Surveillance of virus infection among captive African pygmy hedgehogs in Japan

日本における飼育下ヨツユビハリネズミに感染するウイルスの調査

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September, 2025

1. GENERAL INTRODUCTION

1.1. Exotic animals and hedgehogs as pets in Japan

"Exotic animals" are defined as companion animals except for dogs and cats. The definition of the term "exotic animal" includes board range of animals, such as various species of birds, reptiles, amphibians, and mammals. In particular, African pygmy hedgehogs (*Atelerix albiventris*), also known as four-toed hedgehogs, have become popular exotic pets in America, Europe and Asian countries, including Japan (Díaz-Delgado et al., 2017; Muñoz-Gutiérrez et al., 2018; Okada et al., 2018). African pygmy hedgehog is among the small mammals belonging to genus *Atelerix* and family Erinaceidae, and wild individuals are native to the steppe and savannah regions of Central and Eastern Africa (Doss et al.,2020; Reeve, 1994). This species is well-suited to the housing conditions in Japan due to its manageable body size (average body weight: 300–600 g) and its non-barking nature (Doss et al., 2020). Hedgehog species other than the African pygmy hedgehog are prohibited as pets in Japan, as they are designated invasive alien species.

1.2. Tumors in African pygmy hedgehogs

Recently the description of diseases in African pygmy hedgehogs have increased. (Díaz-Delgado et al., 2017; Gardhouse et al., 2015; Okada et al., 2018). The author previously reviewed medical records of 227 African pygmy hedgehogs presented to a private veterinary hospital in Fukuoka prefecture from 2008 to 2015 (Koizumi et al., 2016), revealing that the most common disease categories were dermatological (45.9%), reproductive (9.1%), and gastrointestinal diseases (8.9%). The most common diseases noted in a retrospective survey of 106 African pygmy hedgehogs kept in America were dermatological (66.04%), gastrointestinal (33.02%), and skeletal diseases (15.09%) (Gardhouse et al., 2015). In addition, this species is known to commonly develop neoplastic diseases, and necropsy reports have indicated a tumor incidence of 29–51.5%

with 85% of the neoplastic diseases being malignant (Heatley et al., 2005; Raymond et al., 2001). A recent histopathological study conducted by two commercial laboratories in Japan reported neoplastic lesions in 60% of samples from captive African pygmy hedgehogs, with 74.6% of these tumors classified as malignant (Okada et al., 2018). Various neoplasms have been reported; in particular, fibrosarcoma, mammary adenocarcinoma, and squamous carcinoma have been more commonly reported (Okada et al., 2018; Raymond et al., 2001). Neoplastic disease is the primary cause of death in African pygmy hedgehogs, accounting for 35.9% of all causes of death in captive hedgehogs (Pei-Chi et al., 2015). The author reported two case series describing clinicopathologic and histopathologic findings, treatments and clinical outcomes of histiocytic sarcoma and leukemia in this species (Koizumi et al., 2019a, 2020a). In addition, the author also reported another case of an unusual form of histiocytic sarcoma localizing to lumbar vertebrae with documentation of the clinical and pathologic features (Koizumi et al., 2020b). These reports highlighted the aggressiveness and poor prognosis of these malignant tumors. More precise knowledge therefore regarding the biological behaviors of neoplastic diseases and advance of treatment in this species is needed. The author reported preputial cystostomy as a novel surgical technique for unresolvable urethral obstruction in a male African pygmy hedgehog, proving effective as a salvage for such emergency condition (Koizumi et al., 2019b).

1.3. Non-virus pathogen diseases in African pygmy hedgehogs and their zoonotic risks

Previous reports have revealed some pathogens causing diseases in African pygmy hedgehogs. Dermatologic disorders caused by *Caparinia tripilis* or fungal organisms (*Trichophyton mentagrophytes*, *Trichophyton erinacei*, and *Microsporum*

species) are very common problems in African pygmy hedgehogs (Doss et al.,2020; Gardhouse et al., 2015). Several strains of *Salmonella*, particularly *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella enterica* serovar Tilene can develop enteritis (Chomel et al., 2005). Intestinal cryptosporidiosis resulting in fatal outcome was reported in a juvenile African pygmy hedgehog (Graczyk et al., 1998). Recently, *Cryptosporidium* spp. was detected from two African pygmy hedgehogs in Japan (Takaki et al., 2020).

In addition to infections in African pygmy hedgehogs themselves, they have also been reported as reservoirs for the transmission of these zoonotic diseases to humans. There is potential risk to keep this species as pets because African pygmy hedgehogs have various zoonotic pathogens and are concerned to act as carriers or hosts (Ruszkowski et al., 2021). In particular, human dermatophytosis and salmonellosis transmitted from pet African pygmy hedgehogs have been frequently reported (Chomel et al., 2005; Hoff et al., 2024; Ruszkowski et al., 2021; Weishaupt et al., 2014). Similarly in Japan, recent case reports also described these zoonoses from pet African pygmy hedgehogs (Ichimi et al., 2018; Kim et al., 2018; Mochizuki et al., 2005)

1.4. Association between viruses and diseases in African pygmy hedgehogs

Previous reports have suggested that viral infections may be a contributing factor for diseases developed in African pygmy hedgehogs. For example, African pygmy hedgehog adenovirus 1 (AhAdV-1) and skunk adenovirus 1 were detected and isolated from the throat/nasal swab and/or tissue samples of African pygmy hedgehogs that showed respiratory clinical signs and died in Japan and USA (Madarame et al., 2019; Needle et al., 2019; Ochiai et al., 2019). Human herpes simplex virus was isolated from an African pygmy hedgehog that showed neurological clinical signs with posterior ataxia and fast progressing paresis. Postmortem examinations revealed randomly distributed

foci of necrosis in the liver, and human herpes simplex virus antigens were detected in neurons and some glial cells using immunolabeling (Allison et al., 2002). Pneumonia virus of mice (PVM) RNA genome and antigen were detected in the brain of an African pygmy hedgehog that showed encephalitis in Japan using next-generation sequencing and immunohistochemical analysis (Madarame et al., 2014), which could be associated to wobbly hedgehog syndrome. The author reported the clinical and pathological characteristics of cutaneous papilloma and multicentric squamous cell carcinoma in two African pygmy hedgehogs with a possible association with papillomavirus (PV) due to evidence of viral antigens within tissue samples (Okumura et al., 2021). Unfortunately, PV DNA was not detected in that study. Recently, Shimazaki et al. (2023) identified and sequenced the full genome of PV in skin lesions of African pygmy hedgehogs, which was named Atelerix albiventris papillomavirus 1 (AalbPV1). The zoonotic viruses or the human case of virus infection transmitted from African pygmy hedgehogs have not been reported.

While various common diseases in African pygmy hedgehogs have been reported, the pathogenesis of those diseases are not well understood. However, as described above, recent reports suggest the association between viruses and diseases in African pygmy hedgehogs. Surveys of infectious agents in African pygmy hedgehogs kept in Japan therefore are expected to provide useful information for the diagnosis, treatment and prevention of emerging infectious diseases in clinical setting. At the same time, it can be also beneficial for human medicine because African pygmy hedgehogs have the potential risks to become reservoirs for zoonotic viruses. Taken together, the author investigated and assessed the correlations between virus infection and various diseases among pet African pygmy hedgehogs with a focus on herpesvirus, adenovirus, and coronavirus in CHAPTER 1. A novel betaherpesvirus (Atelerix albiventris

herpesvirus 1; AAHeV) was identified and found to be significantly associated with neurological disorders, while showing a lower incidence of neoplastic diseases, suggesting a potential role of AAHeV in both neurological and tumor-related pathogenesis. In addition, AhAdV-1-positive hedgehogs were detected with no respiratory signs, indicating that subclinical infection of AhAdV-1 may occur among pet African pygmy hedgehogs kept in Japan (Koizumi et al., 2022).Since AhAdV-1 has already been isolated or detected in various animal species beyond the order with clinical signs (Balic et al., 2020; Bourque et al., 2022; Doszpoly et al., 2020; Gál et al., 2013; Kozac et al., 2015; Needle et al., 2020; Orbay-Cerrato et al., 2024), large-scale epidemiological survey in captive animals including broad species in Japan should be conducted to assess the potential zoonotic risks of this novel unique adenovirus. In particular, pet exotic animals are expected as ideal candidates to evaluate the host range, susceptibility, pathogenicity and current endemicity of AhAdV-1. In CHAPTER 2, the author investigated seroprevalence and evaluated the pathogenicity of AhAdV-1 with clinical samples and medical histories of exotic pets in Japan. It can be concluded that the authors' study has helped to elucidate part of the actual status of viral infections in hedgehogs.

2. CHAPTER 1

Comprehensive surveillance of virus infection among pet African pygmy hedgehogs in Japan

2.1. ABSTRACT

African pygmy hedgehogs (*Atelerix albiventris*) are popular exotic pets in Japan, and their breeding numbers have recently increased. Although various diseases have been reported in hedgehogs, including skin, respiratory, neurological, and neoplastic diseases, most of the causes remain unidentified. In this study, we investigated herpesvirus, adenovirus, and coronavirus infections among 150 African pygmy hedgehogs in Japan and evaluated the correlations between virus infection and diseases. A novel herpesvirus named Atelerix albiventris herpesvirus 1 (AAHeV), and African pygmy hedgehog adenovirus 1 (AhAdV-1) were detected in 14 and 3 oral swab samples, respectively. AAHeV infection may be related to neurological clinical signs. Interestingly, no hedgehog with a neoplastic disorder tested positive for AAHeV. Further research is required to determine the pathogenicity and prevalence of the detected viruses.

2.2. INTRODUCTION

A hedgehog is a small, spiny insectivore that has become popular in recent years as an exotic pet in Japan. Among the 16 hedgehog species, African pygmy hedgehog (*Atelerix albiventris*) is bred as a companion animal. With the increasing number of pet hedgehogs in Japan, various diseases have been reported in hedgehogs, including skin, respiratory, neurological, and neoplastic diseases (Okada et al., 2018). Although the causes of these diseases are not well understood, previous reports have suggested that viral infections may be a contributing factor. As discussed in GENERAL INTRODUCTION, some reports have noted virus infection and possible pathogenesis in African pygmy hedgehogs. African pygmy hedgehog adenovirus 1 (AhAdV-1) was isolated from African pygmy hedgehogs with respiratory clinical signs (Madarame et al., 2019; Needle et al., 2019; Ochiai et al., 2019), while human herpes simplex virus was

isolated from an African pygmy hedgehog with neurological signs (Allison et al., 2002). The PVM RNA genome and antigen were detected in the brain of an African pygmy hedgehog developing encephalitis in Japan (Madarame et al., 2014). Recent reports revealed the association with a novel papillomavirus (PV) and skin lesions in African pygmy hedgehogs, sequenced the full genome of PV and named Atelerix albiventris papillomavirus 1 (AalbPV1) (Okumura et al., 2021; Shimazaki et al. 2023).

In addition, virus infection in hedgehogs other than African pygmy hedgehogs also has reported. Herpesvirus infection has been reported in European hedgehogs (*Erinaceus europaeus*). Examinations of European hedgehogs in the United Kingdom revealed histological changes in the liver and amphophilic inclusion bodies with virus particles in hepatocytes (Stack et al., 1990). Widén et al. (1996) also reported that the cultivation of the liver homogenate from a young European hedgehog led to alpha herpesvirus-like cytopathic effects (CPE) with intranuclear inclusion bodies. Coronavirus was detected in the fecal samples of free-living European hedgehogs in several European countries, including Germany (Corman et al., 2014), France (Monchatre-Leroy et al., 2017), and the United Kingdom (Delogu et al., 2020; Saldanha et al., 2019), as well as in Amur hedgehogs from China (Lau et al., 2019). In these reports, no infectious viruses were isolated, and the animals did not show any clinical signs associated with coronavirus infection.

Taken together, these reports indicate that hedgehogs have the potential to become reservoirs for these pathogens, including zoonotic viruses. Therefore, surveys of infectious agents in African pygmy hedgehogs kept in Japan are expected to provide useful information for the prevention of emerging infectious diseases not only in hedgehogs, but also in humans. In the present study, we investigated the viral infections

among African pygmy hedgehogs in Japan with a focus on herpesvirus, adenovirus and coronavirus, and assessed the correlations between virus infection and various diseases.

2.3. MATERIALS AND METHODS

2.3.1. Sample collection

Oral swabs were collected from 150 domestic hedgehogs at a veterinary hospital between March 2019 and June 2020. Most of them presented some clinical signs. Swab samples were mixed in a vortex mixer in sterilized phosphate-buffered saline and stored at -80°C until examination.

2.3.2. Detection of herpesvirus, adenovirus and coronavirus from hedgehog swab samples

DNA extraction from swab samples was conducted using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Nested polymerase chain reaction (PCR) assays were performed using the TaKaRa Ex Taq kit (TaKaRa, Otsu, Japan) for the detection of herpesvirus and adenovirus. Pan-herpesvirus primers were used for the detection of the herpesvirus DNA polymerase gene (215 to 315 bp in length), as described previously (VanDevanter et al., 1996) (Table 1-1). The first and second rounds of the reaction consisted of an initial denaturation at 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 46°C for 30 s and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. Pan-adenovirus primers were used for the detection of the adenovirus DNA polymerase gene (318 to 324 bp in length), as described previously (Wellehan et al., 2004) (Table 1-1). The first and second rounds of the reaction consisted of an initial denaturation at 94°C for 2 min, followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 46°C for 1 min and

extension at 72°C for 1 min, with a final extension at 72°C for 7 min. RNA was extracted from swab samples using a Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Reverse transcription (RT)-PCR was performed using a QIAGEN OneStep RT-PCR Kit (QIAGEN, Hilden, Germany) and pan-coronavirus primers targeting the coronavirus RNA-dependent RNA polymerase (440 bp), as described previously (Poon et al., 2005) (Table 1-1). The RT-PCR conditions were as follows: an initial step of 30 min at 50°C for reverse transcription; 15 min at 95°C for denaturation; 40 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min and extension at 72°C for 1 min; and a final extension step at 72°C for 10 min. All amplicons were visualized by gel electrophoresis on 2% agarose gels.

2.3.3. Phylogenetic analysis

Sequence analysis was performed using the MEGA 10.0 software (Kumar et al., 2018). The primer-trimmed sequences detected in this study were aligned by ClustalW and compared to previously reported herpesvirus or adenovirus sequences in GenBank using the Basic Local Alignment Search Tool (BLAST). Neighbor-joining trees were constructed using the MEGA 10.0 software with a bootstrap analysis of 1000 replicates and *p*-distance models.

2.3.4. Specific PCR for the detected viruses

Specific nested PCR for the detected viruses was performed with the TaKaRa Ex Taq kit using specific primer pairs constructed based on the sequence data (Table 1-1). The first and second PCR reactions consisted of an initial denaturation at 94°C for 2 min, followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 1 min in the first round and at 55°C for 1 min in the second round, extension at 72°C for 1 min, and a

final extension at 72°C for 7 min. The second-round amplicons were visualized by gel electrophoresis on 2% agarose gels.

