INFLUENCE OF VACCINATION OF PIGS WITH DELETED AUJESZKY`S DISEASE VACCINE ON THE EXCRETION OF VIRULENT HERPESVIRUS SUIS TYPE 1 AND INTENSITY OF LUNG LESIONS AFTER CHALLENGE

ELŻBIETA MIKULSKA–SKUPIEŃ, WOJCIECH SZWEDA AND ZBIGNIEW PROCAJŁO

Department of Infectious and Invasive Diseases, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-718 Olsztyn, Poland
e-mail: skupien@uwm.edu.pl

Received for publication March 11, 2004.

Abstract

Influence of pig vaccination against Aujeszky’s disease (AD) on the level of virulent Herpesvirus suis type 1 (SHV-1) excretion as well as on intensity of lung lesions after challenge was evaluated. Eighteen 8-week-old piglets, divided into 3 equal groups were used. Two groups were vaccinated twice in age of 12 and 16 weeks with deleted Porcilis Begonia vaccine at doses of 2.0 ml (10^6.0 TCID₅₀) administered intramuscularly (I.M.) - group I or 0.2 ml (10^5.0 TCID₅₀) administered intradermally (I.D.) using needleless apparatus – group II. In group K (control) 2.0 ml PBS was given I.M. Seventy days after the first vaccination all pigs were infected intranasally with 10^5.5 TCID₅₀/ml dose of virulent Northern Ireland Aujeszky-3 (NIA-3) strain. During 10 d post infection (d.p.i.) and on the 21st d.p.i. the nasal swabs were taken. SHV-1 titers as –log₁₀ TCID₅₀/100 mg of nasal secretions were expressed. Lung lesions were also evaluated after slaughtering the pigs on the 21st d.p.i. Vaccination of pigs with deleted AD vaccine, irrespective of administering route, radically restricted the extent and duration of virulent SHV-1 excretion after challenge. The vaccination considerably reduced the extent and intensity of inflammatory lesions in lungs, what prove the high protective value of the vaccine in restraining secondary bacterial infections in the respiratory airways.

Key words: swine, Aujeszky’s disease, Herpesvirus suis, vaccination, lung lesions.

In the seventies of the 20th century a sudden increase in the number of Aujeszky’s disease (AD) outbreaks was noted in many countries. It is assumed that the reason of worsening of AD epidemic situation was an increase in the intensity of pig production, followed by overcrowding on farms and uncontrollable animal trade in connection with the appearance of much more virulent strains of Herpesvirus suis type 1 (SHV-1) (27). This situation was the reason that in the late 80s of the last century in many countries, mainly in Europe and in the United States, the implementation of eradication programmes was undertaken (22, 31). Because of different AD epidemic situation in particular countries, eradication methods were also different. Irrespective of the accepted idea a specific prophylaxis played and still plays an essential role in most programmes.

The studies and field observations conducted in many countries revealed that in highly contaminated areas vaccination is the only effective way to restrict economic losses, through the break of piglet morbidity and mortality chain, causing simultaneously reduction of the amount of virulent virus circulating in the pig population (17, 19, 28, 37, 40). Indeed, many studies prove that vaccination does not completely prevent the possibility of wild type virus infection of immunized pigs (5, 7, 9, 20, 42). However, the SHV-1 dose needed to infect vaccinated pigs is many times higher in relation to non-vaccinated ones (37). In sensitive pigs a dose necessary to infection ranges depending on age from 10^1 to 10^5 TCID₅₀ (41), while a dose needed to infect vaccinated pigs has to be 10-1000 times higher (37, 40). It is also important for SHV-1 circulation in the pig population that in immunized individuals the amount and time of virulent virus excretion post infection is considerably shorter (24, 35, 37).

