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Seroprevalence of selected porcine respiratory pathogens in the pig herds in Chungcheong and Gyeongsang provinces in Korea

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Abstract

We studied the seroprevalence of four respiratory pathogens in Korean swine farms located in Chungnam, Chungbuk, Gyeongnam and Gyeongbuk provinces during the period of spring of 2007 to winter of 2008. Serological tests were performed using commercial ELISA kits. A total of 530 serum samples were tested for the antibodies against porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), *Mycoplasma hyopneumoniae* (*M. hyo*) and *Actinobacillus pleuropneumoniae* (*APP*). Seroprevalence for four respiratory pathogens were estimated by ELISA-positive rates of the submitted samples. The overall seropositive rates of PRRSV, *APP*, *M. hyo* and PCV2 were 32.6%, 10.6%, 38.4% and 88.5%, respectively. By production stage, the seropositive rate for PRRSV was highest in nursery pig populations (46.2%). In contrast, the highest seropositive rates of *APP* and *M. hyo* were observed in sow and growing pigs. However, the seroprevalence of PCV2 was ranged from 85.7% to 89.6%, showing no significant difference among the production stages. In the seroprevalence by season, PRRSV, *APP* and *M. hyo* infections revealed typical seasonal patterns that the peaks of the seropositive rates were observed between early winter and late spring. In case of PCV2, no particular seasonal patterns were noticed. The pig herds in Gyeongbuk province where PMWS was endemic during the period of survey showed the highest seropositive rates for PRRSV (44.6%), *M. hyo* (47.5%), and PCV2 (92.7%). Seropositive rates for *APP* of four provinces were approximately 10%. These results might be valuable for control and prevention of the respiratory diseases and helpful to define strategies related to vaccine applications.

Key words : Seroprevalence, PRRSV, *A. pleuropneumoniae*, *M. hyopneumoniae*, PCV2, ELISA

INTRODUCTION

Porcine respiratory disease complex (PRDC) causes a serious health problem in growing and finishing pigs typically around 16~22 weeks of age. PRDC is characterized by slow growth, decreased feed efficiency, lethargy, anorexia, fever, cough, and dysphonia (Halbur, 1998; Thacker et al, 2001). Pneumonia in pigs with PRDC is due to a combination of both viral and bacterial agents, such as porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), swine influenza virus (SIV), *Mycoplasma hyopneumoniae* (*M. hyo*), *Actinobacillus pleuropneumoniae* (*APP*), and *Pasteurella multocida* (Halbur, 1998; Thacker et al, 2001). Although the aetiology of PRDC involves multiple pathogens and varies from farm-to-farm, *M. hyo* and PRRSV are two of the most common pathogens isolated from pigs exhibiting PRDC (Dee, 1996; Thacker et al, 1999). *M. hyo* is one of the most difficult mycoplasmas to isolate and identify. It grows slowly and is

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often overgrown by other organisms that are common secondary invaders in swine pneumonia. Although methods for isolation have been improved, diagnosis by culture is not feasible in most situations. PCV2 has been identified as an aetiological agent for postweaning multisystemic wasting syndrome (PMWS) (Allan and Ellis, 2000; Choi and Chae, 1999; Kim et al, 2001), which was reported in almost all pig-producing countries around the world including Korea. The affected pigs are not really disease-specific and some could easily be attributed to other well-known pathogens, commonly isolated from pigs. *A pleuropneumoniae* is one of the dominant respiratory bacterial pathogens in growing and market weight pigs. Pigs may die in haemorrhagic necrotic pneumonia or fibrinous pleurisy, but the heaviest economic losses are induced by a decreased growth rate in chronically infected pigs (Barnum, 1990; Regula et al, 2000; Taylor, 1999). Still, actinobacillosis may be difficult to diagnose by bacterial isolation in chronically infected herds, but without obvious signs of clinical disease (Dubreuil et al, 2000; Wallgren and Persson, 2000). However, serological methods can be effective in diagnosing the disease and to define patterns for spread of the infection (Sebunya and Saunders, 1983; Harms et al, 2001).