2.3.5. Virus isolation

MDCK cells (JCRB9020; Japanese Collection of Research Bioresources Cell Bank, Osaka, Japan) and BHK-21 cells (JCRB9029; Japanese Collection of Research Bioresources Cell Bank, Osaka, Japan) were used for virus isolation. The cells were cultured in Dulbecco's Modified Eagle Medium (Thermo Fisher Scientific,Waltham, MA, USA) containing 10% heat-inactivated fetal calf serum, 100U/mL penicillin, and 100 g/mL streptomycin (Thermo Fisher Scientific, Waltham, MA, USA). The cells were maintained at 37°C in 5% CO_2. Virus isolation was performed by inoculating filtrated swab suspensions onto monolayers of MDCK and BHK-21 cells. The cells were incubated at 37°C in 5% CO_2 and observed daily for CPE. The cells were blind-passaged five times until CPE were seen.

2.3.6. Statistical analysis

Molecular epidemiological data were analyzed according to sex (male and female), age class (juvenile and adult), and clinical signs (respiratory disorder, digestive disorder, oral disease, neurological disease, skin disease, and neoplastic disorder) using the chi-square test. P values < 0.05 were considered to be statistically significant.

2.4. RESULTS

2.4.1 Detection of herpesvirus and adenovirus from oral swab samples of hedgehogs

Oral swab samples were collected from 150 hedgehogs with various health conditions that were taken to a veterinary clinic in Fukuoka, Japan, between March 2019

and June 2020. First, 50 of the 150 oral swab samples were analyzed for herpesvirus, adenovirus, and coronavirus using universal primer pairs, in accordance with previous studies (VanDevanter et al., 1996; Wellehan et al., 2004; Poon et al., 2005). Among the 50 analyzed samples, 2 were positive for herpesvirus, 2 were positive for adenovirus, and 0 were positive for coronavirus (Table 1-2). Based on sequence analysis, the virus in both of the herpesvirus-positive samples was identified to be a novel betaherpesvirus named Atelerix albiventris herpesvirus 1 (AAHeV, GenBank accession number LC695010). Among viruses with a query coverage of over 80%, AAHeV showed the highest amino acid similarity (with 46% identity and 94% query coverage) to Sorex araneus betaherpesvirus 4 (GenBank accession number AEA39191), which was isolated from a common shrew (Figure 1-1). In addition, in the two adenovirus-positive samples (GenBank accession number LC695010), the virus was 100% identical to African pygmy hedgehog adenovirus 1 (AhAdV-1, GenBank accession number MK937781, Figure 1-2), which was isolated from hedgehogs that showed respiratory clinical signs in Japan (Madarame et al., 2019).

2.4.2 Molecular epidemiology of the detected viruses

For further epidemiological study, we analyzed all 150 swab samples taken from the hedgehogs of the same hospital, including the previously examined 50 samples, using specific primers for AAHeV and AhAdV-1. Of the 150 samples, 14 (9.3%) and 3 (2.0%) were positive for AAHeV and AhAdV-1, respectively (Table 1-2). The sequences of all the partial genomes detected by molecular epidemiology were 100% identical to AAHeV (GenBank accession number LC706228-706240) or AhAdV-1 (GenBank accession number LC706226-706227) detected in the first screening. The data above were further analyzed according to sex (male and female), age class (juvenile and adult), and clinical

signs (respiratory disorder, digestive disorder, oral disease, neurological disease, skin disease, and neoplastic disorder). The prevalence of AAHeV was significantly higher in hedgehogs with neurological clinical signs ($p = 0.016$; Table 1-3). Interestingly, there were no AAHeV-positive hedgehogs among the 49 hedgehogs with a neoplastic disorder ($p = 0.006$; Table 1-3). The prevalence of AAHeV was not significantly related to sex, age class, respiratory disorders, digestive disorders, oral diseases, or skin diseases (Table 1-3). However, the data for AhAdV-1 may not be reliable due to the small number of positive samples. While there was no significant difference according to sex, the AhAdV-1-positive rate was significantly higher in juvenile hedgehogs than in adult hedgehogs ($p < 0.0004$; Table 1-3). The AhAdV-1-positive rate was significantly higher in hedgehogs with a respiratory disorder ($p < 0.045$; Table 1-3), while the other clinical signs showed no significant correlation. The prevalence of AhAdV-1 was not significantly related to sex, neurological diseases, neoplastic disorders, digestive disorders, oral diseases, or skin diseases (Table 1-3). Unfortunately, although we attempted to isolate viruses from the AhAdV-1-positive samples using BHK-21 and MDCK cells, no CPE were observed, and no viral DNA was detected in the inoculated cells (data not shown).

2.5. DISCUSSION

The results of the present study indicated that a novel herpesvirus, AAHeV, and AhAdV-1 are infecting African pygmy hedgehogs bred in Japan with a prevalence of 9.3% and 2.0%, respectively.

Although alpha and gamma herpesvirus infections have been reported in hedgehogs previously (Hydeskov et al., 2018), this is the first report of a betaherpesvirus infecting hedgehogs. Since we only examined a partial sequence of the DNA polymerase gene of AAHeV, additional sequences and/or virus isolation are required for further

characterization of the detected virus, including its pathogenicity, transmission cycle, and growth kinetics. Although there has been no report on the isolation of hedgehog herpesvirus, the isolation of human herpesvirus from hedgehog samples has been described (Allison et al., 2002; Widén et al., 1996). The establishment of optimal cultured cell lines derived from hedgehogs would provide useful tools for hedgehog herpesvirus isolation.

Our study results indicate a significant relationship between AAHeV infection and neurological diseases. A major neurological disease in hedgehogs is wobbly hedgehog syndrome, but its cause has not yet been clarified. Various factors have been suspected to cause the disease, including genetic abnormality (Graesser et al., 2006), malnutrition, stress, and viruses (Madarame et al., 2014). AAHeV infection may be an important factor in the development of neurological diseases in hedgehogs.

It is also notable that none of the hedgehogs with a neoplastic disease were positive for AAHeV infection. Although there has been a report on the detection of a retrovirus in hedgehog sarcomas (Peauroi et al., 1994), the cause of hedgehog tumors remains unclear. Interestingly, herpes simplex virus type 1, belonging to Alphaherpesvirinae, is a well-known oncolytic virus, and it has been studied for potential application as a therapeutic approach for cancer (Varghese et al., 2002). Oncolytic viruses replicate in tumor cells, selectively kill the tumor cells (Liu et al., 2003), and induce anti-tumor immune responses (Lichty et al., 2014). If AAHeV infection has potential effects on the host's response to oncogenicity, further research is necessary to elucidate the developmental mechanisms of tumors in hedgehogs.

In the present study, one of the three AhAdV-1-positive hedgehogs had a respiratory disorder. Although the statistical analysis results suggested a correlation between respiratory signs and AhAdV-1 infection, the low number of positive-testing

hedgehogs and the incidental respiratory disease should be carefully taken into consideration. Recent surveillance data have suggested that AhAdV-1 (Ochiai et al., 2019) and skunk adenovirus 1 (Needle et al., 2019), which is closely related to AhAdV-1, may be the dominant respiratory pathogens among hedgehogs. In particular, skunk adenovirus 1 was reported to have caused fatal bronchopneumonia in an African pygmy hedgehog (Needle et al., 2019), suggesting that AhAdV-1 may also cause fatal respiratory diseases in hedgehogs. However, since AhAdV-1 was detected in hedgehogs with no respiratory diseases, subclinical infection of AhAdV-1 may occur among hedgehogs. Further analyses with a larger number of cases are required to gain more convincing data on the relationship between respiratory diseases and AhAdV-1 infection in hedgehogs.

In conclusion, the results of the present study indicated that a novel betaherpesvirus and AhAdV-1 are infecting African pygmy hedgehogs bred in Japan. The results suggest that AAHeV infection may play a key role in the mechanism of neurological and neoplastic diseases in hedgehogs, but further studies are needed to better assess the relationship between AhAdV-1 and diseases in hedgehogs. Additional investigations are also required to determine the pathogenicity and prevalence of these viruses in hedgehogs.

2.6. LEGENDS FOR FIGURES

Figure 1-1. This phylogenetic tree was constructed based on 49 amino acids of the DNA polymerase gene. Sequences of the novel herpesvirus detected in this study are shown in bold. GenBank accession numbers of the listed viruses are shown in parentheses.

Figure 1-2. This phylogenetic tree was constructed based on 91 amino acids of the DNA polymerase gene. Sequences of the adenovirus detected in this study are shown in bold. GenBank accession numbers of the listed viruses are shown in parentheses.

2.7. FIGURES AND TABLES

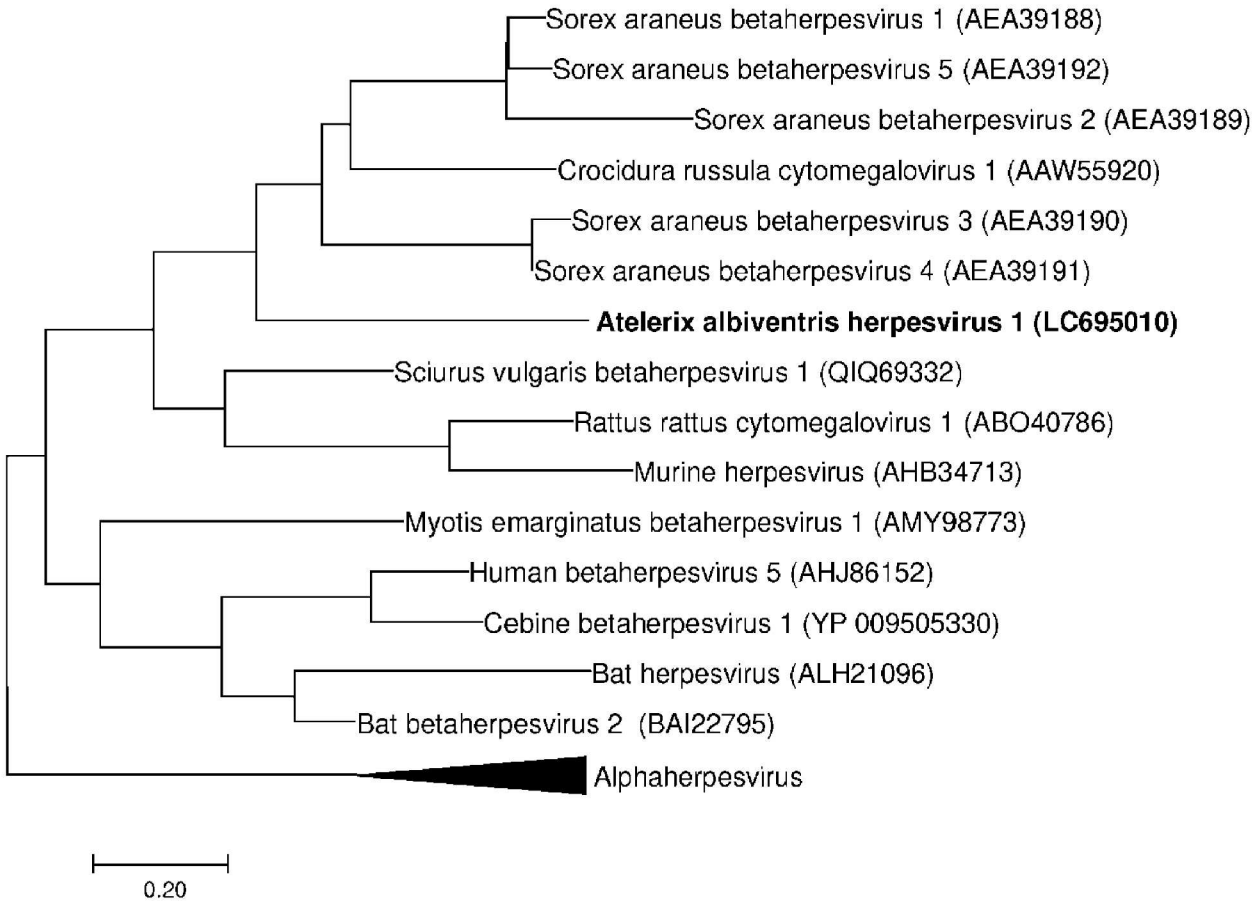


Figure 1-1. Phylogenetic tree of the detected herpesviruses based on the partial amino acid sequences of DNA polymerase.

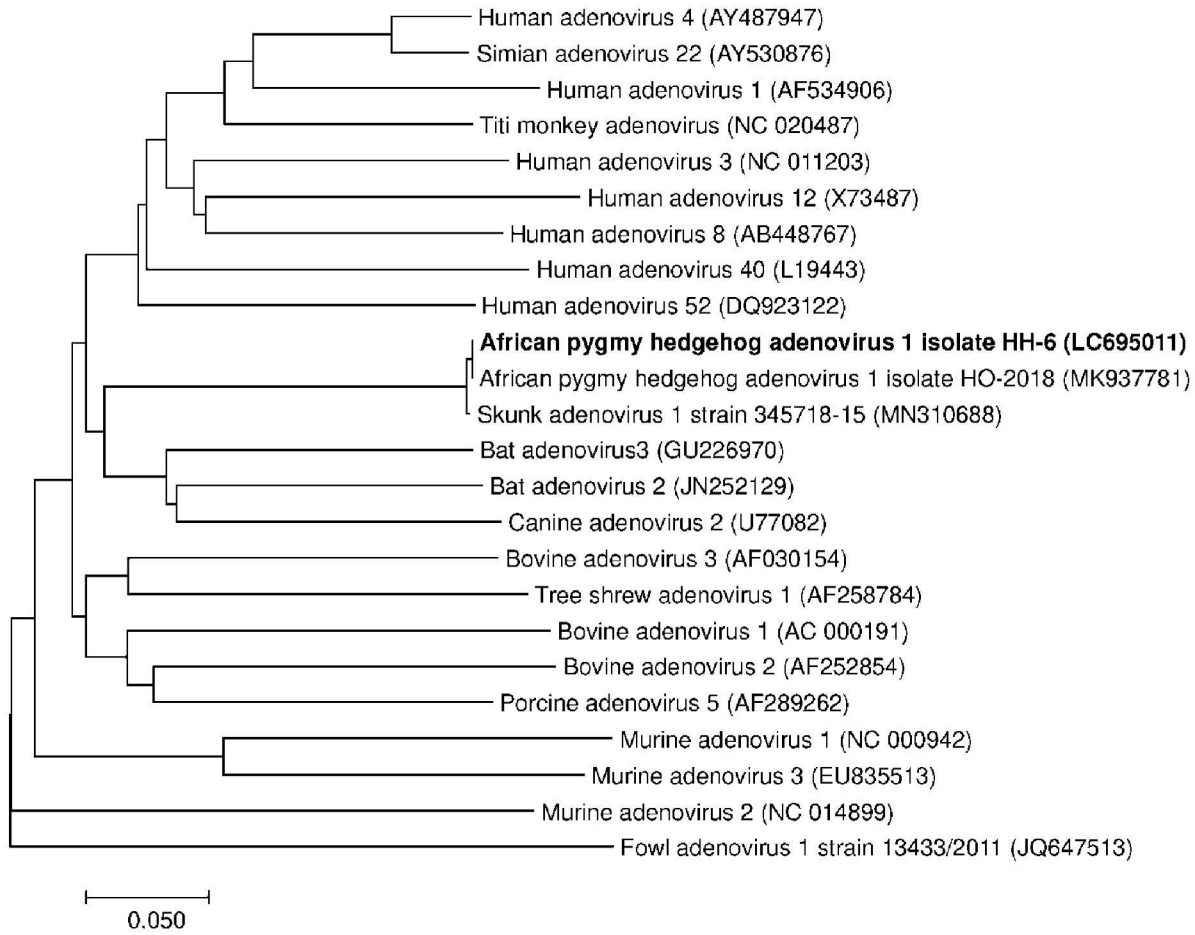


Figure 1-2. Phylogenetic tree of the detected adenoviruses based on the partial amino acid sequences of DNA polymerase.

