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Sero-epidemiology of the major swine infectious disease in Cheju

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제주지역에 대한 돼지 주요 전염병의 혈청학적 역학조사

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초 록 : 제주지역 돼지에서 각종 전염성 질병 원인체에 대한 항체를 조사하여 그간 전염성 병원체에 대한 역학조사가 미진하였던 부분을 보완하여 질병의 분포를 파악하고자 1995년부터 1996년에 걸쳐 제주도 전역에서 돼지의 혈청을 채취하여 각종 병원체에 대한 항체 분포율을 조사하였다. 본 연구에서 검사한 돼지 혈청 시료에서는 돼지 오제스키병 바이러스에 대한 항체는 전혀 검출되지 않았다. 돼지 콜레라바이러스에 대한 항체는 기대 수준 이하로 낮아 백신접종이 원활히 수행되고 있지 않음을 시사하였으며 특히 농장에 따라 항체 보유돈과 항체 음성돈이 혼재하는 농장과 항체가 전혀 검출되지 않는 농장 등 돼지 콜레라 방역의 사각지대가 존재할 가능성이 있음을 보여주었다. 유·사산 원인체인 돼지 파보바이러스 및 뇌심근염에 대한 항체가가 다양하게 나타나 일부 문제가 있을 것으로 사료되었다. 돼지 생식기호흡기증후군(PRRS) 바이러스에 대한 항체 분포율은 내륙 보다 다소 낮게 나타났고, 돼지 influenza virus, 위축성 비염, 흉막 폐염 등 각종 세균성 질환에 대한 항체수준도 다양하게 나타났다. 본 혈청학적인 연구결과는 제주지역에서의 양돈방역 정책수립 및 질병방제의 기초자료로 유용하게 이용될 것으로 사료된다.

Key words : serology, cheju, HCV, ADV, AR, APP, PRRSV, PPV, EMCV, SIV.

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Introduction

Serological profiles of the swine infectious disease provide an important information on the presence of the disease, severity of the disease, efficacy of the vaccine, and economic losses of the farm by affected diseases. The data collected from serological monitoring can be analyzed to understand disease statue and can be used for the preparing effective prevention measures. Disease control plays a key role for the economic improvement of the current pig farm management.

Serological survey data in Cheju island has been limited even though swine industry in Cheju island growing rapidly and local goverment policy support all available measures expanding animal sizes up to 500,000 heads by year 2000. Geographically Cheju island is isolated from main land which may provide a great advantage for the quarantine to protect disease introduction into the island but institutions for the animal disease research and diagnostics is not loctated nearby for the rapid diagnosis and treatment. Antibodies to the major swine bacterial and viral disease agents have been tested. Antibodies to Aujeszky's disease, hog cholera, porcine reproductive and respiratory syndrome virus(PRRSV), porcine parvovirus, encephalomyocarditis virus(EMCV), swine influenza virus H1N1 and H3N2, *Bordetella bronchiseptica*, *Actinobacillus pleuropneumoniae* serotype 1, 2, 5 and 7, and *Mycoplasma hyopneumoniae* were tested by appropriate diagnostic procedures for each disease.

Materials and Methods

To detect antibodies to ADV HerdCheck screening and pseudorabies virus gpI antibody test kit(IDEXX, USA) have been used. The test protocols were followed by manufacturer's recommendation and results have been read at the wave length provided in the protocols for each test using ELISA reader(Biotek UV900C, USA). The test results have been 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[1,2] 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 Cheju were diluted 1:10 with PBS and added on the fixed cells with the virus and incubated for 60 minutes followed by washing three times with PBS. Anti-swine IgG antibody labeled with FITC(KPL, USA) was applied on the washed cells and incubated for 60 minutes followed by washing as same manner as previous step. PRRSV specific cytoplasmic immunofluorescence was observed under the ultra violet light microscope(Fig 1).

