大韓獸醫學會誌(2008) 第48卷 第3號
Korean J Vet Res(2008) 48(3) : 267~274

A comparison of single dose efficacy of *Mycoplasma hyopneumoniae* bacterin in swine farms with different serological patterns of PRRSV and PCV2.

Hye Kwon Kim[1], Hyoung Joon Moon[1], Eun Mi Kim[1], Jeong Sun Yang[1], Seong Jun Park[1], Yuzi Luo[1], Chul Seung Lee[2], Dae Sub Song[2], Bo Kyu Kang[2], Jaebum Lee[3], Bong Kyun Park[1,*]

[1]*College of Veterinary Medicine and BK21 Program for Veterinary Science, Seoul National University, Seoul 151-742, Korea*
[2]*Research Unit, Green Cross Veterinary Products, Yongin 449-903, Korea*
[3]*Hankuk Academy of Foreign Studies, Yongin 449-854, Korea*
(Accepted: August 1, 2008)

Abstracts : This study was to evaluate the efficacy of single dose *Mycoplasma hyopneumoniae* (*M. hyo*)-vaccination in the swine farms which had different serological patterns of porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). A minimum of 240 pigs from each farm was applied, allocating *M. hyo* vaccinated and control groups. The PRRSV and PCV2 infections were analyzed by serological method (commercial ELISA kit). After administrating pigs a single dose of *M. hyo* vaccine or control saline at 3 weeks of age, serum antibodies to *M. hyo*, PRRSV and PCV2 were monitored at 4, 10, 16 and 22 weeks of age. Mortality, weight changes, feed conversion ratio (FCR) and lung score were also evaluated. A single-dose vaccination of *M. hyo* bacterin was efficacious to reduce mycoplasmal lung lesions and induce good humoral immune response. However, FCR was improved only in one of the three farms where showed seronegative status to both PRRSV and PCV2 in the period from 4 to 16 weeks of age. These results might imply that *M. hyo* vaccine alone could not overcome the PRRSV and PCV2 infection-associated wasting in the field condition. Therefore, the control of PRRSV and PCV2 should be considered to obtain the better effects of *M. hyo* vaccination.

Keywords : Efficacy, *Mycoplasma hyopneumoniae*, PCV2, PRRSV, Vaccine

Introduction

Mycoplasma hyopneumoniae (*M. hyo*) is an etiologic agent that causes enzootic pneumonia in the swine population. The common clinical signs of *M. hyo* infection are dry cough, decreased feed conversion ratio (FCR) and *M. hyo*-specific lung lesions [24]. *M. hyo* is also commonly found in complicated respiratory diseases associated with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) [7, 11].

Vaccination is one of the methods for controling *M. hyo* infection. *M. hyo* bacterin, which is commonly used, can effectively induce local and systemic immune responses when administered by intramuscular injection [25]. Recently, several studies revealed no significant differences between one-shot and two-shot vaccination protocols. Along with these reports, other studies showed that a single dose of the *M. hyo* vaccines was efficacious in the field condition [4, 6].

Recently, however, as the PCV2-asscociated diseases becomes one of the important issues in the swine industries, several factors which could potentiate postweaning multisystemic wasting syndrome (PMWS) with PCV2 infection were identified, such as PRRSV and porcine parvovirus (PPV) infection [1, 2, 8, 10, 14] or even *M. hyo* bacterin with mineral oil-based adjuvant [3, 15]. In addition, experimental reproduction of PMWS was established when pigs were dually infected with *M. hyo* and PCV2 [21].