Table 1-1. Primers used in this study.

Target Virus	Primer Name *	Primer Sequence (5′->3′)	Product Size (bp)	Reference
Herpesvirus (universal)	DFA (1st) (Forward)	GAYTTYGCNAGYYTNTAYCC	215–315	(VanDevanter et al., 1996)
	ILK (1st) (Reverse)	TCCTGGACAAGCAGCARNYSGCNMTNAA		
	KG1 (1st) (Reverse)	GTCTTGCTCACCAGNTCNACNCCYTT		
	IYG (2nd) (Forward)	CACAGAGTCCGTRTCNCCRTADAT		
	TGV (2nd) (Reverse)	TGTAACTCGGTGTAYGGNTTYACNGGNGT	168	
African pygmy hedgehog herpesvirus	HHHeV_3F (1st)	GTTACCTTGTTTGCCTGTGGC		This study
	HHHeV_9F (2nd)	GCTTCGGTGACGAAAATCGG		
	HHHeV_9R (1st, 2nd)	TTCATCGTTTGTCTCTGTGGT		
Adenovirus (universal)	polFouter (1st)	TNMGNGGNGGNMGNTGYTAYCC	318–324	(Wellehan et al., 2004)
	polRouter (1st)	GTDGCRAANSHNCCRTABARNGMRTT		
	polFinner (2nd)	GTNTWYGAYATHTGYGGHATGTAYGC		
	polRinner (2nd)	CCANCCBCDRTTRTGNARNGTRA		
African pygmy hedgehog adenovirus	AhAdV-pol-1523F (1st)	CTGGCATACATCCCGCARAT	287	This study
	AhAdV-pol-1976R (1st)	CAGATGGGTTTCCCGCTCTT		
	AhAdV-pol-1601F (2nd)	CCTCGGATACTGGACCTGAC		
	AhAdV-pol-1887R (2nd)	TACGACATCATCCAGCACACC		
Coronavirus (universal)	IN-6	GGTTGGGACTATCCTAAGTGTGA	440	(Poon et al., 2005)
	IN-7	CCATCATCACATAGAATCATCAT		

* In parentheses indicate whether the primer was used in 1st or 2nd PCR.

Table 1-2. Prevalence of virus infection among hedgehogs in Japan.

	Universal Primer			Specific Primer	
	Herpesvirus	Adenovirus	Coronavirus	AAHeV	AhAdV-1
Number of tested samples	50	50	50	150	150
Number of positive samples	2	2	0	14	3
% of positive samples	4	4	0	9.3	2.0

Table 1-3. Comparison of virus infection and status of hedgehogs.

Characteristic	Status	No. of Tested Samples	No. of Positive Samples *		*p* Value	
			AAHeV	AhAdV	AAHeV	AhAdV
sex	Male	83	8 (10%)	0 (0%)	0.813	0.091
	Female	59	5 (8%)	2 (3%)		
Age class	Juvenile (<6 months)	13	1 (8%)	2 (15%)	0.796	0.0004
	Adult (≥6 months)	131	13 (9.9%)	1 (0.8%)		
Neurological disease	Yes	27	6 (22%)	0 (0%)	0.016	0.410
	No	122	8 (6.6%)	3 (2.5%)		
Neoplastic disease	Yes	49	0 (0%)	1 (2%)	0.006	0.987
	No	100	14 (14.0%)	2 (2.0%)		
Respiratory disease	Yes	9	1 (11%)	1 (11%)	0.856	0.045
	No	140	13 (9.3%)	2 (1.4%)		
Digestive disease	Yes	17	2 (12%)	1 (6%)	0.730	0.231
	No	131	12 (9.2%)	2 (1.5%)		
Oral disease	Yes	33	3 (9%)	0 (0%)	0.957	0.353
	No	117	11 (9.4%)	3 (2.6%)		
Skin disease	Yes	40	4 (10%)	1 (3%)	0.878	0.798
	No	109	10 (9.2%)	2 (1.8%)		

* In parentheses indicate the percentage of positive samples.

3. CHAPTER 2

Sero-survey of African pygmy hedgehog adenovirus 1 among captive exotic animals in Japan

3.1. ABSTRACT

African pigmy hedgehog Adenovirus 1 (AhAdV-1) was first isolated in 2020 from a colony of African pigmy hedgehogs which died of severe bronchopneumonia in Japan. AhAdV-1 is closely related to Skunk Adenovirus 1 (SkAdV-1), which was isolated from a wild skunk which died of acute hepatitis and pneumonia in Canada in 2015. Similar viruses have been isolated or detected in a wide variety of animals in many other countries.

In general, adenoviruses are highly species-specific, and the same adenovirus is rarely detected in different animal species. In this study, to determine the host range of AhAdV-1, serological surveillance among 17 species of exotic animals visited a veterinary hospital in Fukuoka Prefecture, Japan, were conducted.

As a result, neutralizing antibodies against AhAdV-1 were detected in a variety of animals with particularly high seroprevalence among meerkats (41%), ferrets (62%) and African pigmy hedgehogs (63%). Ferrets and African pygmy hedgehogs with the history of respiratory clinical signs showed significantly high seroprevalence compared to the animals without the clinical history.

Our result indicated the wide range of host of AhAdV-1, with some animals being high prevalence. Further investigation of the source and route of AhAdV-1 infection, and possibility as zoonotic infection should be examined.

3.2. INTRODUCTION

Adenoviruses have been isolated or detected from various vertebrates. The virions are 70-100 nm in diameter and are non-enveloped icosahedral viruses with a one-segmented linear dsDNA genome. The genome is 26-48 kbp in length and encodes about

40 different proteins. It is morphologically characterized by one or two fibers at the icosahedral vertex (Ison et al., 2016; Russell, 2009).

Adenoviruses are classified into five genera, Mast, Avi, At, Si, and Icht, depending on the biological classification of the host they infect. For mastadenovirus, they are classified into seven species, A to G, according to hemagglutinin properties, DNA homology, oncogenic potential in rodents, transformability, and clinical disease (Ison et al., 2016; Kulanayake et al., 2021). Most are non-pathogenic, and their onset is thought to be associated with an underlying disease that causes immune suppression (Lion, 2014; Crenshaw et al., 2019).

Although natural hosts of adenovirus are generally considered to be species-specific (Borkenhagen et al., 2019; Harrach et al., 2019), canine adenovirus type 1 (CAdV-1) has been exceptionally isolated or detected from a variety of carnivorous animals, including Canidae (Miller et al., 2009; Choi et al., 2014; Hechinger et al., 2017; Ndiana et al., 2022), Mustelidae (Park et al., 2007; Philippa et al., 2008; Hou et al., 2023), Ursidae (García et al., 2018), and Mephitidae (Karstad et al., 1975). Canine adenovirus type 2 (CAdV-2) is also known to have a similar tendency (Zhu et al., 2022). Also, there is a report suggesting that bat adenovirus (BtAdV) also potentially has a broad host range in experimental systems in cultured cells (Kobayashi et al., 2019).

African pigmy hedgehog adenovirus type 1 (AhAdV-1) was first reported in Japan in 2019 (Ochiai et al., 2019). The affected hedgehogs were kept in colonies and showed respiratory clinical signs such as nasal discharge, sniffing, sneezing, coughing, and difficulty in breathing, with some animals died due to severe disease. As a result of histopathological examination, mild to moderate multifocal acute rhinitis characterized by erosion, ulceration or desquamation of the nasal mucosa was observed in the upper respiratory tract. In addition, severe acute diffuse bronchointerstitial pneumonia and

pulmonary edema were observed in the lower respiratory tract and adenoviral amphiphilic nuclear inclusion bodies were found in the nasal epithelium. Virus was isolated using MDCK cells from nasal swabs of dead animals, and sequencing analysis confirmed that it was a novel adenovirus. The full genome sequencing revealed that the isolated virus was 99.88% homologous to skunk adenovirus type 1 (SkAdV-1), which was isolated from a dead wild skunk with acute necrotizing hepatitis and interstitial pneumonia in Canada in 2015 (Kozak et al., 2015 ; Madarame et al., 2019). Our laboratory has also previously succeeded in isolating the virus from meerkats with respiratory clinical signs (manuscript in preparation).

To date, in addition to hedgehogs, AhAdV-1 has already been isolated or detected in various animal species beyond the order, such as skunks, raccoons, meerkats, binturongs, and ferrets of the order Carnivora, common marmosets of the primates, and porcupines of the order Rodentia as listed and illustrated in Table 2-1 and Figure 2-1, respectively. Thus, AhAdV-1 is unique among adenoviruses viruses that there have been a series of reports suggesting outbreaks in animals of phylogenetically distant species.

However, these reports are mainly based on incidental isolation or detection of AhAdV-1 from animals with respiratory clinical signs, and large-scale, comprehensive epidemiological studies have not been conducted to date. Furthermore, the susceptibility and pathogenicity of AhAdV-1 in each animal species, including humans, the route of transmission of the virus and its current endemicity remains unknown. In this study, seroprevalence of AhAdV-1 was examined to assess the host range of AhAdV-1.

3.3. MATERIALS AND METHODS

3.3.1. Cells

Dog-derived MDCK cells (JCRB number; JCRB1957) were cultured in

Dulbecco's Modified Eagle Medium (DMEM; GIBCO, U.S.A) with 10% heat-inactivated fetal bovine serum (FBS; SERANA®, Germany), 100U/ml of penicillin and 100μg/ml of streptomycin (FUJIFILM Wako, Japan) at 37°C in 5% CO_2.

3.3.2. Virus

African pygmy hedgehog adenovirus-1 (AhAdV-1), which was isolated from lung cells of a domestic meerkat with necrotizing bronchopneumonia in 2021 (manuscript in preparation) was used in this study. Its hexon gene sequence was 100% identical to those of AhAdV-1 isolate HO-2018 (Ochiai et al., 2019, GenBank accession number; MK937781) isolated from hedgehogs showing respiratory clinical signs in Japan. Canine adenovirus 1 strain Utrecht (ATCC® number; VR-293™) was purchased from American Tissue culture collection (ATCC, USA). These viruses were propagated in MDCK cells in DMEM with 2% FBS at 37°C in 5% CO_2 until CPE was observed and supernatant were collected. The virus solutions were sorted at -80°C until use.

3.3.3. Serum samples

Serum samples from 17 species, a total of 446 of domestic animals were collected from a veterinary hospital in Fukuoka between January 2020 to January 2024. The author broadly categorized the respiratory symptoms into upper and lower respiratory tract signs. Upper respiratory signs were primarily diagnosed as rhinitis, characterized by evident nasal discharge and sneezing. Lower respiratory signs were defined by abnormal breathing patterns in the absence of cardiac disease, as ruled out by radiographic and ultrasonographic examinations. To avoid a small sample size in each subgroup, these conditions were collectively referred to as "respiratory symptoms" in the manuscript. All sera were stored at -20°C until use, and were inactivated by incubation at 56°C for 30

min before use.

3.3.4. Plaque assay for titration of virus

Virus titers were measured by plaque assay. Serially diluted viruses were inoculated onto MDCK cells in a 6-well plates (Sumitomo Bakelite, Japan). After incubation for 90 min at 37°C in 5% CO_2, the cells were washed twice with DMEM and overlaid with 0.8% agarose (SeaPlaque® agarose, Lonza, Switzerland) in DMEM containing 10% FBS. The plates were then incubated at 37°C in 5% CO_2 for 4 days. The cells were fixed with 5% buffered formaldehyde for 1 hour, and the agarose layers were removed. After staining with crystal violet, plaques were counted.

3.3.5. Virus-neutralization (VN) test

To determine the prevalence of VN antibody against AhAdV-1 among various mammals, 80% plaque reduction VN test was carried out using collected serum samples. For the first screening of AhAdV-1 positive sera, sera were diluted to 1:5 in DMEM containing 2% FBS. To determine VN titer, sera were serially two-fold diluted in DMEM containing 2% FBS. The diluted sera or medium alone were mixed with equal volumes of virus solution containing 80 plaque-forming units (PFU) of AhAdV-1 and then incubated at 37°C for 90 min. After incubation, the mixtures were added to 12-well plates or 24-well plates that were sub-confluent with MDCK cells and the plates were incubated at 37°C for 90 min, washed twice with DMEM and overlaid with 0.8% agarose in DMEM containing 10% FBS. The plates were then incubated at 37°C in 5% CO_2 for 4 days. The cells were fixed with 5% buffered formaldehyde for 1 hour, and the agarose layers were removed. After staining with crystal violet, plaques were counted. Sera that reduced number of plaques by more than 80% comparison with the mine number of plaques in

control wells were considered as seropositive.

The same procedure was applied for the neutralization test of CAdV-1.

3.3.6. Statistical analysis

To analyze the results statistically, Fisher's exact probability and Wilcoxon rank sum tests were performed. Correction of *p*-values in multiple comparison tests was performed with the Benjamini-Hochberg Correction. The significant level was $p<0.05$.

3.4. RESULTS

3.4.1. Serological surveillance of AhAdV-1 among various exotic animals

Of the 446 animals examined, 127 animals were positive for anti-AhAdV-1 antibodies, 7 out of 17 meerkats, 45 out of 73 ferrets, 1 out of 78 chinchillas, 2 out of 58 guinea pigs, 1 out of 19 degus, 1 out of 10 ground squirrels, 3 out of 70 rabbits, 2 out of 3 micro pigs and 65 out of 103 African pigmy hedgehogs, while Fennecs, raccoon dogs, common marmosets, chipmunks, prairie dogs, rats, goats, and sugar gliders were all negative. Especially, meerkats (41%), ferrets (62%) and African pigmy hedgehogs (63%) had significantly higher positive rates for anti-AhAdV-1 antibody than other species (Table 2-2, Figure 2-2).

For further examination, anti-AhAdV-1 antibody titers were determined for positive samples. As a result, antibody titers were significantly higher in meerkats, ferrets, and African pigmy hedgehogs compared to other species of animals, consistent with the results of anti-AhAdV-1 antibody positive rates (Figure 2-3). The mean antibody titers of Meerkats, ferrets, and African pigmy hedgehogs were 1:55.4, 1:144.2, and 1:49.9, respectively.

Among ferrets and African pygmy hedgehogs, anti-AhAdV-1 antibody positive

rates were significantly higher in the group with a history of respiratory clinical signs than in the group without clinical signs ($p<0.05$). Sex was not associated with either anti-AhAdV-1 antibody positive rate or anti-AhAdV-1 antibody titer (Table 2-3, 2-4, Figure 2-4, 2-5).