From the economic point of view the biggest losses in the course of AD result not from death, mainly piglets, which can be substantial in some farms, but first of all from disorders in breeding and fattening (14). Long duration of respiratory symptoms together with fever and listlessness cause the elongation of fattening period by 1-2 weeks and simultaneous worsening the feed conversion ratio (15). These phenomena can be related to pneumotropism of some SHV-1 strains (1, 2, 3) and the relationship to the lymphatic system as well...
as to the immunosuppressive effect in the respiratory tract (15, 16, 21, 32).

Additional evaluation of lung lesions post experimental SHV-1 infection in pigs seems entirely reasonable, because many scientists demonstrated a positive influence of vaccination against AD on reducing lesion intensity in the respiratory airways. These problems were widely discussed in other papers (29, 30).

The objective of the study was to evaluate the influence vaccination against AD using deleted vaccine administrated intradermally versus intramuscularly on the extent and duration of virulent SHV-1 excretion as well as intensity of lung lesions after challenge in pigs.

**Material and Methods**

**Animals.** The study was carried out on 18 piglets, Large-White breed, 8-week old, from a SHV-1 free herd, divided into three equal groups – two experimental and one control. Two weeks after adaptation, the serological examination for the presence of gE SHV-1 antibodies was done using Pseudorabies Virus gpI Antibody Test Kit (Herd Chek Anti-PRV gpI), IDEXX Lab. Inc. (USA).

**Vaccine.** Vaccination against AD was performed using deleted, attenuated vaccine Porcilis Begonia (Intervet), series 97553F, based on gE- and TK-negative deletion mutant Begonia, obtained from virulent SHV-1 Northern Ireland Aujeszky-3 (NIA-3) strain (39). One dose of the vaccine contained at least 10^6.0 TCID50 of the virus. Adjuvantive diluent Diluvac Forte based on alfa-tocopherol was used.

**Vaccination.** At the age of 12 and 16 weeks the piglets were twice vaccinated:
- Group I - Porcilis Begonia at a dose of 2.0 ml/pig (10^6.0 TCID50) intramuscularly (I.M.) at neck muscles.
- Group II - Porcilis Begonia at a dose of 0.2 ml/pig (10^5.0 TCID50) intradermally (I.D.) at neck area.
- Group K – PBS at a dose of 2.0 ml/pig intramuscularly at neck muscles.

For I.D. vaccinations the needleless apparatus SERENA model SD 1-2 (Emplast, Italy) was used.

**Challenge.** Challenge was done using virulent SHV-1 NIA-3 strain of the titer TCID50 = 10^-8.55/ml, kindly provided by the National Veterinary Research Institute in Pulawy. Seventy days after the first vaccination pigs of all groups were intranasally infected at a dose of 10^5.5 TCID50 by instilling of 0.5 ml of virus suspension into each nostril.

**Virological examinations.** Nasal swabs were taken using sterilized sticks 160 mm in length (HAGAMED S. C., Rawa Mazowiecka), according to the method worked out by Vannier et al. (36). Samples from all pigs were taken immediately before infection, during 10 d post infection (d.p.i.) and on the 21st d.p.i.. Sticks were suspended in 2 ml of Eagle’s 1959 (MEM) (Biomed, Lublin) with 10 µl/ml of gentamycin (Gentamicin, Gibco), incubated at 4°C for 1 h and centrifuged (2000 x g for 10 min). Supernatant was stored at –70°C.

The titer of the isolated virus was estimated on 96 well flat microplates (96 – NUNC, Denmark). Non-diluted probe (10^6) and subsequently 10 times diluted (50 µl per well) were set in four repetitions on 24 h continuous swine kidney cell line SK-6 (11). Cell culture was prepared and kept in generally accepted manner (13), using Eagle’s 1959 (MEM) with gentamicin (50 µl/ml) and 7% FCS (Foetal Calf Serum, Sigma, Germany). Plates were incubated at 37°C (5% CO2) and observed for cytopathic effect (CPE) for 5 d. Virus titer as –log10TCID50/100 mg of nasal secretions according to the Kärber method (12) was expressed.

