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Serological survey of infectious agents in domesticated boars

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Abstract

A serological survey was performed to establish basic data for the prevalence of antibodies to some major diseases of domesticated boar serum samples from January to December 2000. Sera collected in breeding farms in Gyeongbuk province were tested for Aujeszky's disease virus(ADV), Porcine reproductive and respiratory syndrome virus(PRRSV), Porcine parvovirus(PPV), Japanese encephalitis virus(JEV), *Bordetella bronchiseptica(B bronchiseptica)*, *Mycoplasma hyopneumoniae(M hyopneumoniae)*, *Actinobacillus pleuropneumoniae(A pleuropneumoniae ;* APP), *Toxoplasma*, and *Brucella*.

There was no antibody to ADV in domesticated boars serum samples detected by Anti-ADV-gpI assay kit. Sero-positive samples to PRRS by IFA were 0.9%(3/330). The HI titers to PPV ranged variously from less than 10 to over 1,280. Two hundred ninety-four out of 330 tested sera showed HI titer of less than 10. In HI test to JEV, 90.3% of the sera (298/330) were below 10.

The majority of the serum samples had low prevalence of the antibody *B bronchiseptica*. ELISA titers to *M hyopneumoniae* ranged variously from $\leq$10 to $\geq$1,280. Antibody titers to *A pleuropneumoniae* type 2(APP2) and type 5(APP5) were 58.2% and 52.7%, respectively, and the tested samples showing ELISA antibody titers of less than 20. There was no significant geographical difference between APP2 and APP5 in this study. In the antibody test of *Toxoplasma*, 11.5%(38/330) were positive and samples were all negative in sera test of *Brucella*.

Key words : Serological survey, Domesticated boars, Serum, Antibody

Introduction

In the swine industry where breeding size becomes larger and multi-dense breeding is generalized, the decrease of productivity due to contagious diseases has become a very important matter. Thus to reduce damage and to gain competitive power, the hygienic

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management of pigs and the systematic prevention and treatment against contagious diseases are in high demand. To prevent the influx of the foreign malignant animal disease and the domestic diffusion of new diseases, it is necessary to intercept the agent of infection. In addition, it is necessary to execute the vaccination against the disease where prevention is possible, and to raise resistance of the disease with improvements to the environment and feed management. But given the case of the Gyeongbuk areas, the treatment against a chronic wasting disease was not sufficient because of the lack of raising technique. As a result, the various disease and low productivity was not resolved.

In spite of such difficulties, many of the raising hogs were monitored for various diseases based on the visual clinical view as the way of preventing the various contagious diseases which cause economic loss, but there are so many difficulties such as hour space restriction, and if not occurring the extra clinical view, it is difficult to judge some diseases. To overcome these difficulties, the sero-monitoring which measures the antibody against the various viral and bacterial diseases was executed. This method enabled us to judge the spreading of diseases in the specific swine herds, the quality of epidemiology, the existence of vaccination and the appropriate times of the vaccination. It will also be used to solve the hour economic problem according to the judgement of the various viral disease, to process the multi examination samples, to establish the counter measures of raising hogs, and to judge the correct hygienic-state of pigs[1~5]. It is reported that many domestic researchers have investigated the distributions of disease through the analysis of the antibody titer against the viral and bacterial diseases[2,5~7].

Based on these facts, in the general swine farms, the high recognition of the inoculation and interceptive prevention against the viral and bacterial diseases were raised and the proper countermeasures were taken. But in the farms of domesticated boar, almost even inoculation was not accomplished due to small size and the farm masters' indifference or insufficient recognition against the diseases. The recognition of this shabby disease prevention means that the various malignant infectious diseases may always occur in Gyeongbuk area. Consequently, although the synthesis investigation and research of diseases against the domesticated boar of Gyeongbuk area is considered very important, these were almost not made.

For the purpose of preparing the basic materials to understand the flows of current various diseases which occur in the boar raised in the Gyeongbuk area and to prevent them, the sero-epidemiology was executed against the important virus diseases of Aujeszky's disease, porcine reproductive and respiratory syndrome, parvovirus infection, Japanese encephalitis, a bacterial disease of atrophic rhinitis, mycoplasmal disease, pleuropneumonia, brucellosis and toxoplasmosis.

Materials and Methods

To detect antibodies to ADV, HerdCheck screening and ADV gpI antibody test kit(IDEXX, USA) were used. The test protocols were followed by manufacturer's recommendation and results were read at the wave length provided in the protocols for each test using ELISA reader(Anthos ht III, Austria). The test results were calculated by using the formula provided by the

manufacturer to identify antibody positive to the virus.