Among the sero-positive animals, 19 animals in 8 families were cohabiting with other animals. In 4 of these families (Family ID: A, B, C, D), all animals living together were seropositive, while seropositive and seronegative animals were cohabiting in 4 other families (Family ID: E, F, G, H) (Table 2-5).

Eight ferrets in the survey continued to retain anti-AhAdV-1 antibodies for more than a year with the longest lasting for 774 days (Table 2-6). One ferret (G.A) finally became negative; 2 ferrets (C.T, F.O) showed an increase in antibody titer on the last day of the study compared to the start of the study; 4 ferrets (R.F, G.A, M.F, K.T) showed a decrease in antibody titer on the last day of the study compared to the start of the study; and 4 ferrets (R.F, F.O, G.A, M.F) showed periodic spikes in antibody titer during the study period. One ferret (C.T) continued to increase in antibody titer throughout the study period, while 6 showed a decreasing trend throughout the entire study period (K.T, R.S) or after spikes in antibody titer (R.F, F.O, G.A, M.F).

3.4.2. Assessment of cross-reaction with canine adenoviruses

Fourteen ferrets and 3 meerkats with antibody titers higher than 1:2560 were examined for anti-CAdV-1 antibody titers to assess the specificity of the AhAdV-1 VN test (Table 2-7). As a result, anti-CAdV-1 antibodies were below the detection limit in 12 ferrets and all meerkat, suggesting that anti-AhAdV-1 antibodies are specifically detected in the VN test. On the other hand, 2 ferrets (KI-18, KI-513) were tested as positive for anti-CAdV-1 antibodies. Those ferrets were cohabiting with dogs, suggesting co-infection

of CAdV-1 among those two ferrets through live-attenuated vaccine.

3.5. DISCUSSION

In this study, we revealed the traces of AhAdV-1 infection in a variety of animals kept as companion animals in general households in Japan. Especially, high prevalence was shown in meerkats (41%), ferrets (62%), and African pigmy hedgehogs (63%), while chinchillas (1%), guinea pigs (3%), rabbits (4%), and degus (5%) showed low prevalence. These results showed clear evidence of frequent AhAdV-1 infection in meerkats, ferrets, and African pigmy hedgehogs in Japan. Interestingly, regarding to their feeding habits, seroprevalence of carnivore and omnivore species, such as hedgehog, ferret and meerkat are higher than herbivore species such as rabbit, chinchilla, guinea pig. In addition to epidemiological studies, future research based on molecular biological perspective, including the search for receptors and differences in affinity between species are required to examine whether these results are due to differences in virus susceptibility among animal species or not.

In 2 ferrets, anti-CAdV-1 antibodies were detected along with high titer of anti-AhAdV-1 antibodies. Notably, these ferrets cohabited with dogs, suggesting potential exposure to CAdV-1 via a live-attenuated canine adenovirus vaccine. The CAdV-1 vaccine used in Japan is mainly attenuated live CAdV-2 vaccine, and vaccinated dogs may continue to shed the virus that can be transmitted, regardless of whether they are infected with the field strain (Kim et al., 2022). A previous report of CAdV-1 infection in mink and otter (Philippa et al., 2008; Hou et al., 2023; Park et al., 2007), and CAdV-2 infection in otter (de Mello et al., 2022), which are members of the weasel family, suggesting that they may also infect ferrets, which are also members of the weasel family. Future research on cross-reactive of anti-AhAdV-1 antibody against CAdV is required to

assess our serological data more precisely.

In previous studies, AhAdV-1 has been isolated or detected from marmosets, hedgehogs, porcupines, raccoons, meerkats, ferrets, and skunks showing respiratory clinical signs and necrotizing hepatitis, which are considered the primary clinical signs caused by AhAdV-1 infection (Gàl et al., 2013; Kozak et al., 2015; Madarame et al., 2016; Needle et al., 2019; Ochiai et al., 2019; Balik et al., 2020; Needle et al., 2020; Bourque et al., 2022; Orbay-Cerrato et al., 2024). On the other hand, as shown in an epidemiological study of hedgehogs kept as domestic companion animals in CHAPTER 2, the gene for AhAdV-1 was detected by PCR in nasal swabs from asymptomatic animals (Koizumi et al., 2022). In the present study, both ferrets and hedgehogs had significantly higher AhAdV-1 antibody positive rates in the group with a history of respiratory clinical signs ($p<0.05$). This suggests that AhAdV-1 infection may be associated with respiratory clinical signs in ferrets and hedgehogs. Further studies on the pathogenicity and mechanisms of onset of diseases in animals are required.

Among households with cohabiting animals, it was shown that not all animals in the same household necessarily have AhAdV-1 antibodies. The result suggests that cohabitation with anti-AhAdV-1 antibody positive animals did not necessarily affect the anti-AhAdV-1 antibody status of other animals. The animals studied in this study were generally kept indoors and were unlikely to have direct contact with AhAdV-1-infected animals living outdoors or in other households. Therefore, contact with anti-AhAdV-1 antibody positive animals that could be expected in the household cannot be considered the primary route of AhAdV-1 infection. Thus, the route of infection for AhAdV-1 may be from animals living in the same household, the pet owners, the groups in a farm at their early age or the dealers. Meerkats, ferrets, and hedgehogs, which showed high prevalence in this study, kept as companion animals in Japan are mainly bred in farms

overseas, with meerkats and hedgehogs coming from Southeast Asia and ferrets from North America. On the other hand, guinea pigs and degus, which showed low prevalence in this study, are mainly bred and distributed in Japan. This fact indicates the possibility that anti-AhAdV-1 antibody positive animals may have been infected with the virus in areas where AhAdV-1 is endemic and imported to Japan.

In conclusion, the results of the present study indicate a wide host range and frequent infection of AhAdV-1 in captive exotic animals in Japan, particularly meerkats, ferrets and African pygmy hedgehogs. The alert for pathogenicity of AhAdV-1 should be taken in ferrets and African pygmy hedgehogs due to the significant association between seroprevalence and the history of respiratory clinical signs. Future research based on molecular biological perspective, including the search for receptors and differences in affinity between species are required.

3.6. LEGENDS FOR FIGURES

Figure 2-1

Phylogenetic tree of mastadenoviruses. The tree was constructed based on the DNA sequences of 1200 bp of DNA polymerase. GenBank accession numbers of the listed viruses are shown in parentheses. For AhAdV-1 and SkAdV-1 are indicated in the bold letter with their host and accession numbers in parentheses. The evolutionary history was inferred using the Neighbor-Joining method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the *p*-distance method and are in the units of the number of base differences per site. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA11.

Figure 2-2

Heatmap of *p*-value comparing seroprevalence of AhAdV-1 in various animal species. Fisher's Exact Test was performed and significant differences were calculated with the Benjamini-Hochberg Correction. The color scale was set as shown in the legend according to the calculated *p*-values ($p<0.05$, $p<0.01$, $p<0.001$, $p<0.0001$).

Figure 2-3

Boxplot showing distribution of the anti-AhAdV-1 antibody titers among various mammals. The central line within each box represents the median, while the box boundaries indicate the interquartile range (IQR; 25th to 75th percentiles). <0: anti-AhAdV-1 antibody negative on VN test screening. +: Mean value of anit-AhAdV-1 antibody titer for each item. Anti-AhAdV-1 antibody negative samples in VN test

screening were calculated as -1 log2 x10 (antibody titer 1:5).

Figure 2-4

Boxplot showing distribution of the anti-AhAdV-1 antibody titers among ferrets regarding to their sex or history of respiratory clinical signs. Significant differences were tested by Wilcoxon rank sum test, and $p<0.05$ was judged to be significant. The item "Ferret" shows the results from all ferret samples. Clinical signs: History of respiratory clinical signs such as rhinitis, pneumonia. ((-): No, (+): Yes). <0: anti-AhAdV-1 antibody negative on VN test screening. +: Mean value of anti-AhAdV-1 antibody titer for each item. Anti-AhAdV-1 antibody negative samples in VN test screening were calculated as -1 log2 x10 (antibody titer 1:5). n.s.: no significant difference ($p\geq0.05$).

Figure 2-5

Boxplot showing distribution of the anti-AhAdV-1 antibody titers among African pygmy hedgehogs regarding to their sex or history of respiratory clinical signs. Significant differences were tested by Wilcoxon rank sum test, and $p<0.05$ was judged to be significant. The item "Hedgehog" shows the results from all African pigmy hedgehog samples. Clinical signs: History of respiratory clinical signs such as rhinitis, pneumonia, etc. ((-): No, (+): Yes). <0: anti-AhAdV-1 antibody negative on VN test screening. +: Mean value of anti-AhAdV-1 antibody titer for each item. Anti-AhAdV-1 antibody negative samples in VN test screening were calculated as -1 log2 x10 (antibody titer 1:5). n.s.: no significant difference ($p\geq0.05$), **: $p<0.01$.

3.7. FIGURES AND TABLES

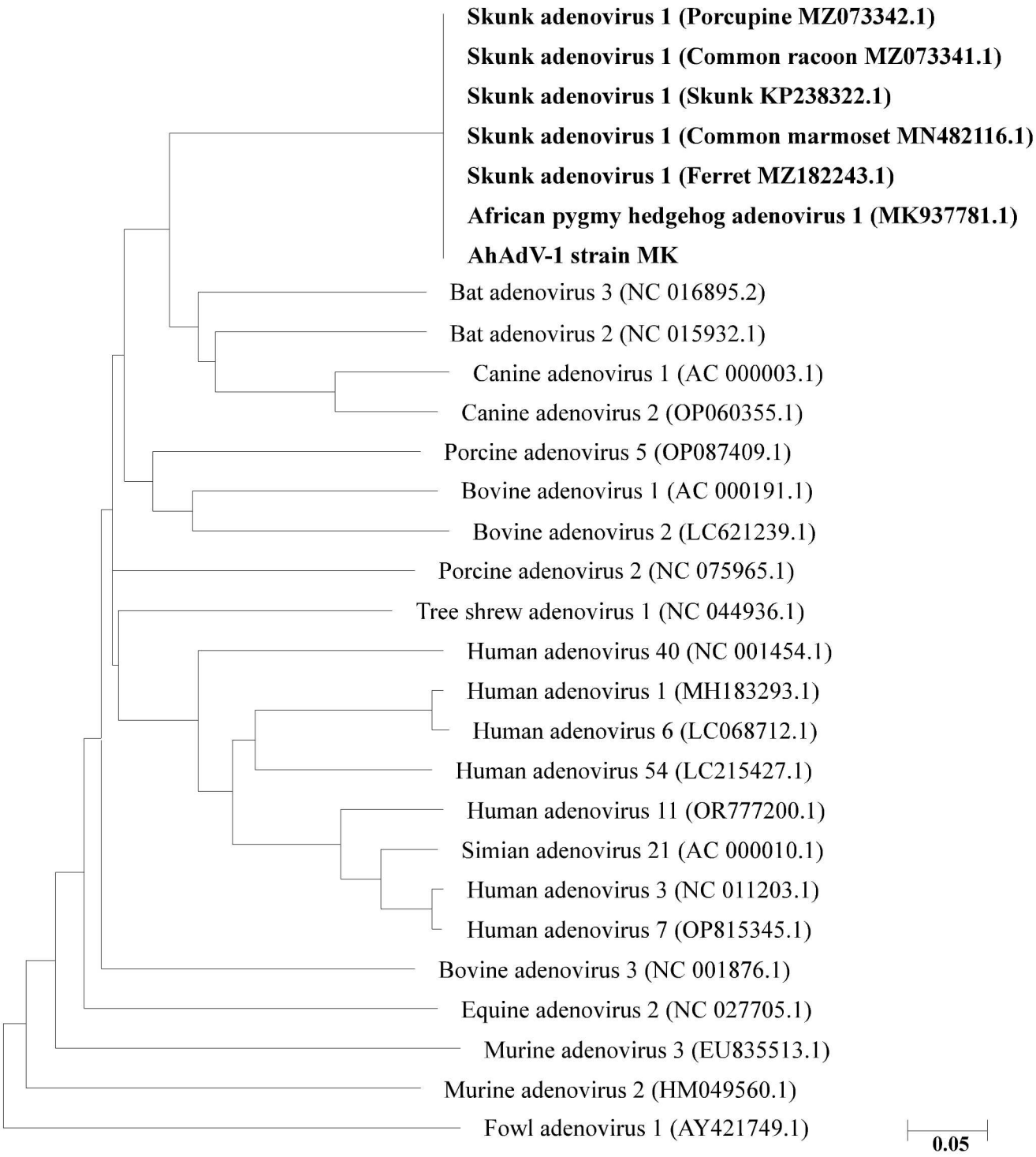


Figure 2-1. Phylogenetic tree containing various mastadenoviruses based on the DNA sequences of DNA polymerase.

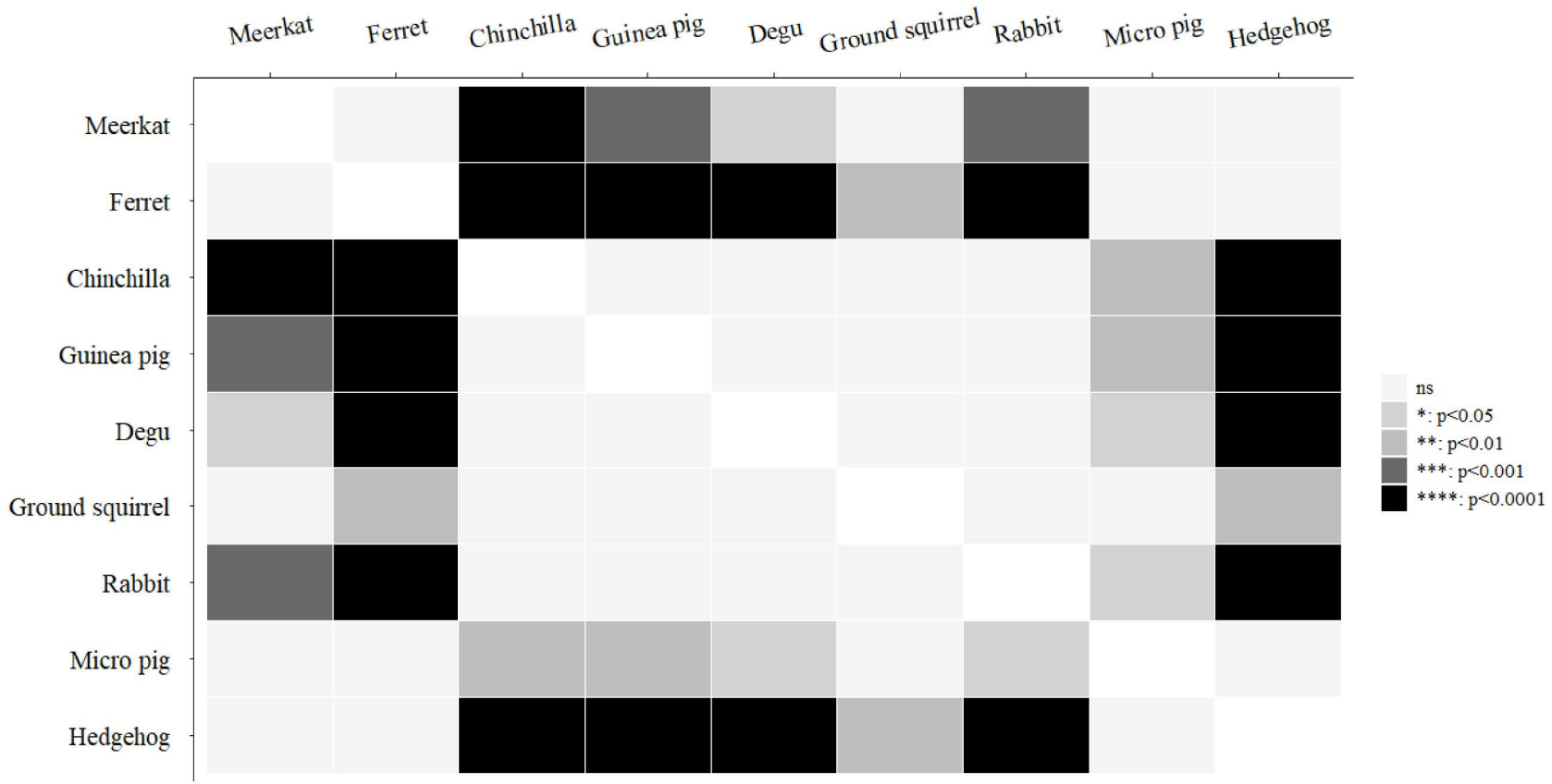


Figure 2-2. Heatmap of *p*-value comparing seroprevalence of AhAdV-1 in various animal species.

