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Isolation of porcine reproductive and respiratory syndrome virus(PRRSV) in Korea

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돼지생식기 및 호흡기증후군(Porcine Reproductive and Respiratory Syndrome ; PRRS) 바이러스의 국내분리주 작성에 관한 연구

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초 록 : 유사산 태아의 폐, 청색증을 나타내는 자돈으로부터 돼지생식기 및 호흡기증후군(PRRS)의 원인체로 추정되는 바이러스주(KPRRSV) 들을 분리하였다. 분리된 바이러스주는 돼지콜레라, 돼지오제스키병, 돼지뇌심근염바이러스에 대한 형광항체반응에서는 음성이었으며 기니픽혈구에 대한 혈구응집 능력을 나타내지 않았다. 그리고 포유 마우스의 뇌내 접종시 이상을 나타내지 않았으나 돼지생식기 및 호흡기증후군에 대한 형광항체검사시 양성반응을 나타내었다. 분리된 바이러스는 돼지폐포탐식세포(porcine alveola macrophages)에서 세포변성효과(cytopathic effect)를 나타내었으며 세포변성효과를 나타내었던 바이러스주중 일주(KPRRSV-1)를 돼지폐포탐식세포에서 7대 연속 계대하여 돼지에 접종한 후 혈청을 분리하여 미국 및 유럽지역에서 분리된 돼지유행성 유사산 및 호흡기증후군의 바이러스를 탐식세포에 감염시켜 효소면역방법(immunoperoxidase monolayer assay)으로 분석한 결과 분리된 바이러스는 미국형 돼지호흡기 및 유사산증후군에 가까운 항원형으로서 판명되었다.

Key words : Porcine reproductive and respiratory syndrome, isolation of KPRRSV strains, antigenic relationship, Lelystad virus, United States isolate.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) or swine infertility and respiratory syndrome (SIRS) was recently identified as the new emerging infectious disease in swine.[1-4] The disease was also known as the mystery swine disease(MSD) because of difficulties in identification of exact causative agent of the syndrome. Initially, the syndrome have been observed in breeding herd, showing late-term abortion including anorexia, pyrexia, lethargy, respiratory signs and slow growth in young pigs.[5-9]

Although the economic losses through the infections of this syndrome were of great significance in swine industry, the exact agent of the disease was not properly characterized until 1991, when Wensvoort et al reported the isolation of Lelystad virus as the causative agent of this syndrome using macrophage cell culture.[1] At present, the PRRS or SIRS virus is known to be a member of Arterivirus and replicate in porcine alveolar macrophage.[10] In addition, it is also known that the antigenic differences are present in various isolates from throughout the world.[11]

Since this country have been importing swines from all those countries having incidence of PRRS or SIRS, there might be a high risk of residence of the disease as well. For this reason, the present study was conducted to isolate and identify the PRRS virus in Korea.

Materials and Methods

Cells : Porcine alveolar macrophages(PAM) were prepared from lung of 3-5 weeks old pig as described by Wensvoort et al.[1] The cells were maintained at 37℃ in 5% of Co_2 incubator. Each batch of PAM was frozen and stored in liquid nitrogens. During the collection of PAM, swine testicle cell of each pig was cultured and tested for the presence of contaminating viral agent through END test by the procedures as described previously.[12]

PK-15 cells were grown in EMEM with 5% of fetal bovine serum and used for isolation of virus.

Sample preparation : The lungs of aborted fetus were aseptically removed and ground for virus isolation. Regularly, 10% suspensions of lung samples were prepared in phosphate buffered saline(PBS) and inoculated into various cells for observation of cytopathic effect(CPE). In addition, serum and lung were also collected from the swine showing blue ear sign in farm. For inoculation into PAM, cells was usually washed with PBS three times and incubated with samples at 37℃ for 1 hour and then washed once with PBS followed by addition of RPMI-1640 medium supplemented with 5% fetal bovine serum.

Virus isolation and identification : To isolate CPE inducing viral agent, the supernatant of ground suspension was inoculated into PAM and PK-15 cells. The inoculated cells were usually passaged three times and observed for the presence of CPE. For the identification of abortive viral agent of fetus, Aujeszky's disease virus(ADV), hog cholera virus(HCV), Japanese encephalitis virus(JEV) and porcine parvovirus(PPV) were tested by indirect immunofluorescence antibody test(IFA) using corresponding monoclonal antibodies and encephalomyocarditis virus(EMCV) by direct FA conjugate, respectively.[13] The monospecific antibody against Lelystad virus(LV) was kindly supplied by Dr. Terpstra and used for the detection of PRRS virus by IFA using avidin-biotin conjugate procedures as described previously.[14,15] The identification on those virus were also checked through observation on the presence of CPE for ADV, END test for HCV, hemagglutinating activity on guinea pig erythrocyte for PPV and EMCV as well as pathogenicity on 2-3 days old mice through intracerebral inoculation for JEV, respectively.