*Corresponding author: Bong Kyun Park
College of Veterinary Medicine and BK21 Program for Veterinary Science, Seoul National University, Seoul 151-742, Korea
[Tel: +82-2-880-1255, Fax: +82-2-885-0263, E-mail: parkx026@snu.ac.kr]

With these concerns, especially PMWS associated with the *M. hyo* or the *M. hyo* vaccine with mineral oil-based adjuvant, field efficacy of the *M. hyo* vaccine might be interpreted ambiguously in the presence of PCV2 or other PMWS-associated pathogens. Therefore, surveillance of the concurrent PCV2 infectious status along with other pathogens should be included to evaluate the efficacy of the *M. hyo* vaccine more practically in the field condition. Although there is a report that PRRSV infection did not affect to the efficacy of a single dose of the *M. hyo* vaccine, especially in daily weight gain and mortality [19], PCV2 infectious status which is the main criteria of PMWS did not included. Therefore, this study was performed to evaluate the field efficacy of the *M. hyo* vaccine with a single-dose protocol in the three commercial farms, observing the serological status of PRRSV and PCV2 of each farm.

Materials and Methods

Experimental farms and animals

Three farrow to finish farms (designated A, B, and C), all of which were located in the Gyeonggi province in Korea, were selected. All farms weaned piglets (crossbreeding, Landrace × Yorkshire × Duroc) at 21 days of age and had been diagnosed to be infected with *M. hyo* in previous serological and post-mortem data. The 276, 240 and 242 of weaned pigs in each farm were selected to perform the efficacy test of the *M. hyo* vaccine by dividing the pigs into vaccinated and non-vaccinated control group with similar sex distribution. Each group consisted of a minimum of 120 pigs with similar distribution according to sex and body weight. In order to avoid inter-building variations, vaccinated and control pigs were grown in the same building, but in separate pens. While the A farm had not used PRRSV-modified live vaccine (MLV) at the time of field study, B and C farms had used the MLV in different vaccine schedules. While B farm had vaccinated MLV to the gilts at 3 weeks before entrance to the farm and piglets at 10-14 days of age, C farm had vaccinated to the gilts at the time of entrance and to the sows at a week after farrowing. All three farms did not use PCV2 vaccines. All animal experiments complied with current Korean law on the care and treatment of animals and were conducted in accordance with the protocols and guidelines of the Seoul National University Institutional Animal Care and Use Committee.

Experimental design

The *M. hyo* vaccine used in this study was a commercialized inactivated whole bacterin vaccine which was combined with light paraffin oil as an adjuvant.

Each pig within the vaccinated group received one dose (2 ml) of the *M. hyo* vaccine, intramuscularly at 3 weeks of age, while control pigs received an equivalent injection of physiological saline.

Collective preventive antibiotic treatment was applied orally, via the feeding route, at entry to the post-weaning and/or fattening unit, but drugs specifically targeted to *M. hyo*, such as macrolides, were avoided.

For each experimental group, 10 blood samples were taken randomly from pigs at 4, 10, 16 and 22 weeks of age to monitor serum antibodies to *M. hyo*, PRRSV and PCV2. In the case of farm A, first blood sampling was performed with pigs at 5 weeks of age.

Animals were individually weighed at 3 and 10 weeks of age and again prior to slaughter. For each experimental group, final weights were taken on the same day at 23 weeks of all pigs of a batch, even if pigs were subsequently sent to the slaughterhouse at slightly different times.

At the time of slaughter, lung lesions were scored in at least 40% of remained pigs. *M. hyo*-specific lesions were described using a standardized drawn map of the lungs that was further used to score lungs as precisely as possible, according to the method of Madec & Kobisch [17].

Enzyme linked immunosorbent assay for *M. hyo*, PRRSV and PCV2

Sera from collected blood samples were obtained by centrifugation at 2,500 rpm for 20 min at 4. An indirect enzyme linked immunosorbent assay kit for *M. hyo* (ELISA kit, Herdcheck *M. hyo*; IDEXX Laboratories, USA) was used to evaluate the antibody titers and titers were presented as S/P ratios. For PRRSV and PCV2, commercial ELISA kit was also used (PRRSV, Herdcheck PRRS 2XR; IDEXX Laboratories, USA) (PCV2, SERELISA PCV2 Ab monoblocking; Synbiotics Europe, France). ELISA kits for *M. hyo* and PRRSV were based on the indirect system, while PCV2 ELISA kit was based on the blocking ELISA system. All protocols were performed as recommended by the

manufacturer. S/P ratio more than 0.4 was regarded as the positive for *M. hyo* and PRRSV. Adjusted O.D (sample O.D/negative O.D) less than 0.15 was regarded positive in the PCV2-ELISA.

