Efficacy of a Mixed Glycoprotein Vaccine against Pseudorabies in Pregnant Sows

Shigeji KATAYAMA, Ken-ichi YOSHIKI, Nobutaka OKADA, Teruaki KOKUBU and Yukio SHIMIZU

Division of Veterinary Microbiology, Kyoto Biken Laboratories, 24–16 Makishima-cho, Uji, Kyoto 611, Japan

(Received 4 June 1997/Accepted 25 August 1997)

ABSTRACT. A pseudorabies virus (PRV) glycoprotein-mixed vaccine was prepared by heparin-affinity chromatography from PRV-infected PK-15 cell lysates. In our previous study [8], the trial vaccine was induced protection with suppression of virus shedding in one-month-old pigs and generation of cytotoxic T lymphocyte (CTL) response in mice. In this study, the effect of the trial vaccine on suppression of both virus shedding and reproductive failure in pregnant sows was examined. Three sows were vaccinated twice until one week before mating. Each of them was infected intranasally with 10^6 $TCID_{50}$ of PRV on day 28, 54, or 85 after mating, respectively. Three other sows were also mated and challenged at the same time as the respective control. The vaccinated sows produced virus-neutralizing antibodies. Sows with high level of VN antibody lowered the level and period of virus shedding after challenge. The maximum level of shed-virus titers in vaccinated sows were $10^{1.25}$ to 10^3 times lower than controls. Vaccinated sows shed virus for 1 or 5 days, while controls shed for 8, 9, or 12 days. No abortion or stillbirth was observed in vaccinated sows during pregnancy. On the other hand, control sow challenged at a late stage of pregnancy showed abortion and stillbirth. The results obtained here indicate that our trial vaccine is effective to prevent reproductive failure by pseudorabies virus. — KEY WORDS: abortion, glycoprotein-mixed vaccine, pseudorabies virus, stillbirth, virus shedding.

J. Vet. Med. Sci. 60(1): 23–27, 1998

Aujeszky's disease (AD) is an economically important disease in pigs which is caused by pseudorabies virus (PRV) [1]. The disease is characterized by symptoms of the nervous system and/ or death in newborn pigs, respiratory disorders in older pigs, and reproductive failures, such as abortions, mummifications and stillbirths in sows [1, 4, 5].

PRV enters the nose of pigs under natural conditions, and then travels to the central nervous system and/or respiratory tract [15]. It is able to replicate in monocyte such as alveolar macrophages (AM) [4, 20, 24]. Monocytes infected with virus can enter the bloodstream, and transport to the pregnant sow's uterus [19, 27]. Abortion is generally the result of intraplacental and intrafetal replication of PRV. Reproductive failures are usually observed between 35 and 75 days of pregnancy [9].

The attempts have been made to control PRV infection using adjuvanted vaccines and attenuated-virus vaccines [2, 6, 8, 10–12, 14, 16, 22, 23]. The period and amount of virus shedding have a tendency to be reduced by use of adjuvanted vaccines rather than attenuated-virus vaccines [10, 21, 26]. Nauwynck and Pensaert reported that cell-associated PRV led to abortion in the presence of circulating antibodies, and suggested that immunity after vaccination with a subunit vaccine could not prevent the passage of PRV from mononuclear cells through the maternal placenta [18]. In our previous study, an oil-adjuvanted vaccine consisting of gC-rich antigen induced protection with suppression of virus shedding in one-month-old pigs and generation of cytotoxic T lymphocytes (CTLs) response in mice [7]. Antibody-dependent cell-mediated cytotoxity (ADCC) activity was induced by anti-gC antibody products [3]. Cell-mediated immunity such as the CTL response and ADCC can suppress viral spreading via cell-to-cell route. The purpose of the study reported here was to test whether the trial vaccine conferred effective protection against PRV infection on pregnant sows.

MATERIALS AND METHODS

Cell and viruses: The porcine kidney cell line (PK-15) was cultured in Eagle's minimum essential medium (Eagle's MEM) supplemented with 5% calf serum (CS).

Iwate strain of pseudorabies virus (PRV) propagated in PK-15 cells was used for vaccine preparation and serum-neutralizing (SN) assay. The virus was isolated initially from tonsil of pig with AD in our laboratory in 1981. For pig challenge experiment, Yamagata S-81 strain kindly provided by Dr. M. Shimizu (National Institute of Animal Health, Tsukuba, Japan) was used.

Animals: Sows (LWD) used for this experiment had delivered between one and three times. All sows were confirmed negative for the presence of PRV-specific antibodies using the SN assay.

