	2	10.40	<i>Aspergillus</i> antigen 1.0 A/G: 0.17	Infiltrative lesion in the pulmonary parenchyma, Multiple nodules in the air sac
Case 10	King	Female	7	12.00	AST: 569 IU/l CK: 967 IU/l	No abnormality
Case 11	King	Male	4	14.00	<i>Aspergillus</i> antigen 0.6	No abnormality
Case 12	King	Male	26	12.02	A/G: 0.69	No abnormality

respectively. Among the gentoo penguins, six were selected based on a blood test. The total number of CT examinations was 15 for 6 penguins. There was no correlation between the air sac volume and body weight (Fig. 1). Among the king penguins, six were selected based on a blood test. The total number of CT examinations was 10 for 6 penguins. There was no correlation between the air sac volume and body weight (Fig. 2).

In case 1, infiltration was detected in the pulmonary parenchyma on CT and treated with the oral administration of itraconazole for 261 days. The lesion was not observed in the third CT examination; itraconazole was therefore stopped. The mean air sac volume increased from 273.9 cm³ before healing to 449.0 cm³ after healing (Fig. 3). This case has remained alive for 2 years since the final CT examination. In case 2, infiltration was detected in the pulmonary parenchyma on CT and treated with the oral administration of itraconazole for 205 days. The lesion was not observed in the third CT examination; itraconazole was thus stopped. The mean air sac volume increased from 329.0 cm³ before healing to 424.6 cm³ after healing (Fig. 4). This case has remained alive for 8 months since the final CT examination. In case 7, multiple nodules were detected in the air sac on CT and treated with the oral administration of itraconazole. This penguin died 53 days after the third CT examination. Despite treatment, the air sac volume decreased with time from 1,556.9 to 920.6 cm³ (Fig. 5). Upon pathological examination, multiple scattered nodules were observed in the air sac, but *Aspergillus* or other pathogenic fungi were not detected using fungal culture. This case

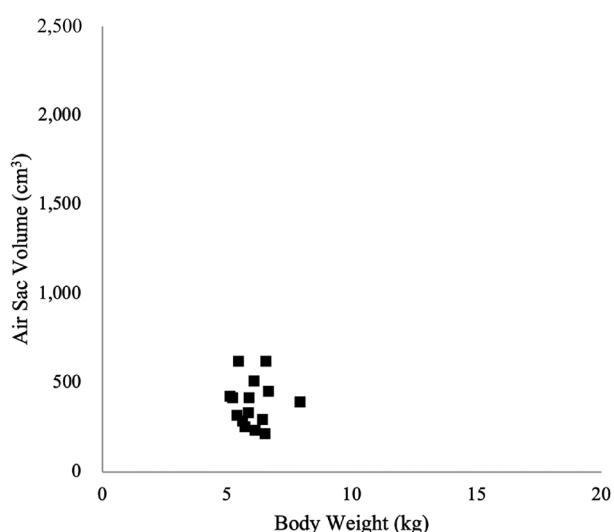


Fig. 1. Scatter plots of the air sac volumes on CT and body weight of Gentoo penguins (15 CT examinations for 6 penguins). There was no correlation between the air sac volume and body weight.

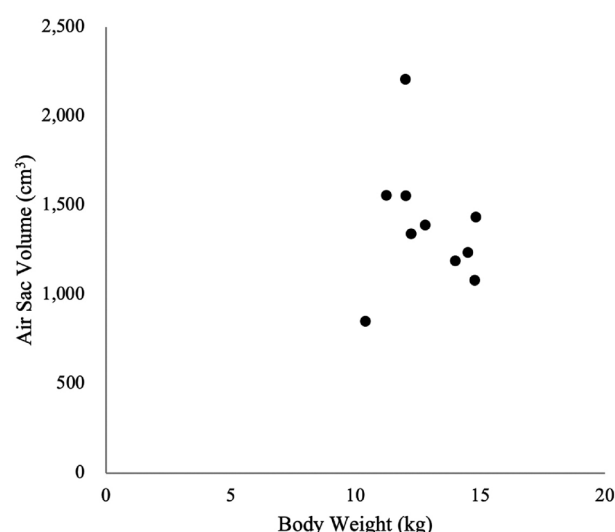


Fig. 2. Scatter plots of the air sac volumes on CT and body weight of King penguins (10 CT examinations for 6 penguins). There was no correlation between the air sac volume and body weight. The dots of the volume of postmortem CT were not included in this graph.

