# IMMUNOGLOBULINS IN PIGS VACCINATED WITH A SUBUNIT E2 AND AN ATTENUATED C STRAIN VACCINE AGAINST CLASSICAL SWINE FEVER

TERZIĆ SVJETLANA*, BILANDŽIĆ NINA*, JEMERŠIĆ LORENA**, LOGOMERAC VESNA*,
CVETNIĆ Ž*, ŠANDOR KSENIJA*, ORŠOLIĆ NADA*** and VALPOTIĆ I****

*Croatian Veterinary Institute, Zagreb, Croatia ; **Food and Agriculture Organisation of the United
Nations, Rome, Italy; ***Faculty of Science, University of Zagreb, Zagreb, Croatia
****Veterinary Faculty, University of Zagreb, Zagreb, Croatia

*The aim of this study was to detect changes in the concentration of serum immunoglobulins following vaccination against classical swine fever (CSF) with an attenuated C strain and a subunit E2 vaccine. Furthermore, the adjuvanticity of an attenuated parapoxvirus ORF virus for the subunit vaccine against CSF was evaluated. Peripheral blood samples were collected before the vaccination and at post-vaccination days 4, 10, 21 and 28. The samples were assessed by a colorimetric method for the detection of total proteins, as well as albumin, IgA and IgM levels and by radial immunodiffusion to record the IgG level. Our findings are in accordance with the normal concentrations of porcine IgG, IgA and IgM. However, a significant increase of some immunoglobulin classes was recorded. The increase of the IgM level in vaccinated pigs confirmed an early development of humoral immunity. Interestingly, the subunit E2 vaccine induced the increase of IgM earlier then did the attenuated C strain. Since the IgG concentratiom was not significantly increased we assumed that the period of 28 days following vaccination was too short to detect any changes in the IgG level, thus reflecting a late humoral immune response. Although, IgA antibodies are mostly responsible for humoral immunity at the mucosal surfaces, in our experiment the attenuated C strain induced a significantly higher production of this immunoglobulin class in the serum very early (on day 4) following vaccination. This could be ascribed to the affinity of IgA antibodies to neutralize or agglutinate virus particles. Early appearance (4 and 10 days after the vaccination) of a significantly higher concentration of IgG and IgM could be induced by the ORF virus strain D1701 applied as an adjuvant.*

*Key words: immunoglobulins, pigs, classical swine fever, vaccination*

490                      **Acta Veterinaria (Beograd), Vol. 59. No. 5-6, 489-501, 2009.**
Terzić Svjetlana *et al.*: Immunoglobulins in pigs vaccinated with a subunit
E2 and an attenuated C strain vaccine against classical swine fever

INTRODUCTION

Classical swine fever (CSF) is a permanent threat for the pig industry. Consequences of the infection cause enormous economical losses. Virulent CSF strains induce severe damage in the humoral, as well as cellular immune response mechanisms in infected pigs (Šuša *et al*., 1992; Pauly *et al*., 1998; Gisler *et al*., 1999; Porntrakulpipat *et al.*, 2001; Polaček *et al.*, 2007; Jamin *et al.,* 2008). Even though, routine vaccination against CSF is banded in the European Union and some other countries, many studies involving different CSF vaccines and different routes of administration were conducted (Bouma *et al.*, 1999; Moormann *et al*., 2000; Dewulf *et al*., 2004; Ganges *et al.*, 2005; Liu *et al.*, 2006; Dortmans *et al*., 2008; Kaden *et al.,* 2008; von Rüden *et al.*, 2008; Holinka *et al*., 2009). The attenuated and subunit vaccines against CSF induce the development of specific antibodies and changes in the peripheral blood leucocyte subpopulation (Pirou *et al.,* 2003; Terzić *et al.*, 2003a, 2004). Humoral immune response studies and investigations of cellular immunity after vaccination showed that both attenuated and subunit E2 vaccines were able to protect pigs against infection with CSF virus (Markowska-Daniel *et al.,* 1999; Piriou *et al*., 2003; Terzić *et al*., 2003a, 2003b; van Oirschot 2003; Dong and Chen, 2007; Voight *et al.,* 2007; Sánchez *et al.,* 2008). The E2 subunit vaccine can protect pigs 7 days after vaccination (de Smit, 2000) and may be an efficient tool during an outbreak of CSF from 10 days after vaccination (Bouma *et al.,* 2000). Specific antibodies after vaccination against CSF with the attenuated C strain that is amplified in minipig kidney (MPK) cell culture protected pigs from challenge 14 days after vaccination (Terzić *et al*., 1997; Polančec *et al.,* 1999; Ballarin-Perharić *et al.,* 2000).