Feed conversion ratio

Pigs from different experimental groups were grown in separate pens and fed separately to allow evaluation of feed conversion rates. After the vaccination, daily feed intake was documented by checking the remaining feed level when new feed was introduced. With the weight data, FCR of pigs were evaluated during total experimental period (3 to last weighted weeks of age) according to the following formula: FCR = kg average daily feed intake / kg average daily weight gain.

Statistical analysis

All data were sorted in the Microsoft Excel software program. Pearson's chi-square test and Fisher's exact test were performed to statistically analyze the mortality and ratio of lungs with mycoplasmal lesion between two groups. For the comparison of mean feed conversion ratio, mean weight and mean lung score between the two groups, a student's *t*-test was performed using the SPSS 12.0 program (SPSS, USA).

Results

Serological status of PRRSV and PCV2 during the study in the three conventional farms

Serological status of PRRSV and PCV2 is different in the 3 farms (Figs. 1 and 2). In the case of PRRSV, there was no obvious difference in serological patterns between *M. hyo* vaccinated and control group in all three farms (data not shown). Farm A showed seronegative status of PRRSV until the last blood sampling which were performed at 22 weeks of age, and the other two farms (B and C) showed seropositive with different serological patterns. In the farm B which applied MLV vaccination to pigs at about 2 weeks of age, titers of anti-PRRSV antibodies were gradually increased as the pigs were growing older. Farm C which vaccinated MLV only to the gilts and sows showed seronegative status in the pigs at 10 weeks of age and seroconversion which could be due to the natural infection of PRRSV was begun to observed at 16 weeks of age.

The serologic pattern of PCV2 was analyzed by

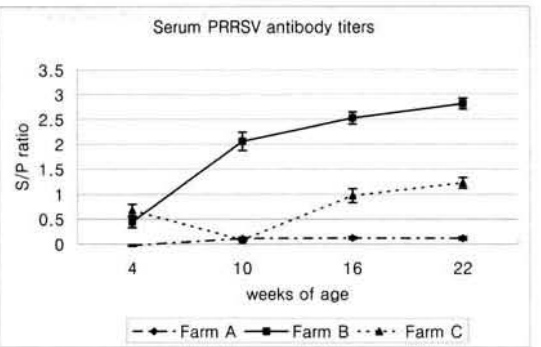

Fig. 1. Serological patterns of porcine reproductive and respiratory syndrome virus (PRRSV) in the three farms were presented using S/P ratio and standard error. S/P ratio more than 0.4 regarded as the PRRSV-positive serum.

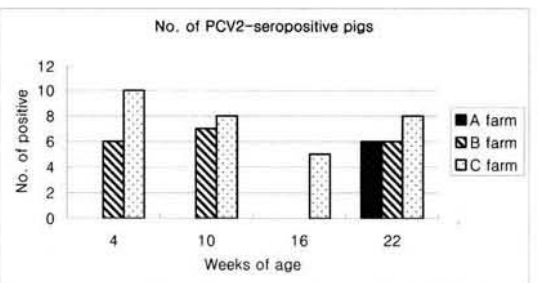

Fig. 2. Number of porcine circovirus type 2 (PCV2)-seroconverted pigs among the 20 tested samples of each farm at 4, 10, 16, 22 weeks of age.

comparing the number of positive sera from 20 pigs of each farm. Farm A had maintained seronegative status of PCV2 until 16 weeks of age followed by seropositive pigs at 22 weeks of age. While farm C had seropositive pigs at all of 4, 10, 16 and 22 weeks of age, farm B showed seropositive status at 4, 10 and 22 weeks of age.

Serum antibody titers against *M. hyo* during the experiment

From 10 weeks of age, the seroconversion rate of vaccinated groups became higher than the control groups and so did the mean titers in all three farms. (Table 1) The control groups showed increased seroconversion at 22 weeks of age in farms A and B, suggesting that natural *M. hyo* infection had occurred. However, in farm C, the control group maintained seronegative status for the experiment duration, although 90% of the vaccinated group displayed seroconversion at 22 weeks of age.