Preparation of glycoprotein-mixed vaccine: The preparation of the trial vaccine was carried out as described in our previous report [7]. PRV-infected cell lysates were chromatographed by Heparin TOYOPEARL 650 M (TOSOH Co., Ltd.). Briefly, cell lysate antigens were applied to the column, which was the washed thoroughly with phosphate-buffered saline (PBS). The antigen was then eluted from the column with 10 mM phosphate buffer (pH7.4) containing 2 M NaCl. The eluent was dialyzed against PBS. The antigen containing 300,000 HA units per dose was homogenized at a rate of 3:7 with MONTANIDE ISA-70 oil adjuvant (Seppic Co., Ltd., France) to make a water-in-oil emulsion.

Assessment of the protective effect of trial vaccine: Six sows were divided into three groups (A, B and C) of two.

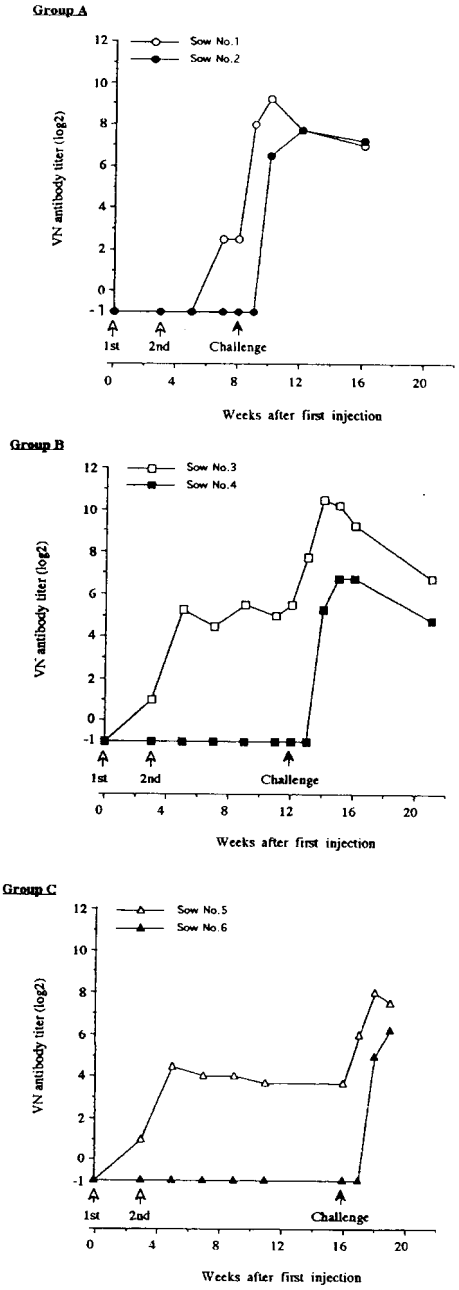

Fig. 1. Virus-neutralizing antibody titers after vaccination and challenge in sows with or without vaccinations. Sows Nos. 1, 3, and 5 were injected intramuscularly with the trial vaccine twice at 3-week intervals. Sows Nos. 2, 4 and 6 were nonvaccination control. All sows were mated at about 4-weeks after 1st injection. A vaccinated sow and control in each group were respectively challenged intranasally at 28 (group A), 54 (group B), or 85 days (group C) after mating. ⇑ ; 1st or 2nd injection with trial vaccine.

Three sows, one from each group, were injected twice intramuscularly with 1 m*l* of vaccine at three-week intervals. These sows together with their respective control were then mated about one week after the final injection. Pregnant sows in each group were challenged intranasally with 10^6 $TCID_{50}$ of Yamagata S-81 strain on day 28 after mating for group A, 54 for group B or 85 for group C. To determine the virus-neutralizing (VN) antibody titer, sera from sows were collected at the times indicated in Fig. 1. Nasal swab samples were collected daily for 2 weeks after the challenge. Clinical observation were also made after challenge. Fever was defined as a rectal temperature ≥ 39.0°C.

Hemagglutination test: Hemagglutination titer was determined by a slight modification of the method of Tetsu *et al.* [25] as described in our previous study [7].