A prevalence of the hemagglutination-inhibition(HI) antibody to porcine parvovirus was determined by HI test[2-4], using porcine parvovirus 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 HA units of the porcine parvovirus for 60 minutes and 0.5% guinea pig RBCs were added to have hemagglutination-inhibition reaction. Antibodies to Japanese encephalitis virus[2,5], EMCV[2,6,7] and swine influenza virus(SIV)[2,8] were detected by HI test described previously. Antibodies to the hog cholera virus were detected by neutralization peroxidase-linked assay(NPLA)[2,9,10]. Test serum samples were diluted with 200 $TCID_{50}$/ml of hog cholera ALD strain and incubated for 60 minutes at 37℃ then 2×10^5/ml of the PK-15 cells were added in 96 well cell culture plates. Serum virus mixture infected cells were incubated for 72 hours at 37℃ followed by fixation with 80% cold acetone, Viral antigens which was not neutralized by test serum were detected by monoclonal antibodies/biotinylated anti-mouse IgG and visualized by staining with Vector stain ABC (Vector PK-4000) and DAB kit (Vector SK-4100).

Plate agglutination test was empolyed for the determination of the antibody titiers to bacterial disease agents such as *Bordetella bronchiseptica*[2,11,12], *Actinobacillus pleuropneumoniae type 1, 2, 5 and 7*[2,13], Antigens for the *Bordetella bronchiseptica, Actinobacillus pleuropneumoniae type 1, 2, 5 and 7* were prepared by inactivated with 0.3% of formalin

and concentration of the antigen was adjusted properly. To test antibodies to *Mycoplasma hyopneumoniae*, ELISA test was used with *Mycoplasma* antigens purified by ion-exchange column. The protocols for the *Mycoplasma* antibody detection was followed by generic procedures described elsewhere[2,14]. The results have been read at 492nm and and P/N ratio 2.0 or greater has been considered as positive to the *Mycoplasma hyopneumoniae*.

Results

There was no antibody to Aujeszky's disease virus in serum samples collected from swine industry in Cheju island was detected by HerdCheck Anti-PRV(S) assay kit (Idexx, USA) and Anti-PRV-gp1 assay kit(Idexx, USA). These data indicate that there is no antibodies to field infection nor antibodies to vaccine are present in Cheju island.

Antibody positive to PRRSV determined by cytoplasmic immunofluorescence of the PRRSV infected MA-104 cells when test serum sample was applied. The immunofluorescence of the PRRSV infected 96 well plate was standardized by using positive polyvalent serum to PRRSV prepared in gnotobiotic pigs. Talbe 1 shows regional distribution of the antibodies to PRRSV and positive rate in the island. There is slight difference of sero-prevalance to PRRSV with geographical difference. But these are not statistically significant because of the small sample size from Seoguipo area. An average of the positive rate to PRRSV in Cheju island was 12.8%.

Table 1. Prevalence of the antibody to PRRSV in pig sera collected from Cheju by IFA test.

Regions	No. samples	No. of positive	Percentage(%)
N. Cheju	223	14	6.3
S. Cheju	113	10	8.8
Cheju city	50	11	22.0
Seoguipo	10	0	0
Cheju*	41	21	51.2
Sum	437	56	12.8(Ave.)

* Samples collected from slaughter house in Cheju but location of the farm has not identified.

A neutralization titiers to HCV by NPLA were≤4 to≤128 in PK-15 cells. Over 70 percent of the tested serum samples were showed neutralization titers of less than 32 and 64% was less than neutralization titer of 1:4. These low neutralization group of animals are susceptible to HCV infection and have great risk to HC infection. There were significant number of farms without antibody to HCV exist in the island and these farms may play an important role in the control of the HC infection. But there is no significant difference among regions where serum samples were collected.

Table 2. Distribution of antibody titers to HCV in serum samples collected from swine industry in Cheju. NPLA test was used to determine antibody titers and HCV specific reactions in the cytoplasm of the infected cells were examined under the light microscope

Regions	No. of samples	Antibody titers to HCV			
		≤4	8~16	32~64	≥128
N. Cheju	255	180	6	26	43
S. Cheju	108	83	4	6	15
Cheju city	55	34	5	4	12
Seoguipo	10	0	2	3	5
Cheju*	51	11	11	5	24
Sum	479	308(64.3%)	28(5.8%)	44(9.2%)	99(20.7%)

* Samples collected from slaughter house in Cheju but location of the farm has not identified.