Serologic examination : 4 weeks old pig was inoculated with isolated virus by intranasal route. The paired sera before and 3 weeks after inoculation were collected for serologic examination against ADV, HCV and PRRSV. Antibody titration against PRRV was performed by the procedures of an immunoperoxidase monolayer assay(IPMA) as described by Wensvoort et al.[1] Sera were tested for the binding activity with Lelystad virus or isolate from United States to identify the antigenic relations. The

highest dilution showing the positive staining of the cytoplasm of infected macrophages was counted as the end titer.

Results

Isolation of PRRSV in PAM culture : Using ground suspension of aborted fetus, one viral agent inducing CPE in PAM culture was isolated after 4th blind passage. second strain was isolated from serum samples from swine showing blue ear symptom in the farm as shown in Fig 1. The third isolate was directly isolated from alveolar macrophage cell culture of suspected pig. The CPE in PAM was usually characterized by lysis of inoculated macrophage cells (Fig 2, b). The isolated viral agents were further subjected to diffeial diagnostic tests for ADV, HCV, PPV, EMCV, JEV and PRRSV. The isolates produce no CPE in PK-15 cells, negative result in END test, no hemagglutinating activity, no signs on mice and negatvie reactions in IFA with monoclonal antibodies against ADV, HCV, PPV and JEV(Table 1). However, when the isolates are examined by IFA test using monospecific porcine sera against Lelystad virus, the strong positive reactions are detected at high dilution as indicated in Table 2 and Fig 3. In addition, it is also worth to note that CPE was not detected clearly in every batch of PAM culture, indicating host cell susceptibilities, but once the lysis started, the CPE usually possible to observe within 24-48 hours. Although even the detection of CPE in inoculated PAM were not evident, the positive reactions were usually observed in 4-5 days with virus titer about 10^3 $TCID_{50}$/ml.

Based on the results of differential diagnostic procedures and IFA test, the isolate was designated as KPRRSV-1, KPRRSV-2 and KPRRSV-3, respectively.

Antigenic relatedness of KPRRSV-1 by IPMA : In order to test the antigenicity of the isolate, the 7th

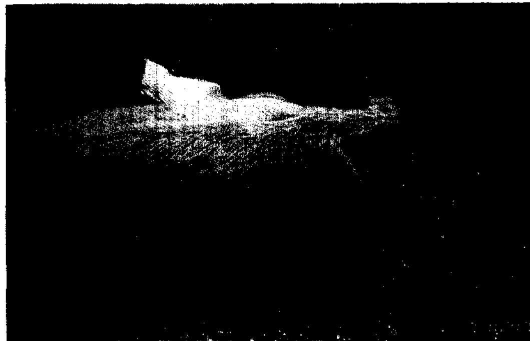
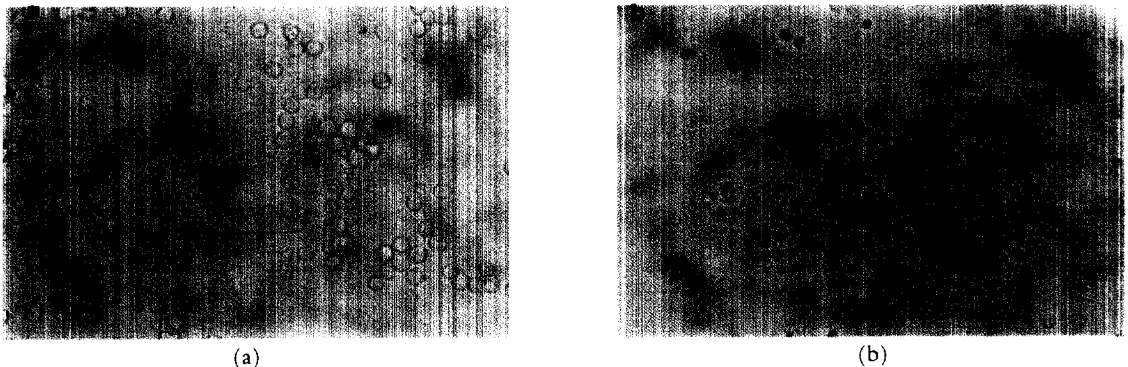


Fig 1. Pig showing blue ear symptom.



