CASE REPORT

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Cross-Reactivity of Porcine Immunoglobulin A Antibodies with Fecal Immunoglobulins of Wild Boar (*Sus scrofa*) and Other Animal Species

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Fecal samples obtained from wild boar habitats are useful for the surveillance of diseases in wild boar populations; however, it is difficult to determine the species of origin of feces collected in natural habitats. In this study, a fecal IgA ELISA was evaluated as a method for identifying the porcine species from fecal samples. Both domestic pigs (*Sus scrofa domestica*) and wild boars (*Sus scrofa coreanus*) showed significantly higher levels of fecal IgA than other animal species. Additionally, age dependent changes in the level of Ig A in wild boars and domestic pigs were identified; Titers of Ig A were highest in suckling period and lowest in weanling period.

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INTRODUCTION

Wild boars can be a reservoir of agents that are infectious to domestic pigs and other livestock (1). The disease status of wild boars has been surveyed using blood samples collected from animals shot during the hunting season (2,3). However, seroprevalence analysis in hunted wild boars is difficult owing to the limited hunting area, the restricted hunting season, and a biased age distribution (2,4). Testing feces obtained from the habitat of wild boars is an alternative to testing serum samples, since fecal samples are easy to collect in large quantities and can also be used for the direct detection of bacteria, viruses, and parasites (4).

Before testing fecal samples for a specific disease, gross examination is commonly used as a method to screen the species of origin. Additionally, the sequence of cytochrome b or chromosomes carrying endogenous retroviruses has been used for scientific proof of the animals' identification (5,6). However, gross examination is not accurate, and the gene sequencing method is complicated and costly, whereas ELISA can be used to screen a large number of samples simultaneously. In the present study, secretory fecal IgA was tested by ELISA as an alternative method for distinguishing the species origin of fecal samples from wild boars (*Sus scrofa coreanus*) and domestic pigs (*Sus scrofa domestica*). IgA was selected as a target protein owing to its presence in large amounts in feces (7) and its conserved domains that allow for comparison across a broad range of species (8,9).

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MATERIALS AND METHODS

Sample collection

Fecal samples from 22 different animals in Korea, including wild boars and domestic pigs, were tested (Table I) for their cross-reactivity with the porcine fecal IgA antibody. Forty fresh fecal samples of farmed wild boars from one farm were collected to evaluate IgA differences in wild boars of different ages. In addition, 50 fecal samples were collected from domestic pigs of different age groups on a commercial pig farm. The samples were transported and stored at 4°C until analyzed.

Quantitative ELISA

Each fecal sample was diluted to a concentration of 0.5 g/mL with phosphate-buffered saline containing 0.05% Tween 20 and 1% BSA, vortexed, and centrifuged at 1,500 *g* for 20 min at 4°C. The supernatants were centrifuged at 16,000 *g* for 15 min to remove large solid particles and then transferred into Eppendorf tubes. Fecal samples from the wild boars and domestic pigs were diluted 200-fold (final dilution) and tested in triplicate using the Pig IgA ELISA Quantitation Set (Bethyl Laboratories, Montgomery, TX, USA). The IgA concentrations were quantified according to the following age groups: 1 day~1 month, 1~3 months, 3~6 months, 6 months~1 year, and >1 year (Table II). The average optical density at 450 nm (OD_{450}) of 1% BSA as a negative control was subtracted from the average OD_{450} values of all other fecal samples. Twelve fecal samples were collected <1 h after excretion from four different individuals of 20 different animals (3 orders, 8 families, 15 genera, 16 species, 19 subspecies) in Seoul Grand Park and analyzed in the same way as described above. Data are presented as the mean and standard deviation (SD).

Statistical analysis

The two-tailed Student's t-test was used to compare differences in the OD_{450} values between *Sus scrofa* and the other animals. The genetic distance based on the cytochrome b peptide sequence from *Sus scrofa* was calculated for each subspecies using the MEGA 6 software with the Jones–Taylor–Thornton amino acid substitution model with gamma distribution (Table III).

