

Kinetics of humoral immune response in pigs vaccinated against Aujeszky's disease in the presence of maternal antibodies

MAŁGORZATA POMORSKA-MÓL, IWONA MARKOWSKA-DANIEL

Department of Swine Diseases, National Veterinary Research Institute, Puławy, Poland

Abstract

Aujeszky's disease virus (ADV) is an alphaherpesvirus that causes Aujeszky's disease (AD) in pigs and is present worldwide. Some countries are free from AD, while others, like Poland, just starting eradication program. The aim of the study was the determination of passive protection's duration, transferred from sows to piglets after using attenuated vaccine, and definition the optimal time for active immunization of piglets derived from immune sows. Several protocols of vaccination of piglets against AD were evaluated with regard to development of humoral immunity in the presence of maternal antibodies. Specific antibodies to the gB and gE of ADV were determined using commercially available ELISA kits. Two weeks after the second vaccination there were no significant differences between sows in the level of anti-gB antibodies in serum and all sows developed humoral immunity. Maternally derived antibodies in the sera of piglets born from vaccinated sows were above level considered to be positive until about 11 weeks of live. The immune response, based on ELISA S/N ratio, was the highest in group vaccinated at 10 and 14 weeks of age. In this age the animals were still protected by passive immunity, but simultaneously were able to develop an active humoral response. It could be also concluded, that the high level of maternally-derived antibodies may successfully blocked developmental of active humoral immunity.

Key words: ADV, vaccination, humoral response, maternal antibodies.

(Centr Eur J Immunol 2009; 34 (2): 72-76)

Introduction

Aujeszky's disease virus (ADV) is an alphaherpesvirus that causes Aujeszky's disease (AD) in pigs and is present worldwide [1-4]. ADV virion has a double-stranded DNA genome in an enveloped capsid capable of encoding approximately 70 proteins [5]. In the envelope, virus-encoded proteins are embedded. Most of the virus-encoded proteins are glycoproteins (g), which mediated important interactions between virion and host cell. Additionally they represent important targets for the host's immune response. The characteristic of ten ADV glycoproteins indentified was shown in Table 1. Of these glycoproteins gC, gE, gG, gI and gM are designated as nonessential, i.e. deletion of the respective gene is not lethal for the virus. In contrast absence of gB, gD, gH, gK or gL abolished productive viral replication [5].

Table 1. Properties and functions of ADV glycoproteins [10]

Designation	Essential	Attachment	Penetration	Cell-cell spread
gB	+	-	+	+
gC	-	[+]	-	-
gD	+	[+]	+	[+]
gE	-	-	-	[+]
gG	-	-	-	-
gH	+	-	+	+
gI	-	-	-	[+]
gK	+	?	?	+
gL	+	-	+	+
gM	-	?	[+]	-

+ essential function; [+] modulating function; - no involvement.

Correspondence: Małgorzata Pomorska-Mól, Department of Swine Diseases, National Veterinary Research Institute, Al. Partyzantów 57, 24-100 Puławy, Poland. Phone number: +48 81 889 30 47, Email: mpomorska@piwet.pulawy.pl

Some countries are free from AD, while others, like Poland, just starting eradication program. In countries where AD is endemic, it causes high economic losses and animal trade restrictions [1, 2]. The consequences of infection depend on the interplay of variety of factors reflecting both, host susceptibility and virus virulence, and range from acute illness and death to subclinical infection [3, 6]. Both humoral and cellular immune response mechanisms appear to be involved in the development of protective immunity to herpes viruses [7, 10, 11]. Several authors describe that it is possible to reduce the prevalence of infection or even to eradicate the disease, using appropriate vaccines and vaccination schedules [1, 2, 6, 8, 9]. In general, attenuated and inactivated vaccines prevent severe clinical signs and death that are often associated with exposure of nonimmune pigs to virulent ADV strain [6, 7, 12, 13]. The aim of the vaccination program is not only to create a good level of immunization in sows but also a high and uniform level of piglets' protection [10]. The most important progress in the eradication of AD has been the elaboration of deleted vaccines allowing differentiation between vaccinated and wild-type virus infected animals. Most of the marker vaccines used for AD control base on the detection of gE surface glycoprotein. More over the discriminatory ELISA tests, which detect the presence or absence of anti-gE antibodies have been developed [5]. Similarly for detection of antibodies in serum of vaccinated animals the anti-gB enzyme-linked immunosorbent assay (ELISA) kit was established. Based on this findings vaccination – eradication program was successfully introduced in several EU countries as well as in the USA. It consists in mass, programmed for many years' vaccinations of reproductive and fattening pigs [9].

