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ANTIBODY RESPONSE FOLLOWING SOW VACCINATION USING CELL AND RECOMBINANT VACCINES, SINGLE AND MULTIPLE APPLICATION

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ABSTRACT

The vaccination of pregnant sows with *E. coli* antigens to increase specific antibodies in colostrum and milk and subsequently the protection of suckling piglets from neonatal *E. coli* diarrhea is a common and efficient method, already used for many years. The vaccination to protect individual animals against erysipelas has been used even for a longer time. There are many vaccines and vaccination techniques available on the market, among them herd specific "stable" vaccines for *E. coli*, inactivated cell vaccines and recombinant DNA vaccines. Unlike vaccines for pet animals, which are mostly polyvalent vaccines, there are very few polyvalent pig vaccines available on the EU market. Recently, Intervet company has developed Diluvac forte (DF) adjuvant which enables mixing of different monovalent vaccines. Porcilis DF vaccine line makes it possible to combine different antigens in the syringe and only one injection of desirable combination of antigens. The difference between two different vaccines (Colisorb, Porcilis DF) was tested to find out the antibody response following vaccination with monovalent recombinant vaccine against bivalent cell vaccine, and the antibody response followed separate and mixed injection of two antigens in one syringe. Statistical evaluation of serum antibody response obtained by ELISA test confirmed the expectations. DNA recombinant vaccines gave significantly higher antibody titers compared to cell vaccine. Porcilis vaccines mixed together prior to application gave surprisingly better response compared to separated application. The result leads to reduction of injections to pregnant sows and consequently to better immune response.

Key words: pigs / sows / immunology / antibody response / vaccination / *E .coli* / erysipelas

IMUNSKI ODZIV SVINJ PO VAKCINACIJI S CELIČNO IN REKOMBINANTNO VAKCINO Z LOČENO IN MEŠANO APLIKACIJO

IZVLEČEK

Vakcinacijo brejih svinj z antigeni *E. coli* uporabljamo že desetletja, da bi povečali specifična protitelesa v kolostrumu in mleku in tako pasivno zaščitili sesne pujske pred drisko, ki jo povzroča *E .coli*. Vakcinacija proti rdečici se uporablja še dlje. Na trgu je na voljo veliko komercialnih vakcin od t.i. hlevskih vakcin, inaktiviranih celičnih vakcin in v zadnjem času rekombinantnih DNK vakcin. Za razliko od vakcin, ki se uporabljajo pri malih živalih in ki so polivalentne, so na trgu EU predvsem monovalentne vakcine za prašiče. Nedavno je firma Intervet razvila DF (diluvac forte) adjuvant, ki omogoča mešanje različnih monovalentnih vakcin in s tem enkratno aplikacijo več poljubnih antigenov. Testirali smo razliko med dvema različnima vakcinama (Colisorb, Porcilis DF) z enakimi antigeni, da bi ugotovili, kakšen je imunski odziv po vakcinaciji z inaktivirano celično in rekombinantno vakcino in kakšna je razlika v količini protiteles po aplikaciji z ločenimi vakcinami v primerjavi z aplikacijo zmešanih antigenov. Statistična analiza nivoja protiteles po ELISA testiranju je potrdila naša pričakovanja. DNK rekombinantna vakcina daje značilno višje titre v primerjavi s celično vakcino. Porcilis DF vakcine, zmešane skupaj pred aplikacijo, presenetljivo spodbudijo boljši imunski odgovor kot

ločena aplikacija. Rezultati testiranja kažejo, da je mogoče zmanjšati število aplikacij vakcin brejim svinjam in hkrati doseči boljši imunski odziv.