The relative concentration of IgM, IgD, IgG, IgE and IgA in swine varies in different body fluids (Butler *et al*., 2006a). Serum IgG levels are 10-fold higher than both IgM and IgA levels in adults. Porcine IgD and IgA can be found in traces and there are still no available, reliable reagents for their determination (Butler *et al.,* 2006a). Large numbers of IgG subclasses are specific for swine. The function, structure and role of B lymphocytes in the immune response was investigated (Butler *et al.,* 2006a, 2006b; Sinkora and Butler 2009; Butler *et al.,* 2009a, 2009b). However, no increase of wCD21$^+$ cells in pigs vaccinated with the attenuated C strain or the subunit E2 vaccine was recorded in our previous work (Terzić *et al.,* 2004). Piriou *et al.,* (2003) reported that pigs vaccinated with the attenuated vaccine against CSF and later on challenged, showed a lower neutralising antibody titre and IgG concentration than pigs that were only challenged.

Many different external factors (stress, viruses), as well as substances, may cause changes in some serum components e.g. immunoglobulins (Tuchscherer *et al.,* 1998; De Groot *et al.,* 2001; Bilandžić *et al.,* 2005; Božić *et al.,* 2006; Janjatović *et al.,* 2008) and influence the humoral and cellular immunity. The attenuated parapox virus strain D1701 (parapoxvirus ORF virus, genus *Parapoxvirus*, subfamily *Chordopoxvirinae*, family *Poxviridae*) was investigated as an immunomodualtor in pigs and other domestic animals (Kyriakis *et al.,* 1998, 2002; Fachinger *et al*., 2000a, 2000b). However, the understanding of the mechanism of its activity is only fragmentary. Parapox virus D1701 was nominated

**Acta Veterinaria (Beograd), Vol. 59. No. 5-6, 489-501, 2009.**
Terzić Svjetlana *et al*.: Immunoglobulins in pigs vaccinated with a subunit
E2 and an attenuated C strain vaccine against classical swine fever

491

as a candidate for the induction of non-specific immunity in pigs. (Büttner, 1993; Rziha *et al.,* 1999; Deane *et al*., 2000; Haig 2001; Haig and Mccines 2002; Haig *et al.,* 2002; Fisher *et al.,* 2003; Weber *et al.,* 2003; Freibe *et al.,* 2004). Our previous study (Terzić *et al.,* 2004) showed that its immunostimulating effect was evident on T lymphocytes but not on B lymphocytes (wCD21$^+$ cells). Results of our *in vitro* proliferation study (Terzić *et al.*, 2007) showed that the attenuated ORF virus, in the applied concentration induced a slight proliferation only in lymphocytes derived from pigs that received the subunit vaccine.

However, changes in immunoglobulin levels after the simultaneous vaccination against CSF and application of the attenuated ORF virus have not been investigated and there are only a few studies that describe the effect of vaccination on serum proteins. This study is a sequence of a large investigation of the cellular and humoral immunity after vaccination against CSF. The results of the study gave additional information on the levels of serum proteins in vaccinated pigs.

The aim of this study was to detect the changes in the concentration of serum immunoglobulins following vaccination against classical swine fever with a subunit or an attenuated vaccine. Also, the adjuvanticity of the attenuated ORF virus for the subunit vaccine against CSF was evaluated.

## MATERIALS AND METHODS

### Pigs

Twelve weeks of age, cross-bred, healthy pigs were randomised into four separately kept groups. Ten pigs from group 1 were vaccinated with the subunit vaccine, the second group of pigs (10) was vaccinated with the subunit vaccine and the attenuated ORF virus strain D 1701 and the third group of ten pigs was vaccinated with the C strain amplified in a minipig kidney cell culture (MPK). All pigs were vaccinated i.m. Ten pigs remained unvaccinated as controls (group 4).

