THE IMPORTANCE OF SAMPLE KINDS FOR
SALMONELLA SPP. ISOLATION

I. STOJANOV, D. ORLIĆ, M. KAPETANOV, R. RATAJAC, DRAGICA
STOJANOVIĆ, NADA PLAVŠA

Scientific Veterinary Institute «Novi Sad», Rumenački put 20, Novi Sad, Serbia

Summary

Poultry and its products are one of the most frequent sources of food born diseases caused by Salmonella. Since it is a dangerous zoonosis, examination of its presence is required by regulations and the control of flocks is compulsory. Salmonella may not be isolated with equal certainty in different material, what may cause suspicion towards the obtained findings.

The subject of our research was to follow presence of Salmonella in different organs and poultry feces with the aim to determine if there is a correlation between positive findings and the type of samples.

Materials for this trial were the poultry samples delivered to our Laboratory as part of regular examinations on Salmonella. In the paper are presented the findings from 2005, 2006 and 2007 year. Isolation of Salmonella was carried out on selective and differentiating media. The isolates were determined by their physiology characteristics, and confirmed by serotypisation of sera.

The obtained results show that in the year 2007, when the majority of materials were feces, the findings of Salmonella were 3.42% comparing to the year 2005 and 2006 when the majority of the samples were from the organs, and positive finds of Salmonella were over 6%. Based on these data it may be concluded that examination of poultry organs was more successful much better comparing to the examination of feces, in determines Salmonella presents in poultry production.

Key words: poultry, organs of poultry, feces of poultry, salmonella

Salmonellosis is one of the most important infectious disorders of animal and humans (5). The findings of salmonella in animals usually are not followed by clinical signs, but are expressed in low morbidity and slight mortality. However, in humans the infection is followed by intensive clinical symptoms: high temperature, diarrhea and vomiting. When the treatment is inadequate or late, the outcomes can be lethal.

The control of salmonella in animals, especially in poultry, represents one of the primary demands for production of good quality food. Poultry and their products are one of the most important infection sources through consummation of food contaminated with salmonella (7). Since it is a dangerous zoonosis, the examination of flocks is compulsory, as required by regulations.

This examination included different material. However, it seems that there is a problem in choosing a representative sample in order to obtain real insight into salmonella presence. The experience has shown that the possibility of salmonella
isolation from different samples (liver, gut, yolk sack, ovary, feces, and paper pads) is different, what raises the question about the obtained results.

The subject of our research was to follow presence of *Salmonella* in different organs and poultry feces with the aim to determine frequency of salmonella isolation in the observed samples and to see if there is a correlation between positive findings and the kind of samples.

**Materials and methods**

In a three year period material from poultry was collected. We examined different material which was divided in two groups. The first group consisted of organs (liver, spleen, hart, lung and yolk sack), while the second group consisted of feces samples (feces, litter, paper pads). In the year 2007 we examined 588 samples of organs and 683 samples of feces, in 2006 1321 samples of organs and 601 samples of feces and in 2005 1387 samples of organs and 254 feces. The isolation was done on selective enrichment medium Selenit broth with cystine (Hi Medium) and selective differentiate medium Salmonella differentiate agar (RajHans Medium). Suspect colonies were streaked on Klinger (TSI agar) (Torlak). Isolated salmonella were identified serologically with fast slide agglutination and determining their biochemical characteristics (6).

**Results and discussions**

During the year 2005 a total of 1387 organ samples and 254 feces samples was examined. There were 120 samples positive on salmonella (6.22%). In the year 2006 we examined 1321 samples of organs and 601 samples of feces and there were 155 positive (6.32%). In 2007 there were 588 organ samples and 683 feces samples, and there were 56 findings positive on salmonella (3.42%). The salmonella isolated in all three years belonged to subgroup I, *Salmonella enterica*. Most present was *Salmonella enterica* serovar. *enteritidis* and *Salmonella enterica* serovar. *typhimurium*. Beside these two mentioned serovars of salmonella, a small number of *Salmonella enterica* serovr. *infantis* and *Salmonella enterica* serovar C₂ group was isolated.

In Table 1 a display of the samples that were examined in three year period and the number of positive findings on salmonella are given.