Serum neutralizing assay: VN antibody titers were determined by the method described in our previous study [7]. Briefly, heat-inactivated serum samples were diluted 2-fold serially. They were then each mixed with the same volume of 200 $TCID_{50}$/0.05 m*l* of PRV Iwate strain in glass tubes and incubated for 60 min at 37°C. Fifty μl aliquots of each sample were added to 4 wells of PK-15 cells in microtiter plate and then incubated for 60 min at 37°C. Two hundred μl of Eagle's MEM supplemented with 1% CS was added to each well. The plates were incubated at 37°C for 7 days, and then the VN antibody titer was determined as the highest reciprocal dilution which inhibited 50% of the cytopathic effect by the Behrens-Kärber method.

Virus isolation from nasal swab and determination of its virus titer: Nasal swab samples were placed in test tube with 2 m*l* of Eagle's MEM containing antibiotics and mixed briefly. After centrifugation at 3,000 rpm, the supernatants were filtrated with 0.45 μm Millipore filter. Ten-fold serial dilutions were then prepared in Eagle's MEM supplemented with 1% CS, and 50 μl of the serial dilutions were added to 4 wells of confluent PK-15 cells in microtiter plates. After adsorption at 37°C for 60 min, 200 μl of Eagle's MEM medium mentioned above was added to each well, and the plates were then incubated at 37°C for 7 days. The virus titer was determined as 50% tissue culture infectious dose ($TCID_{50}$) by the Behrens-Kärber method.

RESULTS

Clinical observation in sows: The clinical signs of each sow after challenge is summarized in Table 1. Vaccinated sows had a tendency to have a reduced time period with a rectal temperature higher than 39°C: being 5, 4 and 4 days for vaccinated sows, and for 16, 7 and 4 days for controls.

Sows in group A had a normal delivery of 12 pigs at 84 days (No. 1) and 8 at 86 days (No. 2) after challenge. Sows in group B had a normal delivery of 9 (No. 3) and 4 (No. 4) at 61 days after challenge. In group C, sow No. 5 delivered 13 healthy pigs at 27 days after challenge, while sow No. 6 had 4 normal-appearing, 8 aborted and 2 mummified fetuses at 25 days after the challenge (Table 1). PRV was isolated from olfactory bulb, lung and tonsil of one out of the 8 aborted fetuses.

Respiratory disorders were severer in the controls as compared to vaccinated sows. Vaccinated sows showed a

Table 1. Clinical signs of vaccinated and nonvaccinated sows and their piglets after challenge exposure

Group	Sow No.	Vaccination	Parity	Time at challenge	Time at farrowing or abortion	Rectal temperature		Aborted fetuses or pigs born			
						Period[b]	Range	Total No.	Normal-appearance	Abored	Mummified
A	1	+	3	28 days[a]	112 days[a]	5 days	39.2–41.2	12	12	0	0
A	2	–	1	28 days	114 days	16 days	39.4–40.0	8	8	0	0
B	3	+	1	54 days	115 days	5 days	39.2–40.4	9	9	0	0
B	4	–	1	54 days	115 days	7 days	39.0–39.6	4	4	0	0
C	5	+	2	85 days	112 days	4 days	39.1–39.8	13	13	0	0
C	6	–	1	85 days	110 days	4 days	39.5–40.5	14	4	8	2

a) Days after mating. b) Days with rectal temperature ≥ 39.0.

loss of appetite for 4 to 5 days, and controls for 14 to 16 days.

PRV-specific virus-neutralizing antibodies: VN antibody titers of all pigs are summarized in Fig. 1. The titers of vaccinated sows were 5.6 (No. 1), 45 (No. 3) and 13 (No. 5) at challenge and these increased to 596, 1420 and 256, respectively after challenge. In contrast, no VN antibody was detected in any of the three controls until challenge-exposure. Their titers reached a peak of 210 (No. 2), 106 (No. 4) and 75 (No. 6) at 3 weeks post-challenge.

Virus-shedding: The periods of virus shedding and the virus titers are shown in Fig. 2. In group A, sow No.1 shed virus until day 5 ($10^{1.50}$ to $10^{5.00}$ $TCID_{50}$/0.05 m*l*), and sow No. 2 did from day 1 to day 8 ($10^{0.50}$ to $10^{6.25}$ $TCID_{50}$/ 0.05 m*l*). In group B (No. 3 and 4), there were distinctions between the vaccinated sows (No. 3) and its control (No. 4) both on the periods and amount of virus shed. A low level of virus was isolated from sow No. 3 on a single day only ($10^{2.5}$ $TCID_{50}$/ 0.05 m*l*), while sow No. 4 shed virus until day 12 ($10^{0.75}$ to $10^{5.50}$ $TCID_{50}$/0.05 m*l*). In group C, sow No. 5 shed virus at low titer until day 5 ($10^{0.75}$ to $10^{4.00}$ $TCID_{50}$/ 0.05 m*l*), while sow No. 6 shed virus until day 9 ($10^{1.50}$ to $10^{6.25}$ $TCID_{50}$/ 0.05 m*l*).