Table 1. Anti-*Mycoplasma hyopneumoniae* antibody titers*

Farm	Group	Weeks of age			
		4	10	16	22
A†	Vaccinated	0.276 ± 0.065‡	0.512 ± 0.104	0.651 ± 0.205	0.965 ± 0.157
		40§	60	50	80
	Control	0.007 ± 0.005	0.015 ± 0.006	0.062 ± 0.027	0.503 ± 0.129
		0	0	0	50
B	Vaccinated	0.058 ± 0.040	1.220 ± 0.159	1.147 ± 0.225	1.599 ± 0.203
		10	100	90	100
	Control	0.089 ± 0.057	0.099 ± 0.095	0.164 ± 0.097	0.715 ± 0.165
		10	10	20	60
C	Vaccinated	0.340 ± 0.156	0.593 ± 0.143	0.486 ± 0.149	0.893 ± 0.114
		20	60	40	90
	Control	0.044 ± 0.026	0.006 ± 0.004	0.011 ± 0.008	0.191 ± 0.047
		0	0	0	0

*IDEXX ELISA kit was used, antibody titers were expressed by S/P ratio (> 0.4 for positive).
†In the case of Farm A, the first blood sampling was performed at 5 weeks of age.
‡Mean S/P ratio ± SE.
§Positive rate (%).

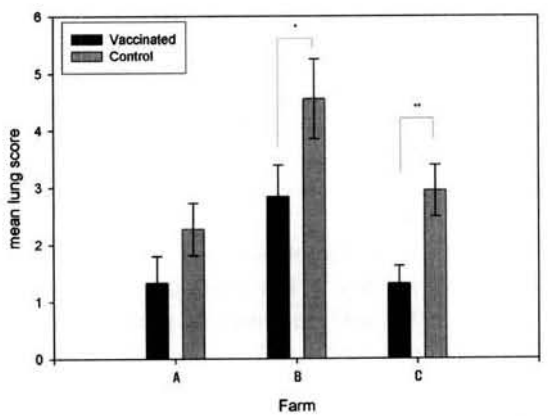

Fig. 3. Mean lung scores of the vaccinated and control groups in each farm. *$p < 0.1$ in t -test; ** $p < 0.05$ in t-test.

Comparison of lung scores between the two groups

Lung scoring was performed in at least 40% of remained pigs. The vaccinated groups from all three farms showed relatively low mean lung scores compared to those of the control groups, although only farm C had a significant difference between two groups in the student's t-test (Fig. 3). Not only was mean lung scores compared, but also the ratio of lungs with mycoplasmal lesions among the investigated lungs was compared between the two groups. All three farms showed that the lungs with mycoplasmal lesions were relatively lower in the vaccinated groups than those of the control groups, but only farm A and C were statistically significant (Table 2).

Comparison of feed conversion ratio

Only farm A had an improved FCR during the total experimental duration ($p = 0.017$, Table 3). The other two farms had relatively high FCR compared to that of farm A and they had no significant difference between the two groups.

Weight changes during the experiment

At the final weighing, farms B and C recorded no significant differences in mean weight between the two groups (Table 4). In farm A, however, the mean weight of the vaccinated group was significantly higher than the control group ($p = 0.018$). Until 10 weeks of age, there were no significant differences in mean weights among the three farms. However, after 10 weeks of age at which the pigs moved to the growing houses, obvious differences in mean weights among the three farms were observed with the data of the last weighing.

Discussion

In Korea, 85.34% of swine farms and 34.81% of pigs

Table 2. Comparison of proportion of pigs having mycoplasmal lung lesion between two groups

Farm	Group	Ratio of investigated lungs (%)	Ratio of lungs having mycoplamsal lesion (%)	p[†]
A	Vaccinated	48/120[‡] (40.0)	15/48 (31.3)	0.046[*]
	Control	51/121 (42.2)	26/51 (51.0)	
B	Vaccinated	69/99 (69.7)	35/69 (50.7)	0.212
	Control	60/100 (60.0)	37/60 (61.7)	
C	Vaccinated	54/116 (46.6)	19/54 (35.2)	0.033[*]
	Control	54/118 (45.8)	30/54 (55.6)	

The proportion of lungs with mycoplasmal lesion in total investigated lungs was compared in each groups. The independence of two variables(vaccinated or control/lungs with mycoplasmal lesion or not) was analyzed by Pearson's chi-square test using SPSS 12.0.