Because maternal immunity not only protects piglets against infection, but it also interferes with the serological response after vaccination, the aim of the present study was the determination of duration of passive protection transferred from sows to piglets after using live attenuated vaccine and definition the optimal time for active immunization of piglets derived from immune sows.

Materials and Methods

Animals

A total of eight multiparous ADV-negative sows, France hybrids FH 900 (mean parity 4.65, range 2-7) and their litters (88 piglets), from local farm were used. The farm has closed production cycle and the basic herd consists of 50 sows. Batches of 8-9 sows were formed every 21 days. Complete management and health data for the sows and their offspring were maintained. The prophylactic program at the time of pregnancy consists of vaccination the sows with inactivated vaccines against atrophic rhinitis and colibacillosis. Production was in all in-all out procedure with a thorough cleaning between batches. Ten days before partum the sows were

moved to the individual farrowing pens (2 × 2.5 m). The piglets were weaned at approximately 28 days. Before commencing the study, sera from all sows were tested by blocking ELISA to confirm their seronegative status.

Serum and colostrum samples

Blood samples (at least 2-3 ml) were collected via *vena cava cranialis* venepuncture into serum separator tubes from all sows at the day of vaccination and 10 and 3 days before parturition and from 6 randomly chosen piglets from each litters, every week in the first month of life and then every two weeks to the end of fattening (about 20 weeks of life). Serum was harvested from the blood samples by centrifugation (3000 g × 15 min).

Colostrum (approximately 10-15 ml) was collected from each sow at about 1 and 24 hours from parturition beginning, using oxytocin, where necessary. Colostrum was centrifuged at 2700 g 20 min, prior frozen. Serum and colostrum samples were stored at -20°C until analysis.

Local Ethical Commission approved all procedures involved in the study.

Vaccine and vaccination schedules

Several vaccination protocols of piglets against AD were evaluated with regard to development of humoral immunity in the presence of maternal antibodies.

All animals were vaccinated intramuscularly with a commercial vaccine containing live Aujeszky's disease virus, gE-deleted Bartha strain, with guaranteed titre $10^{6.3}$ CCID₅₀ in each 2.0 ml dose and oily adjuvant.

Sows were vaccinated twice at 6 and 2 weeks before parturition. Piglets were assigned to six groups: five were vaccinated and one (group 1) was served as unvaccinated control for evaluation the duration of maternal antibodies in piglets' sera. Control pigs received placebo (PBS) instead of vaccine. Vaccination schedule of piglets were as follows: group 2 was vaccinated following vaccine manufacturer recommendation at 10 and 14 weeks of life, group 3 and 4 were vaccinated once at 8 or 12 weeks of life, respectively. Piglets from groups 5 and 6 were vaccinated at 7 days of age and the booster doses were administrated at 8 or 12 weeks of life, respectively.

Serological test

Specific antibodies to the gB and gE (gp1) antigen were determined using a blocking ELISA tests (HerdChek*Anti-PRVgB or HerdChek*Anti-PRVgp1, IDEXX Laboratories, USA), as directed by the manufacturer. All reagents necessary for performing the assay were provided with the kit, and the assay was conducted at ambient temperature. Serum and colostrum samples were diluted 1:2 in a sample diluent. One hundred µl of diluted sample was added to wells coated with vaccine (gB) or viral (gE) antigen. The plates containing positive and negative reference and tested

sera were incubated overnight in 4°C. In next step the plates were washed three times with a wash solution (300 µl/well) using a microplate washer (Autara 1000, Mikura Ltd., UK) and the Anti-PRV-gB or gpl:HPRO conjugate were added for 20 minutes (100 µl/well). After washing three times, potential antigen-antibody reactions were visualized by adding 100 µl/well of TBM (3,3',5,5'-tetramethylbenzidine) substrate solution and incubating for 15 minutes. Color reactions were stopped by adding 100 µl/well of a stop solution. Optical density (OD) was measured at 650 nm wavelength using a computerized microplate reader (Multiskan RC, Labsystems, Finland).