(a) (b)

Fig 2. Cytopathic effect(CPE) of porcine alveolar macrophage(PAM).
a) Uninfected PAM. ×100
b) Infected PAM with KPRRSV-1.×100

passage viral stock of KPRRSV-1 was inoculated into pig by intranasal spray. The paired sera of swine were collected and subjected to IPMA with United States isolate or LV. The results indicated that IPMA titer was increasing by four fold against United State isolate after inoculation. On the other hand, IPMA titer was turned out to be same with Europena LV even after 3 weeks(Table 3 and Fig 4). From the IPMA results, it can be assumed that the KPRRSV-1 is antigenically more related to United States strain than European LV strain. However, there was no detectable antibody titers against HCV and ADV in sera as listed in Table 3.

(a) (b)

Fig 3. Immunofluorescence of PAM infected with KPRRSV-1.
a) Control PAM.×320
b) Infected PAM.×160

Table 1. Identification of cytopathic viral agent by immunofluorescent antibody(IFA), cell culture, hemagglutination(HA) and animal inoculation test

Virus	FA@	CPE in cells#	END test$	HA activity*	Animal inoculation**
ADV	-	+			
HCV	-		+		
PPV	-			+	
EMCV	-			+	+
JEV	-				+
PRRSV	+	-	-	-	-

@:Immunofluorescent antibody test using monoclonal antibody against Aujeszky's disease virus(ADV), Hog cholera virus(HCV), Japanese encephalitis virus(JEV), Porcine parvovirus(PPV) and direct FA conjugate for Encephalomyocarditis virus(EMCV) and indirect FA test using porcine antibody on PRRSV(LV).
#:Inoculation on PK-15 cells.
$:Enhancement of New castle disease virus.
*:HA test on guinea pig red blood cell.
**:Intracerebral inoculation of two days old mice.

Table 2. Indirect fluorescent antibody(IFA) titer against standard PRRSV antibody(LV)

Antibody	dilution	200	400	800	1600
Strains	KPRRSV-1	+*	+	+	-
	KPRRSV-2	+	+	+	-
	KPRRSV-3	+	+	+	-

* : IFA positive reaction.

Table 3. Immunoperoxidase monolayer assay(IPMA) for the identification of antigenic relationship of Korean isolate(KPRRSV-1) to United States isolate and European LV strain

Pig sera	IPMA titer		ADV*	HCV**
	U.S.A. isolate	LV strain		
Before inoculation	40	40	-	-
After inoculation	160	40	-	-

* : Serum neutralization assay to Aujeszky's disease virus.
** : END test to Hog cholera virus(ALD strain).

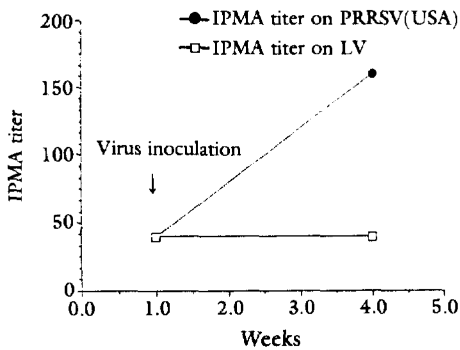


Fig 4. Immune response of Korean Isolate KPRRSV-1

Discussion

Using PAM culture we could isolate at least three viral strains which can be identified as the PRRSV. In addition, the IPMA results on the paired sera from inoculated swine also support our results. First, the conventional swine used for experiment in this study turned out to have the antibody, at least by IPMA, against LV from the Nether lands as well as PRRSV from United States. This finding was rather surprising and unexpected.

However, once assuming the presence of the previous antibody titer as maternal antibody and considering of 2 weeks half life of the maternally acquired passive antibodies, the isolate KPRRSV-1 can be considered as antigenically close to the isolate from United States than European strain.

Although the PRRS was first reported in United States in 1987, similar epidemics in West Europe were also followed[1,4]. It took another several years to identify the exact etiological agent mainly from the difficulties in isolation of causative agent, thus, referring the disease as the MSD until 1991. Nevertheless, the isolation of PRRSV as a causative agent of the mystery swine disease was not succuessful until the PAM culture was known to support the growth of this virus.[1-10]

From those historic backgrounds, it is reasonable to suspect that there must be a high probability that the spread of this disease was rather unnoticed without proper preventive measures mainly because of delayed isolation as well as lack of proper diagnostic measures on etiological agent. In fact, the experimentally inoculated pigs with one of isolate, KPRRSV-1 in this study also showed all the previously reported signs of fever, anorexia, respiratory signs and slow growth as well as interstitial pneumonia(Paper in preparation).

Another finding during the isolation of KPRRSV seems to be the replicating conditions in PAM. Even the CPE was evident during the passage in PAM, the virus titer was not high compared with the reported results of LV or United States isolate. The reason may be rather complicating because we couldn't use SPF or gnotobiotic pigs, which might exclude the environmental effects on alveolar macrophage cells. The delicate PAM susceptibility for PRRSV was also experienced in the case of LV or United States isolate (personal communication with Dr. G. Wensvoort)

Recently. Wensvoort reported that antibodies directed against LV have been widespread throughout the world.[5,6] Moreover, it is also known that there are considerable antigenic variations between American and European isolates, indicatiing mutational diversity among isolated strains.[11]

However, since the virus is now being ablet to

adapt its replications in some kind of line cell, the future characterization will be less laborious than before.[7,16]

At present, the fact of importing swines from all those countries having incidence of PRRSV suggest and necessitate the future task to characterize and differentiate the potential antigenic diversity of those strains reported in this study.