<table>
<thead>
<tr>
<th>Number of examined samples and positive findings on salmonella in three years</th>
<th>Total number of findings positive on salmonella</th>
<th>2007</th>
<th>2006</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organs</td>
<td>588</td>
<td>56</td>
<td>1321</td>
<td>1387</td>
</tr>
<tr>
<td>Feces</td>
<td>683</td>
<td>155</td>
<td>601</td>
<td>254</td>
</tr>
</tbody>
</table>

| Total number of findings positive on salmonella | 3.42% | 6.32% | 6.22% |

Table 1
Our research proves that in the year (2007), when the largest number of examined samples were chicken feces (more than 50% of total number), the number of positive findings was 3.42%. However, in 2005 and 2006 there were more samples from poultry organs (more than 60%) and findings positive on salmonella were more than 6%.

Figure 1 shows the species and number of working material in these three years, while the data on isolated salmonella can be found in figure 2.

Fig. 1. Graph displaying material examined during 2005, 2006 and 2007

Colonization of poultry with salmonella was displayed in papers (2) where the authors in experimental way followed salmonella and it’s spreading through parenchymatous organs. They affirmed that already after one day in poultry infected with salmonella it can be isolated in liver, spleen, caecum, yolk sack, thymus and bursa. These findings point that salmonella passed from digestive tract to parenchymatous organs and colonize it. In the agreement are also the results of other authors (3) who, in similar experiments, detected presence of salmonella in intestine and spleen as well. Antonijević (1) states that pathogenesis of salmonella starts with colonization of digestive tract and continues with invasion and septicemia. Reduced values of pH in stomach and disorders of usual microflora in digestive tract, provide conditions for colonization first of all distal part of thin gut and colon.
Fig. 2. Graphical display of positive findings in three years period in %

Knowing the way of spreading salmonella in infected organism provides better understanding of the problem and determining the material that is referential for analyses and giving the best possible results. Many authors (6, 8) emphasize that salmonella has capability of own enzymatic activity, especially with catalasa and peroxide dismutase, to preserve it against phagocytes and their free radicals. The phagocytes become specific ecological niche where salmonella may be protected and preserved in an organism for a while. Beside this, salmonella has protective mechanism against complement and this is connected to lipopolysaharide O antigen chain. Long chains of cell structures provide better protective cell membrane of salmonella and protect it from contact and damages caused by complements. These data give an explanation how salmonella fights against immunology system of an organism, what provides condition for its spreading in the host, i.e. the cases of septicemia.

The subject of this paper is salmonella pathogenesis (4). It was determined that salmonella can be found in liver, yolk sack, pericardium, intestine, caecum, air sack, ovaries and oviducts. It depends on age, i.e. on production category of poultry.

Considering the obtained results and data from literature, it can be concluded that for accurate insight on the presence of salmonella in poultry it is necessary to determine whether the samples of feces are sufficient or parenchymatous organs are needed as well. This dilemma is due to the fact that
salmonella may survive in phagocyte of infected animals. It is still unknown what the destiny of infected chickens is that do not shed salmonella in feces but may have salmonella in phagocyte.

Conclusions

The examination carried out in three years monitoring showed that the number of positive material was different. In 2005 and 2006 the number of positive findings was more than 6%, while in 2007 it was 3.4%. At the same time the analyses showed that the kind of material was different in three mentioned years. In 2005 and 2006 the samples were predominately from parenchymatous organs (>50%) and well from feces, but in 2007 feces represented the dominant material for sampling.

Less number of isolated salmonella in 2007 can be a consequence of the change in suspect material brought for examination, i.e. these results can be a result of the fact that in our conditions feces presents material for obtaining valid results on the presence of salmonella in poultry production. Based on these results it can be suggested that a change in regulations is necessary.

References

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DETECTION OF CLASSICAL SWINE FEVER VIRUS IN BLOOD SAMPLES IN EXPERIMENTALLY INFECTED PIGLETS OF DIFFERENT IMMUNOLOGICAL STATUS

JASNA PRODANOV¹, R. DOŠEN¹, M. VALČIĆ², T. PETROVIĆ¹, I. PUŠIĆ¹, M. MALJKOVIC¹, DRAGICA STOJANOVIĆ¹

¹ Scientific Veterinary Institute «Novi Sad», Rumenački put 20, Novi Sad, Serbia
² Faculty of Veterinary Medicine, 11000 Belgrade, Serbia

Summary

An experimental study was conducted to investigate the detection of classical swine fever (CSF) infection in blood samples from piglets of different immunological status. The experiment was carried out in 24 piglets (age 28, 35, 44 and 54 days old) from vaccinated sows and in 14 non vaccinated piglets, originated from none CSF vaccinated sows. Two piglets from the each age group originating from vaccinated sows were challenged by intramuscular injection of CSF virus. Four piglets of the same age from vaccinated sows and two piglets derived from unvaccinated sows were added to the challenge-group to determine contact (horizontal) infection. After challenge, blood sampling from every animal was carried out on day 0, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 and 29. Blood samples were examined on presence of CSF virus specific antibodies and for presence of viral antigen (ELISA test) i.e. viral RNA by RT-PCR technique. On the basis of the obtained results it can be concluded that not all piglets born to a vaccinated sows have maternal antibodies at a detectable level, and the issue of the efficiency of passive immunization need to be evaluated in the future.