RESULTS AND DISCUSSION

There were no differences in OD_{450} values between wild boars and domestic pigs (p=0.000) in each age group. Except for the Manchurian leopard cat (*Prionailurus bengalensis* Manchuria), all fecal samples from the

Table I. Biological classification of the 22 different animals, the fecal samples of which were used in this study

Order	Family	Subfamily	Genus	Scientific name	Common name
Artiodactyla	Bovidae	Caprinae	Capra	*Capra hircus coreanae*	Korean native goats
		Caprinae	Ovis	*Ovis aries*	Sheep
	Cervidae	Odocoileinae	Capreolus	*Capreolus pygargus tianschanicus*	Siberian roe deer
		Cervinae	Cervus	*Cervus elaphus*	Red deer
				Cervus nippon hortulorum	Manchurian sika deer
				Cervus nippon nippon	Japanese sika deer
				Cervus nippon yesoensis	Yeso sika dear
		Hydropotinae	Hydropotes	*Hydropotes inermis argyropus*	Korean water deer
	Suidae		Sus	*Sus scrofa coreanus*	Korean wild boar
				Sus scrofa domestica	Domestic pig
Carnivora	Canidae	Caninae	Canis	*Canis lupus familiaris*	Sapsaree
				Canis lupus familiaris	Poongsan dog
				Canis lupus chanco	Korean grey wolf
			Nyctereutes	*Nyctereutes procyonoides*	Raccoon dog
			Vulpes	*Vulpes vulpes peculiosa*	Fox
	Mustelidae	Lutrinae	Lutra	*Lutra lutra lutra*	Eurasian otter
		Mustelinae	Martes	*Martes flavigula*	Yellow throated marten
		Melinae	Meles	*Meles leucurus*	Asian badger
	Felidae	Felinae	Prionailurus	*Prionailurus bengalensis manchuria*	Manchuria leopard cat
	Ursidae	Ursinae	Ursus	*Ursus arctos lasiotus*	Brown bear
				Ursus thibetanususs uricus	Asiatic black bear
Rodentia	Myocastoridae	Myocastorinae	Myocastor	*Myocastor coypus*	Nutria

Table II. Concentration of IgA in different age groups of wild boars and domestic pigs

Age of wild boar (months)	Wild boar (*Sus scrofa coreanus*)			Domestic pig (*Sus scrofa domestica*)		
	Number of samples	Mean±Standard deviation		Number of samples	Mean±Standard deviation	
		Porcine-IgA ELISA OD_{450}	Concentration of fecal IgA (μg/g of fresh fecal sample)		Porcine-IgA ELISA (OD_{450})	Concentration of fecal IgA (μg/g of fresh fecal sample)
<1	6	2.8604±0.0788	184.81±17.56	10	2.8404±0.1728	183.72±32.37
1-3	8	0.0900±0.0300	4.07±0.59	10	0.1453±0.0285	5.17±0.58
3-6	9	0.8369±0.2640	22.97±8.86	10	0.9425±0.2499	24.99±8.67
6-12	7	2.0857±0.4843	91.83±40.99	10	2.1579±0.4063	95.32±36.92
>12	10	2.5186±0.3680	136.71±44.16	10	2.5786±0.3680	146.08±47.81

In the suckling period (<1 month), the fecal IgA concentration peaked, followed by a decrease during the weanling period (1~3 months). The level of IgA began to rise again after the weanling period. No statistically significant differences in OD_{450} values between wild boars and domestic pigs (p=0.000) of each age group was detected.

Table III. Cross-reactivity of porcine IgA with immunoglobulin from 20 animal species in Korea