With the intensification in pig production, veterinary services that are provided have moved from the so-called first-aid practice into planned prevention and control programs. A successful application of these programs largely depends upon a thorough understanding of the epidemiology of diseases. This is especially apparent for the prevention of respiratory disorders because they may be multifactorial (Hurniket al, 1994; Ross, 1999).

The aim of this study was to determine the seroprevalence of the selected infectious respiratory pathogens (PRRSV, PCV2, *M. hyopneumoniae* and *A. pleuropneumoniae*) in the pig farms located in Chungnam, Chungbuk, Gyeongnam and Gyeongbuk provinces during the period of spring of 2007 to winter of 2008.

MATERIALS AND METHODS

Serum samples

A total of 546 blood samples were submitted for diagnosis from 37 pig herds located in Chungnam, Chungbuk, Gyeongnam and Gyeongbuk provinces during the period of spring of 2007 to winter of 2008. Serum was obtained by centrifugation at 3,000 rpm for 15min and frozen at −20°C until examination. Only sera with limited haemolysis (530 samples out of 546) and absence of protein denaturation were selected for analysis.

Detection of antibody to PRRSV

HerdCheck® PRRS Virus Antibody Test Kit 2XR (IDEXX, Westbrook, ME, USA), an indirect enzyme immunoassay for the detection of antibody to PRRSV in swine serum using PRRSV and normal host cell antigens, was used and performed by following the manufacturer's instructions. When the S/P ratio was greater than or equal to 0.4, then the sample was classified as positive for PRRSV antibodies. This test was reported with 97.5% sensitivity and 99.5% specificity for detection of PRRSV antibody (Ruiz-Fons et al, 2006).

Detection of antibody to PCV2

SERELISA® PCV2 Ab Mono Blocking detection kit (Synbiotics Co., Europe, France), a single well blocking ELISA for the detection of anti-PCV2 antibodies in swine feces or serum, was used and performed by following the manufacturer's instructions. Any sample presenting a ratio ($\overline{OD}$ sample/$\overline{OD}$ Negative) ≤ 0.15 was considered positive for the presence of antibodies in serum.

Detection of antibody to *M. hyopneumoniae* (*M. hyo*)

An indirect enzyme-linked immunosorbent assay kit (HerdChek* *M. hyopneumoniae*, IDEXXL Laboratories, USA) for the detection of antibody to *M. hyop-*

neumoniae was used and performed by following the manufacturer's instructions. Samples with S/P ratios greater than 0.4 were considered positive.

Detection of antibody to *A. pleuropneu-moniae* (*APP*)

CHEKIT® APP-ApxIV (IDEXX Switzerland AG, Switzerland), an enzyme-linked immunosorbent assay based on the newly discovered *APP* toxin, ApxIV, was used and performed by following the manufacturer's instructions. The CHEKIT® APP-ApxIV assay was reported with 99~100% specificity and 95% sensitivity for the detection of *APP* infections, with no cross-reactivity. It is possible to differentiate the vaccinated from infected animals. Samples with value (OD_{sample}- $OD_{negative}$/ $OD_{positive}$-$OD_{negative}$ × 100%) greater than 40% were considered positive.

Statistical analyses

All data were sorted in the Microsoft Excel software program. Positive rates of antibodies against the infectious pathogens were analyzed according to production stages, season and provinces. The statistical analysis of data and generation of Row stats graph were performed using GraphPad Prism version 5.00 for Windows, GraphPad Software (San Diego, California, USA, www.graphpad.com). Statistical significance was assumed at $P<0.05$.