Hemagglutination inhibition titers to porcine parvovirus were varies ranging from less 5 to over 2560. One hundred forty three out of 472 tested sera showed HI titier of less than 5 which indicate that these population need to vac-

Table 3. Hemagglutination inhibition(HI) titers to porcine parvovirus in sera collected from Cheju island

Regions	No. of samples	Antibody titers to PPV			
		≤5	10~80	160~1280	≥2560
N. Cheju	251	88	59	12	92
S. Cheju	109	28	25	11	45
Cheju city	56	4	14	6	32
Seoguipo	10	0	0	0	10
Cheju*	46	23	11	7	5
Sum	472	143(30.3%)	109(23.1%)	36(7.6%)	184(39.0%)

* Samples collected from slaughter house in Cheju but location of the farm has not identified.

cinate extensively when pigs are selected for the breeding stock. Variety of the antibody titers indicate that there is outbreak of PPV infection in Cheju island.

Serum samples from Cheju island had high titers to EMCV and HI titers were ranged from 1:4 to over 128. This result shown in the table 4 does not imply that EMCV causes any clinical disease.

Table 4. Distribution of the HI titers to EMCV in serum samples collected from Cheju island

Regions	No. of samples	Antibody titers to EMCV			
		≤4	8~16	32~64	≥128
N. Cheju	67	1	21	27	18
S. Cheju	20	1	0	16	3
Cheju city	6	0	0	6	0
Cheju*	10	9	0	0	1
Sum	103	11(10.7%)	21(20.4%)	49(47.6%)	22(21.3%)

*Samples collected from slaughter house in Cheju but location of the farm has not identified.

Table 5 shows HI titiers to H1N1 type of the swine influenza virus in serum samples colected from Cheju island and 53% was negative to the virus. This is the first serological survey for the influenza virus in the island even with liminted number of the samples submited.

Table 5. Distribution of the HI titers to swine influenza virus sero-type H1N1 in serum samples collected from Cheju island

Regions	No. of samples	Antibody titers to SIV(H1N1)			
		≤20	40~80	160~320	≥640
N. Cheju	64	34	21	6	3
S. Cheju	20	14	6	0	0
Cheju city	6	3	2	1	0
Cheju*	10	2	7	1	0
Sum	100	53	36	8	3

*Samples collected from slaughter house in Cheju but location of the farm has not identified.

Antibodies to H1N1 type of the swine influenza virus (Table 5) were less prevalent than H3N2 type(Table 6). HI titers to serotype H3N2 of swine influenza virus were evenly distributed and no significant difference among regions have been detected.

Table 6. Distribution of the HI titers to swine influenza virus H3N2 in serum samples collected from Cheju island

Regions	No. of samples	Antibody titers to SIV(H3N2)			
		≤20	40~80	160~320	≥640
N. Cheju	63	16	17	14	16
S. Cheju	27	1	7	7	12
Cheju city	6	3	0	0	3
Cheju*	10	3	2	0	5
Sum	106	23(21.7%)	26(24.5%)	21(19.8%)	36(30.0%)

*Samples collected from slaughter house in Cheju but location of the farm has not identified.

The table 7 shows results of the antibody titers and prevalence of the atrophic rhinitis in Cheju island. Majority of the serum samples had high prevalence of the antibody to *B bronchiseptica*. But we did not have vaccination history which may important for the epidemiological analysis of the serological data obtained. Based on our knowledge high levels of the antibody titer imply that there is an infection by *B bronchiseptica* and may have atrophic rhinitis problem in swine industry in Cheju island.

Table 7. Antibody titers to *Bordetella bronchiseptica was tested by plate agglutination test*

Regions	No. of samples	Antibody titers to *B bronchiseptica*			
		≤20	40~80	160~320	≥640
N. Cheju	255	7	80	126	42
S. Cheju	109	0	27	53	29
Cheju city	56	2	20	28	6
Seoguipo	10	0	2	7	1
Cheju*	51	5	39	6	1
Sum	481	14(2.9%)	168(35.0%)	220(45.7%)	79(16.4%)

*Samples collected from slaughter house in Cheju but location of the farm has not identified.

This result showed interesting sero-epidemiology in Cheju island. There is significantly high level of the antibody to APP1 in serum samples collected from Cheju island compare to the serological data obtained from other parts of the country. Over 70% of the samples were showed antibody titer over 40 and this result is different from other area of the nation.