The presence or absence of antibodies to investigated antigen was determined by calculating the sample to negative (S/N) ratio (OD of test serum/mean OD of negative reference serum).

Samples were considered to be positive for gB antigen if S/N ratio was less or equal to 0.5, while for gE antigen if S/N ratio was lower or equal to 0.6.

Results

No adverse local or systemic reactions were evidenced in all pigs. All tested animals were negative to the gE antibodies.

Taking into consideration the S/N ELISA ratio, four weeks after the first vaccination of sows, only one sow didn't developed humoral response at the level which could be considered as positive. The biggest differences in S/N

ratio between sows were observed up to administration of booster dose. Two weeks after the second vaccination there were no significant differences between sows in the level of anti-gB antibodies in serum.

In colostrum the ELISA S/N ratio was also similar in all sows.

The development of humoral immunity in sows vaccinated twice during pregnancy was shown in Fig. 1.

Maternally derived antibodies in the sera of piglets born from vaccinated sows were above level considered to be positive until about 11 weeks of live. However, from 8-10 weeks of life, maternal antibodies S/N ratio decreased quickly.

The active immune response in piglets vaccinated once at 8 or 12 weeks of age, was developed only in group vaccinated at later age. In group vaccinated at 8 weeks of age there were no humoral response to vaccine antigen. Piglets vaccinated twice at 7 days and 8 weeks of age responded similar like piglets from group 3, which were vaccinated once at 8 weeks of life. Piglets from group 6 had an ELISA S/N ratio considered to be positive during whole period of study, but starting from 10 weeks of life it was lower than in group 2. Piglets from groups 3, 4 and 5 become negative against gB antigen from about 12 weeks of age. Decline of ELISA S/N ratio in groups 3 and 5 and unvaccinated were similar. There were no significant differences in ELISA S/N ratio between piglets from the same group.

Antibody responses of piglets from all groups to gE-attenuated vaccine were shown on Fig. 2.

Discussion

All experimental pigs were seronegative to the gE antibodies, which indicate that they were not infected with field strain of ADV, during the period of study.

As published previously, if all sows are vaccinated at the same time, there will be only few differences in the level of antibodies in their serum [1]. The results obtained in our study were confirmed that statement. Two weeks after booster dose the level of anti-gB antibodies in serum of all sows were similar.

Active immune responses in piglets vaccinated once at 8 or 12 weeks of age was evidenced only in group 4, which corresponded approximately with the time at which passive antibodies usually disappear. However antibodies ELISA S/N ratio in next sampling periods, were lower than those detected in group 2, but still considered to be positive. Also Vannier et al. [8] was shown that piglets vaccinated earlier (in his study at 4 and 8 weeks of age) were not able to develop fully humoral response. Piglets vaccinated at 4 weeks of life were protected worst then the second one.

In our study piglets vaccinated at 1 and 12 weeks of life were better protected than those vaccinated with booster dose four weeks earlier. As a matter of fact, the humoral

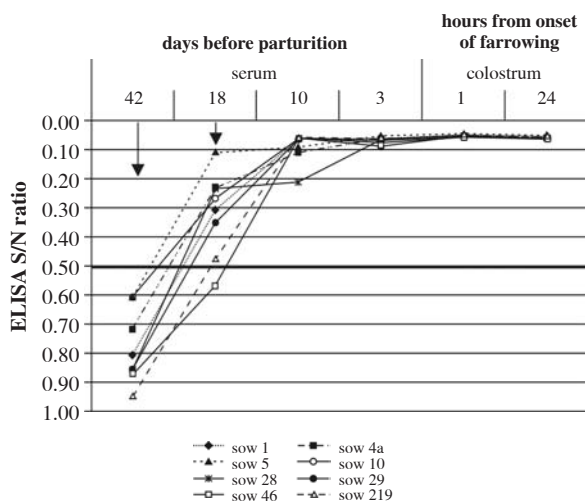


Fig. 1. Antibodies S/N ratio in serum and colostrum of individuals sows. Bold line at 0.5 ELISA S/N ratio, indicates border-line between positive and negative samples. Arrows indicate moment of vaccination