### Vaccines and vaccination

The CSF subunit E2 vaccine (32 μg of gp E2 in water/oil/water emulsion, Bayer AG, Leverkusen, Germany) was administered as a single i.m. injection (2 mL). The attenuated freeze-dried vaccine against CSF (C strain virus amplified in minipig kidney cell culture $10^{4\pm0.15}$ TCID$_{50}$/mL, produced by Veterina d.o.o., Rakov Potok, Croatia) was applied i.m. The lyophilized ORF virus strain D1701 of a minimum $10^{6.45}$ TCID$_{50}$/dose (Bayer AG, Leverkusen, Germany) was applied as an i.m. injection. The first dose was given two days before vaccination, and the second dose on the day of vaccination.

### Blood sampling

Peripheral blood samples were collected by venepuncture before the vaccination (day 0) and days 4, 10, 21 and 28 after vaccination.

492                    **Acta Veterinaria (Beograd), Vol. 59. No. 5-6, 489-501, 2009.**
Terzić Svjetlana *et al*.: Immunoglobulins in pigs vaccinated with a subunit
E2 and an attenuated C strain vaccine against classical swine fever

*Total serum proteins, albumins and immunoglobulins*

Total serum proteins (g/L) were determinated by a colorimetric method (spectrophotometer HACH DR/4000U, $\lambda$ of 546 nm) with commercially available kits (Herbos Diagnostika d.o.o., Sisak, Croatia and Thermo Trace Ltd., Australia).

Albumins of vaccinated and control pigs (g/L) were assessed by a colorimetric method (spectrophotometer HACH DR/4000U, $\lambda$ of 630 nm) with commercially available kits (Herbos Diagnostika d.o.o., Sisak, Croatia and Thermo Trace Ltd., Australia).

Radial immunodiffusion (RID) was used for the determination of pig IgG (Mancini *et al*., 1965). The concentrations of immunoglobulins A and M were determined using commercially available Pig IgA and IgM VET-RID kits (Bethyl Laboratories, Inc., Montgomery, Texas, USA). Test plates for the determination of IgG were prepared by dilution of 2 g of agarose in 100 mL of barbiturate buffered saline (0.1 M) with the addition of anti-pig IgG antiserum (1:10) and 0.1% sodium azide. Five microliters of reference standard solutions of IgG and diluted serum samples (1:20) were pipetted to a separately identified well of the test plates. After incubation (48 to 72 hours at room temperature), the plates were removed and placed over a source of illumination in order to clearly see precipitation rings. The external diameters of the rings were measured to the nearest 0.1 mm by using an ocular scale. A reference curve was plotted using the diameters measured from standard solutions. From the reference curve, the IgG concentration of each diluted test sample was calculated by multiplying the concentration read from the curve by the dilution factor to obtain the actual concentration.

Student's *t*-test was used to determine the statistical significance of total proteins, albumins and serum immunoglobuline concentration.

## RESULTS

The total protein concentration was statistically significantly higher in pigs vaccinated with the attenuated C strain at the beginning of the trial than at the end. Control pigs had statistically significantly higher protein concentrations on day 4 after vaccination than pigs vaccinated with the C strain, as well as pigs that have received the subunit vaccine and ORF virus strain D1701.

A significantly higher level of albumins was recorded in the vaccinated groups at the beginning of the trial in comparison with days 10, 21 and 28. Differences between the groups were detected only in pigs vaccinated with the C strain in comparison with pigs that have received the subunit vaccine and ORF virus strain D1701.

IgG concentration increased in pigs vaccinated with the subunit vaccine and ORF virus strain D1701 4 days after vaccination. On day 28 after vaccination differences between vaccinated groups were recorded and pigs that received the attenuated vaccine had significantly higher concentrations of IgG in comparison to other vaccinated pigs.

**Acta Veterinaria (Beograd), Vol. 59. No. 5-6, 489-501, 2009.**     493
Terzić Svjetlana *et al*.: Immunoglobulins in pigs vaccinated with a subunit
E2 and an attenuated C strain vaccine against classical swine fever