Ključne besede: prašiči / svinje / imunologija / protitelesa / odziv / vakcinacija / *E. coli* / rdečica

INTRODUCTION

Neonatal *Escherichia coli (E. coli)* diarrhea is an important animal health concern for pig producers. It is responsible for significant economic losses. *E. coli* diarrhea is observed most commonly in piglets aged 1–4 days. Causative strains of *E. coli* are adhered by fimbrial adhesins (K88, K99, 987P and F41) (Hall, 1989; Nakazawa *et al*., 1987) to the small intestinal mucosa and produce one or more enterotoxins (ST, LT) (Bertschinger *et al*. 1992; Hall, 1989). Immunity to *E. coli* diarrhea is humoral and is initially provided via maternal colostrum. Specific antibodies inhibit bacterial adherence to the gut wall and neutralize the activity of the enterotoxins produced by the attached *E. coli*. Maternal vaccination has been one of the most effective ways of controlling neonatal *E. coli* diarrhea (Genovese *et al*., 2001; Osek *et al*., 1995). The application of recombinant DNA technology has resulted in more efficient vaccines (Riising *et al*., 2005; Larsen *et al*., 2004b; Moon and Bunn, 1993; Nagy, 1986; Clarke *et al*., 1985). Commercially available vaccines are given parenterally at about 6 and 3 weeks prior to parturition. These may be killed whole cell bacterins or purified fimbrial vaccines.

The purpose of the trial was to determine whether new recombinant monovalent vaccines (Porcilis) give higher antibody response compared to the old cell vaccine (Colisorb). Secondly, the aim was to find out if different monovalent Porcilis vaccines containing DF (diluvac forte) adjuvants can be mixed together in the syringe prior to vaccination and obtain impaired results compared to separated vaccination (Larsen *et al*., 2004a).

MATERIAL AND METHODS

The purpose of the trial was to determine possible difference in blood serum antibody levels following vaccination with three different vaccines and two different application techniques. Tested vaccines were: Colisorb (Hoechst, Intervet, bivalent *E. coli* and erysipelas vaccine), Porcilis Coli DF (Intervet, monovalent *E. coli* vaccine) and Porcilis Ery DF (Intervet, monovalent erysipelas vaccine). On a commercial farm 120 pregnant sows and gilts (min. 5 gilts per group) inseminated in the same week were divided into three separated groups (A, B, C) containing 40 animals each. They were vaccinated at week 7 and week 3 prior to expected farrowing. On average 6 days before expected farrowing (approx. 15 days after the second – booster vaccination) the blood samples were collected and sent to the lab for antibody level determination. Group A: animals vaccinated with Colisorb 2 ml intramuscular (i/m). Group B: vaccinated with Porcilis coli and Porcilis ery separately 2 ml each vaccine – i/m. Group C: vaccinated with Porcilis coli and Porcilis ery mixed together in one bottle/syringe (DF adjuvant) 4 ml i/m. Antibody response against the following antigens was tested with the following standard ELISA tests: ECO - K88ab, ECO - K88ac, ECO - K99, ECO - 987P, ECO - LT (labile toxin) and *Erysipelothrix rhusiopathiae* ELISA - ERY IRPC value (Cypress diagnostics). Laboratory analyses were done at Intervet R&D Service Lab, 5830 AA Boxmeer, The Netherlands.

The data evaluation was done in two steps. In the first step the means and standard deviations for every experimental group (A, B and C) were estimated. In the second step the data were evaluated with the following statistical model:

$$Y_{ijk} = \mu + G_i + P_j + b\left(\overline{P} - P_{ijk}\right) + e_{ijk}$$

where Y_{ijk} is an observed trait (adsorbance for every studied concentration of antibodies in blood serum), μ is an average value of the model, G_i is a fixed effect of the group (group A, B an C), P_j is a fixed effect of the parity, b is a linear regression coefficient of the time period between the date of vaccination and the date of farrowing and e_{ijkl} is the residual for the observation ijk.

Means and standard deviations were estimated with SAS/BASIC procedure MEANS. The statistics for linear statistic model were estimated with SAS/STAT procedure GLM.

RESULTS AND DISCUSSION

The number of treated animals per group, the average value and standard deviations are presented in the Table 1. The number of observations laid between 34 and 38. The sizes of groups were relatively good weighted. The values for adsorbances, connected with immunity against different strains of *E. coli,* were between 8.89 and 13.64. The differences were larger between different strains than between different groups inside the same stain. The values for group A were generally slightly lower than the values for the other two groups.