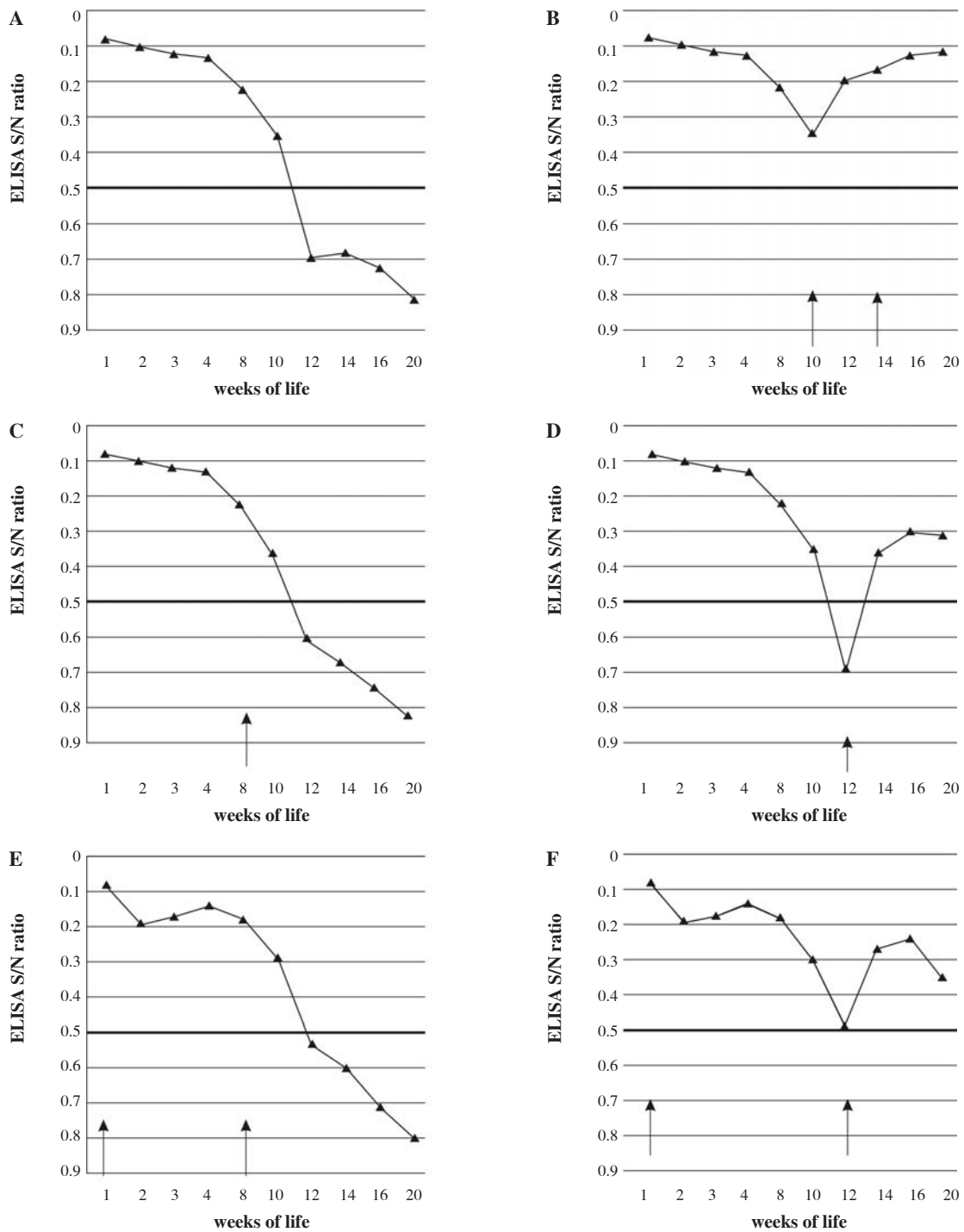


Fig. 2. Antibody responses of piglets to gE⁻ attenuated vaccine: serum of piglets from group 1(A); 2 (B); 3 (C); 4 (D); 5 (E) and 6 (F). Bold line and arrows – explanations as on Fig. 1

response was appeared only in group 6 and only after booster dose because the first vaccination was performed at the time when passive immunity was still high.