Table 8. Distribution of antibody titers to APP1 from serum samples collected from different region of the Cheju by plate agglutination test

Regions	No. of samples	Antibody titers to APP1			
		≤20	40~80	160~320	≥640
N. Cheju	19	3	9	4	3
S. Cheju	29	10	8	11	0
Cheju city	17	5	9	3	0
Sum	65	18(27.7%)	26(40.0%)	18(27.7%)	3(4.6%)

Low levels of antibody to APP2 have been detected from samples collected from Cheju island and 95% of the tested serum samples showed antibody titer less than 20. This result may indicate that APP2 in Cheju island is not prevalent compare with other pathogens. But we believe that this limited research result may not represent overall situations of the swine health statue in Cheju island.

Table 9. Distribution of antibody titers to APP2 from serum samples collected from different region of the Cheju by plate agglutination test

Regions	No. of samples	Antibody titers to APP2			
		≤20	40~80	160~320	≥640
N. Cheju	186	181	2	2	1
S. Cheju	89	83	4	1	1
Cheju city	47	41	2	4	0
Seoguipo	10	10	0	0	0
Cheju*	41	41	0	0	0
Sum	373	356(95.4%)	8(2.1%)	7(1.9%)	2(0.5%)

*Samples collected from slaughter house in Cheju but location of the farm has not identified.

Antibody titers to APP5 were lower than other serotypes and 93% of the tested samples showed plate agglutination antibody titer of less than 20(Table 10). But there is no significant geographical difference was exit in this experiment. These antibodies may not represent APP infection in the farm but there was no way to differentiate field infection from vaccination in this study.

Majority of the antibody level to the APP7 belong to plate agglutination titer of under 80(81%). This result indicated that the APP7 in Cheju swine herd was not considered to be a major problem as a respiratory pathogen.

Table 10. Distribution of antibody titers to APP5 from serum samples collected from different region of the Cheju by plate agglutination test

Regions	No. of samples	Antibody titers to APP5			
		≤20	40~80	160~320	≥640
N. Cheju	186	177	8	0	1
S. Cheju	89	80	6	2	1
Cheju city	47	41	4	1	1
Seoguipo	10	10	0	0	0
Cheju*	41	40	1	0	0
Sum	373	348(93.3%)	19(5.1%)	3(0.8%)	3(0.8%)

*Samples collected from slaughter house in Cheju but location of the farm has not identified.

Table 11. Distribution of antibody titers to APP7 from serum samples collected from different region of the Cheju by plate agglutination test

Regions	No. of samples	Antibody titers to APP7			
		≤20	40~80	160~320	≥640
N. Cheju	186	23	113	50	0
S. Cheju	89	20	62	7	0
Cheju city	47	13	28	4	2
Seoguipo	10	0	6	4	0
Cheju*	41	35	6	0	0
Sum	373	91(24.4%)	215(57.6%)	65(17.4%)	2(0.5%)

*Samples collected from slaughter house in Cheju but location of the farm has not identified.

ELISA titirs to *Mycoplasma* was varied ranging from ≤ 50 to≥ 3200 which is common in swine industry and no difference was found in Cheju swine herd compare to the results published previously.

Table 12. Distribution of antibody titers to *Mycoplasma* by ELISA test

Regions	No. of samples	Antibody titers to *Mycoplasma hyopneumoniae*			
		≤50**	100~400	400~1600	≥3200
N. Cheju	156	55	98	3	0
S. Cheju	59	17	40	2	0
Cheju city	18	9	9	0	0
Seoguipo	10	0	9	1	0
Cheju*	41	3	36	1	1
Sum	284	84(29.6%)	192(67.6%)	7(2.5%)	1(0.3%)

*Samples collected from slaughter house in Cheju but location of the farm has not identified.
**Dilution factor