Table 1. Total serum proteins (g/L) in pigs vaccinated with subunit vaccine (group 1), subunit vaccine and attenuated ORF virus strain D1701 (group 2), attenuated vaccine (group 3) and of non-vaccinated control pigs (group 4)

|  |  | Total proteins (g/L) | | | |
|---|---|---|---|---|---|
|  |  | Group 1 | Group 2 | Group 3 | Group 4 |
| Day 0 | M | 31.3* | 24.7 | 34.3** | 34.4 |
|  | SEM | 2.2 | 1.9 | 2.5 | 3.5 |
| Day 4 | M | 32.3 | 29.6 | 29.5 | 37.7# |
|  | SEM | 2.0 | 2.8 | 3.1 | 1.8 |
| Day 10 | M | 25.4 | 24.6 | 26.1 | 20.9 |
|  | SEM | 3.0 | 3.9 | 4.0 | 2.9 |
| Day 21 | M | 25.3 | 30.9 | 30.5 | 33.7 |
|  | SEM | 3.2 | 3.6 | 3.5 | 3.3 |
| Day 28 | M | 17.1 | 17.2 | 32.7** | 20.0 |
|  | SEM | 4.6 | 4.0 | 2.7 | 4.0 |

 * = statistically significant higher values (p<0.05) between days in the same group;
** = statistically significant higher values (p<0.05) between vaccinated groups;
 # = statistically significant higher values (p<0.05) between vaccinated and control groups.
 M = mean; SEM = standard error of the mean

Table 2. Serum albumins (g/L) in pigs vaccinated with subunit vaccine (group 1), subunit vaccine and attenuated ORF virus strain D1701 (group 2), attenuated vaccine (group 3) and of non-vaccinated control pigs (group 4)

|  |  | Albumins (g/L) | | | |
|---|---|---|---|---|---|
|  |  | Group 1 | Group 2 | Group 3 | Group 4 |
| Day 0 | M | 25.3* | 22.9* | 26.1* ** | 25.5 |
|  | MSE | 1.3 | 1.3 | 1.0 | 1.5 |
| Day 4 | M | 25.0 | 22.1 | 29.3 | 26.7 |
|  | SEM | 1.4 | 1.5 | 4.1 | 1.3 |
| Day 10 | M | 21.6 | 19.7 | 18.3 | 19.3 |
|  | SEM | 1.7 | 1.0 | 1.5 | 1.6 |
| Day 21 | M | 19.7 | 20.8 | 23.2 | 26.0 |
|  | SEM | 1.7 | 2.5 | 1.8 | 1.8 |
| Day 28 | M | 16.8 | 13.8 | 22.1 | 18.4 |
|  | SEM | 2.2 | 2.2 | 2.6 | 1.9 |

 * = statistically significant higher values (p<0.05) between days in the same group;
** = statistically significant higher values (p<0.05) between vaccinated groups;
 M = mean; SEM = standard error of the mean

494
**Acta Veterinaria (Beograd), Vol. 59. No. 5-6, 489-501, 2009.**
Terzić Svjetlana *et al*.: Immunoglobulins in pigs vaccinated with a subunit
E2 and an attenuated C strain vaccine against classical swine fever

Table 3. IgG (g/L) in pigs vaccinated with subunit vaccine (group 1), subunit vaccine and attenuated ORF virus strain D1701 (group 2), attenuated vaccine (group 3) and of non-vaccinated control pigs (group 4)

| | | Ig (g/L) | | | |
|---|---|---|---|---|---|
| | | Group 1 | Group 2 | Group 3 | Group 4 |
| Day 0 | M | 23.4 | 20.9 | 22.8 | 24.8 |
| | SEM | 0.7 | 1.2 | 1.2 | 0.7 |
| Day 4 | M | 24.1 | 23.8* | 23.4 | 26.4 |
| | SEM | 0.9 | 1.8 | 1.5 | 0.7 |
| Day 10 | M | 22.9 | 23.7* | 22.4 | 26.6 |
| | SEM | 1.1 | 1.3 | 1.6 | 0.2 |
| Day 21 | M | 23.8 | 24.4 | 25.8 | 26.1 |
| | SEM | 1.1 | 1.8 | 1.1 | 0.4 |
| Day 28 | M | 21.6 | 20.9 | 27.4** | 23.3 |
| | SEM | 1.2 | 1.9 | 1.0 | 1.4 |

 * = statistically significant higher values ($p<0.05$) between days in the same group;
** = statistically significant higher values ($p<0.05$) between vaccinated groups;
 M = mean; SEM = standard error of the mean