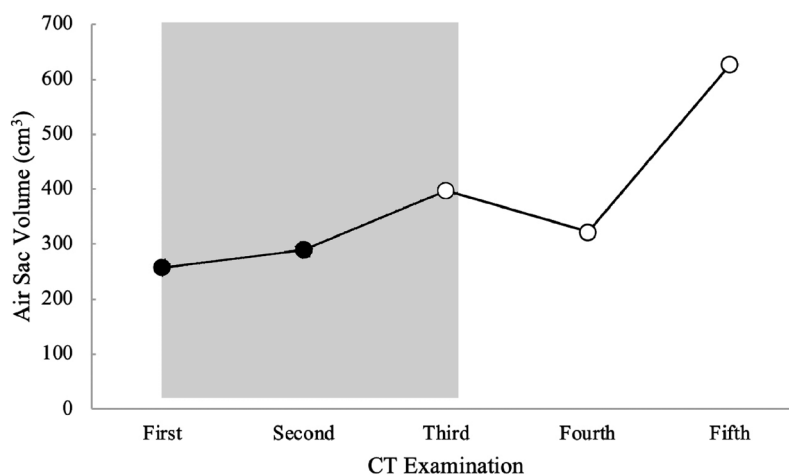


Fig. 3. Changes in the air sac volumes in case 1. Area-colored background is during the administration of itraconazole. The abnormal CT findings showed in closed circle; no abnormality showed in open circle.

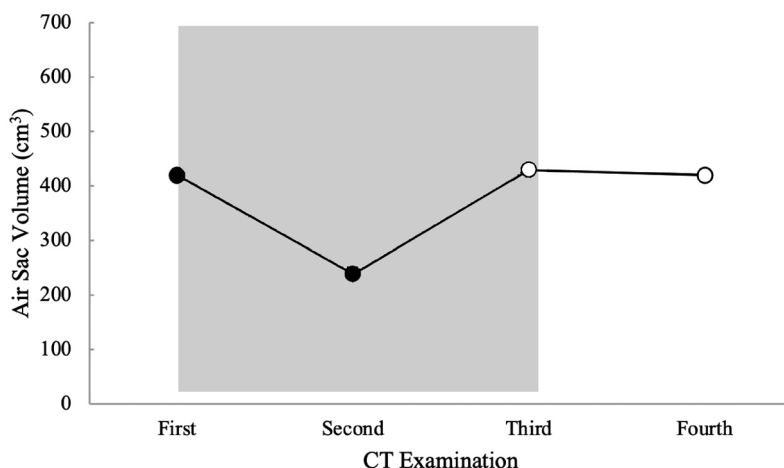


Fig. 4. Changes in the air sac volumes in case 2. Area-colored background is during the administration of itraconazole. Abnormal CT findings showed in closed circle; no abnormality showed in open circle.