[*]Significant independence of two variables ($p < 0.05$).

[†]2-sided p values of Pearson's chi-square test.

[‡]No. of investigated lungs/no. of total pigs used in this study.

Table 3. Feed conversion ratio during the study

Farm	Group	Feed Conversion Ratio[†]
A	Vaccinated	2.004 ± 0.018[*]
	Control	2.065 ± 0.018[*]
B	Vaccinated	2.800 ± 0.103
	Control	2.715 ± 0.071
C	Vaccinated	2.725 ± 0.048
	Control	2.638 ± 0.028

[*]Significant with student's *t*-test.

[†]Mean feed conversion ratio ± SE.

were seropositive to *M. hyo* infection [9]. This data indicates that vaccination could be one of the choices to control the *M. hyo* infection. Although maternal antibodies against *M. hyo* can persist to 9 weeks of age in pigs [20], there is an evidence that these antibodies do not interfere with the immune reaction to the vaccine [18]. Thus, early vaccination with a one-shot protocol, which would have both practical and economic advantages, could theoretically be applied in swine farms. However, as PRRSV and PCV2 infection

Table 4. Weight changes during the experiment

Farm	Group	Weeks of age		
		3	10	23
A	Vaccinated	6.84 ± 1.39[‡]	27.22 ± 3.09	112.3 ± 9.60
		138[§]	130	120
	Control	6.78 ± 1.27	27.77 ± 3.09	108.5 ± 9.18
		138	133	121
		0.701[‖]	0.155	0.018[*]
B[†]	Vaccinated	5.52 ± 1.09	24.12 ± 5.41	88.99 ± 17.48
		120	113	99
	Control	5.22 ± 0.70	23.51 ± 4.66	92.83 ± 16.96
		120	106	97
		0.012[*]	0.374	0.120
C	Vaccinated	5.79 ± 1.10	24.91 ± 3.83	103.9 ± 13.22
		121	116	112
	Control	6.01 ± 1.29	25.15 ± 4.34	104.4 ± 10.69
		121	119	117
		0.159	0.659	0.754

[*]Significantly different between two groups ($p < 0.05$).

[†]In the case of farm B, the last weighing was performed at 24 weeks of age.

[‡]Mean weight ± SD.

[§]No. of weighed pigs.

[‖]*p* value of student's *t*-test.

are endemic in Korean swine farms, practical efficacy of the M. hyo vaccine should also be estimated in the field condition. Therefore, this study was performed to evaluate the efficacy of commercial *M. hyo* vaccine in the three commercial Korean swine farms with a one-shot vaccination schedule at 3 weeks of age, observing serological status of PRRSV and PCV2.

In this study, one-shot vaccination with *M. hyo* bacterin produced a good serum antibody induction in all farms. Even though many other studies have shown that it takes 3 to 8 weeks to achieve seroconversion status after *M. hyo* infection [13, 20], this study showed that there was an increase in mean S/P ratio and seroconversion rate of vaccinated groups at 4 or 5 weeks of age (1 or 2 weeks after vaccination) in two of the three farms. Although pigs of this age might retain some maternal antibodies, this difference indicated that *M. hyo* bacterin can induce humoral immunity effectively.