Key words: classical swine fever, passive immunity, viraemia, RT-PCR

Classical swine fever (CSF) is an important viral disease caused by an RNA-virus, belonging to the family Flaviviridae, genus Pestivirus (4). The control of CSF in the European Union (EU) has been based on a policy of non-vaccination and stamping-out since 1980 (2). In countries in which CSF is endemic, prevention and control depend primarily on vaccination programs, using attenuated live-virus vaccines (4, 14). Diagnosis of CSF in a herd in the early phase of infection is of great importance for both economical and epidemiological points of view (3, 6).

At present, in Serbia the detection of classical swine fever virus (CSFV) antibodies and antigen (viraemia) in blood samples from piglets relies on enzyme-linked immunosorbent assay (antigen ELISA test). The immunological status (passive immunity) can possibly influences on the detection of the virus / viral antigen in blood samples in the early phase of infection. This fact can be important for regions where the CSF is present as an endemic infection, despite the intensive vaccination program with modified live virus vaccines (China strain).

The aim of the research was to examine the detection of the presence of CSFV antigen (viraemia) in experimentally infected piglets of different immunological status (passive immunity). Emphasis was put on detection of CSFV
antigen and genome in blood samples of piglets during the 30 days post-infection (dpi), in order to obtain more detailed information about viral replication kinetics.

Materials and methods

Experimental animals and virus

The experiment was carried out on 38 clinically healthy, conventional weaner pigs, divided in four groups (group A, B, C, and D). The piglets were of mixed sex, originating from the same herd, known as CSF-free. The experimental group A consisted of 8 piglets, out of which 2 piglets were aged 21, 28, 37 and 47 days old. The experimental group B consisted of 16 piglets, out of which piglets were aged 21, 28, 37 and 47 days (4 piglets for every age). The experimental animals from group A and B originated from the sows that were several time vaccinated with China (C)-strain of CSFV. The experimental groups C and D consisted of 8 i.e. 6 piglets respectively, aged 35-40 days, not vaccinated against CSF and they originated from unvaccinated sows (susceptible piglets).

For challenge infection the CSFV (strain Baker) was used. The isolate was verified as free from bovine viral diarrhoea virus (BVDV) by means of reverse transcriptase–polymerase chain reaction (RT-PCR). The 14 selected pigs of different age and immunological status were challenged with a dose of 1 ml intramuscular (i/m) while the remaining animals were exposed to horizontal (contact) infection. The titer was $2 \times 10^5$ median tissue-culture-infective doses (TCID$_{50}$/ml).

Experimental design. The experiment was divided in three subsequent periods: the acclimatisation, the challenge and the post-challenge (observation) period. Upon arrival, the animals were allocated systematically (based on ear-tag numbers) to pens within separate compartments. After acclimatization period of 7 days, when the piglets originating from vaccinated sows were 28, 35, 44 and 54 days old, 8 piglets from the group A and 6 piglets from the group D were i/m inoculated with CSFV. On the same day, piglets from the groups B and C (No.N/1-8) were added to the groups of directly infected piglets in order to imitate and to examine contact infection with the same CSFV strain.

After experimental inoculation, in each newly formed group (A, B, C, D) there were three sub-groups: two directly infected piglets, i/m challenged on day 28, 35, 44 and 54 of age, originating from vaccinated sows, 4 piglets of the same age originating from vaccinated sows that were exposed to contact infection and 2 susceptible pigs originating from unvaccinated sows, exposed to horizontal infection. The newly formed E group (control) consisted of 6 remaining susceptible piglets (ear tags No. K/1-K/6) where experimental infection was done on the same day, according to the same model (i/m, 1ml). The newly formed groups of piglets were placed in five separate compartments. Each compartment consisted of a well isolated area of 35 m$^2$, with independent ventilation, assigned for experimental work with the animals. Technical design and biosafety measures entirely prevented the possibility of mechanic transmission of the virus from one compartment into the other. All materials necessary for rectal temperature monitoring, cleansing of the
Sample collection and analyses

The heparinized blood samples were collected from the jugular vein of all piglets on 0, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 and 29 days post-infection. On 30 dpi, the piglets that survived the trial were euthanized. During the experiment 271 samples of unclotted blood and 126 blood sera samples were taken and were placed into a freezer (-70 °C) until time for examination.