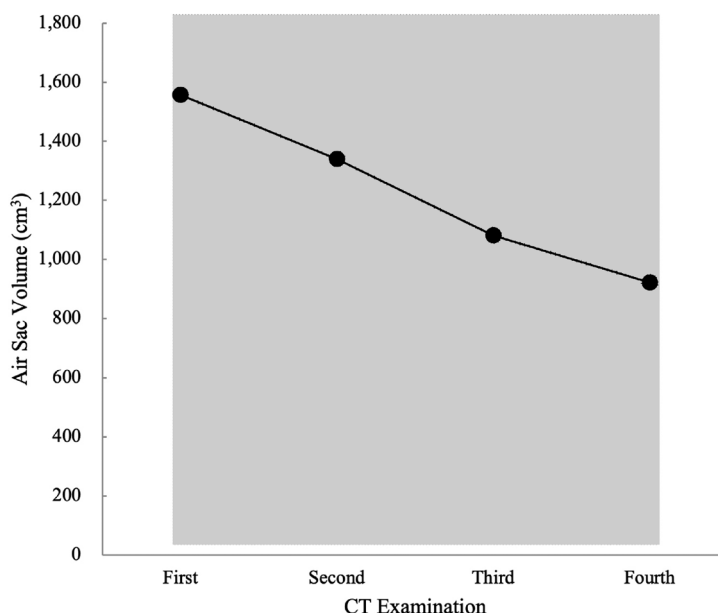


Fig. 5. Changes in the air sac volumes in case 7. Area-colored background is during the administration of itraconazole. Abnormal CT findings showed in closed circle. Fourth CT was performed after death.

was diagnosed with airsacculitis. In case 9, infiltrative lesion in the pulmonary parenchyma and multiple nodules in the air sac were detected on CT 19 days before death, and its air sac volume was 851.3 cm³. Case 9 was definitively diagnosed with aspergillosis via polymerase chain reaction.

DISCUSSION

There was no significant correlation between the air sac volume and body weight in either the gentoo or king penguins. In this study, the body weight of penguins was measured once a month for health monitoring. It is known that captive penguins often gain weight and lose around 25% of their body weight during the molt, breeding, and egg-laying periods [1]. Therefore, it is difficult to use the ratio of air sac volume to body weight as a means for evaluating respiratory conditions.

In gentoo penguin cases 1 and 2, in which infiltration in the pulmonary parenchyma on CT was detected and then healed, the mean air sac volume during the period of abnormal CT findings was lower than the volume during the period of non-abnormal CT findings. It is reported that breathing capacity decreases in human pneumonia patients [5, 14]. In avians, the air sacs have an important role in respiration. Inspiration and expiration of air during breathing facilitate the air passing through the lungs [7]. It is

considered that mean air sac volume indicates respiratory conditions. In addition, the air sac volume increased after healing. This demonstrated that the volume of air sacs is reversible depending on respiratory conditions. Therefore, the monitoring of air sac volumes could be a useful clue for evaluating an appropriate treatment method.

We suspected that the dead king penguin case 7 had aspergillosis as per the blood test results of low A/G and CT findings of multiple nodules in the air sac. However, it was not pathologically diagnosed with aspergillosis. Despite itraconazole administration, the air sac volumes decreased continuously. This suggested that the case did not involve aspergillosis. The air sac volume could be useful for evaluating the effect of diagnostic treatment on penguins with or without aspergillosis. The pathological findings revealed multiple scattered nodules in the air sac. It is considered that airsacculitis leads to a decrease in air sac volume, so the decreasing air sac volume is not specific for aspergillosis. The air sac volume might be a useful clue for not only aspergillosis but also other respiratory diseases in penguins.

In king penguin case 9, which died 19 days after CT, aspergillosis was confirmed in pathological examination. Its air sac volume was 851.3 cm³, and this volume was less than the third CT air sac volume (1,080.9 cm³) and postmortem CT air sac volume (920.6 cm³) of case 7. Hence, there might be a threshold of air sac volume for survival or death, and low air sac volume may predict poor prognosis.

The limitation of this study is the low number of cases that underwent CT. Ideally, the more samples we have, the more accurate our evaluation will be. Fortunately, the occurrence of aspergillosis in the aquarium was eradicated after implementing this study. Early diagnosis using CT facilitates the initiation of treatment early. As a result of early treatment, the number of aspergillosis cases will not increase.

In conclusion, changes of the air sac volume in the same individual evaluated using CT could be a useful and objective clue for the prognosis and diagnostic evaluation of respiratory condition of penguins.

CONFLICT OF INTEREST. The authors have no conflicts of interest.

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