

Seroepidemiological Survey of Aujeszky's Disease Virus in Wild Boar (*Sus scrofa*) and Raccoon Dogs (*Nyctereutes procyonoides koreensis*) in Korea

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Aujeszky's disease caused by Aujeszky's disease virus (ADV) is one of the most important diseases in the pig industry. In this study, we conducted a seroepidemiological survey of ADV in wild boars and raccoon dogs in South Korea. In total, 217 wild boar sera collected between March and August 2013, and 96 raccoon dogs between 2011 and 2012 were screened for the presence of antibodies against ADV. The sero-positive rates in wild boars and raccoon dogs tested for ADV were found to be 3.55% (8/225) and 0% (0/96), respectively. The presence of virus neutralization antibody titer against ADV means that small number of wild boars was infected with ADV and AD may be circulated continuously in Korean wild boar populations, and that wild boars may act as a potential reservoir of ADV. Therefore, to achieve the declaration of AD free, effective preventive measures to block transmission of AD should be taken to the wild boars.

Key Words: Sero-prevalence, Aujeszky's disease virus, Wild boar, Raccoon dogs

INTRODUCTION

Aujeszky's disease (AD) known as pseudorabies is caused by suid herpesvirus-1, a member of the subfamily *Alphaherpes virinae* that possesses a positive double strand DNA genome about 145 kb in length (1). The virulent Aujeszky's disease virus (ADV) has an ability to infect several kinds of organs in variety of animals including cattle, sheep, goat, raccoon, opossum, rat and mouse. ADV has a relation mainly to pigs considered as natural host, but remains latently infected after recovery. Symptoms of AD

depend on pig's age; piglets under 2 weeks old died from encephalitis showing hyperthermia, severe neurological disorders. Grower-finisher pigs infected with ADV develop mainly respiratory signs and the naturally infected sows in the second or third trimester manifest clinical symptoms of reproductive failure such as abortion, still birth and weak piglets (2).

The declaration of AD free allows swine industry to grow up continuously and pork producers to sell their products internationally. So, ADV has been eliminated from domestic pigs in many European countries such as Denmark, Finland, France, Germany, Hungary, Luxembourg, the Netherlands,

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Sweden, Switzerland, Scotland and Wales after conducting national AD eradication program (3). In July 1987, the first outbreak of AD was identified in Korean swine farm located in Yangsan district of Gyoungnam Province. ADV infection was confirmed by isolating virus from the nasal fluid of the infected pigs. All 954 pigs were slaughtered and buried from the infected farm and neighboring farms. The ADV isolate named as Yangsan strain was characterized by restriction endonuclease analysis and banding patterns of the isolate were very similar to that of the Taiwanese ADV strain, suggesting that the ADV was probably introduced from Taiwan (4). As AD caused economic loss in Korean swine industry, Veterinary Authority made a decision to launched AD-eradication program with inactivated marker vaccine and stamping out policy since 2000. This intensive control program against AD has resulted in dramatic decrease of incidence rate in domestic pigs. Even though this kind of strict program to eliminate the disease in domestic pigs is carried out in many countries, ADV infections seem to be widespread in wild boars (*Sus scrofa*) worldwide (5~7).

The ADV infection can be transmitted by several kinds of routes and is spread by direct contact with infected animals and fomites including contaminated bedding, carcasses of raccoons (8). The mucosae of oral cavity and nasal discharge from infected animals are the main carriers. The ADV infection in wild boar populations has been reported through serological evidence in the USA and in many European countries (9, 10). Transmission of ADV to wild carnivores via direct contact with infected animals has been reported in Austria and USA, which indicates that ADV is widespread with easy among wild boars (11). Wild animals such as wild boars and raccoon dogs (*Nyctereutes procyonoides koreensis*) as a potential reservoir or transmitter can play a key role transmitting ADV to domestic pigs. The AD status of a country or zone can be divided into three statuses, i.e., AD free country, AD provisionally country and AD infected country in accordance with the Terrestrial Code of OIE (12). A country may be considered free from the disease if the disease has not been reported at least for 25 years. If AD has been reported in the country, a surveillance and control program should be carried out to

detect every infected establishment and eradicate AD completely. In AD free country with wild and feral pigs, government should take measures to prevent any transmission of the ADV from wild and feral pigs to domestic pigs. Sero-surveillance for the presence of antibodies with respect to ADV is important for the maintenance of an AD free status and can provide determination of the AD status of a country. Although serological evidence for ADV infections in wild animals has been reported in many countries, sero-surveillance of ADV in wild animals has not been performed in Korea yet. In this study, sera from wild boars and raccoon dogs were screened for the presence of antibodies against ADV using virus neutralization (VN) assay.