DISCUSSION

The results obtained in this study using a mixed glycoprotein vaccine showed that all vaccinated sows had a reduced period and quantity of virus shedding and had no abortions or stillborns in comparison to their unvaccinated controls.

Vaccines to control AD in pigs should completely prevent virus entry or suppress virus shedding to arrest subsequent virus reactivation. Our trial vaccine in sows is characterized by the latter. In our previous study, one-month-old pigs immunized with the trial vaccine induced protection with complete suppression of virus shedding [7]. In this study, the immunized sows could not completely prevent virus shedding. VN antibody titers could be related to the reduction of virus shedding [10, 21, 26]. Several authors have reported that pigs vaccinated with various Aujeszky's disease vaccines shed virus following challenge [10, 11, 17, 21, 26]. The oil-adjuvanted vaccine could accomplish suppression of virus shedding in contrast with live-modified vaccines [10, 21, 26]. A higher level of VN antibodies are generated in pigs vaccinated with oil-adjuvanted subunit vaccines than any other vaccines [10, 21]. Therefore virus neutralization by antibody may be considered as one of the mechanisms for suppression of virus shedding.

It was confirmed that pigs immunized with our trial vaccine mainly produced anti-gC serum antibodies [7]. The periods of virus shedding after challenge in pigs were reduced by administration of PRV-specific mouse monoclonal antibodies against gC [13]. Anti-gC antibodies are the main contributor of antibody-dependent cell-mediated cytotoxicity (ADCC) against virus-infected cells [3]. Thus the antibodies may play an important role in prevention of virus shedding.

PRVs are able to replicate in AM or peripheral blood mononuclear cell (MC) [18, 20, 24], where the cell-associated virus but not cell-free virus might then be transported by these cells from nose to uterine or fetuses even in the presence of circulating antibodies [18]. In the present study, none of the vaccinated sows aborted or had stillbirths after challenge in spite of partial shedding of virus. Only control sow No. 6 bore 10 stillborn fetuses, and 2 out of 10 were mummified. PRV-uninfected AM had ADCC activity [3]. As anti-gC antibodies participated in ADCC, our trial vaccine may induce these immune response. PRV gC-specific CTL were also generated in mice and pigs that had been injected with an attenuated vaccine [28]. In previous study, we demonstrated that the trial vaccine induced anti-gC CTLs in mice [7]. Nauwynck and Pensaert discussed the possibility that pigs immunized with a subunit vaccine could not prevent transplacental infection with PRV in the form of infected MC by inoculation of intra-arterial route, but could prevent cell-free virus inoculated by the same route [18]. The results of this study indicate that pigs injected with the trial vaccine were protected from transplacental infection with intranasally challenged cell-free PRV. It remains to be determined how our mixed glycoprotein vaccine prevents the spread of PRV infection in the body of pigs.

