Determination of optimal age for single vaccination of growing pigs with foot-and-mouth disease bivalent vaccine in South Korea

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ABSTRACT. In South Korea, pigs were vaccinated once between 8 and 12 weeks of age because of the injection-site granulomas. Therefore this study was performed to determine the optimal age for single vaccination of growing pigs with the currently used type O FMD vaccine. With 498 pigs divided into four groups, seroprevalence of the antibody was analyzed with enzyme-linked immunosorbent assay. Although double vaccination is necessary to completely protect growing pigs from FMD virus infection with the current vaccine, the age of 8 weeks can be considered as the optimal age for piglet vaccination if the booster injection is unavailable.

KEY WORDS: foot-and-mouth disease, pig, seroprevalence, vaccine

A widespread foot-and-mouth disease (FMD) outbreak caused severe economic damage in South Korea during November 2010–April 2011 [9]; consequently, a vaccination policy was implemented across the country. Since March 2015, the Korean government has started using a bivalent vaccine, consisting of O1 Manisa and O 3039, for routine vaccination in pigs. Although double vaccination with an interval of 4 weeks at ages between 2 and 3 months has been recommended for other FMD-susceptible animals, growing pigs were vaccinated only once between 8 and 12 weeks of age due to the costs involved, vaccine shortage, and the occurrence of injection-site granulomas [8]. Therefore, this study was performed to determine the optimal age for vaccination of pigs in case of single vaccination with the currently used FMD vaccine consisting of O1 Manisa and O 3039 virus strains, adjuvanted by double oil emulsion (DOE).

At the beginning of this study, a total of 498 pigs were kept on a total of five farms in three provinces (one farm in Jeonnam, one farm in Jeonbuk, and three farms in Gyeongbuk) in South Korea. The sows that gave birth to these pigs had been vaccinated at least twice with an FMD type O vaccine containing O1 Manisa and O 3039 strains and additionally vaccinated 3–4 weeks before parturition. The pigs on each farm were randomly distributed into four groups (n=24–25 for each; Fig. 1) that were classified as per age at the time of vaccination: group I, 8 weeks; group II, 10 weeks; group III, 12 weeks; group IV, 14 weeks.

All pigs were vaccinated once with an oil-adjuvanted inactivated FMD vaccine (GCVP FMD Vaccine, Yongin, South Korea) that had been imported from Merial Animal Health Ltd., Harlow, U.K. and filled in the bottle at Green Cross Ltd. (batch no. 16FMD27P) in South Korea. Pigs were regularly bled by jugular vein puncture as shown in Fig. 1.

PrioCHECK FMDV Type O ELISA (Prionics, Zurich, Switzerland), a blocking ELISA that detects antibodies against FMDV type O, was used to detect type O antibodies in the serum samples. Raw data have been expressed as percentage inhibition (PI) values. If the PI value of the sample was more than 50%, the result was considered positive. If the PI was below 50%, the result was considered negative. The seroprevalence (%) has been expressed as a ratio of the number of positive samples divided by the total number of samples tested.

The Z test and *P*-value calculation were conducted using Microsoft Excel. *P*-values below 0.05 (95% confidence interval) were

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No. of pigs

Group 1
125   124(-1)   121(-3)   116(-5)   113(-3)

Group 2
125   124(-1)   124   123(-1)   122(-1)   122

Group 3
125   123(-2)   123   122(-1)   120(-2)

Group 4
123   122(-1)   120(-2)   117(-3)   117

8   10   12   14   16   20   24 weeks-old
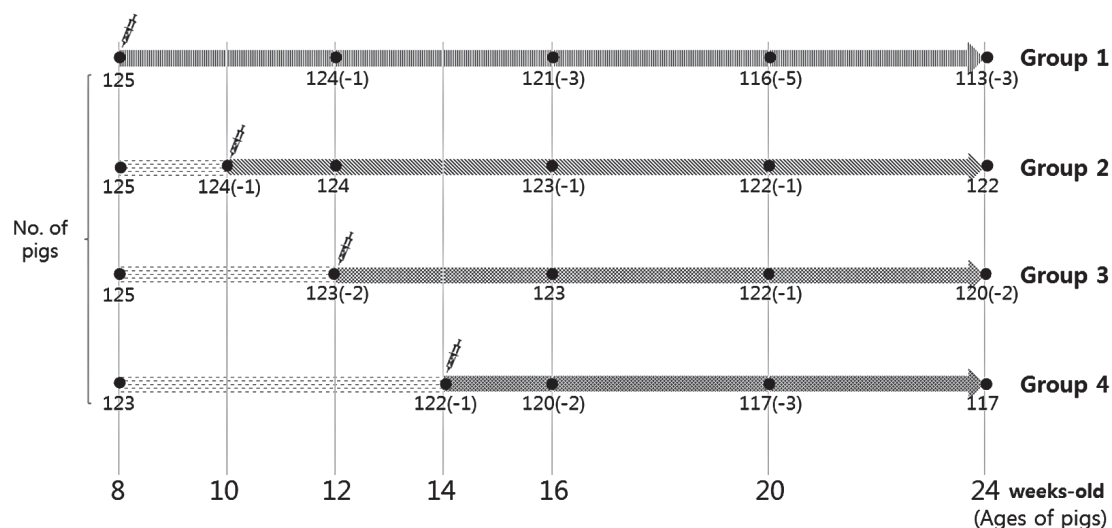(Ages of pigs)