**Macroscopic lung examination.** Intensity of lung lesions after slaughtering the pigs on the 21st d.p.i. using point method according to Vannier and Cariolet (34) were evaluated. Depending on the area of lung tissue changes, 0 to 4 points for every lobe (max. 28 points) were recognized.

**Statistical estimation.** The results were statistically estimated with the application of variance analysis test for several means comparison (NIR test) at P < 0.05 and P < 0.01 with standard deviation statement.

**Results**

The results of reisolation of SHV-1 NIA-3 strain used for challenge from the nasal mucus in the period of 1-10 d.p.i. and on the 21st d.p.i. are presented in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Days post infection</th>
<th>Group I (I.M.)</th>
<th>Group II (I.D.)</th>
<th>Group K</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*0/6</td>
<td>*0/6</td>
<td>*1/6</td>
</tr>
<tr>
<td>2</td>
<td>0/6</td>
<td>3/6</td>
<td>2/6</td>
</tr>
<tr>
<td>3</td>
<td>0/6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>4</td>
<td>0/6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>5</td>
<td>0/6</td>
<td>4/6</td>
<td>6/6</td>
</tr>
<tr>
<td>6</td>
<td>2/6</td>
<td>3/6</td>
<td>6/6</td>
</tr>
<tr>
<td>7</td>
<td>3/6</td>
<td>1/6</td>
<td>5/6</td>
</tr>
<tr>
<td>8</td>
<td>6/6</td>
<td>1/6</td>
<td>5/6</td>
</tr>
<tr>
<td>9</td>
<td>4/6</td>
<td>1/6</td>
<td>3/6</td>
</tr>
<tr>
<td>10</td>
<td>4/6</td>
<td>1/6</td>
<td>3/6</td>
</tr>
<tr>
<td>21</td>
<td>0/6</td>
<td>0/6</td>
<td>1/6</td>
</tr>
</tbody>
</table>

* number of pigs with isolated virus / number of pigs examined
In group I, vaccinated I.M., virus isolation failed in the period of the first five d.p.i.. Excretion was barely found on the 6th d.p.i. and remained till the 10th d.p.i. Only on the 8th d.p.i. excretion was found in all pigs examined in this group, on other days only in two-three pigs.

In group II, vaccinated I.D., virus excretion was found already on the 2nd d.p.i. On the d.p.i. 3-4 excretion was recorded in all pigs examined in this group, on d.p.i. 5-6 in three-four pigs and in remaining 7-10 d.p.i. only in one pig.

In group K virus excretion was the most intensive. It started already on the 1st d.p.i. and remained till the 10th d.p.i. with various intensity. On d.p.i. 3-6 all examined pigs excreted virus, and on d.p.i. 7-8 five out of six pigs. On the 2nd d.p.i. and d.p.i. 9-10 was found excretion in two-three pigs in this group.

On the 21st d.p.i. in all groups virus excretion was not found except one pig in group K.

Total day number of SHV-1 excretion during 1-10 d.p.i. in group I was 19, in group II – 26 and in group K – 43, whereas the mean time of excretion in this period, being quotient of excretion day number and pig number in groups was 3.2 d in group I, 4.3 d in group II and 7.2 d in group K.

Formation of mean SHV-1 NIA-3 strain titers in the nasal mucus after challenge is presented in Table 2 and Fig. 1.