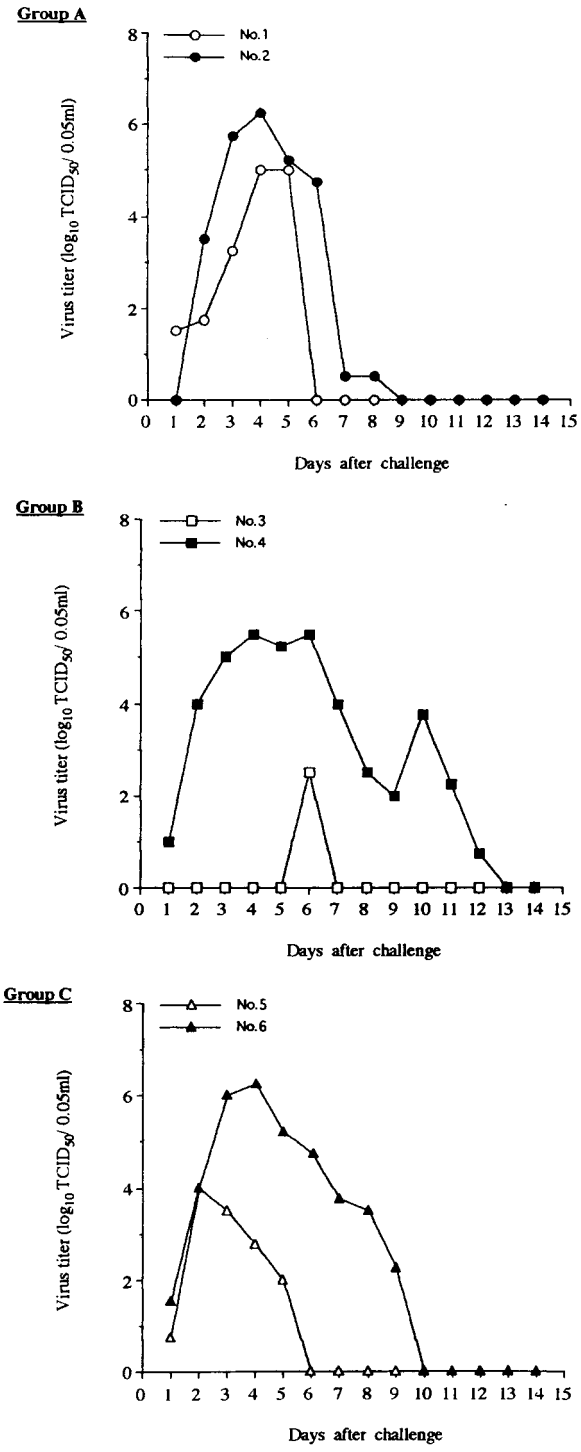

Fig. 2. Period and quantity of virus shedding from vaccinated and nonvaccinated sows after challenge-exposure. Sows Nos. 1, 3 and 5 were divided into 3 groups and vaccinated with trial vaccine. Sows Nos. 2, 4 and 6 were nonvaccinated control of each group. Two sows in each group were respectively challenged on day 28 for group A, 54 for group B, or 85 for group C after mating. Nasal-swab samples were collected daily for 14 days, and virus titers were measured.

REFERENCES

1. Baskerville, A., McFerran, J. B. and Dow, C. 1973. Aujeszky's disease in pigs. *Vet. Bull.* 43: 465–480.
2. Elvinger, F., Reeves, D. E., Pursell, A. R., Cole, J. C. and Liauw, H. 1994. Antibody titers to pseudorabies virus in piglets immunized with gIII deleted pseudorabies vaccine in a pseudorabies infected herd. *Vet. Microbiol.* 42: 341-348.
3. Iglesias, G., Molitor, T., Reed, D. and L'Italien, J. 1990. Antibodies to Aujeszky's disease virus in pigs immunized with purified virus glycoproteins. *Vet. Microbiol.* 24: 1–10.
4. Iglesias, G., Trujano, M., Lokensgard, J. and Molitor, T. 1992. Study of the potential involvement of pseudorabies virus in swine respiratory disease. *Can. J. Vet. Res.* 56: 74–77.
5. Iglesias, G. J. and Harkness, J. W. 1988. Studies of transplacental and perinatal infection with two clones of a single Aujeszky's disease (pseudorabies) virus isolate. *Vet. Microbiol.* 16: 243–254.
6. Ishii, H., Kobayashi, Y., Kuroki, M. and Kodama, Y. 1988. Protection of mice from lethal infection with Aujeszky's disease virus by immunization with purified gVI. *J. Gen. Virol.* 69: 1411–1414.
7. Katayama, S., Okada, N., Yoshiki, K. and Shimizu, Y. 1997. Protective effect of glycoprotein gC-rich antigen against pseudorabies virus. *J. Vet. Med. Sci.* 59: 657–663.
8. Kit, S., Kit, M. and Pirtle, E. C. 1985. Attenuated properties of thymidine kinase-negative deletion mutant of pseudorabies virus. *Am. J. Vet. Res.* 46: 1359–1367.
9. Kluge, J. P. and Mare, C. J. 1974. Swine pseudorabies: abortion, clinical disease, and lesions in pregnant gilts infected with pseudorabies virus (Aujeszky's disease). *Am. J. Vet. Res.* 35: 911–915.
10. Maes, R. K. and Schutz, J. C. 1983. Evaluation in swine of a subunit vaccine against pseudorabies. *Am. J. Vet. Res.* 44: 123–125.
11. Marchioli, C. C., Yancey, R. J. Jr., Wardley, R. C., Thomsen, D. R. and Post, L. E. 1987. A vaccine strain of pseudorabies virus with deletions in the thymidine kinase and glycoprotein X genes. *Am. J. Vet. Res.* 48: 1577–1583.
12. Marchioli, C. C., Yancey, R. J. Jr., Petrovskis, E. A., Timmins, J. G. and Post, L. E. 1987. Evaluation of pseudorabies virus glycoprotein gp50 as a vaccine for Aujeszky's disease in mice and swine: Expression by vaccinia virus and Chinese hamster ovary cells. *J. Virol.* 61: 3977–3982.
13. Marchioli, C. C., Yancey, R. J. Jr., Timmins, J. G., Post, L. E. and Young, B. R. 1988. Protection of mice and swine from pseudorabies virus induced mortality by administration of pseudorabies virus-specific mouse monoclonal antibodies. *Am. J. Vet. Res.* 49: 860–864.
14. McFarland, M. D. and Hill, H. T. 1987. Vaccination of mice and swine with a pseudorabies virus mutant lacking thymidine kinase activity. *Can. J. Vet. Res.* 51: 340–344.
15. McFerran, J. B. and Dow, C. 1965. The distribution of the virus of Aujeszky's disease (pseudorabies virus) in experimentally infected swine. *Am. J. Vet. Res.* 26: 631–635.
16. McGregor, S., Easterday, B. C., Kaplan, A. S. and Ben-Porat, T. 1985. Vaccination of swine with thymidine kinase-deficient mutants of pseudorabies virus. *Am. J. Vet. Res.* 46: 1494–1497.
17. Mengeling, W. L., Lager, K. M., Volz, D. M. and Brockmeier, S. L. 1992. Effect of various vaccination procedures on shedding, latency, and reactivation of attenuated and virulent