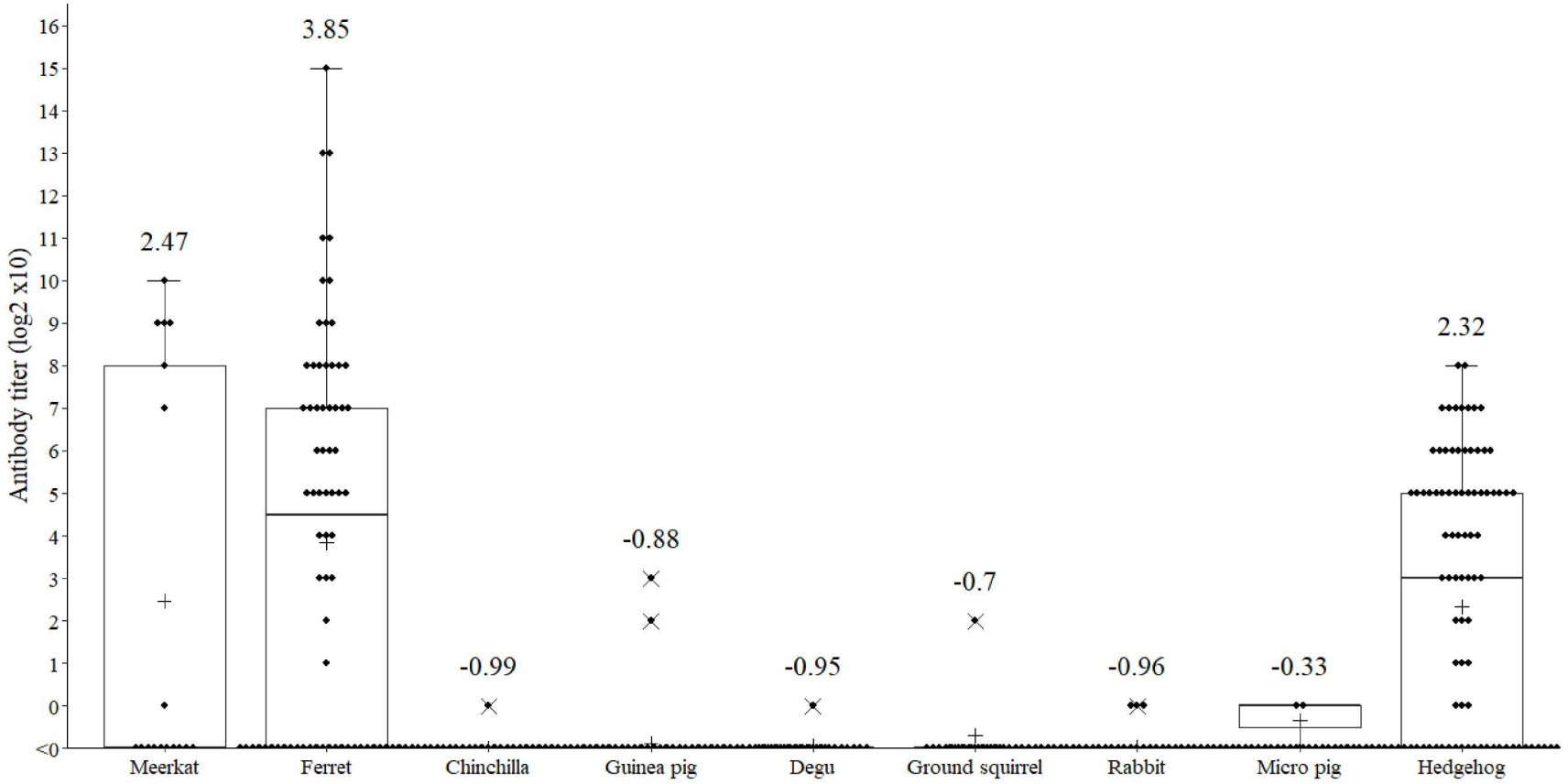
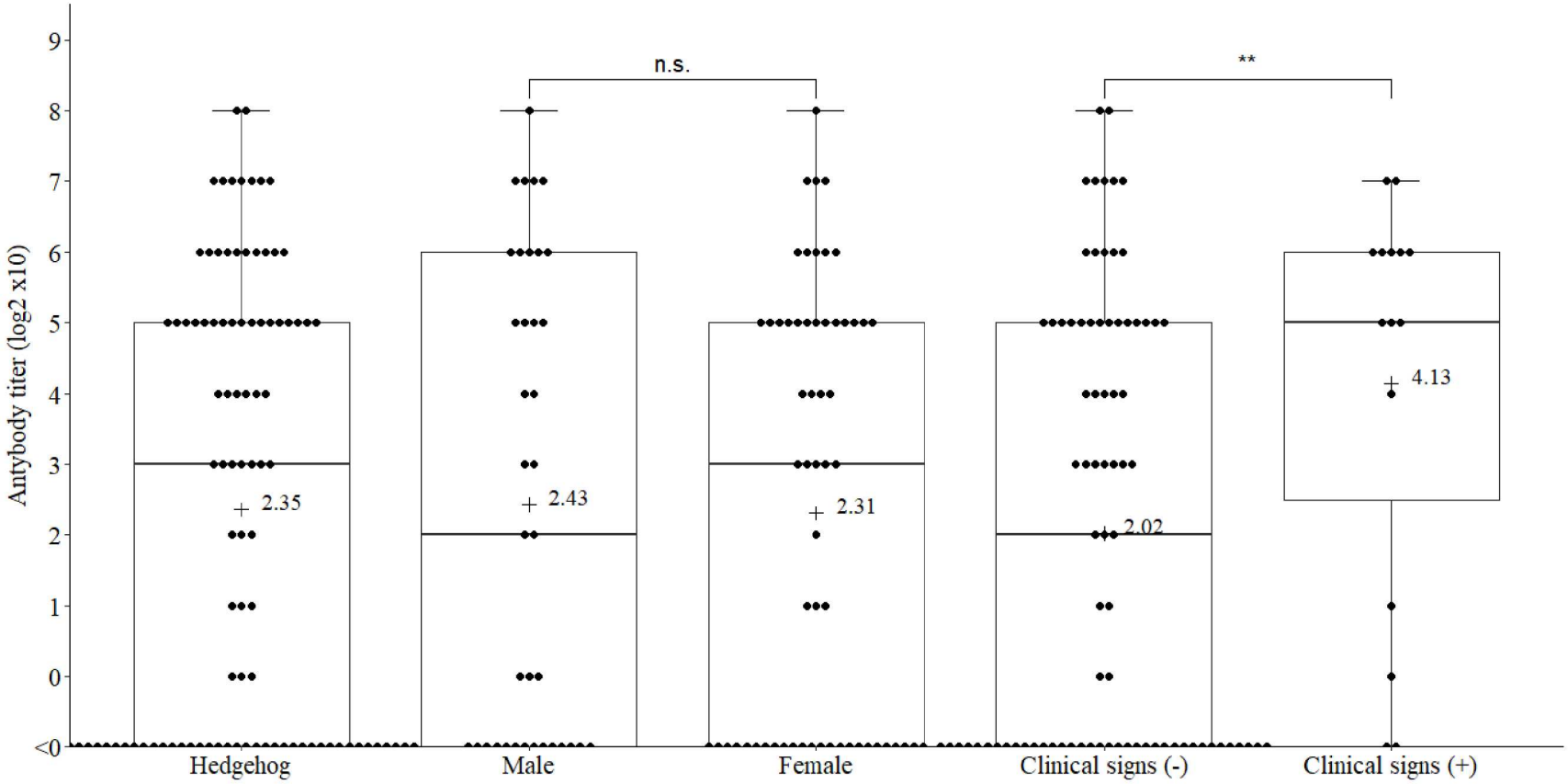


Figure 2-3. Boxplot showing distribution of the anti-AhAdV-1 antibody titers among various mammals.

Figure 2-4. Boxplot showing distribution of the anti-AhAdV-1 antibody titers among ferrets regarding to their sex or history of respiratory clinical signs.



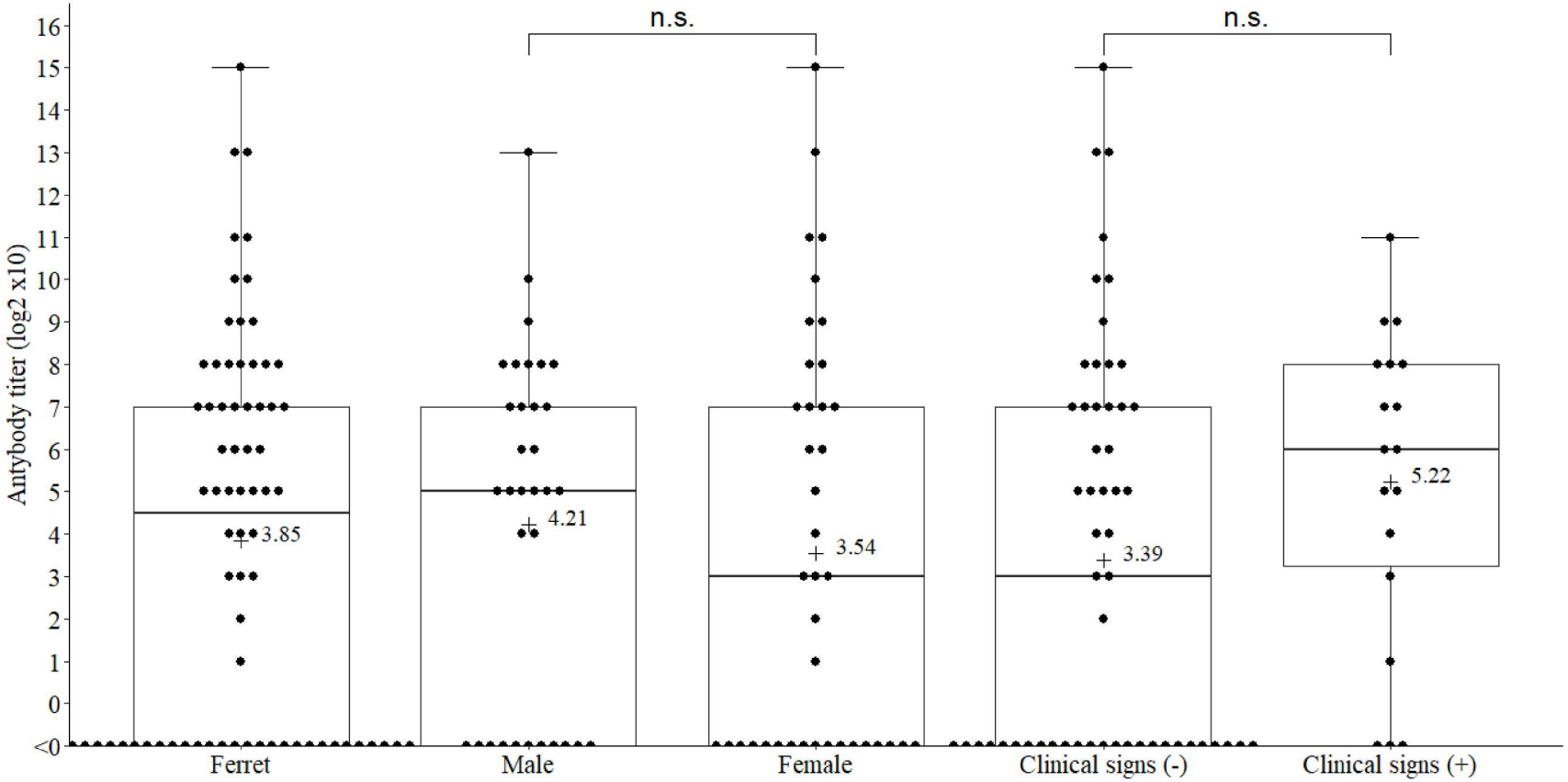


Figure 2-5. Boxplot showing distribution of the anti-AhAdV-1 antibody titers among African pygmy hedgehogs regarding to their sex or history of respiratory clinical signs.

Table 2-1. Previously published reports on the isolation or detection of AhAdV-1 and SkAdV-1.

No.	Article	Target Animal	Farming Type	Clinical Signs	Note
1	Gál et al., 2013 Doszpoly et al., 2020	Common marmoset	Exhibit	Catarrhal bronchopneumonia	Virus isolation with ST, MA-104, Vero, Bot-2
2	Kozac et al., 2015	Skunk	Wild	Acute hepatitis Interstitial pneumonia	Virus isolation with MDCK The first report of SkAdV-1
3	Madarame et al., 2016 Madarame et al., 2019	African pigmy hedgehog	Companion	Subclinical tracheitis Congestive heart failure	Virus isolation with MDCK The virus was reported as SkAdV-1
4	Needle et al., 2019	African pigmy hedgehog	Companion	Acute necrotizing bronchopneumonia	Virus detection from lung
5	Ochiai et al., 2019	African pigmy hedgehog	Companion	Multifocal acute rhinitis Bronchointerstitial pneumonia	Virus isolation with MDCK The first report of AhAdV-1
6	Balic et al., 2020	Porcupine	Wild	Respiratory diseases	Virus isolation with MDCK
7	Needle et al., 2020	Gray fox	Exhibit	Bronchointerstitial pneumonia	Virus detection from lung
8	Bourque et al., 2022	Porcupine, Skunk, Racoon	Wild	Necrotizing bronchopneumonia	Virus isolation from porcupine and common racoon with Vero C1008
9	Koizumi et al., 2022	African pigmy hedgehog	Companion	No significant clinical sign	Virus detection from nasal swab
10	Shimoda et al. (manuscript in preparation)	Meerkat, Binturong	Exhibit	Necrotizing bronchopneumonia	Virus isolation from meerkat with MDCK
11	Orbay-Cerrato et al., 2024	Ferret	Companion	Respiratory diseases	Virus detection from lung

Table 2-2. Seroprevalence of AhAdV-1 among various household exotic mammals.