CSF antibody detection. For CSF antibody detection in the serum the commercial indirect immunoenzyme test (ELISA) kit (Herd Check CSFV Ab-ELISA test; IDEXX Scandinavia, Osterbybruk, Sweden) was used according to the manufacturer’s instruction.

BVDV antibody detection. Seven days before the experimental infection blood sera from all experimental animals were examined on presence of BVDV antibodies. For establishing presence of BVDV antibodies we used virus-neutralisation (VN) test. The test was carried out using 100 TCID<sub>50</sub>/0.1ml NADL (BVDV-1) and 178003 (BVDV-2) strains of BVDV, on BT cell culture (previously proved as BVDV free).

Detection of CSFV antigen in blood samples. For CSFV antigen detection in heparinised blood samples the commercial direct Elisa test kit (Herd Check CSFV Ag ELISA Test Kit; IDEXX Laboratories, Scandinavia, Osterbybruk, Sweden) were used according to the manufacturer’s instruction.

Detection of viral RNA in blood samples. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) test was applied to detect genomic RNA of CSFV in heparinised blood samples. This test was used for direct detection of CSFV in blood samples that gave negative or doubtful result on CSFV antigen in ELISA test. A total of 52 blood samples were examined by RT-PCR test. RNA was extracted tissues using TRizol reagent ("Gibco BRL", Invitrogen "Life Technologies",UK) according to manufacturer’s recommendation. The “one-tube” or “one-step RT-PCR” assay was performed by using reagents supplied in a commercial “Access RT-PCR System” (Promega Corporation, UK), according to the manufacturer’s instruction.

Results and discussions

The results of the clinical examination and pathomorphological changes, detection of viral antigen in tissue samples in piglets deriving from vaccinated sows have been described earlier (10, 11). The achieved results of CSFV antibody and antigen detection in blood samples (viraemia) by ELISA test and RT-PCR technique are presented in tables 1 and 2, according to the experimental groups.
In pre-challenge sera samples from piglets 28, 35 and 54 days old, in each age group only in 50% of the examined samples maternally derived antibodies (MDA) were detected (Table 1). The smallest number of MDA positive sera samples were detected in piglets 44 days of age, i.e. MDA was detected only in one piglet (No. III/5). According to records data, sows whose piglets were used in our trial had been vaccinated with C-strain at least 5 times in life. Maternal antibodies ingested through colostrum protect young piglets against mortality due to CSF (14). This protection declines as piglets grow older, and maternal antibody titres decrease. It is assumed that passive immunity is primarily dependent on the antibody titre of the mother and on the amount of colostrum ingested by the newborn (13). In i/m challenged piglet (No. IV/5) and in two piglets age 54 days that survived contact infection (No.IV/2, IV/4), in which colostral antibodies were detected, in all the examined sera samples (from 11-29 dpi) presence of CSFV antibodies was detected.

Table 1

<table>
<thead>
<tr>
<th>Group A</th>
<th>No.</th>
<th>Days post infection</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
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<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>I/m challenge</td>
<td>I/1</td>
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<tr>
<td></td>
<td>I/6</td>
<td>-</td>
</tr>
<tr>
<td>Contactly infected piglets</td>
<td>I/2</td>
<td>±</td>
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<tr>
<td></td>
<td>I/3</td>
<td>±</td>
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<tr>
<td></td>
<td>I/4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>I/5</td>
<td>±</td>
</tr>
<tr>
<td>Susceptible piglets</td>
<td>N/1</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>N/2</td>
<td>-</td>
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<tr>
<td></td>
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<td></td>
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<tr>
<td>Group B</td>
<td>I/m challenge</td>
<td>II/2</td>
</tr>
<tr>
<td></td>
<td>II/3</td>
<td>-</td>
</tr>
<tr>
<td>Contactly infected piglets</td>
<td>II/1</td>
<td>±</td>
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<tr>
<td></td>
<td>II/3</td>
<td>±</td>
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<tr>
<td></td>
<td>II/5</td>
<td>±</td>
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<tr>
<td></td>
<td>II/6</td>
<td>±</td>
</tr>
<tr>
<td>Susceptible piglets</td>
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<td></td>
<td>N/4</td>
<td>-</td>
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<td>Group C</td>
<td>I/m challenge</td>
<td>III/3</td>
</tr>
<tr>
<td></td>
<td>III/4</td>
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<td></td>
<td>III/5</td>
<td>±</td>
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<td>Susceptible piglets</td>
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<td>N/6</td>
<td>-</td>
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<td></td>
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<tr>
<td>Group D</td>
<td>I/m challenge</td>
<td>IV/3</td>
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<tr>
<td></td>
<td>IV/5</td>
<td>-</td>
</tr>
</tbody>
</table>