RESULTS

Overall prevalence of antibodies

A total of 530 serum samples were investigated the prevalence of antibodies to PRRSV, *APP*, *M. hyo* and PCV2 using the commercial ELISA kits. These serum samples were submitted from 37 pig herds located in Chungnam, Chungbuk, Gyeongnam and Gyeongbuk provinces during the period of spring of 2007 to winter of 2008. As seen in Table 1, 10 (38.5%) of 26 pig herds tested were positive to PRRSV antibody, and 125 (32.6%) of 383 serum samples were antibody-positive to PRRSV. Three (15%) of 20 farms tested were seropositive for APP, and 23 (10.6%) of 265 serum samples were antibody-positive to *APP*. Seven (70%) of 10 farms tested for *M. hyo* antibody were positive, and 48 (38.4%) of 125 serum samples tested were antibody-positive to *M. hyo*. Twenty (95.2%) of 21 farms tested were antibody-positive for PCV2, and, of 209 serum samples tested, 185 samples (88.5%) were positive to PCV2 antibody.

Seroprevalence by production stages

As shown in Table 1 and Fig. 1, the seroprevalence of antibody varied with production stage except for PCV2. The highest seroprevalence of PRRSV antibody was observed in nursery pigs and growing pigs ($P<0.05$). In contrast, seroprevalence of *APP* antibody was relatively

Table 1. Summary of seroprevalence for four selected respiratory pathogens in the swine farms in Chungnam, Chungbuk, Gyeongnam and Gyeongbuk provinces during the period of 2007 to 2008

Production stage	PRRSV		*APP*		*M. hyo*		PCV2	
	Farms[a]	Serum	Farms	Serum	Farms	Serum	Farms	Serum
Sow	5/10 (50)	32/118 (27.1)	2/7 (28.6)	11/80 (13.8)	4/6 (66.7)	16/25 (64)	14/15 (93.3)	50/56 (89.3)
Sucking piglet (<30days)	3/8 (37.5)	7/51 (13.7)	1/5 (20)	1/45 (2.2)	2/5 (40)	6/26 (23.1)	8/11 (72.7)	24/28 (85.7)
Nursery pig (30-70days)	6/7 (85.7)	36/78 (46.2)	1/4 (25)	3/36 (8.3)	3/6 (50)	4/16 (25)	13/13 (100)	43/48 (89.6)
Growing pig (>70days)	5/10 (50)	50/136 (36.8)	2/7 (28.6)	13/104 (12.5)	4/6 (66.7)	22/58 (37.9)	11/12 (91.7)	68/77 (88.3)
Total	10/26 (38.5)	125/383 (32.6)	3/20 (15)	28/265 (10.6)	7/10 (70)	48/125 (38.4)	20/21 (95.2)	185/209 (88.5)

[a]Number of positive farms/Number of tested farms (%)
PRRSV: porcine reproductive and respiratory syndrome virus
APP: *Actinobacillus pleuropneumoniae*, *M. hyo*: *Mycoplasma hyopneumoniae*
PCV2: porcine circovirus type 2

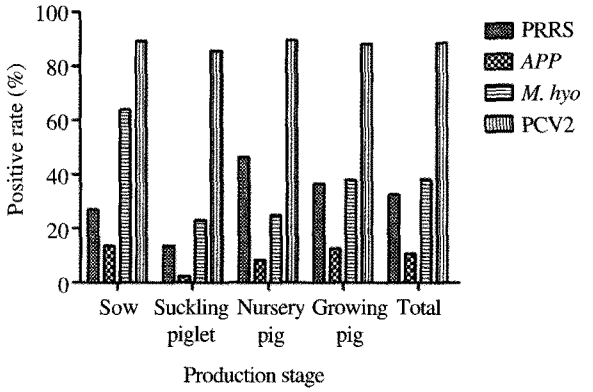

Fig. 1. Seroprevalence of the selected respiratory pathogens by production stages in the pig herds in Chungnam, Chung-buk, Gyeongnam and Gyeongbuk provinces during the period of 2007 to 2008.