Order	Animal species	Number of examined animals	Number of positive animals	% of positive animals
Carnivora	Ferret	73	45	62
	Meerkat	17	7	41
	Fennec fox	1	0	0
	Raccoon dog	1	0	0
Primates	Common marmoset	2	0	0
Rodentia	Chinchilla	78	1	1
	Guinea pig	58	2	3
	Degu	19	1	5
	Ground squirrel	10	1	10
	Chipmunk	1	0	0
	Prairie dog	1	0	0
	Rat	3	0	0
Lagomorpha	Rabbit	70	3	4
Artiodactyla	Micro pig	3	2	67
	Goat	2	0	0
Diprotodontia	Sugar glider	4	0	0
Erinaceomorpha	African pigmy hedgehog	103	65	63
	Total	446	127	28

Table 2-3. Seroprevalence of ferrets regarding their sex and the history of respiratory clinical sign.

	Sex		Respiratory clinical sign		Total
	Male	Female	No	Yes	
Number of examined animals	34	39	55	18	73
Number of positive animals	23	22	30	15	45
% of positive animals	68	56	55	83	62

Table 2-4. Seroprevalence of African pygmy hedgehogs regarding their sex and the history of respiratory clinical sign.

	Sex		Respiratory clinical sign		Total
	Male	Female	No	Yes	
Number of examined animals	41	62	85	18	103
Number of Positive animals	27	38	49	16	65
% of positive animals	66	61	58	89	63

Table 2-5. Anti-AhAdV-1 Antibody status of animals living in the same house.

Family ID	Animal ID	Animal species	Anti-AhAdV-1 antibody titer	Sex	Age during survey period (y)
A	KI-4	Ferret	>1:10	M	2.7~5.0
	KI-5	Ferret	1:2560	F	2.5~4.5
B	KI-307	Ferret	1:160	M	3.3~4.6
	KI-308	Ferret	1:10240	F	2.6
C	KI-101	Ferret	1:80	F	2.6~3.4
	KI-509	Ferret	1:40	F	3.5~4.3
D	KI-11	Ferret	1:1280	M	4.3~6.7
	KI-97	Ferret	1:640	F	4.3
E	KI-14	Ferret	<1:10	M	7.1~7.8
	KI-164	Ferret	<1:10	M	5.7
	KI-380	Ferret	1:640	M	4.0
F	KI-91	Ferret	1:1280	F	6.7~7.1
	KI-96	Ferret	<1:10	F	5.5~6.3
G	KI-480	Meerkat	1:10240	M	1.8~2.0
	KI-481	Meerkat	<1:10	M	0.8
	KI-482	Meerkat	<1:10	F	0.6
H	KI-15	Ferret	1:320	M	5.2~7.2
	KI-206	Micro pig	1:10	M	0.7
	KI-353	Meerkat	<1:10	M	0.7

Table 2-6. Animals continued to retain anti-AhAdV-1 antibodies for more than a year.

	C. T	R. F	F. O	K. K	G. A	M. F	K. T	R. S
Animal species	Ferret	Ferret	Ferret	Ferret	Ferret	Ferret	Ferret	Ferret
Sex	F	M	M	M	M	F	F	M
Anti-AhAdV-1 antibody titer (number of days elapsed (days))	1:20480 (0)	1:40 (0)	1:10 (0)	1:5120 (0)	1:640 (0)	1:80 (0)	1:5120 (0)	1:160 (0)
	1:20480 (18)	1:320 (291)	1:80 (29)	1:5120 (631)	1:640 (5)	1:40 (78)	1:20480 (299)	1:40 (457)
	1:40960 (182)	1:160 (361)	1:20 (50)		1:2560 (133)	1:20 (80)	1:10240 (423)	1:20 (484)
	1:40960 (210)	1:10 (479)	1:320 (134)		1:2560 (225)	1:20 (83)	1:20480 (497)	
	1:40960 (239)	1:20 (610)	1:20 (179)		1:1280 (522)	1:40 (134)	1:10240 (544)	
	1:81920 (267)	1:10 (727)	1:20 (213)		<1:10 (574)	1:10 (269)		
	1:81920 (309)		1:10 (269)			1:20 (304)		
	1:163840 (386)		1:10 (352)			1:10 (317)		
	1:81920 (456)		1:10 (434)			<1:10 (346)		
	1:81920 (497)		1:160 (520)			1:40 (415)		
	1:163840 (513)		1:20 (609)			1:10 (458)		
	1:163840 (534)		1:20 (665)			1:20 (500)		
	1:327680 (613)					1:20 (535)		
	1:327680 (652)					1:20 (574)		
	1:327680 (713)							
	1:327680 (774)							

Table 2-7. Assessment of cross-reaction of anti-AhAdV-1 antibodies against CAdV-1.

ID	Animal species	Anti-AhAdV-1 antibody titer	Anti-CAdV-1 antibody titer	Cohabit with dogs	Other mammals living together
KI-42	Meerkat	1:2560	<1:10	-	Sugar gliders (2)
KI-170	Ferret	1:2560	<1:10	-	Ferret
KI-447	Ferret	1:2560	<1:10	-	Ferret
KI-692	Ferret	1:2560	<1:10	-	-
KI-766	Ferret	1:2560	<1:10	-	Ferret
KI1009	Ferret	1:2560	<1:10	-	Ferrets (many)
KI-401	Ferret	1:5120	<1:10	-	Ferret
KI-529	Ferret	1:5120	<1:10	-	Ferret
KI-1013	Ferret	1:5120	<1:10	-	-
KI-1052	Meerkat	1:5120	<1:10	○	-
KI-308	Ferret	1:10240	<1:10	-	Ferret
KI-600	Meerkat	1:10240	<1:10	-	Meerkats (5)
KI-772	Ferret	1:10240	<1:10	-	-
KI-512	Ferret	1:20480	<1:10	-	-
KI-894	Ferret	1:81920	<1:10	-	-
KI-18	Ferret	1:81920	1:320	○	Rabbit
KI-513	Ferret	1:81920	1:5120	○	-

7. GENERAL CONCLUSION

In this thesis, the author focused to understand 1) the viral infections status and relevance to various diseases in pet African pygmy hedgehogs, 2) the host range, pathogenesis and prevalence of AhAdV-1 in various exotic pets kept in Japan.

In CHAPTER 1, the author investigated herpesvirus, adenovirus, and coronavirus infections among pet African pygmy hedgehogs in Japan. A novel herpesvirus named Atelerix albiventris herpesvirus 1 (AAHeV) was identified and indicated a significant relationship to neurological signs. AAHeV infection also might have negative effect to develop neoplastic disorder because no hedgehog with a neoplastic disorder tested positive for AAHeV. These results suggest that AAHeV infection may play a key role in the mechanism of neurological and neoplastic diseases. On the other hand, because AhAdV-1 was detected in hedgehogs with no respiratory signs, subclinical infection of AhAdV-1 may occur among pet African pygmy hedgehogs in Japan. Given the unique nature of this novel adenovirus, this result highlighted the necessity of large-scale epidemiological survey in Japanese captive animals including broad species.

In CHAPTER 2, serological surveillance among 17 species of exotic animals were conducted by plaque reduction virus neutralization test. Antibodies against AhAdV-1 were detected in a variety of animals with particularly high seroprevalence among meerkats (41%), ferrets (62%) and African pigmy hedgehogs (63%). These results underscored the wide range of host and an epidemic of AhAdV-1 in meerkats, ferrets, African pygmy hedgehog kept in Japan. Because Ferrets and African pygmy hedgehogs with the history of respiratory clinical signs showed significantly high seroprevalence compared to the animals without the clinical history, the alert for pathogenicity of AhAdV-1 therefor should be taken particularly in these two species. The present study

revealed that not all animals in the same household necessarily have AhAdV-1 antibodies, suggesting contact with anti-AhAdV-1 antibody positive animals in the household cannot be considered the primary route of AhAdV-1 infection. Possibility that anti-AhAdV-1 antibody positive animals may have been infected with the virus in areas where AhAdV-1 is endemic and imported to Japan, because meerkats, ferrets, and hedgehogs are mainly bred in farms overseas. Future research based on molecular biological perspective, including the search for receptors and differences in affinity between species are required.

8. ACKNOWLEDGMENTS

The present studies were carried at the Laboratory of Veterinary Microbiology, Joint Faculty of Veterinary Medicine, Yamaguchi University, Japan, from 2021 to 2025.

First of all, the author would like to show his greatest appreciation to his supervisor, **Dr. Daisuke Hayasaka** (Laboratory of Veterinary Microbiology, Yamaguchi University) for providing him this precious opportunity to study as a Ph.D. student with the invaluable support and advice.

The author is very grateful to all of his co-supervisors, **Dr. Hiroshi Shimoda** (Laboratory of Veterinary Microbiology, Yamaguchi University) for giving him encourages and critical advices on his experiment.

The author wants to thank to his laboratory members for their support in his experiments. The author is thankful to veterinary technicians in his own hospital (Koizumi Nest Animal hospital) for supporting him collecting clinical samples.

Finally, the author would like to show the deepest appreciation to his family who supported him and sacrificed themselves to allow him start and finish his study as a Ph.D. student.

9. REFERENCES

Allison N, Chang TC, Steele KE, Hilliard JK (2002) Fatal herpes simplex infection in a pygmy African hedgehog (*Atelerix albiventris*). *J Comp Pathol,* 126:76-78

Balik S, Bunting E, Dubovi E, Renshaw R, Childs-Sanford S (2020) Detection of skunk adenovirus 1 in two North American porcupines (*Erethizon dorsatum*) with respiratory disease. *J Zoo Wildl Med,* 50:1012-1015

Borkenhagen LK, Fieldhouse JK, Seto D, Gray GC (2019) Are adenoviruses zoonotic? A systematic review of the evidence. *Emerg Microbes Infect,* 8:1679-1687

Bourque L, Nielsen O, Jager M, Ojkic D, Provost C, Gagnon CA, Lockerbie B, Snyman H, Stevens B, Needle D, Nakagun S, Cox S, Khodadad J, Jones MEB (2022) In situ hybridization and virus characterization of skunk adenovirus in North American wildlife reveals multisystemic infections in a broad host range. *J Wildl Dis*, 58:356-367

Chomel BB, Riley PY (2005) Hedgehog Zoonoses. *Emerg Infect Dis,* 11:1146

Choi JW, Lee HK, Kim SH, Kim YH, Lee KK, Lee MH, Oem JK (2014) Canine adenovirus type 1 in a fennec fox (*Vulpes zerda*). *J Zoo Wildl Med,* 45:947-950

Corman VM, Kallies R, Philipps H, Göpner G, Müller MA, Eckerle I, Brünink S, Drosten C, Drexler JF (2014) Characterization of a novel betacoronavirus related to middle East respiratory syndrome coronavirus in European hedgehogs. *J Virol,* 88:717-724

Crenshaw BJ, Jones LB, Bell CR, Kumar S, Matthews QL (2019) Perspective on adenoviruses: Epidemiology, pathogenicity, and gene therapy. *Biomedicines,* 7:61

Delogu M, Cotti C, Lelli D, Sozzi E, Trogu T, Lavazza A, Garuti G, Castrucci MR, Vaccari G, De Marco MA, Moreno A (2020) Eco-virological preliminary study of potentially emerging pathogens in hedgehogs (*Erinaceus europaeus*) recovered at a wildlife treatment and rehabilitation center in Northern Italy. *Animals,* 10:407

de Mello Zanim Michelazzo M, Martinelli TM, de Amorim VRG, Silva LE, Silva FHP, Xavier AAC, Cubas ZS, de Almeida RF, de Moraes W, Headley SA (2022) Canine distemper virus and canine adenovirus type-2 infections in neotropical otters (*Lontra longicaudis*) from Southern Brazil. *Braz J Microbiol,* 53:369-375

Doss AG, Carpenter JW (2020) African pygmy hedgehogs. In: Ferrets, rabbits, and rodents clinical medicine and surgery, 4th ed. (Quesenberry EK, Orcutt JC, Mans C, Carpenter WJ eds.) pp. 401-415. Elsevier, Amsterdam

Doszpoly A, Hornyák Á, Bányai K (2020) Complete genome analysis confirms that the pygmy marmoset adenovirus is a variant of the skunk adenovirus 1 - Short communication. *Acta Vet Hung,* 68:323-327

Díaz-Delgado J, Pool R, Hoppes S, Cerezo A, Quesada-Canales Ó, Stoica G (2017) Spontaneous multicentric soft tissue sarcoma in a captive African pygmy hedgehog (*Atelerix albiventris*): case report and literature review. *J Vet Med Sci*, 79:889-895

Gál J, Hornyák Á, Mándoki M, Bakonyi T, Balka G, Szeredi L, Marosán M, Ludányi T, Forgách P, Sós E, Demeter Z, Farkas SL (2013) Novel mastadenovirus infection and clinical disease in a pygmy marmoset (*Callithrix* [*Cebuella*] *pygmaea*). *Vet Microbiol,* 167:695-699

García Marín JF, Royo LJ, Oleaga A, Gayo E, Alarcia O, Pinto D, Martínez IZ, González P, Balsera R, Marcos JL, Balseiro A (2018) Canine adenovirus type 1 (CAdV-1) in free-ranging European brown bear (*Ursus arctos arctos*): A threat for Cantabrian population? *Transbound Emerg Dis,* 65:2049-2056

Gardhouse S, Eshar D (2015) Retrospective study of disease occurrence in captive African pygmy hedgehogs (*Atelerix albiventris*). *Isr J Vet Med,* 70:32-36

Graczyk TK, Cranfield MR, Dunning C, Strandberg JD (1998) Fatal cryptosporidiosis in a juvenile captive African hedgehog (*Ateletrix albiventris*). *J Parasitol,* 84:178-180

Graesser D, Spraker TR, Dressen P, Garner MM, Raymond JT, Terwilliger G, Kim J, Madri JA (2006) Wobbly hedgehog syndrome in African pygmy hedgehogs (*Atelerix* spp.). *J Exot Pet Med,* 15:59-65

Harrach B, Tarján ZL, Benkő M (2019) Adenoviruses across the animal kingdom: a walk in the zoo. *FEBS Lett,* 593:3660-3673

Heatley JJ, Mauldin GE, Cho DY (2005) A review of neoplasia in the captive African hedgehog (*Atelerix albiventris*). *Semin Avian Exot Pet Med,* 14:182-192

Hechinger S, Scheffold S, Hamann HP, Zschöck M (2017) Detection of canine adenovirus 1 in red foxes (*Vulpes vulpes*) and raccoons (*Procyon lotor*) in Germany with a TaqMan real-time PCR assay. *J Vet Diagn Invest,* 29:741-746