Fig. 1. The test schedule for vaccination and bleeding in each group. A total of 498 pigs in five farms (approximately 100 pigs per each farm) were divided into 4 groups (approximately 25 pigs for each group per farm) depending on the vaccination age. A syringe symbol indicates the age of vaccination, and the period before vaccination was shown in dotted lines. Black dots mean the points of bleeding, and the number of blood samples at each time point was presented under each arrow; the number of dead pigs at each sampling point was shown in parentheses.

Table 1. Average seroprevalence at 8–24 weeks of age in five farms

Age (weeks)	Item	Group I	Group II	Group III	Group IV
8	No. tested	125	125	125	123
	No. positive	35	46	51	39
	Seroprevalence (%)	28.0 [a]	36.8	40.8	31.7
10	No. tested	-	124	-	-
	No. positive	-	31	-	-
	Seroprevalence (%)	-	25.0 [a]	-	-
12	No. tested	124	124	123	123
	No. positive	26	31	11	14
	Seroprevalence (%)	21.0	25.0	8.9 [a]	11.4
14	No. tested	-	-	-	122
	No. positive	-	-	-	8
	Seroprevalence (%)	-	-	-	6.6 [a]
16	No. tested	121	123	123	120
	No. positive	57	40	15	30
	Seroprevalence (%)	47.1	32.5	12.2	25.0
20	No. tested	116	122	122	117
	No. positive	71	56	46	36
	Seroprevalence (%)	61.2	45.9	37.7	30.8
24	No. tested	113	122	120	117
	No. positive	67	49	43	45
	Seroprevalence (%)	59.3	40.2	35.8	38.5

a) Seroprevalence due to maternally derived antibody at the time of vaccination.

considered statistically significant.

The average level of maternally derived antibodies (MDAs) of pigs in groups I, II, III and IV just before vaccination was 28, 25, 8.9 and 6.6%, respectively (Table 1). The difference in the MDA levels of groups I and II was not statistically significant ($P>0.05$), but the difference between groups I and III and groups I and IV was statistically significant ($P<0.001$ for both). Group I exhibited the highest antibody level throughout the test period (Fig. 2). The antibody levels of groups I, II and III kept increasing up to 20 weeks and that of group IV showed further increase up to 24 weeks (Fig. 2). The peak antibody level of groups I, II, III and IV was 61.2% (at 20 weeks), 45.9% (20 weeks), 37.7% (20 weeks) and 38.5% (24 weeks), respectively (Table 1). The antibody level did not significantly differ at 20 and 24 weeks of age ($P>0.05$ for all) for all four groups. At the slaughter age (24 weeks), only
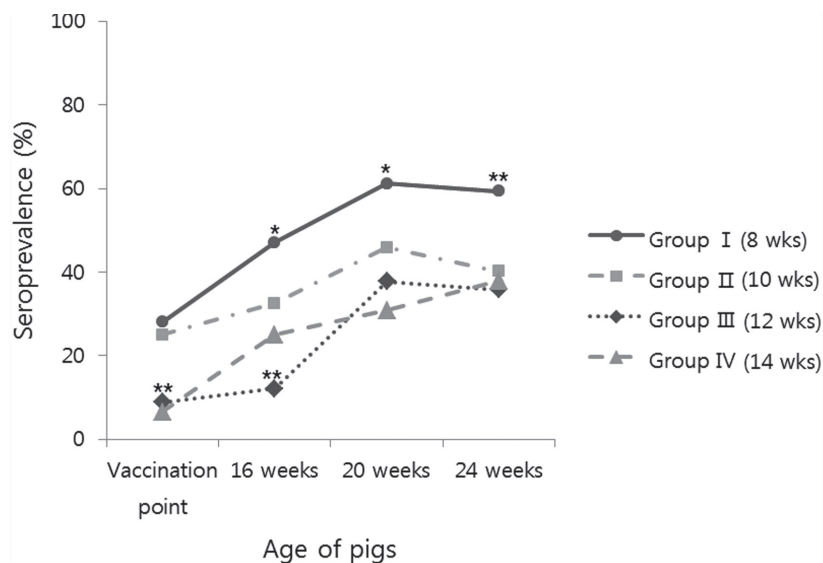
Fig. 2. Seroprevalence of type O FMDV antibodies, depending on the pig's age at the time of vaccination. Although the maternally derived antibody level in group I was the highest among the four groups, the pigs in group I showed seroprevalence greater than 50% only during the later period of fattening (20–24 weeks). Group I, vaccination at 8 weeks; Group II, vaccination at 10 weeks; Group III, vaccination at 12 weeks; Group IV, vaccination at 14 weeks of age. At each time point of the test, n=119 ± 6 samples. Group I or group III exhibited significantly different values (*$P<0.05$, **$P<0.01$) when compared to group II at the respective sampling point.