The results of the examined blood sera of experimental animals by ELISA test on presence of CSFV antibodies

139
On the contrary, in the pre-challenge sera sample from piglet age 28 days (No. I/3) that survived horizontal infection, doubtful result on the presence of MDA by ELISA test were detected. This finding is in agreement with the opinion that there is a “grey” zone of antibody titers, in which the outcome of a challenge is unpredictable (13). Serological finding in piglet that originated from unvaccinated sow and was exposed to contact infection (No. N/7) should be underlined since CSFV antibodies were detected from 13 dpi until death. Similar results are reported by Dewulf (2), i.e. after challenge the first CSFV antibodies were detected from 11 and 13 dpi. Immune response after infection with CSFV is mostly determined with appearance of neutralizing antibodies, and most often may be found in pigs that survived the infection with highly virulent CSFV (9). It is assumed that neutralizing antibodies against CSF are detectable 2 weeks after infection at the earliest (4).

Detection of CSFV antigen in blood samples of experimental animals by IDEXX ELISA (viraemia) and detection of viral RNA by RT-PCR technique

<table>
<thead>
<tr>
<th>No.</th>
<th>Group A</th>
<th>Days post infection</th>
<th>i/m challenge</th>
<th>Contactly infected piglets</th>
<th>Susceptible piglets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>7</td>
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<tr>
<td>I/1</td>
<td></td>
<td>-</td>
<td>±</td>
<td>+</td>
<td>+</td>
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<td>I/2</td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>I/3</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>I/4</td>
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<tr>
<td>I/5</td>
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<td>-</td>
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<td>-</td>
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<tr>
<td>I/6</td>
<td></td>
<td>-</td>
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<tr>
<td>N/7</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>N/8</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

No. – ear-tag number of pigs; (-) negative ELISA result; (±) doubtful ELISA result; (+) positive ELISA result; † - death of experimental animal

Table 2
In blood samples of the most piglets that originate from vaccinated sows viraemia was detected by using one of the applied methods (Table 2). In all i/m inoculated piglets (by RT–PCR technique) and in piglets from the control (E) group (by ELISA) viraemia was detected already on 3 dpi. In blood samples of piglet No. IV/5 that survived challenge infection, later on only on the 15 dpi doubtful reaction on presence of CSFV antigen (by ELISA test) was detected. It is assumed that it takes on average 4 days for a challenged pig to become infected, although initial viraemia occurs approximately 16-24 hours after infection (2). As earlier mentioned, the experimental infection was carried out through i/m inoculation of CSFV. The use of i/m route for the experimental inoculation was unnatural and thus the time of viraemia might be shorter than under natural circumstances. Laevens et al. (5) cited that even after i/m inoculation the influence of the inoculation route on the time to viraemia may be less important than infection dose.

Despite the fact that two piglets (No IV/2 and IV/4) survived contact infection in the period from 11 to 13 dpi doubtful reaction was detected by ELISA test. Applying RT-PCR technique presence of viral RNA was detected in blood samples. This finding is in accordance with the opinion that in piglets with maternal antibodies after infection, CSFV can multiply without showing visible clinical signs and they may act as a source of further dissemination of the virus (13). Our results support the conclusion that piglets with maternal antibodies do not succumb when they get infected with virulent CSFV. The presence of maternal antibodies influences the clinical course of CSF in terms that the outcome is rather transient than lethal. Such animal could play a crucial role in spreading of CSFV and might contribute to the maintenance of long lasting epizootics (2, 9). In this experiment, positive results on presence of CSFV in blood may present sufficient evidence that survived piglets may represent a source of infection. In the piglets age 28, 35, 44 and 54 days, that were exposed to contact infection, viraemia was at the earliest detected by ELISA test on 7 and 9 dpi. Applying RT-PCR technique on the samples