Discussion

Serological survey was carried out to determine prevalence of antibodies to infectious disease agents in pig serum samples collected from Cheju island. Seropositive to the disease agents indicate that the animals were exposed to the disease agents and/or vaccination and these result would be a useful information for the analysis of the health statues of the swine industry where samples collected from. There is no antibodies to Aujeszky's disease virus found by ELISA test in serum samples tested. Intensive test and quarantine for the animals introduced from outside of the island may be albe to keep the island free from the ADV infection and economic loses. Presence of an antibodies to hog cholera virus were tested by neutralization peroxidase-linked assay(NPLA) and 70% of the tested serum showed titers less than 1:16 which may susceptible to hog cholera virus infection(Table 2). Based on the antibody titers and prevalence of the antibody to hog cholera virus, poor vaccination was carried out in the island even though government campaigning contagious disease eradication policy. This serological results may indicate that Cheju island has a risk of a hog cholera outbreak anytime in the near future if there is no proper quarantine measures practiced. A great risk factor for the disease control in the island is the presence of many farms without any antibody to HCV even in the finishers and breeders. This result also indicate that Cheju has an advantage for the controlling HCV because there are many famrs without antibody to HCV had no clinical HCV outbreak. We believe that if there is any introduction of the HCV from other farm or abroad Cheju swine industry prior to massive vaccination may face catastrophic risk. The antibody to PRRSV, a new emerging viral disease in pig also has been detected in Cheju and 12.8% of the tested sample showed sero-positive by indirect immunofluorescent test(Table 1). Relatively low prevalence of the antibody to PRRSV was detected in Cheju island compare to any part of the world. The presence of the PRRSV in swine herd might influence other disease statue such as bacterial respiratory disease. The HI titers to porcine parvovirus varied ranging from ≤ 5 to ≥ 2560 and 143(30.3%) samples out of 472 had HI titer less than 1:5. If we select replacement stock for the breeding from these animals there are high demand for the vaccination to porcine parvovirus to prevent the disease. EMCV which cause reproductive failure in swine also exist is Cheju and high HI titers were detected(Table 4). Swine influenza may play an important role in respiratory disease in swine and low levels of antibody to H1N1 and relatively high level of the antibody to H3N2 has bee detected(Table 5, 6). Swine influenza virus with other bacterial agent could aggrevate respiratory disease in pigs. It may need to considering development of the vaccine to swine influenza virus to prevent primary respiratory disease pathogen. It was interesting to have significantly high level of antibodies to *Actinobacillus pleuropneumoniae* type 1 compare to other inland pig industry. But there is no clear evidence exist which might support this result directly. We suppose that this difference in the result may due to the geopraphical difference and/or other factors could influence the disease statue in the Cheju island. The vaccination program in the Cheju island is not well established and much less vaccination has been practiced. This poor vaccination and isolation from inland may affect the sero-epidemiology of the swine population in Cheju island. Antibodies to *Bordetella bronchiseptica* and *Mycoplasma hyopneumoniae* also tested by plate agglutination and ELISA test but there is no significant difference has been found compare to other places in the nation. This result would provide an information for the preparation of prevention measures to infectious agents in Cheju island.

Legends for figures

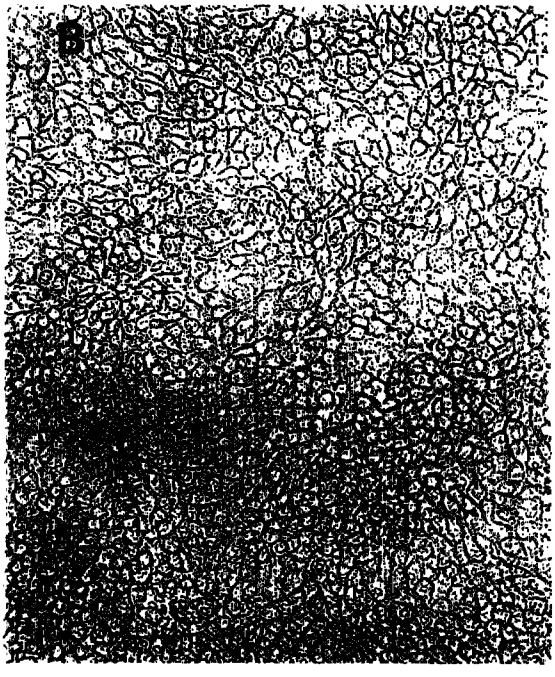
Fig 1. Indirect immunofluorescent test in the PRRSV infected MA-104 cells. The PRRSV specific cytoplasmic immunofluorescence using hyper-immune serum is evident in the cells infected with virus (A) and no specific flu-

orescent showed in the MA-104 cells with mock infection (B).

Fig 2. Neutralization peroxidase linked assay (NPLA) to determine presence of the antibodies to HCV and cell with cytoplasmic dark brown reaction indicated no antibodies(A). The picture B shows no color reaction because of the virus neutralization resulting no viral infection in the PK-15 cells.

A

B



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