group I showed seroprevalence greater than 50%. The difference in antibody levels of groups I and II at the ages of 8 and 12 weeks was not statistically significant ($P=0.591$ and 0.454, respectively; Fig. 2). Starting from the age of 16 weeks, the differences in antibody levels of groups I and II became statistically significant ($P<0.05$) until slaughter age (24 weeks). Thus, group I showed higher seroprevalence than the other three groups after 16 weeks of age. Group II, however, did not show statistically significant differences from group III at the ages of 20 and 24 weeks ($P>0.05$ for both; Fig. 2).

The result was contrary to our supposition that the antibody response would increase with increase in age at the time of vaccination because the lower MDA level would not interfere with active immunization with the FMD vaccine. The reasons underlying this unexpected result seem elusive, but this finding might be attributable to the body weight of pigs at the time of vaccination. The biggest change in the weight of piglets occurs at an early stage, when piglets grow from a weight of 1–2 kg to approximately 10 kg within a month and the next 10-fold increase takes more than 5 months [3]. At 8 weeks of age, pigs weigh less than those at later weeks of age. If the pigs receive the same dose of the vaccine, the functional vaccine dose per body unit mass would be much higher in the younger pigs with lesser body weight and could cause an overdose effect [6, 7]. Some researchers postulate that MDAs do not always interfere with the development of inactivated vaccine-induced immunity. The reasons for this are, however, not clear and given the complexity of the immune response, particularly the dynamic nature of the immune system in a rapidly developing animal, it is not surprising that contradictory data have been obtained [5]. Besides, a previous report revealed that the DOE adjuvanted vaccines could induce an antibody response even in the high level of MDA presence (A. Dekker, presented at the symposium on the global control of FMD–Tools, ideas and ideals–Erice, Italy, 14 to 17 October 2008). In the current study, the antibody response continued to increase up to 20 or 24 weeks, regardless of vaccination time. The serological response pattern in the current study differed from that in a previous study wherein pigs without vaccination history exhibited an antibody response that peaked within 1 month after vaccination and then waned slowly [1]. In the current study, even groups III and IV with a low MDA level (8.9 and 6.6% seroprevalence, respectively) exhibited increasing antibody levels up to 20 or 24 weeks, rather than an immediate increase in the antibody level within 1 month after vaccination. The finding that group I showed higher seroprevalence than the other groups, indicates that the vaccination time needs not to be delayed until the MDA level decreases sufficiently. Early vaccination at 8 weeks could also help avoid a gap in immunity due to late vaccination, for example, at 12 or 16 weeks.

Although vaccination at the age of 8 weeks led to better seroprevalence than that in other groups, pigs that had been immunized once with the current vaccine did not achieve the antibody level required for protection against FMDV. The PrioCHECK FMDV Type O ELISA is known to have sufficient sensitivity for the detection of type O FMDV antibodies in vaccinated pigs, based on its high consistency with the results of the virus neutralization test [2]. To achieve complete protection of pigs at the herd level, the seroprevalence in pigs should be maintained at least at more than 80% because it is generally considered that vaccination of not less than 80% of the herd is necessary to provide herd immunity [4]. In this regard, double vaccination is necessary to completely protect pigs from FMDV infection with the current FMD bivalent vaccine. However, the age of 8 weeks can be considered as the optimal age for piglet vaccination if the booster is unavailable because of several realistic reasons. Although the results are paradoxical considering general theoretical perspectives, they are of practical value to researchers as they have been proven by

empirical study performed under field conditions.

Although the mechanism by which the vaccine antigens avoid interference of MDAs and elicit stronger immunity at the earlier age remains elusive, further extensive study would provide in-depth information on the correlation between the MDA level and initial vaccination time. Furthermore, because the present study is confined to the use of a specific commercial vaccine, additional tests using commercial vaccines other than the Merial bivalent vaccine are required in the future.

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