Antibody to PRRSV was detected by indirect immunofluorescent test[5] using PRRSV Korean isolate PL96-1 infected MA-104 cells. Briefly, the virus was infected on the fluent monolayer of the MA-104 cells and fixed with cold methanol at 48 hours after the virus infection. Serum samples collected from in breeding farms in Gyeongbuk province were diluted 1:10 with PBS and added on the fixed cells with the virus and incubated for 60 minutes. Afterward, they were washed three time with PBS. Antiswine IgG antibody labeled with FITC (KPL, USA) was applied on the washed cells, incubated for 60 minutes, and washed in the same manner as the previous step[8].

A prevalence of the hemagglutination-inhibition(HI) antibody to PPV was determined by HI test using PPV-PV9 in 96 well U bottom plate(Corning, USA). Serum samples were pre-treated with 25% Kaolin to remove non-specific agglutinins in the serum. Serial dilution of the field serum samples were reacted with 4 hemagglutination (HA) units of the PPV for 60 minutes and 0.5% guinea pig RBCs were added to have HI reaction. Antibodies to JEV were detected by HI test described previously[8,9].

Plate agglutination test was employed for the determination of the antibody titiers to bacterial disease agent such as *B bronchiseptica*. Antigen for the *B bronchiseptica* was by inactivated with 0.3% of formalin and the concentration of the antigen was adjusted properly. To test antibodies to *M hyopneumoniae,* APP2 and APP5, ELISA test was used with each antigens purified by ion-exchange column. The protocols for the *Mycoplasma* antibody detection was followed by generic procedures described elsewhere[8,10]. The results have been read at 492 nm and P/N ratio 2.0 or greater has been considered as positive to the *M hyopneumoniae,* APP2 and APP5.

Antibody titers of *Toxoplasma* were investigated by Latex agglutination test described previously[11]. Those of *Brucella abortus* were investigated by plate and tube agglutination tests.

Results

A serological survey was carried out to determine the prevalence of antibodies to infectious disease agents in domesticated boar serum samples collected from January to December 2000 in Gyeongbuk area.

There was no antibody to Aujeszky's disease virus in 330 serum samples collected

Table 1. Seroprevalence of PRRSV in domesticated boar sera by IFA test

Region	No of farms	No of positive farms(%)	No of samples	No of positive samples(%)
North*	8	0 (0)	43	0 (0)
East	8	0 (0)	51	0 (0)
West	8	0 (0)	48	0 (0)
South	31	2 (6.5)	188	3 (1.6)
Total	55	2 (3.6)	330	3 (0.9)

*Area in Gyeongbuk

Table 2. Hemagglutination inhibition titers to PPV in domesticated boar sera

Region	No of samples (farms)	Antibody titers(%)			
		≤10	20~80	160~640	≥1280
North*	43(8)	37	2	2	2
East	51(8)	48	0	2	1
West	48(8)	44	2	0	2
South	188(31)	165	8	5	10
Total	330(55)	294(89.1)	12(3.6)	9(2.7)	15(4.6)

*Area in Gyeongbuk

Table 3. Antibody titers to JEV by Hemagglutination Inhibition test in domesticated boar sera

Region	No of samples (farms)	Antibody titers(%)			
		≤10	20~80	160~640	≥1280
North*	43(8)	39	2	2	0
East	51(8)	47	2	2	0
West	48(8)	40	4	2	2
South	188(31)	172	8	4	4
Total	330(55)	298(90.3)	16(4.9)	10(3.0)	6(1.8)

*Area in Gyeongbuk

from 55 pig farms in Gyeongbuk area by HerdCheck Anti-ADV(S) assay kit(IDEXX, USA) and Anti-ADV-gpI assay kit(IDEXX, USA). These data indicate that neither antibodies to field infection nor antibodies to vaccine are present in Gyeongbuk area.

Antibody positive to PRRSV in serum samples was investigated by IFA. Table 1 shows the antibodies and positive rate to PRRSV in Gyeongbuk area. Sero-positive samples to PRRS by IFA were 0.9%(3/330). This result indicates that infection had occurred in a small degree.

Hemagglutination inhibition(HI) titers to PPV ranged variously from less than 10 to over 1280. 294(89.1%) out of 330 tested samples showed HI titer of less than 10 and the variety of antibody titers indicated that there is outbreak of PPV infection in domesticated boar in Gyeongbuk area(Table 2).

Table 3 shows the HI titers against the JEV. Among 330 tested serum of domesticated boars, HI titer of less than 10 was 90.3% and over 1280 was only 1.8%. The positive antibody of serum samples between 20 and 640 ranged variously in different areas.

In the result of investigating the antibody distribution of the *B bronchiseptica* with the Plate agglutination test, antibody less than 20 was 87.6% and positive antibody was relatively low(Table 4). This result shows that there may be a possibility to occur atrophic rhinitis problem infected with *B bronchiseptica* in domesticated boars of Gyeongbuk area.