<table>
<thead>
<tr>
<th>Group D</th>
<th>i/m challenge</th>
<th>Infected</th>
<th>Contactly infected</th>
<th>Susceptible piglets</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>IV/3</td>
<td>-</td>
<td>±</td>
<td>+</td>
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<tr>
<td>Susceptible piglets</td>
<td>N/6</td>
<td>-</td>
<td>±</td>
<td>+</td>
</tr>
</tbody>
</table>

No. – ear-tag number of pigs; (-) negative ELISA result; (±) doubtful ELISA result; (+) positive ELISA result; (⊕) - positive RT-PCR result; (∅) - negative RT-PCR result; † - death of experimental animal
of blood two days earlier (from the first positive result by ELISA test) the presence
the viral RNA was detected only in 3 piglets. In the piglet age 28 days that survived
contact infection, the viral antigen was detected at the latest (13 dpi). Positive
results were confirmed again on 23 i 27 dpi. A transient disappearance of CSFV
from the blood corresponded with the clinical improvement, and by the end of the
experiment this piglet recovered completely. On the contrary, in the piglet No I/5
CSFV antigen was not detected in blood samples during the trial and this piglet had
succumbed the earliest in the group. After experimental infection of piglets Laevens
et al. (5) found that the latency period from inoculation to viraemia in the
experimentally inoculated pig lasted 4 days, while in the piglets that were exposed
to horizontal infection, the first viraemia was detected on 12 and 14 dpi. After
experimental infection of piglets Lowings et al. (7) detected viraemia by
examination of blood (ELISA test) from 6th and 7th dpi, up to 21 dpi, but non
nested RT-PCR was able to detect virus from 6 dpi. The obtained results are,
certainly, influenced by different strains of CSFV (high and moderate virulence)
applied for inoculation, as well as the fact that the above mentioned experiments
were performed in piglets originated from unvaccinated sows. The different days of
establishing viraemia in piglets may be due to the fact that a viraemia with short
duration may be missed when samples are only taken every two days, or that
viraemia remains under detection limit (2, 3). The negative results could be due to
neutralisation of the virus by antibodies in the bloodstream, thus underestimating
the viral load (12).

Within the subgroup III, findings of piglets No N/7 should be mentioned,
where at the same time CSFV antigen and antibodies in blood on 13 dpi was
detected. In further course of the disease, viraemia was not detected, but specific
antibodies were confirmed in all the examined samples until death (19 dpi). The
simultaneous detection antibodies and virus has been reported from Depner et al.
(2000), while other authors (13) assume that only in the case of chronic infection
detecting the virus and specific antibodies in blood is possible after a longer period.
Antibodies may be temporarily detected in serum samples, as the immune system
starts to produce antibodies although they are not able to eliminate the virus from
the host. Consequently the antibodies are neutralised by the virus and cease to be
detectable (1). Considering the results of examination viraemia of naïve (susceptible) piglets in all groups, in contactly infected animals viral RNA in blood
samples was at the earliest detected on 9 dpi. Applying the ELISA test for
examination, viraemia was detected later, i.e. the earliest on 11 dpi and in the most
of animals on 13 dpi. This points out that RT-PCR technique detected viraemia in
susceptible piglets 2 days prior to detection of viraemia by the use of ELISA.
Determining viraemia is considered useful in early detection of CSF in a herd, since
this can be done before fever or clinical signs are noticed (3). It is also highly
important that the number of samples have to be taken spread over the farm and it
should be focusing young piglets to be sure to sample viraemic animals (7, 8).
Since it is difficult to recognize the disease clinically in its early stage, our findings
confirm the results of Dewulf (2) that RT-PCR technique detected viraemia in pigs, and that the beginning of viraemic period highly corresponds with the period of fever (10).

Conclusions

The results of our experiment suggest that not all piglets born to a vaccinated sow have MDA at a detectable level and this arise the question of the efficiency of passive immunity. Also, the obtained results indicate that infection of piglets is possible despite of presence of MDA and that these piglets may be a source of spreading the CSF infection. The survived piglets were clinically but not virologically protected, and it may be considered that secondary outbreaks are not prevented as long as sources of infection remain present. Because subclinically infected pigs may live for months and shed virus, they represent a major impediment to the control of epizootics (13). It seems justified to conclude that such courses appear under field conditions, too and this has to be considered in discussions on the most effective way for the eradication of CSFV in Serbia. The key to CSF control during outbreak is early identification of such herds and their elimination (3). Having in mind the results of our trial, and for the purpose of early detection of infection, RT-PCR technique certainly has to be included in all the cases when it is not possible to establish accurate laboratory diagnosis of CSF using other available methods.

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References