Hoff C, Nichols M, Gollarza L, Scheftel J, Adams J, Tagg KA, Francois Watkins L, Poissant T, Stapleton GS, Morningstar-Shaw B, Signs K, Bidol S, Donovan D, Basler C (2022) Multistate outbreak of Salmonella Typhimurium linked to pet hedgehogs, United States, 2018-2019. *Zoonoses Public Health,* 69:167-174

Hou J, Xu J, Wang B, Zhang H, Yin B, Li G, Lei F, Cai X, Zhu Y, Wang L (2023) First identification of canine adenovirus 1 in mink and bioinformatics analysis of its 100 K protein. *Front Microbiol,* 14:1245581

Hydeskov HB, Dastjerdi A, Hopkins KP, Ryser-Degiorgis MP, Widén F, Cunningham AA, Lawson B (2018) Detection and characterisation of multiple herpesviruses in free-living Western European hedgehogs (*Erinaceus europaeus*). *Sci Rep,* 8:13942

Ichimi R, Yoshino A, Higashigawa M (2018) Salmonella Stanley bacteremia transmitted from a pet hedgehog. *Pediatr Int,* 60:606-607

Ison MG, Hayden RT (2016) Adenovirus. *Microbiol Spectr,* 4

Karstad L, Ramsden R, Berry TJ, Binn LN (1975) Hepatitis in skunks caused by the virus of infectious canine hepatitis. *J Wildl Dis,* 11:494-496

Kim J, Tsuchihashi H, Hiruma M, Kano R, Ikeda S (2018) Tinea corporis due to *Trichophyton erinacei* probably transmitted from a hedgehog: The second case report from Japan. *Med Mycol J,* 59:E77-E79

Kim YJ, Lee SY, Kim YS, Na EJ, Park JS, Oem JK (2022) Genetic characteristics of canine adenovirus type 2 detected in wild raccoon dogs (*Nyctereutes procyonoides*) in Korea (2017-2020). *Vet Sci,* 9:591

Kobayashi T, Matsugo H, Maruyama J, Kamiki H, Takada A, Maeda K, Takenaka-Uema A, Tohya Y, Murakami S, Horimoto T (2019) Characterization of a novel species of adenovirus from Japanese microbat and role of CXADR as its entry factor. *Sci Rep,* 9:573

Koizumi I†, Tsukada H†, Hayasaka D, Shimoda H (2022) Comprehensive surveillance of virus infection among captive African pygmy hedgehogs in Japan. *Viruses,* 14:857 (†Equally contributed)

Koizumi I, Hernandez-Muguiro D, Chu SAA, Stokol T, Asakawa MG (2020a) Clinicopathologic findings of spontaneous leukemia in 9 pet African hedgehogs (*Atelerix Albiventris*). *Front Vet Sci,* 7:54

Koizumi I, Okumura N, Kondo H (2020b) Primary localized histiocytic sarcoma of the lumbar vertebrae in a four-toed hedgehog (*Atelerix albiventris*). *J Vet Med Sci,* 82:1511-1514

Koizumi I, Kondo H (2019a) Clinical management and outcome of four-toed hedgehogs (*Atelerix albiventris*) with histiocytic sarcoma. *J Vet Med Sci,* 81:545-550

Koizumi I, Kondo H (2019b) A case of preputial cystostomy performed on an African pygmy hedgehog (*Atelerix albiventris*) with urethral obstruction. *J Exot Pet Med,* 30:1-6

Koizumi I, Takaki Y, Watanabe T, Takeyama K, Takami Y (2016) Retrospective study of

disease occurrence in captive African pygmy hedgehogs (*Atelerix albiventris*) in Japan (in Japanese). *JSEPM,* 18:27-34

Kozak RA, Ackford JG, Slaine P, Li A, Carman S, Campbell D, Welch MK, Kropinski AM, Nagy É (2015) Characterization of a novel adenovirus isolated from a skunk. *Virology,* 485:16-24

Kulanayake S, Tikoo SK (2021) Adenovirus Core Proteins: Structure and Function. *Viruses,* 13:388

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol,* 35:1547-1549

Lau SKP, Luk HKH, Wong ACP, Fan RYY, Lam CSF, Li KSM, Ahmed SS, Chow FWN, Cai JP, Zhu X, Chan JFW, Lau TCK, Cao K, Li M, Woo PCY, Yuen KY (2019) Identification of a novel betacoronavirus (merbecovirus) in amur hedgehogs from China. *Viruses,* 11:980

Lichty BD, Breitbach CJ, Stojdl DF, Bell JC (2014) Going viral with cancer immunotherapy. *Nat Rev Cancer,* 14:559-567

Lion T (2014) Adenovirus infections in immunocompetent and immunocompromised patients. *Clin Microbiol Rev,* 27:441-462

Liu BL, Robinson M, Han ZQ, Branston RH, English C, Reay P, McGrath Y, Thomas SK, Thornton M, Bullock P, Love CA, Coffin RS (2003) ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. *Gene Ther,* 10:292-303

Madarame H, Ogihara K, Kimura M, Nagai M, Omatsu T, Ochiai H, Mizutani T (2014) Detection of a pneumonia virus of mice (PVM) in an African hedgehog (*Atelerix arbiventris*) with suspected wobbly hedgehog syndrome (WHS). *Vet Microbiol,* 173:136-140

Madarame H, Ogihara K, Ochiai H, Omatsu T, Mizutani T (2016) Detection of skunk adenovirus 1 (SkAdV-1) in an African pigmy hedgehog (*Atelerix albiventris*). *Vet Rec Case Rep,* 4:e000321

Madarame H, Uchiyama J, Tamukai K, Katayama Y, Osawa N, Suzuki K, Mizutani T, Ochiai H (2019) Complete genome sequence of an adenovirus-1 isolate from an African pygmy hedgehog (*Atelerix albiventris*) exhibiting respiratory symptoms in Japan. *Microbiol Resour Announc,* 8:e00695-19

Miller DL, Schrecengost J, Merrill A, Kilgo J, Ray HS, Miller KV, Baldwin CA (2009) Hematology, parasitology, and serology of free-ranging coyotes (*Canis latrans*) from South Carolina. *J Wildl Dis,* 45:863-869

Mochizuki T, Takeda K, Nakagawa M, Kawasaki M, Tanabe H, Ishizaki H (2005) The first isolation in Japan of *Trichophyton mentagrophytes* var. *erinacei* causing tinea manuum. *Int J Dermatol,* 44:765-768

Monchatre-Leroy E, Boué F, Boucher JM, Renault C, Moutou F, Ar Gouilh M, Umhang G (2017) Identification of alpha and beta coronavirus in wildlife species in France: bats, rodents, rabbits, and hedgehogs. *Viruses,* 9:364

Muñoz-Gutiérrez JF, Garner MM, Kiupel M (2018) Primary central nervous system neoplasms in African hedgehogs. *J Vet Diagn Invest,* 30:715-720

Ndiana LA, Lanave G, Vasinioti V, Desario C, Martino C, Colaianni ML, Pellegrini F, Camarda A, Berjaoui S, Sgroi G, Elia G, Pratelli A, Buono F, Martella V, Buonavoglia C, Decaro N (2022) Detection and genetic characterization of canine adenoviruses, circoviruses, and novel cycloviruses from wild carnivores in Italy. *Front Vet Sci,* 9:851987

Needle DB, Selig MK, Jackson KA, Delwart E, Tighe E, Leib SL, Seuberlich T, Pesavento PA (2019) Fatal bronchopneumonia caused by skunk adenovirus 1 in an

African pygmy hedgehog. *J Vet Diagn Invest,* 31:103-106

Needle DB, Marr JL, Park CJ, Andam CP, Wise AG, Maes RK, Wilkes RP, Anis EA, Sidor IF, Agnew D, Ellis JC, Tate P, Mathewson A, Benton C, Gibson R (2020) Concurrent infection of skunk adenovirus-1, listeria monocytogenes, and a regionally specific clade of canine distemper virus in one gray fox (*Urocyon cinereoargenteus*) and concurrent listeriosis and canine distemper in a second gray fox. *Pathogens,* 9(7):591

Ochiai H, Tamukai K, Akabane Y, Oba M, Omatsu T, Okumura A, Mizutani T, Madarame H (2019) An African pygmy hedgehog adenovirus 1 (AhAdV-1) outbreak in an African pygmy hedgehog (*Atelerix albiventris*) colony in Japan. *Vet Anim Sci,* 9: 100083

Okada K, Kondo H, Sumi A, Kagawa Y (2018) A retrospective study of disease incidence in African pygmy hedgehogs (*Atelerix albiventris*). *J Vet Med Sci,* 80:1504-1510

Okumura N†, Koizumi I†, Iwata Y, Yoneya S, Koba R, Kondo H, Shibuya H (2021) Cutaneous papilloma and multicentric squamous cell carcinoma in four-toed hedgehogs (*Atelerix albiventris*). *J Vet Med Sci,* 83:1726-1729 (†Equally contributed)

Orbay-Cerrato ME, Nilsen RA, Gottdenker N, McManamon R, Elbert JA, Stilwell JM, Ard MB, Ritchie BW, McHale B (2024) Detection of skunk adenovirus-1 in domestic ferrets (*Mustela putorius*). *Vet Pathol,* 61:135-139

Park NY, Lee MC, Kurkure NV, Cho HS (2007) Canine adenovirus type 1 infection of a Eurasian river otter (*Lutra lutra*). *Vet Pathol,* 44:536-539

Peauroi JR, Lowenstine LJ, Munn RJ, Wilson DW (1994) Multicentric skeletal sarcomas associated with probable retrovirus particles in two African hedgehogs (*Atelerix albiventris*). *Vet Pathol,* 31:481-484

Pei-Chi H, Jane-Fang Y, Lih-Chiann W (2015) A retrospective study of the medical status

on 63 African hedgehogs (*Atelerix albiventris*) at the Taipei Zoo from 2003 to 2011. *J Exot Pet Med,* 24:105-111

Philippa J, Fournier-Chambrillon C, Fournier P, Schaftenaar W, van de Bildt M, van Herweijnen R, Kuiken T, Liabeuf M, Ditcharry S, Joubert L, Bégnier M, Osterhaus A (2008) Serologic survey for selected viral pathogens in free-ranging endangered European mink (*Mustela lutreola*) and other mustelids from South-western France. *J Wildl Dis,* 44:791-801

Poon LL, Chu DK, Chan KH, Wong OK, Ellis TM, Leung YH, Lau SK, Woo PC, Suen KY, Yuen KY, Guan Y, Peiris JS (2005) Identification of a novel coronavirus in bats. *J Virol,* 79:2001-2009

Raymond JT, Garner MM (2001) Spontaneous tumours in captive African hedgehogs (*Atelerix albiventris*): a retrospective study. *J Comp Pathol,* 124: 128-133

Reeve N (1994) An introduction to Hedgehogs. In: Hedgehogs. pp. 1-33. T & AD Poyser Ltd, London

Russell WC (2009) Adenoviruses: update on structure and function. *J Gen Virol,* 90:1-20

Ruszkowski JJ, Hetman M, Turlewicz-Podbielska H, Pomorska-Mól M (2021) Hedgehogs as a potential source of zoonotic pathogens- A review and an update of knowledge. *Animals (Basel),* 11:1754

Saldanha IF, Lawson B, Goharriz H, Rodriguez-Ramos FJ, John SK, Fooks AR, Cunningham AA, Johnson N, Horton DL (2019) Extension of the known distribution of a novel clade C betacoronavirus in a wildlife host. *Epidemiol Infect,* 147, e169

Shimazaki Y, Yoneya S, Fujita S, Nakashima T, Nabeshima K, Sudoh S, Matsubara K, Okumura N, Kondo H, Nishifuji K, Koba R, Tohya Y (2023) Identification and characterization of the genome of a papillomavirus from skin lesions of four-toed hedgehogs (*Atelerix albiventris*). *Virus Genes,* 59:234-239

Stack MJ, Higgins RJ, Challoner DJ, Gregory MW (1990) Herpesvirus in the liver of a hedgehog (*Erinaceus europaeus*). *Vet Rec,* 127:620-621

Takaki Y, Takami Y, Watanabe T, Nakaya T, Murakoshi F (2020) Molecular identification of *Cryptosporidium* isolates from ill exotic pet animals in Japan including a new subtype in *Cryptosporidium fayeri*. *Vet Parasitol Reg Stud Reports,* 21:100430

Varghese S, Rabkin SD (2002) Oncolytic herpes simplex virus vectors for cancer virotherapy. *Cancer Gene Ther,* 9:967-978

VanDevanter DR, Warrener P, Bennett L, Schultz ER, Coulter S, Garber RL, Rose TM (1996) Detection and analysis of diverse herpesviral species by consensus primer PCR. *J Clin Microbiol,* 34:1666-1671

Weishaupt J, Kolb-Mäurer A, Lempert S, Nenoff P, Uhrlaß S, Hamm H, Goebeler M (2014) A different kind of hedgehog pathway: tinea manus due to *Trichophyton erinacei* transmitted by an African pygmy hedgehog (*Atelerix albiventris*). *Mycoses,* 57:125-127

Wellehan JF, Johnson AJ, Harrach B, Benkö M, Pessier AP, Johnson CM, Garner MM, Childress A, Jacobson ER (2004) Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. *J Virol,* 78:13366–13369

Widén F, Gavier-Widén, D, Nikiila T, Mörner T (1996) Fatal herpesvirus infection in a hedgehog (*Erinaceus europaeus*). *Vet Rec,* 139:237–238

Zhu Y, Xu J, Lian S, Zhang R, Hou J, Wang M, Yan X (2022) Difference analysis between canine adenovirus types 1 and 2. *Front Cell Infect Microbiol* 12:854876

10. PUBLICATION LIST

10.1. Original Papers

Koizumi, I., Shigenaga, C., Kiuno, K., Mitsunaga, S., Nishizato, M., Nochide, K., Hayasaka, D. and Shimoda, H. 2025. Serological evidence of African pygmy hedgehog adenovirus 1 in exotic companion animals. *J. Vet. Med. Sci.* In press.

Inanaga, S., Shimoda, H., Igase, M., Kondo, H., **Koizumi, I.** and Mizuno, T. 2024. Establishment of a histiocytic sarcoma cell line and anti-tumor effect of bortezomib in the African pygmy hedgehog (*Atelerix albiventris*). *J. Vet. Med. Sci.* **86**:833-840.

Koizumi, I., Okumura, N. and Kondo, H. 2023. Pyometra in a sugar glider (*Petaurus breviceps*): surgical treatment and postoperative complications. *J. Vet. Med. Sci.* **85**:1252-1256.

Koizumi, I. 2022. Diagnosis and surgical treatment of colonic obstructive foreign bodies in chinchillas (*Chinchilla lanigera*): a case series (2017-2020). *J. Vet. Med. Sci.* **84**:1121-1127.

Ikeda, M., Kondo, H., Iwata, Y., **Koizumi, I.**, Tsunesumi, N. and Shibuya, H. 2022. Vascular tumors in two Argentine horned frogs (*Ceratophrys ornata*). *J. Herpetol. Med. Surg.* **32**:119–122.