\[
\text{Table 2} \\
\text{Mean titers of SHV-1 NIA-3 strain in the nasal mucus after challenge} \\
\begin{array}{cccc}
\text{Days} & \text{Group I (I.M.)} & \text{Group II (I.D.)} & \text{Group K} \\
\text{post} & \text{Mean} & \text{Mean} & \text{Mean} \\
\text{infection} & \text{SHV-1 titers (-log}_{10}\text{TCID}_{50}) & \text{SHV-1 titers (-log}_{10}\text{TCID}_{50}) & \text{SHV-1 titers (-log}_{10}\text{TCID}_{50}) \\
1 & 0.00 & 0.00 & 0.43 \\
2 & 0.00 & 1.67 & 1.79 \\
3 & 0.00^{\text{A}} & 3.80^{\text{B}} & 6.13^{\text{C}} \\
4 & 0.00^{\text{A}} & 5.23^{\text{B}} & 6.51^{\text{C}} \\
5 & 0.00^{\text{A}} & 3.70^{\text{B}} & 6.25^{\text{B}} \\
6 & 1.33^{\text{A}} & 2.23^{\text{A}} & 4.87^{\text{hb}} \\
7 & 1.90 & 0.70 & 2.07 \\
8 & 3.11^{\text{A}} & 0.59^{\text{ib}} & 2.43^{\text{a}} \\
9 & 3.38^{\text{A}} & 0.45^{\text{ib}} & 1.55 \\
10 & 1.53 & 0.39 & 1.57 \\
21 & 0.00 & 0.00 & 0.53 \\
\end{array}
\]

Explanations: differences between groups A, B, C at P < 0.01; a, b at P < 0.05

In groups of vaccinated pigs mean virus titers were from 1.0 to 2.0 -log_{10}TCID_{50} less than in control group. The highest mean virus titer in group K was 6.51 -log_{10}TCID_{50}/100 mg of nasal mucus on the 4th d.p.i., whereas in groups I and II it was 3.38 -log_{10}TCID_{50}/100 mg on the 9th d.p.i. and 5.23 -log_{10}TCID_{50}/100 mg on the 4th d.p.i., respectively. In individual pigs the highest virus titer in group K was: 6.98 -log_{10}TCID_{50}/100 mg on the 4th d.p.i., in group I: 5.8 -log_{10}TCID_{50}/100 mg on the 9th d.p.i. and in group II: 6.05 -log_{10}TCID_{50}/100 mg on the 5th d.p.i. The results of macroscopic lung examination according to the point method of Vannier and Cariolet (34) are presented in Table 3.

In group I the presence of small macroscopic lung lesions was found only in two pigs. The number of points assigned to individual pigs ranged from 0 to 5, with mean 1.7. In group II in five pigs lung lesions after slaughter were revealed with the number of points in individuals from 0 to 8, with mean 4.0. In group K the presence of lung lesions was found in all pigs and the number of points for individual pigs was extremely higher than in vaccinated groups, in the range from 1 to 21, with mean 8.3.

\[
\text{Discussion} \\
\]

The studies carried out in many countries proved that vaccinations do not completely prevent the penetration and limited multiplication of virulent SHV-1 in vaccinated pigs, as well as its excretion during several days (18, 20, 24, 35). In connection with this, the ability of immunized individuals to restrict virulent SHV-1 excretion after infection is an unusually important criterion of the evaluation of vaccine efficacy and necessity in AD eradication programmes (6, 33, 37). In our studies the titer and period of NIA-3 strain excretion after challenge in both vaccinated and control groups were compared. Virus excretion was found in all infected animals, but time and extent of excretion differed between groups. The obtained results indicate that both routes of vaccinations in comparable degree influenced the reduction of both the amount of SHV-1 excreted and duration of virus excretion after challenge.
# Table 3
Point evaluation of macroscopic lesions in lungs according to the method of Vannier and Cariolet (34)

<table>
<thead>
<tr>
<th>Group</th>
<th>Pig number</th>
<th>Cranial lobes</th>
<th>Middle lobes</th>
<th>Caudal lobes</th>
<th>Accessory lobe</th>
<th>Mean</th>
<th>Mean for group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>left</td>
<td>right</td>
<td>left</td>
<td>right</td>
<td>left</td>
<td>right</td>
</tr>
<tr>
<td>I (I.M.)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>II (I.D.)</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>K</td>
<td>13</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

(0 – 5)

(0 – 8)