Scientific name	Common name	Genetic distance from *Sus scrofa*[a]	Mean±Standard deviation	
			Porcine-IgA ELISA (OD_{450})[b]	Concentration of porcine IgA-like molecules (μg/g fresh fecal sample)
Canis lupus chanco	Korean grey wolf	0.149	0.0060±0.0058	0.8613±0.1178
Canis lupus familiaris	Sapsaree	0.139	0.0055±0.0036	0.8507±0.0727
Canis lupus familiaris	Poongsan dog	NA	0.0075±0.0032	0.8912±0.0633
Capra hircus coreanae	Korean native goats	0.138	0.0115±0.0022	0.9690±0.0440
Capreolus pygargus tianschanicus	Siberian roe deer	0.115	0.0090±0.0051	0.9204±0.1019
Cervus elaphus	Red deer	0.108	0.0063±0.0044	0.8669±0.0874
Cervus nippon hortulorum	Manchurian sika deer	0.102	0.0063±0.0041	0.8677±0.0814
Cervus nippon nippon	Japanese sika deer	0.125	0.0082±0.0028	0.9033±0.0562
Cervus nippon yesoensis	Yeso sika dear	0.108	0.0090±0.0037	0.9204±0.0739
Hydropotes inermis argyropus	Korean water deer	0.114	0.0020±0.0048	0.7836±0.0946
Lutra lutra lutra	Eurasian otter	0.153	0.0077±0.0040	0.8944±0.0780
Martes flavigula	Yellow throated marten	0.163	0.0143±0.0116	1.0232±0.2279
Meles leucurus	Asian badger	0.137	0.0145±0.0048	1.0268±0.0941
Myocastor coypus	Nutria	0.207	0.0128±0.0133	0.9933±0.2611
Nyctereutes procyonoides	Raccoon dog	0.155	0.0090±0.0024	0.9203±0.0477
Ovis aries	Sheep	0.130	0.0095±0.0039	0.9301±0.0773
Prionailurus bengalensis manchuria	Manchuria leopard cat	0.151	0.1197±0.0444	4.1388±0.9125
Ursus arctos lasiotus	Brown bear	0.143	0.0075±0.0040	0.0889±0.0810
Ursus thibetanususs uricus	Asiatic black bear	0.153	0.0096±0.0092	0.9312±0.1803
Vulpes vulpes peculiosa	Fox	0.159	0.0084±0.0055	0.9073±0.1073

[a]*Cytochrome b* sequences were downloaded from GenBank under the following accession numbers: *Canis lupus chanco* (NC_010340), *Canis lupus familiaris breed Sapsaree* (AY656755), *Capra hircus* (EU259132), *Capreolus pygargus tianschanicus* (EF139144), *Cervus elaphus* (JF489133), *Cervus Nippon hortulorum* (GU377266), *Cervus Nippon Nippon* (AB021093), *Cervus Nippon yesoensis* (AB160860), *Hydropotes inermis argyropus* (EF139155), *Lutra lutra* (EF689067), *Martes flavigula* (EF987749), *Meles leucurus* (HQ711951), *Myocastor coypus* (AF422919), *Nyctereutes procyonoides* (NC_013700), *Ovis aries* (DQ903227), *Prionailurus bengalensis* (AB210238), *Sus scrofa coreanus* (AY692029), *Ursus arctos* (NC_003427), *Ursus thibetanususs uricus* (AY522430), *Vulpes vulpes* (AY928669). NA, Not available.
[b]Twelve samples were tested from each species. OD_{450}, Optical density at 450 nm.

other animal species had low reactivity with porcine IgA antibodies, showing statistically significant difference with that of wild boar in all age groups (p<0.001). Despite its similar genetic distance from *Sus scrofa*, the OD_{450} of the Manchurian leopard cat was 10 times higher than that of the other animal species (Table III). This

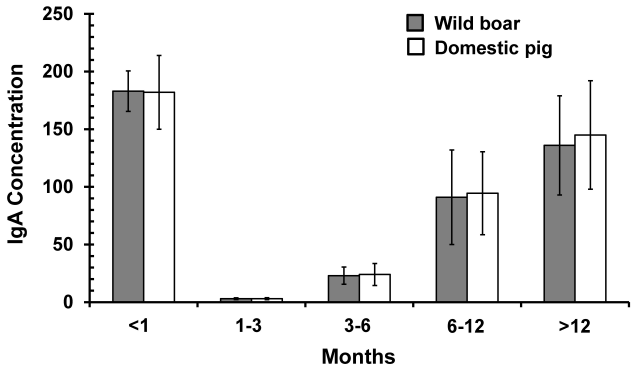

Figure 1. Fecal IgA level of wild boars and domestic pigs in each age group.

result contradicts the finding of the previous report (10) showing the significant correlation between cytochrome b sequence and cross-reactivity with dolphin Ig G antibodies. However, there has been no known report that the structure of Ig A is evolutionarily related with cytochrome b sequence. Therefore, to elucidate the high affinity of pig Ig A antibodies with immunoglobulin-like molecules of Manchurian leopard cat, further studies about the genetic relationship between Ig A and cytochrome b sequence will be needed.