Koizumi, I.†, Tsukada, H.†, Hayasaka, D. and Shimoda, H. 2022. Comprehensive surveillance of virus infection among captive African pygmy hedgehogs in Japan. *Viruses.* **14**:857. (†Equally contributed)

Iwata, Y., Kondo, H., **Koizumi, I.** and Shibuya, H. 2021. Tracheal chondrosarcoma in a blue tegu (*Salvator merianae*). *J. Vet. Med. Sci.* **83**:981-983.

Okumura, N.†, **Koizumi, I.**†, Iwata, Y., Yoneya, S., Koba, R., Kondo, H. and Shibuya, H. 2021. Cutaneous papilloma and multicentric squamous cell carcinoma in four-toed hedgehogs (*Atelerix albiventris*). *J. Vet. Med. Sci.* **83**:1726-1729. (†Equally

contributed)

Koizumi, I. and Kondo, H. 2020. Diagnostic challenge. *J. Exot. Pet. Med.* **32**:43-46

Koizumi, I., Okumura, N. and Kondo, H. 2020. Primary localized histiocytic sarcoma of the lumbar vertebrae in a four-toed hedgehog (*Atelerix albiventris*). *J. Vet. Med. Sci.* **82**:1511-1514.

Koizumi, I., Hernandez-Muguiro, D., Chu, SAA., Stokol, T. and Asakawa, MG. 2020. Clinicopathologic findings of spontaneous leukemia in 9 pet African hedgehogs (*Atelerix Albiventris*). *Front. Vet. Sci.* **7**:54.

Koizumi, I. and Kondo, H. 2019. Clinical management and outcome of four-toed hedgehogs (*Atelerix albiventris*) with histiocytic sarcoma. *J. Vet. Med. Sci.* **81**:545-550.

Koizumi, I. and Kondo, H. 2019. A case of preputial cystostomy performed on an African pygmy hedgehog (*Atelerix albiventris*) with urethral obstruction. *J. Exot. Pet. Med.* **30**:1-6.

Kondo, H., Koizumi, I., Yamamoto, N. and Shibuya, H. 2018. Thyroid adenoma and ectopic thyroid carcinoma in a guinea pig (*Cavia porcellus*). *Comp. Med.* **68**:212-214.

10.2. Reviews

小泉伊織. 2023. カメの 3 大疾患：下部呼吸器感染症（肺炎）. In: エキゾチックアニマルの 3 大疾患診断ガイド, 監修: 小嶋篤史. pp218-226, Eduward Press

小泉伊織. 2022. ウサギ. In: 伴侶動物の処方ガイド, 監修: 馬場健司. pp. 344-362, 緑書房

小泉伊織. 2022. フェレット. In: 伴侶動物の処方ガイド, 監修: 馬場健司. pp. 363-374, 緑書房

小泉伊織. 2022. ハムスター. In: 伴侶動物の処方ガイド, 監修: 馬場健司. pp. 375-388, 緑書房

小泉伊織. 2022. 爬虫類. In: 伴侶動物の処方ガイド, 監修: 馬場健司. pp. 397-410, 緑書房

小泉伊織. 2020. 知っておきたいエキゾチックアニマルの診療: ハリネズミ. Clinic Note. 16:55-71, インターズー

小泉伊織, 田向健一. 2018. ゼロから始める爬虫類診療 (カメ): 第 11 回 画像検査: 消化管造影 X 線検査, 超音波検査. エキゾチック診療. 10:123-131, インターズー

小泉伊織, 田向健一. 2018. ゼロから始める爬虫類診療 (カメ): 第 10 回 画像検査: X 線検査. エキゾチック診療. 10:111-117, インターズー

小泉伊織, 田向健一. 2018. ゼロから始める爬虫類診療 (カメ): 第 9 回 血液検査 (解釈編: 血液生化学検査). エキゾチック診療. 10:100-110, インターズー

小泉伊織. 2017. 爬虫類の輸液療法. エキゾチック診療. 9:17-34, インターズー

小泉伊織. 2017. カメの 3 大疾患 その 1 下部呼吸器感染症 (肺炎). エキゾチック診療. 9:61-68, インターズー

小泉伊織, 田向健一. 2017. ゼロから始める爬虫類診療 (カメ): 第 8 回 血液検査 (解釈編: 血液学的検査). エキゾチック診療. 9:64-71, インターズー

小泉伊織, 田向健一. 2017. ゼロから始める爬虫類診療 (カメ): 第 7 回 血液検査 (採血編). エキゾチック診療. 9:87-94, インターズー

小泉伊織, 田向健一. 2017. ゼロから始める爬虫類診療 (カメ): 第 6 回 一般身体検査 (後編). エキゾチック診療. 9:90-96, インターズー

小泉伊織, 高木佑基, 渡邉岳大, 武山 航, 高見義紀. 2016. 飼育下のヨツユビハリネズミに発生した疾患に関する回顧的調査. エキゾチックペット研究会誌. 18:27-34

小泉伊織, 田向健一. 2016. 爬虫類の皮膚疾患. エキゾチック診療. 8:54-61, インターズー

小泉伊織, 田向健一. 2016. ゼロから始める爬虫類診療 (カメ): 第 5 回 一般身体検査 (前編). エキゾチック診療. 8:79-85, インターズー

小泉伊織, 田向健一. 2016. ゼロから始める爬虫類診療 (カメ): 第 4 回 問診. エキゾチック診療. 8:89-94, インターズー

小泉伊織, 田向健一. 2016. ゼロから始める爬虫類診療 (カメ): 第 3 回 カメの解剖と生理 (後編) . エキゾチック診療. 8:69-74, インターズー

小泉伊織, 田向健一. 2016. ゼロから始める爬虫類診療 (カメ): 第 2 回 カメの解剖と生理 (前編) . エキゾチック診療. 8:105-111, インターズー

小泉伊織. 2016. カメ医療の基礎: 第 6 回. カメの治療: 支持治療 2 強制給餌, 投薬, 注射. Biophilia. 5:73-80, アドスリー

小泉伊織. 2016. カメ医療の基礎: 第 5 回. カメの治療: 支持治療 1 温度, 湿度管理. Biophilia. 5:75-83, アドスリー

小泉伊織, 武山 航, 高見義紀. 2015. フェレットおよび小型エキゾチックアニマルの背臥位保定における心エコー検査の有用性. エキゾチックペット研究会誌. 17:21-30

小泉伊織, 田向健一. 2015. ゼロから始める爬虫類診療 (カメ): 第 1 回 カメの分類学と種, 法規制. エキゾチック診療. 7:3-10, インターズー

小泉伊織. 2015. カメ医療の基礎: 第 4 回 ペットとしてのカメを取り巻く状況と法規制. Biophilia. 4:45-53, アドスリー

小泉伊織. 2015. カメ医療の基礎: 第 3 回 カメの臨床検査 (後編) . Biophilia. 4:131-141, アドスリー

小泉伊織. 2015. カメ医療の基礎: 第 2 回 カメの臨床検査 (前編) . Biophilia. 4:123-131, アドスリー

小泉伊織. 2015. カメ医療の基礎: 第 1 回 カメの解剖, 生理, 飼育概論. Biophilia. 4:60-70, アドスリー

小泉伊織 (訳) , 藤田桂一 (監訳) . 2013. 高齢動物のデンタルケア. J-Vet. 26:78-84, インターズー

10.3. Case reports

小泉伊織. 2023. モルモット (*Cavia porcellus*) のリンパ腫/ 白血病の 1 例. Veterinary

Oncology. 10:112-116, EDUWARD Press

小泉伊織. 2023. フトアゴヒゲトカゲ（*Pogona vitticeps*）の粘液性黒色素胞腫の 1 例. Veterinary Oncology. 10:116-120, EDUWARD Press

小泉伊織, Hernandez-Muguiro, D., Chu, SAA., Stokol, T., 浅川翠. 2020. ペット飼育されている 9 例のヨツユビハリネズミに自然発生した白血病の臨床病理学的所見. 日本獣医エキゾチック動物学会誌. 22:34-42

小泉伊織. 2014. 膀胱亜全切除術を行った膀胱脱のビルマホシガメ（*Geochelone platynotan*）の 1 例. エキゾチックペット研究会誌. 16:20-24

小泉伊織. 2014. 鼠径ヘルニアと腹壁ヘルニアを併発したウサギの 1 例. エキゾチック診療. 6:113-116, インターズー

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Surveillance of virus infection among captive African pygmy hedgehogs in Japan

日本における飼育下ヨツユビハリネズミに感染するウイルスの調査

エキゾチックアニマルとは犬と猫以外の伴侶動物を示す用語であり、広範な動物種を含む。日本では以前から多様なエキゾチックアニマルが飼育されているが、中でもヨツユビハリネズミは近年非常に一般的に飼育されている動物種である。ヨツユビハリネズミに発生する疾患に関するデータベースは徐々に大きくなりつつあるが、その本質的な病因は詳しく明らかにされていない。本研究は全 2 章からなり、第 1 章ではヨツユビハリネズミの疾患とウイルス感染の関係性を明らかにすることを目的としている。第 2 章では、第 1 章の研究過程で明らかになった飼育下ヨツユビハリネズミにおける African pygmy hedgehog adenovirus 1 感染に関して、日本で飼育される様々なエキゾチックアニマルにおけるその蔓延を明らかにすることを通じて、本ウイルスの生態を解析することを目的としている。

第 1 章　日本における飼育下ヨツユビハリネズミのウイルス感染に関する網羅的調査

ヨツユビハリネズミに感染するウイルスはわずかながら過去に報告されているが、疾患との関連性に関してはほとんどわかっていない。また、そのほとんどが少数例における症例報告であることから、より大規模な調査が望まれる。本章では、本種におけるアデノウイルス、ヘルペスウイルス、コロナウイルスの感染を調査し、その疾患との関連性を評価した。

動物病院に来院したヨツユビハリネズミから口腔スワブを計 150 例採取し、そのうち 50 例についてユニバーサルプライマーを用いて各ウイルスゲノムを検出した。その結果、新規のベータヘルペスウイルスが 50 例中 2 例（4%）で検出され、Atelerix albiventris herpesvirus 1（AAHeV）と命名された。また、既報である African pygmy hedgehog adenovirus 1（AhAdV-1）が 50 例中 2 例（4%）で検出された。これらのウイルスに対する特異的なプライマーを用いた nested PCR の結果、AAHeV 感染は 150 例中 14 例(9.3%)で認められ、AhAdV-1 感染は 150 例中 3 例（2.0%）で認められた。

各症例の臨床情報を用いてこれらのウイルスと疾患の関連性を探索したところ、神経学的異常を呈するヨツユビハリネズミでは AAHeV 感染率が有意に高かった（p=0.016）。ヨツユビハリネズミにおいて、wobbly hedgehog syndorome を含む神経学的疾患は一般的に

認められるが、その明確な発生原因はわかっていない。本研究結果から、AAHeV 感染はヨツユビハリネズミの神経学的疾患発生における重要な因子である可能性が考えられた。また、AAHeV に感染した個体において、腫瘍性疾患の発生および既往が認められず、AAHeV 感染群では有意にその発生率が低かった（p=0.006）。腫瘍性疾患はヨツユビハリネズミに非常に好発し、そのほとんどが悪性であることからヨツユビハリネズミの最も一般的な死因である。AAHeV 感染はヨツユビハリネズミの腫瘍発生に対して抑制的に作用する可能性が示唆された。

AhAdV-1 感染が認められた 3 例のうち、2 例では不顕性感染を呈していた。これまでに、呼吸器症状を主体とする AhAdV-1 感染症が非常に広範な種において報告されてきたが、本研究結果は日本の飼育動物における AhAdV-1 の水面下での伝播を評価する必要性を喚起するものであると考えられた。

第 2 章　日本飼育下エキゾチックアニマルにおける AhAdV-1 抗体保有状況調査

AhAdV-1 感染症は、重度の気管支肺炎によって斃死したヨツユビハリネズミから本邦で 2019 年に分離された新規アデノウィルスによる新興感染症である。本ウイルスは、食肉目に属するスカンク、アライグマ、ミーアキャット、ビントロング、フェレット、霊長目に属するコモンマーモセット、齧歯目に属するヤマアラシなど、目を超えた非常に広範な種から分離されており、その蔓延が危惧されている。日本の伴侶動物における AhAdV-1 の大規模な疫学的調査はなされていない。本邦で飼育されているエキゾチックアニマルは、非常に多様な動物種を含むことから、AhAdV-1 の宿主域、各動物種における感受性と病原性、および近年の日本の蔓延状況を評価するうえで理想的な研究対象であると考えられる。

筆者の一般開業病院（福岡県）に来院した計 17 種のエキゾチックアニマルから採取した計 446 症例の血清を用いて、プラーク減数ウイルス中和試験によって AhAdV-1 抗体保有状況調査を実施した。AhAdV-1 抗体は計 9 種の動物種でその保有が確認されたが、そのうち保有率が特に高かった動物種はミーアキャット、フェレット、ヨツユビハリネズミであり、それぞれ 17 例中 7 例（41%）、73 例中 45 例（62%）、103 例中 65 例（63%）であった。これらの動物の抗体保有率および抗体価はそのほかの動物種に比べて有意に高かった。フェレットとヨツユビハリネズミにおいては、呼吸器症状の既往がある群では AhAdV-1 抗体保有率が有意に高かったことから、AhAdV-1 感染はこれら 2 種において呼吸器病原性を呈する可能性が示唆された。

AhAdV-1 抗体陽性であった動物の飼育環境や同居動物を調査したところ、すべての動物は室内飼育であり、8 家庭で飼育されている 19 個体がほかの動物と同居していた。このうち、4 家庭では同居動物すべてが AhAdV-1 抗体陽性であったが、一方で 4 家庭では AhAdV-1 抗体陽性個体と陰性個体が同居していた。この結果から、同一家庭内で飼育されている伴侶動物同士の接触は AhAdV-1 感染の主経路ではない可能性が考えられた。AhAdV-1 抗体保有率が特に高かったミーアキャット、フェレット、ヨツユビハリネズミは海外繁殖された個体が日本国内に輸入されており、一方で抗体保有率が低かったそのほかの動物種は日本国内で繁殖されていることから、海外の繁殖施設における AhAdV-1 感染が主たる感染経路として推測された。

AhAdV-1 抗体陽性であった動物のうち、抗体価が 1:2560 以上であった 14 例のフェレッ

トと 3 例のミーアキャットにおいて、canine adenovirus type 1 （CAdV-1）抗体価を調べたところ、12 例のフェレットとすべてのミーアキャットで検出限界以下であった。このことから、本研究の AhAdV-1 ウイルス中和試験は特異的にその抗体を検出していることが確認された。また、複数回検体を採取した動物のうち、AhAdV 抗体陽性であった 8 例のフェレットの抗体価を継続的に評価したところ、すべての動物で 1 年以上の期間抗体が検出された。

本研究から、日本国内飼育エキゾチックアニマルにおける AhAdV-1 の蔓延が示唆され、フェレットとヨツユビハリネズミは呼吸器疾患を主体とした健康被害が懸念された。AhAdV-1 の宿主域とそれぞれの動物における病原性に関して、レセプター親和性の種差に着目したさらなる分子生物学的研究が必要であり、潜在的なズーノーシスとしてリスクを評価することが今後の課題である。