The immune response, based on ELISA S/N ratio, was the highest in group vaccinated at 10 and 14 weeks of age. So, the recommendations for administration of vaccine

given by manufacturer were in agree with results of our study. It seems that passive immunity, the level of which is relatively low at 10 weeks after birth, not interfered already with the development of an active post-vaccinal immunity. Similar results were obtained previously by Vannier [8] and Wittman [12] who used an inactivated commercial vaccine, and by Bouma et al. [2] who used an attenuated 783 strain without gE.

Our results have shown that an active immunization of piglets with using live attenuated vaccine, when they were derived from vaccinated sows, could be successful when maternal antibodies levels are about 0.35 ELISA S/N ratio. Mentioned value was observed in unvaccinated group about 9-10 weeks of life. This indicates that the animals are still protected by passive immunity, but simultaneously are able to develop an active humoral immunity. It could be also concluded, that the high level of maternally-derived antibodies may successfully blocked developmental of active humoral immunity. Therefore there is a need for careful consideration of optimal moment for the piglets' vaccination.

Acknowledgements

This work is supported by Project No NN 308 275934 founded by Ministry of Science and Higher Education.

References

1. Casal J, Planasdemunt L, Varo JA, Martin M (2004): The use of different vaccination schedules for sows to protect piglets against Aujeszky's disease. *J Vet Med B* 51: 8-11.
2. Bouma A, De Jong MCM, Kimman TG (1997): The influence of maternal immunity on the transmission of pseudorabies virus and on the effectiveness of vaccination. *Vaccine* 15: 287-294.
3. Biuk-Ruban N, Sven L, Valpotic I et al. (2004): Effect of Baypamum treatment on Aujeszky's Disease Virus (ADV) transmission in pigs. *Acta Vet Brno* 73: 59-68.
4. Fischer L, Barzu S, Andreoni C et al. (2003): DNA vaccination of neonate piglets in face of maternal immunity induces humoral memory and protection against a virulent pseudorabies virus challenge. *Vaccine* 21: 1732-1741.
5. Mattenleiter TC (1996): Immunobiology of pseudorabies (Aujeszky's Disease). *Vet Immunol Immunopathol* 54: 221-229.
6. William LM, Pirtle EC (1990): Sequential changes in the humoral immune response of pigs to pseudorabies virus after vaccination, exposure to virulent virus, and reactivation of latent virus. *J Vet Diagn Invest* 2: 35-43.
7. Gutekunst DE, Pirtle EC (1979): Humoral and cellular immune responses in swine after vaccination with inactivated pseudorabies virus. *Am J Vet Res* 40: 1343-1346.
8. Vannier P (1986): Vaccination against Aujeszky's diseases by different routes using live attenuated and inactivated virus vaccines in pigs with or without passive immunity. *J Vet Med B* 33: 704-712.
9. Mikulska-Skupień E, Szweda W, Procajło Z, Plat-Samoraj A (2004): Indices of non-specific immune response in pigs after intradermal vaccination with deleted Aujeszky's disease vaccine and after experimental infection. *Bull Vet Inst Pulawy* 48: 347-354.
10. De Bruin MGM, De Visser YE, Kimman TG, Bianchi ATJ (1998): Time course of the porcine cellular and humoral immune responses in vivo against pseudorabies virus after inoculation and challenge: significance of in vitro antigenic restimulation. *Vet Immunol Immunopathol* 65: 75-87.
11. Martin S, Wardley RC, Donaldson AI (1983): Serological response of pigs infected with Aujeszky's disease virus. *Res Vet Sci* 35: 227-233.
12. Wittman G (1981): Die Beeinträchtigung der Schutzimpfung von saugferkeln mit inaktivierter Aujeszky-Vakzine durch kolostrale Immunität. *Tierarzt. Umschau* 36: 523-528.
13. McFerran JB, Dow C (1975): Studies on immunization of pigs with the bartha strain of Aujeszky's disease virus. *Res Vet Sci* 19: 17-22.