Notably, all vaccinated groups showed lower lung scores and less pigs with mycoplasmal lesions than their respective controls, although there were some differences in significancy. Interestingly, mycoplasmal lung lesions were observed in farm C, even though the antibody titers were maintained to negative status in the control group during the experiment. This might be due to that that natural infection that occured at the end of the growing period. A previous report showed that the natural infection of *M. hyo* could occurred at the beginning of fattening period (around 100 days of pigs) and median inhibition percentage (IP) was remained negative until 150 days of age [16]. Therefore, this might explain why *M. hyo*-associated lung lesions were observed in the control group. Besides, lung lesions were significantly lower in the vaccinated group than the control group in this farm. Thus, vaccination with *M. hyo* bacterin reduced mycoplasmal lung lesions, even though putative natural infection seemed to occur in the late part of the growing period, quite some time after vaccination.

Aside from the good effects of the *M. hyo* vaccine in the *M. hyo*-specific measuring criteria (such as humoral response and mycoplasmal lung lesions), other broad range criteria (such as mean weights and FCR) did not always reflect the positive effects of the *M. hyo* vaccine. With diverse conditions in field context, these criteria could be affected by other disease status, especially PRRSV and PCV2 infections which are associated with pig's wasting in the field. Since dual infection of PRRSV and PCV2 was one of the most potential causes of PMWS among other pathogens [22], this study was focused on the infectious status of PRRSV and PCV2 to assay the relationship of these viruses with efficacy of *M. hyo* vaccination, using serological method.

The serological status of PRRSV and PCV2 was evaluated by indirect ELISA and blocking ELISA systems, respectively. While serological data of PRRSV was interpreted with S/P ratio, PCV2 was done by total number of positive pigs because PCV2-ELISA was based on the blocking ELISA system which had narrow window to quantify the PCV2 antibodies. In the case of PRRSV, farm A had been seronegative during total period, but farm B and C showed seroconversion as the pigs grew up with different pattern that could be resulted from MLV vaccinating to the pigs at 10-14 days of age in farm B. However, increased S/P ratio was observed in pigs at 16 weeks of age and this obviously represented that farms B and C were exposed to the natural PRRSV infection. As no farms had adopted the PCV2-related vaccines, the PCV2-positive serum of the pigs could be resulted from natural infection. From the fact that the age mostly affected by the PMWS was regarded as about 11-17 weeks of age [5], we could assume that the seroconversion of PCV2 at 10 and 16 weeks of age in farm B and C might be mostly associated with PCV-associated diseases. In addition, seroconversion related to the PCV2 infection was detected in pigs of 22 weeks of age in all 3 farms.

Notably, the mean weight and the FCR of the vaccinated group were improved in farm A throughout the total experimental period, but no significant difference was observed in farms B and C. Although *M. hyo* bacterin with mineral oil-based adjuvant could potentiate PMWS [3, 15], there were no significant differences of FCR between two groups in farm B and C. This indicates that the *M. hyo* vaccine (light paraffin oil as an ajuvant) used in this study did not at least have a negative effects to the FCR possibly associated with PMWS. This was along with the previous report which proved the other three commercial *M. hyo*-vaccines did not induce any PCV2-associated disease [23]. Interestingly, farm A was PRRSV-negative during the whole period of study and PCV2-negative at least until 16 weeks of age compared to other 2 farms which

showed no significant improvement of mean weight and FCR in vaccinated group. Moreover, mean weight of 23 weeks of age was much higher in the farm A than farm B and C, although mean weights at 10 weeks of age were similar among the three farms. Besides, standard deviation (SD) of weights in 23 weeks of age pigs was higher in farm B and C than farm A. This implied that this higher SD resulted from irregular weight distribution of growing pigs associated with wasting symptoms. In Korea, most PMWS case was observed in pigs of 60-80 days of age [12]. As the farm B and C had serological evidence of PRRSV and PCV2 infection at 10 to 16 weeks of age, these viral infections which are known to cause PMWS might contribute to the reduced efficacy regarding to mean weight and FCR in *M. hyo* vaccinated groups.