Table 1. Number ob observations per group (n), means and standard deviations (SD) for groups A, B and C for traits: period (time interval between the day of blood collection and farrowing day, in days), litter size, and adsorbances for antibodies of *E. coli* strains K88ab, K88ac, K99, 987p, for labile toxin (LT) and adsorbances for antibodies for erysipelas (Ery)

Trait	group A			group B			group C		
	n	mean	SD	n	mean	SD	n	mean	SD
period	36	9.44	1.75	38	8.89	2.61	37	9.27	1.22
litter size	36	13.64	2.53	38	13.45	2.60	37	14.11	2.45
K88ab	36	12.04	1.21	38	12.49	1.25	36	13.17	1.15
K88ac	36	12.56	0.97	38	13.04	1.37	36	13.64	1.03
K99	36	9.51	0.98	38	11.05	0.96	36	11.21	1.04
987p	36	10.66	0.92	38	13.14	1.00	36	13.11	1.06
LT	34	8.02	1.05	38	10.11	1.13	36	10.29	1.32
Ery	36	116.73	37.43	37	157.13	27.14	36	158.49	22.80

Standard deviations (SD) presented about 10% of average value. The group with older bivalent vaccine showed on average a little lower variability compared to both other groups. The cause of larger variability after group with the recombinant vaccine cannot be explained with the results from this experiment.

The immunity against *Erysipelothrix rhusiopathiae* was also much larger in groups B and C than in group A. In that case, the variability following vaccination with the old vaccine was also much larger.

In the Table 3 the results of analysis of variance according to the model described in chapter Material and methods are presented. The R^2's were middle to large that is between 0.33 and 0.65.

The group means the type of vaccine combined with the type of application. It statistically significantly influenced the quantity of antibodies measured as absorbance for every strain. The parity influenced the immunity against both strains K88 – ab and ac as well as the immunity against *Erysipelothrix rhusiopathiae*. The immunity against two other strains (K99 and 987p) did not depend on parity – the immunocompetence did not change during the productive life of sows and high immunity was expected also at the first parity. This was not the case for both K88

strains where the immunity increased from first to second parity. Some higher incidence of diarrhea in first little piglets can be expected because of lower immunity in gilts. The time interval between the time of vaccination and farrowing influenced only the immunity against strain 987p. In this particular case, late vaccination resulted in lower piglet protection.

Table 2. Analysis of variance for observed adsorbaces for antibodys of *E. coli* strains K88ab, K88ac, K99, 987p, for labile toxin (LT) and adsorbances for antibodies for erysipelas (Ery)

	model					Effects						
	df					T_i		P_j		regresion		
Trait	mod.	error	F	P	R^2	df	P	df	P	df	P	b
K88ab	10	99	4.96	<0.0001	0.3336	2	0.0004	7	0.0004	1	0.5212	−0.0360
K88ac	10	99	5.96	<0.0001	0.3756	2	<0.0001	7	<0.0001	1	0.9226	0.0050
K99	10	99	7.10	<0.0001	0.4178	2	<0.0001	7	0.8157	1	0.0750	0.0918
987p	10	99	18.56	<0.0001	0.6521	2	<0.0001	7	0.1166	1	0.0007	0.1691
LT	10	97	12.62	<0.0001	0.5654	2	<0.0001	7	0.0018	1	0.0449	0.1109
Ery	10	98	7.19	<0.0001	0.4232	2	<0.0001	7	0.0106	1	0.3580	1.3310

The LSMEANS values for the group corresponded to the statistical model. The old bivalent vaccine (group A) generally caused lower immunoresponse compared to the new recombinant vaccines. Only in case of K88ab strain the group A was as good as group B, but both mentioned groups were worse than group C with mixed monovalent recombinant vaccines.

Table 3. LSMEANS for groups A, B and C according to the statistical model*

Trait	group A	group B	group C
K88ab	12.03[a]	12.48[a]	13.27
K88ac	12.36	12.91	13.78
K99	9.37	10.95[a]	11.22[a]
987p	10.34	12.99[a]	13.09[a]
LT	7.49	9.68	10.37
Ery	118.59	161.66[a]	158.16[a]

* the values with the same letter are not statistically significantly different (P > 0.05)

The differences between the old bivalent vaccine and the recombinant new type of vaccines were expected. The group C – mixed monovalent recombinant vaccines surprisingly caused better immune response compared to the vaccination with the same but unmixed vaccines applied separately (group B). The effect is difficult to explain, but is known from the previous studies.