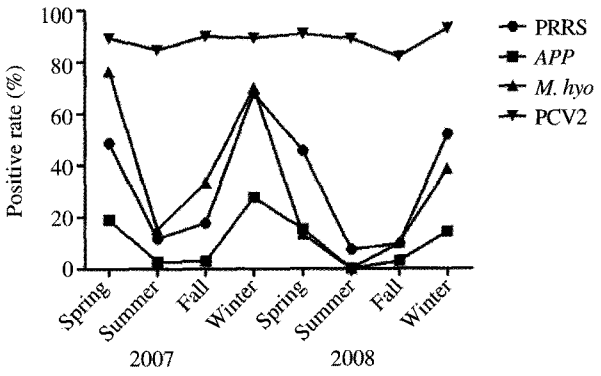

Fig. 2. Seroprevalence of the selected respiratory pathogens by season in the pig herds in Chungnam, Chung-buk, Gyeongnam and Gyeongbuk provinces during the period of spring of 2007 to winter of 2008 (Spring; Mar-May, Summer; Jun-Aug, Fall; Sep-Nov, Winter; Dec-Feb).

low in suckling piglets ($P<0.05$). In the case of *M. hyo*, sows had the highest antibody-positive rate (64%), followed by growing pigs (37.9%) and nursery pigs (25%). However, PCV2 antibody level was extremely high (approximately 90%) in all production stage.

Seroprevalence by season

As shown in Fig. 2, seroprevalence of antibodies to PRRSV, *APP* and *M. hyo* shown the higher positive rates in winter (68.1%, 27.8% and 70% in 2007, respectively; 51.9%, 14.3% and 38.5% in 2008, respectively) and spring (48.8%, 19% and 76.2% in 2007, respectively; 45.8%, 15.4% and 13.3% in 2008, respectively). However, the high prevalence (approximately 90%) of PCV2 antibody was observed regardless of seasons throughout the year of 2007 and 2008.

Seroprevalence by provinces

As shown in Table 2, Gyeongbuk province showed the highest seropositive rates for PRRSV (44.6%), *M. hyo* (47.5%), and PCV2 (92.7%). Seropositive rates for *APP* of four provinces were approximately 10%. The lowest seropositive rates for PRRSV, *APP*, *M. hyo* and PCV2 were found in Chungbuk (15.9%), Gyeongnam (7.0%), Gyeongnam (24.0%) and Chungnam (78.9%), respectively.

DISCUSSION

The interactions between different pathogens play an important role in developing PRDC. For example, pigs infected with *M. hyo* have an increased duration of PRRSV-induced pneumonia (Choi and Chae, 1999). In contrast to the potentiation observed with dual infection with *M. hyo* and PRRSV, pigs infected with both SIV and *M. hyo* lacked the significant increase in severity and duration of SIV-induced pneumonia (Thacker et al, 1999). There is experimental evidence that synergism occurs between PCV2 and PRRSV (Kim et al, 2001; Ross, 1999). Pigs dual-infected with respiratory pathogens has been also reported in Korea (Regula et al, 2000; Kim et al, 2003a; Kim et al, 2003b). The co-infec-

Table 2. Summary of seroprevalence for the selected respiratory pathogens by provinces during the period of 2007 to 2008

Provinces	PRRSV[a]	*APP*	*M. hyo*	PCV2
Chungnam	17/66 (25.8)	5/48 (10.4)	6/22 (27.3)	30/38 (78.9)
Chungbuk	13/82 (15.9)	7/55 (12.7)	8/19 (42.1)	37/41 (90.2)
Gyeongnam	17/60 (28.3)	4/57 (7.0)	6/25 (24.0)	42/48 (87.5)
Gyeongbuk	78/175 (44.6)	12/105 (11.4)	28/59 (47.5)	76/82 (92.7)
Total	125/383 (32.6)	28/265 (10.6)	48/125 (38.4)	185/209 (88.5)

[a]Number of positive serum/Number of tested serum (%)

tion may cause more severe respiratory problems. Economic losses can be considerable when these secondary infections occur. In order to prevent and control these respiratory diseases, serological status of these respiratory pathogens in pig herds should be well understood. Therefore, in this study, seroprevalence survey of four selected respiratory pathogens (PRRSV, *M. hyo*, *APP* and PCV2) was performed using serum samples collected from 37 different swine farms located in Chungnam, Chungbuk, Gyeongnam and Gyeongbuk provinces during the period of spring of 2007 to winter of 2008.