Summary

Three viral strains, causing CPE in porcine alveolar macrophage cell, were isolated from aborted fetus, serum from young pig showing blue-ear sign and lung of suspected pig, respectively. The differential diagnostic results showed no characteristics of Aujeszky's disease virus(ADV), hog cholera virus (HCV), Japanese encephalitis virus(JEV), porcine parvovirus(PPV) and encephalomyocarditis virus (EMCV). However, positive reactions were demonstrated by IFA using monospecific porcine antibodies against Lelystad virus.

When the paired sera of experimentally inoculated swine with one of isolate, KPRRSV-1 were tested by IPMA, the result indicated that the isolate was related to United States isolate than European LV.

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References

1. Wensvoort G, Terpstra C, Pol JMA et al. Mystery swine disease in the Netherlands : the isolation of Lelystad virus. *The Vet Quartery* 1991; 13(3):121-130.
2. Terpstra C, Wensvoort G, Pol JMA et al. Experimental reproduction of porcine epidemic abortion and respiratory syndrome(mystery swine disease) by infection with Lelystad virus : Koch's postulates fulfilled. *The Vet Quartery* 1991; 13(3):131-136.
3. Pol JMA, van Dijk JE, Wensvoort et al. Pathological, ultrastructural, and immunohistochemical changes caused by Lelystad virus in experimentally induced infections of mystery swine disease (synonym : porcine epidemic abortion and respiratory syndrome(PEARS). *The Vet Quartery* 1991; 13(3):137-144.
4. Keffaber KK, Reproductive failure of unknown etiology. *Am Assoc Swine Pract Newsl* 1989; 1:1-9.
5. Wensvoort G. Lelystad virus and the porcine epidemic abortion and respiratory syndrome. *Vet Res* 1993; 24:117-124.
6. Wensvoort G, de Kluyver EP, Pol JMA et al. Lelystad virus, the cause of porcine epidemic abortion and respiratory syndrome : a review of mystery swine disease research at Lelystad. *Vet Microbiol* 1992; 33:185-193.
7. Benfield DA, Nelson E, Collins JE et al. Characterization of swine infertility and respiratory syndrome (SIRS) virus(isolate ATCC VR-2332). *J Vet Diagn Invest* 1992; 4:127-133.
8. Christianson WT, Collins JE, Benfield DA et al. Experimental reproduction of swine infertility and respiratory syndrome in pregnant sows. *Am J Vet Res* 1992; 53:485-488.
9. Collins JE, Benfield DA, Christianson WT et al. Swine infertility and respiratory syndrome(SIRS) virus(isolate ATCC VR-2332) in North America and experimental reproduction of the disease in gnotobiotic pigs. *J Vet Diagn Invest* 1992; 4:117-126.
10. Janneke JMM, Marcel MH, Emile JM et al, Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome(PEARS), is related to LDV and EAV.*Virol* 1993; 192:62-72.
11. Wensvoort G, de Kluyver EP, Luijtze et al. Antigenic comparison of Lelystad virus and swine infertility and respiratory syndrome(SIRS) virus.*J Vet Diagn Invest* 1992; 4:134-138.
12. Kumagai T, Shimizu T, Ikeda S et al. A new in vitro method(END) for detection and measurement of hog cholera virus and its antibody by means of effect of HC virus on newcastle disease virus in swine tissue culture. I. Establishment of standard procedure.*J Immunol* 1961; 87:245-256.
13. Kim BH, Kweon CH, An SH et al. Pig viral

diseases causing reproductive failure in Korea.*Korean J Vet Res* 1992; 32(3):365-368.

14. Kweon CH, Kwon BJ, Jung TS et al. Isolation of porcine epidemic diarrhea virus(PEDV) in Korea. *Korean J Vet Res* 1993; 33(2):249-254.
15. Yoon IJ, Joo HS, Christianson WT et al. An indirect fluorescence antibody test for the detection to swine infertility and respiratory syndrome virus in swine sera.*J Vet Diagn Invest* 1992; 4:144-147.
16. Bautista EM. Sagar M, Goyal IJ et al. Comparison of porcine alveolar macrophages and CL 2621 for the isolation of porcine epidemic abortion and respiratory syndrome(PRRS) virus and anti-PRRS antibody.*J Vet Diagn Invest* 1993; 5:163-165.