Table 4. IgM (g/L) in pigs vaccinated with subunit vaccine (group 1), subunit vaccine and attenuated ORF virus strain D1701 (group 2), attenuated vaccine (group 3) and of non-vaccinated control pigs (group 4)

| | | IgM (g/L) | | | |
|---|---|---|---|---|---|
| | | Group 1 | Group 2 | Group 3 | Group 4 |
| Day 0 | M | 1.4** | 1.5 | 2.9** | 2.3 |
| | SEM | 0.2 | 0.2 | 0.4 | 0.2 |
| Day 4 | M | 5.6*[#]** | 3.7[#] | 3.5 | 1.6 |
| | SEM | 0.6 | 0.8 | 0.4 | 0.2 |
| Day 10 | M | 3.9*[#] | 4.5[#] | 1.1 | 2.4 |
| | SEM | 0.3 | 0.7 | 0.2 | 0.3 |
| Day 21 | M | 3.8*[#]** | 4.5[#] | 4.9[#] | 1.8 |
| | SEM | 0.6 | 0.4 | 0.5 | 0.2 |
| Day 28 | M | 5.2*[#]** | 1.3 | 4.0**[#] | 1.6 |
| | SEM | 0.4 | 0.2 | 0.4 | 0.2 |

 * = statistically significant higher values ($p<0.05$) between days in the same group;
** = statistically significant higher values ($p<0.05$) between vaccinated groups;
 # = statistically significant higher values ($p<0.05$) between vaccinated and control groups.
 M = mean; SEM = standard error of the mean

**Acta Veterinaria (Beograd), Vol. 59. No. 5-6, 489-501, 2009.**                 495
Terzić Svjetlana *et al*.: Immunoglobulins in pigs vaccinated with a subunit
E2 and an attenuated C strain vaccine against classical swine fever

The majority of differences were recorded in IgM concentrations. IgM concentrations were increased on day 4 in pigs that received the subunit vaccine and subunit vaccine and ORF virus strain D1701. The concentrations remained significantly higher until the end of the trial in comparison with the two other vaccinated groups. A significantly higher level of IgM was recorded in pigs vaccinated with the attenuated vaccine on day 28 in comparison with pigs that received the subunit vaccine and ORF virus strain D1701. Results between the vaccinated groups showed that IgM concentrations were significantly higher in pigs vaccinated with the subunit vaccine in comparison with pigs that received the C strain and control pigs. In all of vaccinated groups statistically higher concentrations of IgM were recorded in comparison to the control group.

Table 5 shows a statistically significant decrease of IgA level on day 4 and 28 in pigs that received the attenuated vaccine in comparison with the results obtained at the beginning of the trial and in comparison with pigs that received the subunit vaccine and the subunit vaccine and ORF virus.

Table 5. IgA (g/L) in pigs vaccinated with subunit vaccine (group 1), subunit vaccine and attenuated ORF virus strain D1701 (group 2), the attenuated vaccine (group 3) and of non-vaccinated control pigs (group 4)

| | | IgA (g/L) | | | |
| --- | --- | --- | --- | --- | --- |
| | | Group 1 | Group 2 | Group 3 | Group 4 |
| Day 0 | M | 2.0 | 1.9 | 2.0 | 2.9 |
| | SEM | 0.2 | 0.2 | 0.3 | 0.2 |
| Day 4 | M | 1.5 | 1.6 | 2.8*, ** | 2.2[#] |
| | SEM | 1.4 | 0.3 | 0.4 | 0.2 |
| Day 10 | M | 2.1 | 1.5 | 2.4 | 2.7 |
| | SEM | 0.5 | 0.3 | 0.3 | 0.2 |
| Day 21 | M | 2.6 | 2.6 | 1.9 | 2.9 |
| | SEM | 0.3 | 0.4 | 0.2 | 0.2 |
| Day 28 | M | 1.2 | 1.4 | 2.4*, ** | 2.3[#] |
| | SEM | 0.2 | 0.2 | 0.3 | 0.2 |

 * = statistically significant higher values (p<0.05) between days in the same group;
** = statistically significant higher values (p<0.05) between vaccinated groups;
 # = statistically significant higher values (p<0.05) between vaccinated and control groups.
 M = mean; SEM = standard error of the mean

## DISCUSSION

The specific antibody development after vaccination against CSF using differently prepared vaccines and different routes of vaccination were described in many studies. However, only a few authors reported changes in serum