CONCLUSIONS

The newer recombinant vaccines against different *E. coli* antigens and against *Erysipelothrix rhusiopathiae* were compared with the old bivalent cell vaccine (group A). The recombinant vaccines were applied separately (group B) or they were mixed (group C). The recombinant vaccines caused stronger antibody response compared to the old bivalent vaccine. Group C was

in absolute values better than groups A and B. In cases of antibodies against K88ab and LT toxin the application of mixed vaccines resulted in higher antibody response than the application of two monovalent vaccines separately. In the other six cases the application of mixed or separated vaccines was equivalent. The effect of parity influenced the level of antibodies against strains K88ab, K88ac and *Erysipelothrix rhusiopathiae* as well as the concentration of LT. Higher incidence of diarrhea in first litter piglets was expected. The time period between the vaccination and the day of blood samples collection influenced only the level of antibodies against 987p strain and LT toxin. Vaccination with mixed recombinant vaccines is recommended due to powerful antibody response, less injections required and lower labor input.

REFERENCES

Bertschinger, H.U./ Fairbrother, J.M./ Nielson, N.O./ Pohlenz, J.F. *Escherichia coli* infections. In: Diseases of Swine, Seventh ed. (Eds.: Leman, A.D. *et al*.). Ames, IA, Iowa State Press, 1992, 570–583.

Clarke, S./ Cahill, A./ Stirzaker, C./ Greenwood, P./ Gregson, R. Prevention by vaccination-animal bacteria. In: Infectious Diarrhea in the Young. Amsterdam, Elsevier Science Publishers, 1985, 481.

Genovese, J.K./ Harvey, R.B./ Anderson, R.C./ Nisbet, D.J. Protection of Suckling Neonatal Pigs Against Infection with an Enterotoxigenic *Escherichia coli* Expressing 987P Fimbriae by the Administration of a Bacterial Competitive Exclusion Culture. Microbial Ecology in Health and Disease, 13(2001), 223–228.

Hall, W. A review of colibacillosis in neonatal swine. Vet. Med., 84(1989), 428–431.

Larsen, A.B./ Riising, H.J./ Taneno, A./ Chanter, N./ Ohishi, S. Serological responses after vaccination of pigs with Porcilis Ery or Porcilis Glaeser. In: Proceedings of the 18th International Pig Veterinary Society Congress, Hamburg, 2004-06/07-27/01. Hamburg, Germany, IPVS Veranstaltungs GmbH, 2004(a), 447.

Larsen, A.B./ Murmans, M./ Witvliet, M. Serological responses after vaccination of pigs with Porcilis Porcoli DF and other *E.coli* vaccines. In: Proceedings of the 18th International Pig Veterinary Society Congress, Hamburg, 2004-06/07-27/01. Hamburg, Germany, IPVS Veranstaltungs GmbH, 2004(b), 446.

Moon, H.W./ Bunn, T.O. Vaccines for preventing enterotoxigenic *Escherichia coli* infections in farm animals. Vaccine, 11(1993): 213–220.

Nagy, B. Vaccines against enterotoxic Escherichia coli disease in animals. In: Development of Drugs and Vaccines against Diarrhea. 11th Nobel Conf., Stockholm, 1986 (Eds.: Holmgren, J./ Molby, R). Lund, Studentliteratur, 53–61.

Nakazawa, M./ Sugimoto, C./ Isamaya, Y. Virulence factors in *Escherichia coli* isolated from piglets with neonatal and post-weaning diarrhea in Japan. Vet. Microbiol., 13(1987), 291–300.

Osek, J./ Truszcynski, M./ Tarasiuk, K./ Pejsak, Z. Evaluation of different vaccines to control of pig colibacillosis under large scale farm conditions. Comp. Immun. Microbiol. Infect. Dis., 18(1995), 1–8.

Riising, H.J./ Murmans, M./ Witvlivet, M. Protection Against Neonatal *Escherichia coli* diarrhoea in Pigs by Vaccination of Sows with a New Vaccine that Contains Purified Enterotoxic *E.coliE.coli* Virulence Factors F4ac, F4ab, F5 and F6 Fimbrial Antigens and Heat-Labile *E.coli* Enterotoxin (LT) Toxoid. J. Vet. Med., ser B, 52(2005)6, 296–300.