MATERIALS AND METHODS

Blood samples from wild boars and raccoon dogs

Wild boars were captured for the annual surveillance of classical swine fever virus from all provinces in Korea. In total, 225 blood samples were randomly selected from wild boars hunted from March to August 2013. For the seroprevalence survey, blood samples were obtained from 96 wild raccoon dogs that were 0.6 month to 2 years old. Twenty two raccoon dogs were being housed in Gyeonggi-do rescue centers in 2011 and 74 samples were obtained from wild raccoon dogs in Gangwon-do in 2012. The blood was taken from cephalic vein. Clotted blood samples were centrifuged ($3,000 \times g$, 10 min), and sera were stored at -20°C until use.

Cell and virus

Vero cells (Africa green monkey kidney cell line: ATCC CCL81) were maintained in α -minimum essential medium (MEM; Gibco BRL, USA) containing 5% fetal bovine serum (Gibco BRL, USA), penicillin (100 IU/ml), streptomycin (100 $\mu\text{g/ml}$), and amphotericin B (0.25 $\mu\text{g/ml}$) at 37°C in a 5% CO_2 incubator. The ADV used for VN test was Yangsan strain, which was isolated from pig in the July 1987. The virus was propagated in Vero cells cultivated in α -MEM. Uninfected cell cultures were used as negative cell controls.

Virus neutralization (VN) test

The VN test was carried out in 96-well microplates using Vero cells. Each wild boar serum sample as well as the positive and negative controls were distributed in two consecutive wells, and then serially diluted by two fold. The ADV (Yangsan strain) containing around 100 TCID₅₀/50 µl was then added to each well. After 60 min of incubation at 37°C, a volume of 50 µl of Vero cells suspension containing 4×10^5 cells/ml was added to each well. The microplates were incubated for 72 h in a humidified incubator with 5% CO₂ at 37°C and microscopic evaluation of virus-induced cytopathic effect (CPE) was conducted. The titers of serum samples were expressed as the reciprocal of the highest serum dilution completely inhibiting CPE in the wells. The serum dilution ranged from 1:2 to 1:256. An antibody titer of > 1:2 was considered positive.

RESULTS

The results of sero-surveillance of ADV were shown in Table 1 and 2. The positive rate against ADV in 225 serum samples collected from wild boars was found to be 3.55% (8/225). The regional distribution of the sero-prevalence was negative in Ganwon and Gyeonggi provinces, 1.64%

(1/61) in Gyeongsang-do, 8.57% (6/70) in Chungcheong-do, 8.33% (1/12) in Jeolla-do. Five wild boars from the Chungju city and three from Gongju, Geochang and Gumi city showed positive reaction to ADV. The VN titer in 8 positive sera ranged from 1:4 to 1:128 (Table 3). All serum samples of Korean wild raccoon dogs were negative (0/96) to ADV.

DISCUSSION

Since the first outbreak of AD in Korea was identified in 1987, a lot of ADV infection occurred in the several provinces. A nationwide survey conducted in 2001 showed that 9,780 pigs from 278 cases had serological evidence of ADV infection in four Provinces according to information provided by the KAHIS program (www.kahis.go.kr) of the Animals and Plant Quarantine Agency (QIA). In detail regional outbreaks were 27 cases in Gyeonggi, 4 cases in Gangwon, 3 cases in Chungnam and 244 cases in Jeonbuk

Table 1. Seroprevalence rate of ADV by neutralization assay in blood samples of wild boars and raccoon dogs

Animals	Positive	Negative	Positive rate (%)
Wild boars	8	217	3.55 (8/225)
Raccoon dogs	0	96	0 (0/96)

Table 2. Regional distribution of Aujeszky's disease virus antibody from Korean wild boars and raccoon dogs

Animals	Province					Total
	GW*	GG	GS	CC	JL	
Wild boars (%)	0/37 (0)	0/45 (0)	1/61 (1.64)	6/70 (8.57)	1/12 (8.33)	8/225 (3.55)
Raccoon dogs (%)	0/74 (0)	0/22 (0)	–	–	–	0/96 (0)

*GW: Ganwon-do, GG: Gyeonggi-do, GS: Gyeongsang-do, CC: Chungcheong-do, and JL: Jeolla-do.