496 **Acta Veterinaria (Beograd), Vol. 59. No. 5-6, 489-501, 2009.**
Terzić Svjetlana *et al*.: Immunoglobulins in pigs vaccinated with a subunit
E2 and an attenuated C strain vaccine against classical swine fever

immunoglobulins after vaccination (Piriou *et al.,* 2003). Apart from detecting the IgG, IgA and IgM concentrations, total serum proteins and albumins were measured in our experiment, also. The obtained results did not show unexpected values of serum proteins and albumins. However, changes in globulin fractions were found. Higher values of serum albumins and total serum proteins at the beginning of the experiment could be explained by individual differences. Namely, as we consider that all pigs in our trial belong to the same breed, originated from the same herd, and were of the same age, the above mentioned explanation seems to be logical. Since there are no available reagents for the determination of most IgG subclasses (Van der Stede *et al.,* 2004; Butler *et al.,* 2006a) the investigation of the influence of the attenuated C strain, the subunit E2 CSF vaccine and combination of the subunit vaccine and ORF virus strain D1701 was based only on the concentration of the main immunoglobulin classes.

The mechanism of action of the attenuated parapox virus strain D1701 as an immunomodulator is not completely clear. Considering its immunomodulatory ability parapox virus strain D1701 is also used as a vector in vaccine production. New parapox vector vaccine has proven a successful protection in different models, as well as in CSF vaccines. The vector virus (recombinant ORF virus, ORFV VrV-E2) vaccine can induce early onset protection against virulent CSF virus and single shot of vector vaccine provided similar efficacy as the live C strain vaccine (Voight *et al.,* 2007). There are relatively few studies describing the synergistic action of the vaccine and attenuated ORF virus as an immunomodulator in pigs. The immunostimulatory effect of inactivated ORF virus strain D1701 (Baypamun®, Bayer AG, Leverkusen, Germany) in piglets simultaneously administered with an Aujeszky's disease vaccine has been described (Valpotić *et al*., 1998). Despite of the rather unknown mechanism of action of ORF virus as an immunomodulator, its effect was evident on T lymphocytes but not on B lymphocytes (wCD21^{+} cells) (Terzić *et al.,* 2004). Early appearance (4 and 10 days after vaccination) of significantly higher concentrations of IgG and IgM could be a result of the application of the ORF virus strain D1701 as an adjuvant. However, the concentration of IgG was in the normal range for swine serum (17- 29 g/L) (Tizard, 2000; Butler 2006a) and represents 65 to 80% of the total serum immunoglobulins. The main changes in IgG levels were expected considering our previous results. Even though the specific antibodies increase 10 days after vaccination with the subunit vaccine and subunit vaccine and ORF virus (Terzić *et al.,* 2004), as well as the attenuated vaccine (Terzić *et al.,* 2003a), the IgG concentration was significantly increased only in pigs that received the subunit vaccine and ORF virus. In some occasions the absence of detectable specific CSF antibodies was referred suggesting that cellular immunity may be involved in protection against infection (Suradhat *et al*., 2001). Piriou *et al.* (2003) reported that the intranasal and intramuscular challenge with the CSF virus Alfort strain, $10^{4.5}$ $TCID_{50}$ induced high T cell responses while the cell mediated response was low in vaccinated/challenged pigs. Also, the challenged pigs developed higher concentrations of IgG1 and IgG2 while vaccinated/challenged pigs 21 days after infection showed IgG2 response only. These results are in accordance with results reported by Terzić *et al.,* 2003a which showed that the

**Acta Veterinaria (Beograd), Vol. 59. No. 5-6, 489-501, 2009.**            497
Terzić Svjetlana *et al*.: Immunoglobulins in pigs vaccinated with a subunit
E2 and an attenuated C strain vaccine against classical swine fever

subunit vaccine produced a better stimulation of B cells and CD11b+ monocytes/macrophages/granulocytes/NK cells, whereas the attenuated vaccine induced a higher response of Th cells, naive/memory cells and macrophages/neutrophils. We assume that the period of twenty-eight days following the vaccination was too short to detect any changes in IgG level, reflecting a late humoral immune response. However, the concentration of IgM increased in accordance to our expectation between 4 and 21 days post vaccination. IgM is the second highest concentration immunoglobulin in the serum. Its concentration in swine is 1-5 g/L and it is produced during a primary immune response. The statistically significant increase of IgM in vaccinated pigs confirmed an early development of humoral immunity. Interestingly, the subunit E2 vaccine alone and in the combination with the attenuated ORF virus induced an increase of IgM earlier then did the attenuated C strain. Thus, it is likely that both types of vaccines are capable to provoke significant changes in IgM concentration.