pseudorabies virus in swine. *Am. J. Vet. Res.* 53: 2164–2173.
18. Nauwynck, H. J. and Pensaert, M. B. 1992. Abortion induced by cell-associated pseudorabies virus in vaccinated sows. *Am. J. Vet. Res.* 53: 489–493.
19. Nauwynck, H. J. and Pensaert, M. B. 1994. Virus production and viral antigen expression in porcine blood monocytes inoculated with pseudorabies virus. *Arch. Virol.* 137: 69–79.
20. Nauwynck, H. J. and Pensaert, M. B. 1995. Cell-free and cell associated viremia in pigs after oronasal infection with Aujeszky's disease virus. *Vet. Microbiol.* 43: 307–314.
21. Pensaert, M. B., De Smet, K. and De Waele, K. 1990. Extent and duration of virulent virus excretion upon challenge of pigs vaccinated with different glycoprotein-deleted Aujeszky's disease vaccine. *Vet. Microbiol.* 22: 107–117.
22. Platt, K. B. 1984. The evaluation of a lectin-agarose based subunit vaccine and complementary diagnostic antigen for Aujeszky's disease (pseudorabies) in the pig. *Vet. Microbiol.* 9: 35–51.
23. Riviere, M., Tartaglia, J., Perkus, M. E., Norton, E. K., Bongermino, C. M., Lacoste, F., Duret, C., Desmettre, P. and Paoletti, E. 1992. Protection of mice and swine from pseudorabies virus conferred by vaccinia virus-based recombinants. *J. Virol.* 66: 3424–3434.
24. Shibata, I., Uruno, K., Samegai, Y., Okada, M. and Inaba, Y. 1994. Replication of virulent and attenuated strains of Aujeszky's disease virus in swine alveolar macrophages. *J. Vet. Med. Sci.* 56: 465–468.
25. Tetsu, N., Inaba, Y., Yukawa, M., Yoshiki, K., Hirahara, T., Furuya, T., Ito, S., Yonemochi, A. and Ishikawa, H. 1989. Hemagglutination with pseudorabies virus. *Arch. Virol.* 106: 321–326.
26. Vannier, P., Hutet, E., Bourgueil, E. and Cariolet, R. 1991. Level of virulent virus excreted by infected pigs previously vaccinated with different glycoprotein deleted Aujeszky's disease vaccines. *Vet. Microbiol.* 29: 213–223.
27. Wittmann, G., Jakubik, J. and Ahl, R. 1980. Multiplication and distribution of Aujeszky's disease (pseudorabies) virus in vaccinated and non-vaccinated pigs after intranasal infection. *Arch. Virol.* 66: 227–240.
28. Zuckermann, F. A., Zsak, L., Mettenleiter, T. C. and Ben-Porat, T. 1990. Pseudorabies virus glycoprotein gIII is a major target antigen for murine and swine virus-specific cytotoxic T lymphocytes. *J. Virol.* 64: 802–812.