The fecal IgA concentrations in the suckling period were high, whereas they were lower in weanling pigs (1~3 months old) and higher again in pigs older than 6 months (Fig. 1, Table II), which agrees with previous reports of lower porcine secretory fecal IgA during the weanling period (7). Additionally, it was identified that the OD_{450} of the weanling pigs did not show a statistical difference with that of Manchurian leopard cat (Table I, p=0.0652). For this reason, in case of a fecal sample not showing statistically significant difference in OD_{450} with that of weanling pigs, we cannot convince it as droppings from wild boar. However, the IgA concentration in fecal samples of wild boars of all ages, except those 1~3 months old, was distinguishable from that of all wild animal species used for comparison in this paper, which means that the porcine IgA ELISA could be a useful method for differentiating wild boar feces from the feces of other wild animal species.

Fecal IgM concentrations are higher than IgA concentrations in weanling pigs (7) and could therefore be more useful than IgA for species identification in pigs and wild boars at 1~6 months of age. Conversely, the low level of fecal IgA in animals at 1~3 months of age could be useful to differentiate feces of the weaning period from those of the adult period. The prevalence of many infectious diseases in wild boar populations depends on the density and abundance of juveniles (11). In this situation, the population structure of weaners, as estimated from the IgA concentration, may contribute to understanding the disease status of wild boars.

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CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

REFERENCES

1. Meng, X. J., D. S. Lindsay, and N. Sriranganathan. 2009. Wild boars as sources for infectious diseases in livestock and humans. *Philos. Trans. R. Soc. Lond. B Biol. Sci*. 364: 2697-2707.
2. Albina, E., A. Mesplede, G. Chenut, M. F. Le Potier, G. Bourbao, G. S. Le, and Y. Leforban. 2000. A serological survey on classical swine fever (CSF), Aujeszky's disease (AD) and porcine reproductive and respiratory syndrome (PRRS) virus infections in French wild boars from 1991 to 1998. *Vet. Microbiol*. 77: 43-57.
3. Jacobson, M., L. M. Gerth, N. Holmgren, N. Lundeheim, and C. Fellstrom. 2005. The prevalences of Brachyspira spp. and Lawsonia intracellularis in Swedish piglet producing herds and wild boar population. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 52: 386-391.
4. Seo, S., S. Sunwoo, B. Hyun, and Y. S. Lyoo. 2012. Detection of antibodies against classical swine fever virus in fecal samples from wild boar. *Vet. Microbiol*. 161: 218-221.
5. Hsieh, H. M., H. L. Chiang, L. C. Tsai, S. Y. Lai, N. E. Huang, A. Linacre, and J. C. Lee. 2001. Cytochrome b gene for species identification of the conservation animals. *Forensic Sci. Int*. 122: 7-18.
6. Nikitin, S. V., N. S. Iudin, S. P. Kniazev, R. B. Aitnazarov, V. F. Kobzev, V. A. Bekenev, M. A. Savvina, and V. I. Ermolaev. 2008. Frequency of chromosomes carrying endogenous retroviruses in the populations of domestic pig and wild boar. *Genetika* 44: 789-797.
7. Franz, J. and G. Corthier. 1981. Measurement of porcine faecal IgA, IgG and IgM levels by a competitive enzyme-linked

immunosorbent assay. *Clin*. *Exp*. *Immunol*. 43: 645-649.
8. Asada, Y., Y. Kawamoto, T. Shotake, and K. Terao. 2002. Molecular evolution of IgG subclass among nonhuman primates: implication of differences in antigenic determinants among Apes. *Primate*. 43: 343-349.
9. Omatsu, T., Y. Ishii, S. Kyuwa, E. G. Milanda, K. Terao, and Y. Yoshikawa. 2003. Molecular evolution inferred from immunological cross-reactivity of immunoglobulin G among Chiroptera and closely related species. *Exp*. *Anim*. 52: 425-428.
10. Nollens, H. H., C. Ruiz, M. T. Walsh, F. M. Gulland, G. Bossart, E. D. Jensen, J. F. McBain, and J. F. Wellehan. 2008. Cross-reactivity between immunoglobulin G antibodies of whales and dolphins correlates with evolutionary distance. *Clin*. *Vaccine Immunol*. 15: 1547-1554.
11. Kern, B., K. R. Depner, W. Letz, M. Rott, S. Thalheim, B. Nitschke, R. Plagemann, and B. Liess. 1999. Incidence of classical swine fever (CSF) in wild boar in a densely populated area indicating CSF virus persistence as a mechanism for virus perpetuation. *Zentralbl*. *Veterinarmed*. B 46: 63-67.