Table 3. Distribution of virus neutralizing antibody titers against ADV in Korean wild boars and raccoon dogs

Animals	No. of samples	Virus neutralizing antibody titer against ADV							
		<1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Wild boars	225	217	0	2	1	2	0	1	2
Raccoon dogs	96	96	0	0	0	0	0	0	0

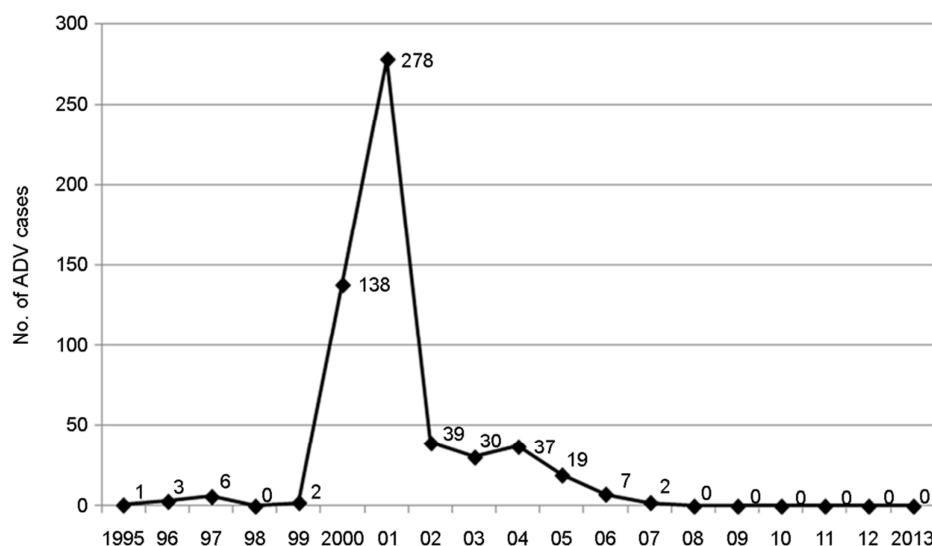


Figure 1. The number of Aujeszky's disease cases in Korea between 1995 and 2013.

Provinces. Korean Veterinary Authority made a decision to carry out AD-eradication program in domestic pigs using eradication measures such as the restriction of movement of pigs on infected farms, the slaughter of pigs showing positive for ADV gE, and the use of gE-deleted marker vaccine in the ADV risk region. As the result of conducting AD-eradication program, ADV infection case dramatically has decreased in domestic pigs and there has been no ADV case since 2008 (Fig. 1).

ADV infection has been reported in many countries, and its sero-positivity has been estimated to range from 30% in Czech Republic to 36% in Spain (9, 13, 14). Ruiz-Fons *et al.* (15) reported that AD viral detection prevalence was approximately 30% in European wild boars, suggesting that it is important to consider wild boar's role as reservoir for the domestic pigs. Since AD is included as an OIE notifiable disease and has not been reported in Korean domestic pigs since 2008, we conducted the sero-surveillance of wild animals with ADV by performing a VN test on sera from wild boars and raccoon dogs (12). Our sero-prevalence data revealed that the overall sero-positive rates against ADV in wild boars and raccoon dogs were very low (3.55%) and negative, respectively. In addition, the regional sero-prevalence against ADV ranged from 8.57% to negative.

This result deduces that wild boars infected with wild ADV in Chungcheong-do can transmit pathogen to animals residing in other regions. On the other hand, it is noted that ADV was transmitted by contact to uninfected raccoons and all raccoons infected with virulent ADV strain died (16). Weigel *et al.* reported that raccoons exposed to ADV gene deleted vaccine result in an immune response (17). In our study, raccoon dogs captured from Gyeonggi and Gangwon provinces did not show any sero-positivity against ADV, assuming that raccoon dogs did not contact the infected wild boars directly.

In this sero-prevalence of ADV it is necessary to choose an antibody titer level indicating infection. Our results show that eight wild boars had VN titer ranging from 1:4 to 1:128. It has been accepted that animals with natural infection develop VN titer level higher than 1:32 (18). However, since 2010 domestic pigs in Korea have not been inoculated with gE-deleted vaccine, which allows us to differentiate between infected and vaccinated pigs. Therefore, it is possible to assume that wild boars with antibody titer more than 1:2 were estimated as ADV infected. OIE also indicates that neutralization at any dilution (even undiluted, equivalent to a final dilution of 1:2) is considered to be positive (12).

In order to meet requirements of OIE provisions associated

with AD free country, Veterinary Authorities of each nation should conduct as follows. To begin with, AD vaccination should be prohibited for all domestic and captive wild boars in the country at least two years. Secondly, sero-surveillance directed at the detection of antibodies to the whole virus should be conducted on a representative sample of all pigs. If AD has been reported in the country, both sero-survey and control program should be performed to detect infection and eradicate AD. Finally, if the country has wild and feral pigs, preventive measures should be implemented to block any transmission of ADV from wild boars to domestic pigs.

In conclusion, antibodies against ADV in Korean wild boars were identified, indicating that preventive measures be taken to the wild boars. In addition to banning the movement of wild boars from the AD risk regions, oral immunization with bait vaccine containing gE deleted vaccine strain may be an option (19). Therefore, it is necessary to develop bait vaccine for AD to immunize wild animals including wild boars in the near future.

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