Table 4. Distribution of antibody titers to *Bordetella bronchiseptica* by agglutination test in domesticated boar sera

Region	No of samples	Antibody titers(%)			
		≤20	40~80	160~320	≥640
North*	43	40	0	3	0
East	51	47	2	2	0
West	48	46	2	0	0
South	188	156	14	10	8
Total	330	289(87.6)	18(5.5)	15(4.5)	8(2.4)

*Area in Gyeongbuk

Table 5. Distribution of antibody titers to *Mycoplasma hyopneumoniae* by ELISA test in domesticated boar sera

Region	No of samples	Antibody titers(%)			
		≤10	20~80	160~640	≥1280
North*	43	21	18	4	0
East	51	21	22	6	2
West	48	18	20	8	2
South	188	62	88	28	10
Total	330	122(37.1)	148(44.8)	46(13.9)	14(4.2)

*Area in Gyeongbuk

Table 6. Antibody titers to *Actinobacillus pleuropneumoniae* serotype 2 by ELISA

Region	No of samples	Antibody titers(%)			
		≤20	40~80	160~320	≥640
North*	43	24	17	2	0
East	51	38	7	6	0
West	48	20	18	10	0
South	188	110	52	20	6
Total	330	192(58.2)	94(28.5)	38(11.5)	6(1.8)

*Area in Gyeongbuk

Table 6-1. Antibody titers to *Actinobacillus pleuropneumoniae* serotype 5 by ELISA

Region	No of samples	Antibody titers(%)			
		≤20	40~80	160~320	≥640
North*	43	21	12	10	0
East	51	31	12	8	0
West	48	18	16	14	0
South	188	104	58	26	0
Total	330	174(52.7)	98(29.7)	58(17.6)	0(0)

*Area in Gyeongbuk

The distribution of the serum antibody against the *M hyopneumoniae* using ELISA appears in Table 5. This table shows that antibody negative was 37.1% and the distribution of the antibody ranged variously from 20 to over 1280.

ELISA antibody titers against the *A pleuropneumoniae* serotype 2 and the *A pleuropneumoniae* serotype 5 appeared in Table 6 and Table 6-1. In both Tables, antibody negative was 58.2%, 52.7% respectively. The distribution of the antibody was from 40 to 320 and the distribution of the antibody in serotypes did not show a big difference.

In antibody titers against the *Toxoplasma gondii* with Latex agglutination test, 38 out of 330 samples(11.5%) were positive(Table 7) and in the serum agglutination test to *Brucella*, the antibody to *Brucella* were all negative in 330 samples. These results indicated that there was no occurrence of Brucellosis in domesticated boars of Gyeongbuk area(Table 8).

Discussion

In the growing swine industry, hygienicswine herds have to be raised to diminish the occurrence of contagious diseases andincrease productivity. Sero-monitoring, one of the useful ways of managing pigs, enables us to predict various diseases, to inoculate the proper vaccination, and to better understand the occurring diseases. The raising of domesticated boars in Gyeongbuk area is prone to malignant infectious diseases due to small-size farms and the insufficient recognition of diseases and prevention.

Since the initial occurrence was reported in 1987, the Aujeszky's disease occurred every year is the systemic infectious disease by ADV and it appears in neurosis and respiratory diseases as characteristics. It

Table 7. Antibody titers to *Toxoplasma gondii*

Region	No of samples	No of positive	% positive
North*	43	4	9.3
East	51	2	3.9
West	48	5	10.4
South	188	27	14.4
Total	330	38	11.5

*Area in Gyeongbuk

Table 8. Antibody titers to *Brucella*

Region	No of samples	No of positive	% positive
North*	43	0	0
East	51	0	0
West	48	0	0
South	188	0	0
Total	330	0	0

*Area in Gyeongbuk

shows various symptoms of abortion and stillbirth of pregnant pigs, and the high lethal ratios and the growth obstacle of young pigs according to the age of pigs. In addition, it causes many infection with no clinical condition and a serious economic damage to the occurring swine farm. The Gyeonggi and Chungcheong provinces are the area where the Aujeszky's disease always occurs. If careless movement of pigs occurs, there is a possibility that this disease could spread to unaffected areas. In the research of the serological survey of domesticated boar of Gyeongbuk province using the ELISA kit to diagnose the infected antibody. the infected antibody was not discovered in the 330 serum samples. This result shows that the Gyeongbuk area has not exposure to the Aujesky's disease. To prevent the spreading of the Aujesky's disease, it is judged that there must be the through search for carrying pigs and effective measures for early discovery of infected pigs.