There have been some previous studies demonstrating that one-shot *M. hyo* vaccination improves pig performance compared to non-vaccinated pigs [4, 6]. In this study, a single-dose vaccination of *M. hyo* bacterin to the pigs at 3 weeks of age was efficacious to reduce mycoplasmal lung lesions and induce a good humoral immune response. However, mean weight and FCR was not always improved in *M. hyo*-vaccinated groups in the three conventional farms. This might be due to the difference in PRRSV and PCV2 infectious status in the three farms, which could be a negative effect for *M. hyo* vaccination to overcome the virus-associated growing factors. Although, PRRSV infection did not affect daily weight gain in *M. hyo* vaccinated group in the previous study [19], dual infection of PRRSV and PCV2 could affect to the efficacy of *M. hyo* vaccination, especially in mean weight and FCR in this study. As this study did not provide information whether the FCR was affected by PRRSV and PCV2 infection directly or indirectly concerning with other swine pathogens, and did not perform histopathological tests to confirm the PMWS, more study in restricted condition, rather than in the field condition will be needed to support the result of this study.

In conclusion a single dose of the M. hyo vaccine induced good humoral immune responses and reduced mycoplasmal lesions in the field condition. However, a possible influence of dual infection of PRRSV and PCV2 should be considered to improve the feed conversion ratio by the *M. hyo* vaccination.

Acknowledgments

This work was supported by a grant(Code # 20070401034009) from BioGreen21 Program, Rural Development Administration and by Research Institute for Veterinary Science, Republic of Korea.

References

1. **Allan GM, Kennedy S, McNeilly F, Foster JC, Ellis JA, Krakowka SJ, Meehan BM, Adair BM.** Experimental reproduction of severe wasting disease by co-infection of pigs with porcine circovirus and porcine parvovirus. J Comp Pathol 1999, **121**, 1-11.
2. **Allan GM, McNeilly F, Ellis J, Krakowka S, Meehan B, McNair I, Walker I, Kennedy S.** Experimental infection of colostrum deprived piglets with porcine circovirus 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) potentiates PCV2 replication. Arch Virol 2000, **145**, 2421-2429.
3. **Allan GM, McNeilly F, Kennedy S, Meehan B, Ellis J, Krakowka S.** Immunostimulation, PCV-2 and PMWS. Vet Rec 2000, **147**, 170-171.
4. **Baccaro MR, Hirose F, Umehara O, Gonçalves LCB, Doto DS, Paixão R, Shinya LT, Moreno AM.** Comparative efficacy of two single-dose bacterins in the control of *Mycoplasma hyopneumoniae* in swine raised under commercial conditions in Brazil. Vet J 2006, **172**, 526-531.
5. **Blanchard P, Mahé D, Cariolet R, Truong C, Le Dimna M, Arnauld C, Rose N, Eveno E, Albina E, Madec F, Jestin A.** An ORF2 protein-based ELISA for porcine circovirus type 2 antibodies in post-weaning multisystemic wasting syndrome. Vet Microbiol 2003, **94**, 183-194.
6. **Dawson A, Harvey RE, Thevasagayam SJ, Sherington J, Peters AR.** Studies of the field efficacy and safety of a single-dose *Mycoplasma hyopneumoniae* vaccine for pigs. Vet Rec 2002, **151**, 535-538.
7. **Ellis J, Clark E, Haines D, West K, Krakowka S, Kennedy S, Allan GM.** Porcine circovirus-2 and concurrent infections in the field. Vet Microbiol 2004, **98**, 159-163.
8. **Harms PA, Sorden SD, Halbur PG, Bolin SR, Lager KM, Morozov I, Paul PS.** Experimental reproduction of severe disease in CD/CD pigs concurrently infected

with type 2 porcine circovirus and porcine reproductive and respiratory syndrome virus. Vet Pathol 2001, **38**, 528-539.