Our findings are in accordance with the normal concentrations of porcine IgA (0.5 -5 g/L) (Butler, 2006a). Although, IgA antibodies are mostly responsible for humoral immunity at the mucosal surfaces, in our experiment the attenuated C strain induced a significantly higher production of this immunoglobulin class very early following vaccination. This could be ascribed to the affinity of IgA antibodies for virus neutralisation or agglutination of virus particles. Two other vaccinated groups (pigs that received the subunit vaccine and subunit vaccine and ORF virus) had normal concentrations of IgA during the whole period of observation and it is obvious that the subunit E2 vaccine, as well as ORF virus did not influence the changes of IgA in the peripheral blood during the twenty-eight days after vaccination.

The mechanism of protection after vaccination against CSF is well known. Many trials included challenge as the most important proof of efficacy of the vaccine. Even though we did not find completely comparable situations with immunoglobulins we detected the expected development of serum immunoglobulins in the vaccinated pigs. The results obtained by enzyme immunoassay show specific antibody increase (Terzić *et al.,* 2003a, 2003b) even though the absence of dramatical changes in immunoglobulin concentration was recorded.

Our results from previous experiments (specific antibodies, cellular immunity) and these results of blood serum immunoglobulins can prove a better understanding of changes in pigs after vaccination against CSF and application ORF virus strain D1701 as imunomodulator. However, further studies are necessary to solve all aspects of development of immunity.

Address for correspondence:
Svjetlana Terzić, Ph.D., DVM
Croatian Veterinary Institute
Savska cesta 143
P.O. Box 883
10000 Zagreb
Croatia
E-mail: terzic@veinst.hr

498 **Acta Veterinaria (Beograd), Vol. 59. No. 5-6, 489-501, 2009.**
Terzić Svjetlana *et al*.: Immunoglobulins in pigs vaccinated with a subunit
E2 and an attenuated C strain vaccine against classical swine fever

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**Acta Veterinaria (Beograd), Vol. 59. No. 5-6, 489-501, 2009.** 499
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Terzić Svjetlana *et al*.: Immunoglobulins in pigs vaccinated with a subunit
E2 and an attenuated C strain vaccine against classical swine fever

**IMUNOGLOBULINI SVINJA VAKCINISANIH PROTIV KLASIČNE KUGE SUBJEDINIČNOM E2 I ATENUIRANOM VAKCINOM C SOJA**

TERZIĆ SVJETLANA, BILANDŽIĆ NINA, JEMERŠIĆ LORENA, LOGOMERAC VESNA, CVETNIĆ Ž, ŠANDOR KSENIJA, ORŠOLIĆ NADA i VALPOTIĆ I

SADRŽAJ

Cilj ovih ispitivanja je bio da se utvrde promene u koncentraciji serumskih imunoglobulina posle vakcinacije protiv klasične kuge svinja atenuisanim C sojem i subjediničnom E2 vakcinom. Osim toga, ispitivan je i adjuvantni efekat atenuisanog parapoks virusa ORF. Uzorci periferne krvi su prikupljani pre vakcinacije, a zatim 4, 10, 21. i 28. dana. Koncentracija ukupnih proteina i albumina je određivana kolorimetrijskim metodom, a koncentracija IgA, IgM i IgG radijalnom imunodifuzijom. Dobijene vrednosti su bile u skladu sa referentnim vrednostima za svinje. Međutim, u našem ogledu je zapažen značajan porast koncentracije pojedinih klasa imunoglobulina. Porast koncentracije IgM kod vakcinisanih svinja je ukazivao na rani razvoj humoralnog imuniteta. Zanimljivo je, da je subjedinična E2 vakcina, ranije prouzrokovala porast koncentracije IgM nego atenuirana vakcina dobijena od soja C. Koncentracija IgG nije bila značajno povećana, verovatno zbog toga što period od 28 dana posle vakcinacije nije bio dovoljan da dođe do ove promene. Bez obzira na činjenicu da su IgA antintela odgovorna za imunitet na sluzokožama, atenuisana vakcina poreklom od soja C dovela je do značajnog povećanja njihove koncentracije u serumu (4. dan po vakcinaciji). Ovo se može objasniti sposobnošću IgA antitela da neutrališu ili aglutinuju virusne čestice. Upotrebom virusa ORF, soja D1701 kao adjuvansa, značajan porast u koncentraciji IgG i IgM je registrovan znatno ranije (4. i 10. dana po vakcinaciji).