The PRRS reported with separated virus at first in 1993[12] domestically is presumed to have occurred since late 1980 years[2]. The causative agent of PRRSV is propagated rapidly through respiratory organ infection, and is a propagation obstacle of pigs because it causes stillbirths as well as respiratory disease at the early stage. As time goes by, several respiratory diseases were deteriorated through complex infection with the agent of other respiratory diseases. When we examine the situation of antibody titers' investigation in our country, since the first report of antibody titer in serum using IFA test by Shin[2], the research of IFA test has been actively advanced and many researchers have reported 10.6~49.3% antibody titer[4~7].

Like this, nationwide diffusion of PRRS causes consuming disease by complex infection in our swine industry filled with the various respiratory diseases and it is presumed to give a big damage. Therefore, the continuous research of PRRS has to be carried out to prevent the complex infection of respiratory organs. To prevent an inflow into farms using Nursery depopulation(ND) or medicated early weaning(MEW) which is very effective in diminishing the circulation of the PRRSV and in eliminating the circulation of the bacterial respiratory disease is very important for prevention[3,13].

In the antibody test from the sera of domesticated boar using PRRSV separated in domestic area with IFA, it will be able to confirm the antibody from 0.9% of tested samples. Although this is much lower than 49.3% of general farm investigated by Kim & Gong[4], when considering non-vaccination of domesticated boars in Gyeongbuk province, it confirmed that there is an infection even in domesticated boars. Therefore, to protect hogs from exposure to PRRSV and stop an influx of PRRS, interceptive prevention of pig farms, continuous surveillance setup by sero-monitoring, and proper prevention counter-measures have to be taken.

The PPV infection causes abortions and stillbirths to early pregnancy sows. while most of swine industry is executing the vaccination, the farms of domesticated boars do not vaccinate. Consequently the fact that the antibody is detected from the sera of 4~10 month pigs becomes the base data of grasping the degree of infection against the objective farm. In the result of this research, the sera of domesticated boar mostly shows antibody negative and domesticated boar retaining over 160 antibody titer shows low distribution of 7.3% relatively.

The JE is a disease transmitted the *Culex tritaeniorhynchus* mosquito and mainly it causes a problem from a mosquito occurrence time[14]. The sows of JE have an abortion, and the aborted fetus shows the various view of stillborn fetus, mummified fetus, and the infected piglets die of neurosis symptoms like convulsions, revolutions and paralysis. In this research, in the case of tested domesticated boar, most of the objects show negative antibody and the objects retaining over 160 antibody titer show relatively low level of 4.8% as much as knowing there was an infection. Like this, since farms showing positive antibody titer are in danger of spreading reproductive disturbances of the sow by JE, it is needed for the prevention policy to this.

The AR of the pig is the disease *B bronchiseptica*. It is multiplied primarily in the nasal cavity of 3~4 week old piglets and damages the mucous membrane. *P multocida* (mainly capsular type D) increased secondarily secretes necrosis toxin and causes nasal turbinate atrophy[15]. In this study investigating the distribution of the antibody titer against the *B bronchiseptica* with the plate agglutination test, it is judged that the AR of domesticated boar is not significant because 20 below of negative antibody was 87.6%.

The mycoplasmal pneumonia of the pig occurs by the infection of the *M hyponeumoniae* with very contagious disease, and mortality is low but the damage is very serious in the management of raising hogs due to growth rate or decrease of the feed efficacy. Pigs infected with mycoplasmal pneumonia do not always show clinical conditions, and become progressed chronicity without special clinical sign[16]. In the result of investigating the distribution of the serum antibody titer against the *M hyponeumoniae* using ELISA, antibody negative shows 31.7%, the various distribution from 20 to 1,280.

Actinobacillus pleuropneumonia occurs by the infection of *A pleuropneumoniae*. It is known to be the serotype 1, 2, 5 and 7 in worldwide and among them serotype 2 and 5 cause serious damage in domestic pigs. In this study, antibody titer against the serotype 2, 5 of domesticated boar of Gyeongbuk area showed 58.2% and 52.7% in antibody negative respectively and did not show a big difference in antibody positive.

Toxoplasmosis of the pigs is zoonosis and is considered one of the most important diseases for public hygiene. In the research of the domesticated boars of Gyeongbuk province, 38 sera of all 330 showed antibody positive. This study found that the infection is caused by the contact of wild cats domesticated boars. Accordingly, the farms showing positive antibody gave to prepare countermeasures to prevent the contact of cats around the farm. Brucellosis of pigs is also zoonosis and us a very important disease for public hygiene requiring a continuous watch. This research confirmed that there was no occurrence of this disease because whole sera of tested samples appeared in antibody negative.

To conclude, executing sero-monitoring periodically and preparing the basic data for prevention by the occurrence situation and epidemiology investigation of the diseases will be very helpful for the management of pig farms and prevention of diseases.

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