(1 – 21)
The longest and the most intensive virus excretion was found in control group – the mean period of excretion for the range of 0-10 d.p.i. was estimated on 7.2 d. In this group the highest mean NIA-3 strain titers were also found – in individual pigs on the culminating day of excretion (the 4th d.p.i.) the titers of virus isolated ranged from 5.98 to 6.98 –log_{10}TCID$_{50}$/100 mg of nasal mucus. In groups I and II the mean period of virus excretion was reduced to 3.2 and 4.3 d, respectively. In group I SHV-1 excretion was not noticed during the first five d.p.i. It is possible that virus in very low titers could be present in the examined samples, but the applied method did not reveal it. Despite the effort to determine virus titer from non-diluted material (10$^0$), it was possible to observe CPE only at dilution 10$^1$, because of the toxic influence of non-diluted sample of nasal mucus on the cell culture. In group vaccinated I.M., the virus did not reach the highest mean titer 3.38 –log$_{10}$TCID$_{50}$/100 mg of nasal mucus until the 9th d.p.i., whereas in group vaccinated I.D. the maximum of virus excretion was found on the 4th d.p.i. with mean titer 5.23 –log$_{10}$TCID$_{50}$/100 mg of nasal mucus. The results of our studies related to titer levels of SHV-1 reisolated from nasal secretions are in agreement with other authors’ observations. Visser et al. (38), who used virulent 75V19 SHV-1 strain for pig infection demonstrated the highest virus excretion in pigs of control group – in culminating the 3rd d of excretion the mean titer was 6.5 –log$_{10}$TCID$_{50}$/ml, while in group vaccinated I.D. 5.5 –log$_{10}$TCID$_{50}$/ml and vaccinated I.M. 4.2 –log$_{10}$TCID$_{50}$/ml. The authors also found the shortening of excretion period in vaccinated animals – in both immunized groups virus isolation failed already on the 6th d.p.i., whereas in the control group excretion remained till the 8th d.p.i. Our studies showed that virus excretion in all groups lasted longer – in each group on the 10th d.p.i. virus excretion was demonstrated at least in one animal. Yet on the 21st d.p.i. virus was isolated from one pig of control group. Longer virus excretion was also noticed by other authors. Nauwynck et al. (23), comparing efficacy of various AD vaccines in restriction of SHV-1 excretion after challenge, found that mean excretion period of control group was 12.5 d, in the group of young pigs immunized with inactivated vaccine 7.3 d, whereas in vaccinated group with oil adjuvant this period was shortened to 5.6 d. However, De Smet et al. (8) observed SHV-1 excretion in control group during 13 d.p.i., whereas in vaccinated groups during 8 d.p.i. at the longest. McFerran and Dow (8) demonstrated that in seronegative pigs infected with virulent SHV-1 strain, virus excretion lasts on the average till the 10th d.p.i., but in individuals it remained by 17 d.p.i. These authors suggest that duration of excretion period in individual pigs depends on the level of seroneutralizing (SN) antibodies appearing in sera at that time. The correlation between SN antibody titer and reduction of virus excretion after challenge was also emphasized in other papers (24, 35, 36). Bourqueil et al. (4) found that excreted virus titer and duration depends on animal’s immunological status on the day of infection. In groups of pigs with high level of SN antibody titers at the moment of infection, the virus was excreted shorter and less intensively as a result of vaccination. These observations confirm the results of our studies, where considerably higher SN antibody titers were found in the group vaccinated I.M., which can be linked with shorter, weaker and late virulent SHV-1 excretion after challenge. Moreover, Eliot et al. (10) revealed the strict dependence between production of antibody against main SHV-1 envelope glycoproteins after vaccination and amount of virus excreted after infection. Cited formerly Nauwynck et al. (23) paid also attention to the influence of multiple revaccinations on shortening of SHV-1 excretion period. They found that in groups of older sows, immunized 8-10 times with the Bartha vaccine with an oil adjuvant, the excretion period was considerably shortened compared to groups of young sows immunized 2-3 times with the same vaccine. In groups of older sows, the virus was excreted at low titers between d.p.i. 4 and 7 and in individual pigs trials the virus isolation failed, just as in our studies in group I in the period of the first 5 d.p.i. These scientists also paid attention to the fact that although SN antibody titers in all groups immunized with attenuated vaccine with an oil adjuvant were higher than in pigs of other groups without vaccinations, the essential difference in titer levels between sows vaccinated 2-3 times and those after multiple vaccinations was not found. These results inclined the cited authors to express an opinion that shortening of virulent SHV-1 excretion period after infection is influenced not only by mechanisms of humoral immunity, but also by cellular immunity stimulated unspecifically by adjuvant.