It is clear that PRRSV, *M. hyo*, *APP* and PCV2 are endemic in Korean swine farms. It has been reported that 85.34% of swine farms and 34.81% of pigs were seropositive to *M. hyo* infection (Kim et al, 2008). In 2001, 89.8% farms and 52.1% pigs were seropositive to PRRSV (Kim et al, 2002). The incidence of the respiratory diseases have been frequently reported by the National Veterinary Research & Quarantine Service of Korea. In Canada and Costa Rica, 82.4% and 14.6% of pigs were seropositive to PCV2 (Liu et al, 2002), respectively. In Korea, 50 to 100% of commercial swine farms were positive to PCV2, as tested by polymerase chain reaction method (Oh et al, 2006). An epidemiologic study on pleuropneumonia in the slaughter pigs showed that 5.2% pigs were infected with *APP* (Lee et al, 1996). In the present study, the overall seropositive rates of PRRSV, PCV2, *M. hyo* and *APP* were 32.6%, 88.5%, 38.4% and 10.6%, respectively. In the present study, it is difficult to estimate the significance of the data because the seroprevalence could depend upon the origins of the samples submitted to laboratory. However, it might demonstrate epidemiological patterns of the major respiratory diseases in swine in the regions.

The vaccination status of pig herds is one of the important factors to evaluate the results of sero-epidemiological survey. In the present study, the informations (including vaccination history, production stages and farm's location) about the submitted blood samples were obtained from the field veterinarians using questionnaires. Most of the blood samples were collected from the unvaccinated pig herds for PCV2. Vaccination against PRRSV and *APP* were practised in approximately 10% to 20% of the pig herds in Chungnam, Chungbuk, Gyeongnam and Gyeongbuk provinces. Over 60% of the pig herds of these regions were practised vaccination against *M. hyo*. In case of *APP*, the CHEKIT® APP-ApxIV assay is possible to differentiate the vaccinated from infected animals, resulting in increase of the specificity of the antibody survey. Therefore, the data for seroprevalence of *APP* may represent the natural infection of the agent in the herds. Among the provinces investigated, Gyeongbuk province showed the highest seroprevalence for all of the pathogens tested. It is conjectured that the results might be closely related with the particular status of epidemiology of the pig farms in the region where PMWS was seriously endemic during the period of survey.

The age distribution of the seroprevalence reflects differences in the risk for respiratory pathogens infections by pig ages. The seroprevalence of PRRSV, *APP* and *M. hyo* were relatively low in the population of suckling pigs and nursery pigs, suggesting that the pigs in the age groups might be vulnerable to the pathogenic strains of the diseases. In the case of PCV2, the positive rate reaches approximately 90% of the pig population during all of the production stages (Fig. 1). As no farms had adopted the PCV2-related vaccines, the PCV2-positive serum of the pigs could be resulted from natural infection.

The bacterial and viral infections in pigs have exhibited seasonal patterns in incidence. In general, some pathogen infections peak in the winter, whereas others peak in the summer, and respiratory infections spread more readily in the winter. In our study, seroprevalence of PRRSV, *APP* and *M. hyo* were higher between early winter and late spring. In contrast, there seems no seasonal relationship in the seroprevalence of PCV2.

The results obtained in this study could help for controlling the respiratory pathogens in swine operations in Korea and to define the role of the potential agents that can be involved in the porcine respiratory syndrome.

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