9. **Kim HK, Kim TY, Lim JS, Lee YH, Park BK.** Seroprevalence of *Mycoplasma hyopneumoniae* infection in Korean swine herds. Korean J Vet Res 2005, **45**, 55-61.
10. **Kim J, Choi C, Chae C.** Pathogenesis of postweaning multisystemic wasting syndrome reproduced by co-infection with Korean isolates of porcine circovirus 2 and porcine parvovirus. J Comp Pathol 2003, **128**, 52-59.
11. **Kim J, Chung HK, Chae C.** Association of porcine circovirus 2 with porcine respiratory disease complex. Vet J 2003, **166**, 251-256.
12. **Kim J, Chung HK, Jung T, Cho WS, Choi CS, Chae C.** Postweaning multisystemic wasting syndrome of pigs in korea: Prevalence, microscopic lesions and coexisting microorganisms. J Vet Med Sci 2002, **64**, 57-62.
13. **Kobisch M, Blanchard B, Lepotier MF.** *Mycoplasma hyopneumoniae* infection in pigs: duration of the disease and resistance to reinfection. Vet Res 1993, **24**, 67-77.
14. **Krakowka S, Ellis JA, Meehan B, Kennedy S, McNeilly F, Allan G.** Viral wasting syndrome of swine: Experimental reproduction of postweaning multisystemic wasting syndrome in gnotobiotic swine by coinfection with porcine circovirus 2 and porcine parvovirus. Vet Pathol 2000, **37**, 254-263.
15. **Kyriakis SC, Saoulidis K, Lekkas S, Miliotis CC, Papoutsis PA, Kennedy S.** The effects of immuno-modulation on the clinical and pathological expression of postweaning multisystemic wasting syndrome. J Comp Pathol 2002, **126**, 38-46.
16. **Leon EA, Madec F, Taylor NM, Kobisch M.** Seroepidemiology of *Mycoplasma hyopneumoniae* in pigs from farrow-to-finish farms. Vet Microbiol 2001, **78**, 331-341.
17. **Madec F, Kobisch M.** Bilan lésionel des porcs charcutiers à l'abattoir, Journées de la Recherche porcine en France. Institut Techinique du Porc 1982, **14**, 405-412.
18. **Martelli P, Terreni M, Guazzetti S, Cavirani S.** Antibody response to *Mycoplasma hyopneumoniae* infection in vaccinated pigs with or without maternal antibodies induced by sow vaccination. J Vet Med B Infect Dis Vet Public Health 2006, **53**, 229-233.
19. **Moreau IA, Miller GY, Bahnson PB.** Effects of *Mycoplasma hyopneumoniae* vaccine on pigs naturally infected with *M-hyopneumoniae* and porcine reproductive and respiratory syndrome virus. Vaccine 2004, **22**, 2328-2333.
20. **Morris CR, Gardner IA, Hietala SK, Carpenter TE, Anderson RJ, Parker KM.** Seroepidemiologic study of natural transmission of *Mycoplasma hyopneumoniae* in a swine herd. Prev Vet Med 1995, **21**, 323-337.
21. **Opriessnig T, Thacker EL, Yu S, Fenaux M, Meng XJ, Halbur PG.** Experimental reproduction of postweaning multisystemic wasting syndrome in pigs by dual infection with *Mycoplasma hyopneumoniae* and porcine circovirus type 2. Vet Pathol 2004, **41**, 624-640.
22. **Pogranichniy RM, Yoon KJ, Harms PA, Sorden SD, Daniels M.** Case-control study on the association of porcine circovirus type 2 and other swine viral pathogens with postweaning multisystemic wasting syndrome. J Vet Diagn Invest 2002, **14**, 449-456.
23. **Rapp-Gabrielson VJ, Hoover T, Sornsen S, Kesl L, Taylor L, Jolie R, Runnels P, Weigel D, Yu S, Opriessnig T, Ruebling-Jass K, Strait E, Halbur PG.** Effects of Mycoplasma hyopneumoniae vaccination in pigs co-infected with M hyopneumoniae and porcine circovirus type 2. J Swine Health Prod 2008, **16**, 16-26.
24. **Thacker EL.** Mycoplasmal diseases. In: Straw BE, Zimmerman JJ, Sylvie D'Allaire, Taylor JJ. (eds.). Diseases of Swine. 9th ed. pp.701-719, Blackwell, Iowa, 2006.
25. **Thacker EL, Thacker BJ, Kuhn M, Hawkins PA, Waters WR.** Evaluation of local and systemic immune responses induced by intramuscular injection of a *Mycoplasma hyopneumoniae* bacterin to pigs. Am J Vet Res 2000, **61**, 1384-1389.