Our studies demonstrated that the period of existence and intensity of increase in body temperature were correlated with clinical status of infected pigs. The longest maintenance and the most serious clinical signs were found in the control group, whereas, in both vaccinated groups the course of disease was short and mild. More serious course of disease in the control group finds confirmation in the results of macroscopic lung evaluation done after slaughter, which revealed the occurrence of lung lesions in all pigs with high intensity in some of them (21 out of 28 points). It must be emphasized that point lung evaluation on the 21st d.p.i. was done, when no clinical signs were found in any of the animals. On the basis of the obtained results it can be accepted that lung lesions lasting longer than clinical signs could be the reason of growth inhibition being the result of worse feed conversion ratio (FCR) and body weight gains (BWG), which cause considerable economic losses.

In the absence of death cases, which were not noticed in our experiment, evaluation of lung lesions, in principle the level of its reduction in vaccinated pigs compared to control ones in the post-slaughter study, can be a valuable, additional tool for assessment of protective value of AD vaccines. Performance of such evaluation seems reasonable in the light of the known fact of pneumotropism of SHV-1 strains and their immunosuppressive effect in the respiratory tract, activating secondary bacterial infections. The question is, if lack of death, even in the control group, can be explained with relatively low dose of the virus used for
challenge. In our study the virus dose used for challenge (10^{5.5} TCID_{50}) was similar to that, what pigs meet in the natural conditions resulting 10^{1.0} - 10^{7.0} TCID_{50} in fattening and adult pigs (41). Doses of SHV-1 used in challenges are quite different and higher mortality rate can happen when they extremely exceed the field dose. An example maybe the study of Vannier and Cariolet (34), in which fattening pigs about 80 kg of body weight were infected with a dose of ≥ 10^{7.0} TCID_{50} of 75V19 strain by instilling of 2 ml in each nostril. As a result 5 out of 8 fatteners died. That is why the dose of virus used for challenge should be similar to the field dose. It is taken into consideration by the majority of scientists, because in fatteners with very low mortality rate (≤ 5%), the evaluation of other parameters is more important than mortality rate, which, however, should not be neglected. In our study just accurately well-chosen challenge parameters enabled the demonstration of high protective value of assessed vaccine and technique of vaccination.

The own study demonstrated that vaccinations both I.M. and I.D. routes do not completely restrain the development of inflammatory lesions in lungs, but considerably reduce their extent and intensity. The obtained results can be confirmation of pneumotropism of NIA-3 SHV-1, however, weaker postinfection reaction as lesser intensity of lung lesions in both groups of vaccinated pigs (1.7 and 4.0 points) comparing to the control group (8.3 points) can be the proof of high efficacy of applied vaccine. Slaughtering of pigs on the 21st d.p.i. leaves enough time for the development of efficacy of applied vaccine. Slaughtering of pigs on the 21st d.p.i. leaves enough time for the development of pulmonary airways in pigs infected with a strain of Aujeszky’s disease virus. Res Vet Sci 1972, 12, 590-592.

References
27. Szweda W.: Studies on application of various methods of Aujeszky’s disease prophylaxis and eradication in pigs. Hab. dissertation, Faculty of Veterinary Medicine, University of Agriculture and Technology, Olsztyn, 1993.