Neutralizing Antibody Titres in Pig Serum after Revaccination with an Inactivated Aujeszky Disease Virus (ADV) Vaccine

By

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With 3 figures and one table

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Introduction

In a previous publication we described that in 6 to 8 week old pigs a partial reduction of protection against challenge infection had been noticed 3 weeks after the application of a vaccine, consisting of ethylene-inactivated ADV and DEAE dextran as an adjuvant (Wittmann and Jakubik, 1977). In spite of the fact that the clinical reactions in the control animals were significantly severer, we concluded from those experiments that a single vaccine dose not evoke optimal immunity and therefore a second vaccination should be recommended.

To ascertain this we performed experiments in which young pigs were revaccinated with inactivated vaccine. The neutralizing antibody titres in the sera were determined as a measure of the immune response. Unfortunately, the immune state of the vaccinated animals could not be tested by challenge infection because of the irregular behaviour of older pigs against challenge (Wittmann and Jakubik, 1977), and since we had only a limited number of pigs available. In the present paper we describe the humoral immune response and the influence on this response related to the interval between the first and the second vaccination.

Material and Methods

Virus, cell cultures, virus multiplication and titrations

The virulent ADV strain Phylaxia, 5th passage in BHK-21 cells, clone CT (Schwöbel and Ahl, 1972) was used throughout the experiments.

These cells were also employed for virus multiplication in 70 × 350 mm roller (one rotation per min.) tubes and for seeding cells into test tubes for
virus titrations and the neutralization tests. The CT cells were grown at 37 °C in Eagle's minimal essential medium (MEM) supplemented with 10 % calf serum and antibiotics. For virus production the medium was replaced by serum-free MEM. Infection of the cultures with ADV was done at a multiplicity of 10 to 100 TCD50 ADV per cell. The cells were usually destroyed within 24 hr. The virus-containing medium was clarified by centrifugation at 3000 rpm for 30 min., and the virus medium was stored at -65 °C. The titre of the stock virus was 10^8.0 TCD50/ml.

For titrations and the neutralizing tests 5 × 10^6 cells/ml. in MEM containing 10 % calf serum were seeded into test tubes and the virus dilutions or the serum/virus mixtures immediately added. Five test tubes were used per dilution and the cultures were incubated at 37 °C without agitation. The TCD50 values and the ND50 values were calculated according to Kärber (1930) per 0.1 ml.

Preparation of the vaccine and animal immunization

The virus was inactivated by 0.15 % ethyleneimine (Schuchard, München) and DEAE dextran MW 5 × 10^5 (Pharmacia, Uppsala, Sweden) was added to give a final concentration of 0.5 g. per vaccine dose (5 ml.). Details of vaccine preparation and innocuity testing have been given (Wittmann and Jakubik, 1977). Thirty-nine 8 week old pigs (race Deutsches Landschwein) from 5 different farms were used for immunization. They were mixed and divided into groups of 1 × 9 and 3 × 10 animals, housed in isolated units and vaccinated by injection of 5 ml. of vaccine intramuscularly deeply into the neck. The second vaccine injection was made at the same site at intervals of 1 week (group I), 2 weeks (group II), 3 weeks (group III) and 4 weeks (group IV). During the experiment 1 pig from group I and group II, respectively, died after 8 and 9 weeks from unknown causes.

Neutralization test

Serum samples were taken from each of the animals before vaccination, at the time of revaccination, 1 week after revaccination and at intervals of 2 weeks thereafter. The sera were stored at -20 °C before use. Fourfold serum dilutions in PBS were made and 50 TCD50/ml. of ADV added. The mixture was incubated in the presence of 10 % guinea pig complement at 37 °C for 30 min. (Wittmann et al., 1976). In some experiments mixtures without complement were run in parallel. One ml. of a cell suspension containing 5 × 10^6 CT cells in MEM supplemented with 10 % calf serum was added to each test tube and 0.1 ml. of ADV/serum mixture was inoculated immediately afterwards. Per serum dilution 5 test tubes were used. The cultures were observed as to the appearance of a cytopathic effect for 6 days.

Double immunodiffusion test

For the determination of non-viral antibodies against vaccine components at the time of revaccination, the double immunodiffusion test was applied. We followed the procedure outlined by Cowan and Graves (1966). Undiluted inactivated calf serum (CS), undiluted supernatant of non-infected CT cell cultures, lysed by repeated cycles of freezing and thawing, and ethyleneimine-treated after clarification by low speed centrifugation, and DEAE dextran, MW 5 × 10^5, in a concentration of 1,600 μg/ml (concentrations between 780 μg/ml. and 1,200 μg/ml. had been found to give optimal precipitation bands in a pretest) were used as antigens. Agar medium was sufficient
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for testing the CS and CT system, whereas 1% agarose medium was necessary for testing the DEAE dextran system, since DEAE dextran formed unspecific halos in agar. Some of the sera were also tested against various amounts (see results) of dextran sulfate, MW $5 \times 10^5$, and dextran T 500 (both from Pharmacia). The diluent for antigens and serum was veronal buffered saline.

Results

Anaphylactic reactions after revaccination

Anaphylactic reactions occurred with revaccination group I, which was revaccinated 1 week post vacc. Seven out of 9 revaccinated animals showed dyspnöea, cyanosis of the skin, vomiting and motor disorder. These symptoms appeared within 5 min. after revaccination and disappeared without medical treatment within 10 to 20 min. Very slight anaphylactic symptoms could also be seen with one of the 10 pigs of group II, revaccinated 2 weeks post vacc. The other animals of this group and the animals of group III and IV, which were revaccinated 3 and 4 weeks post vacc., respectively, did not show any symptoms of anaphylaxis.

Sera from the animals before the first vaccination and immediately before revaccination were tested in the double immunodiffusion test against non-viral vaccine constituents, namely CT antigen, calf serum (CS) and DEAE dextran (DD). The results of this examination are shown by the following schedule:

1 week post vacc., 9 sera tested.
Positive against CT, 2 sera, titre range $1 : 2$
Positive against CS, 2 sera $1 : 2$
Positive against DD, 9 sera $1 : 1 - 1 : 4$

2 weeks post vacc., 10 sera tested.
Positive against CT, 3 sera, titre range $1 : 1 - 1 : 4$
Positive against CS, 5 sera $1 : 128 - 1 : 256$
Positive against DD, 10 sera $1 : 2 - 1 : 4$

3 weeks post vacc., 10 sera tested.
Positive against CT, 6 sera, titre range $1 : 1 - 1 : 2$
Positive against CS, 6 sera $1 : 128 - 1 : 256$
Positive against DD, 10 sera $1 : 2 - 1 : 4$

4 weeks post vacc., 10 sera tested.
Positive against CT, 6 sera, titre range $1 : 1 - 1 : 2$
Positive against CS, 7 sera $1 : 128 - 1 : 256$
Positive against DD, 10 sera $1 : 2 - 1 : 4$

Prevaccinal sera (before the first vaccination), 39 sera tested.
Positive against CT, 0 sera
Positive against CS, 0 sera
Positive against DD, 37 sera, titre range $1 : 1 - 1 : 2$

Some of the DEAE dextran positive sera were also tested against twofold dilutions of dextran sulfate and dextran T 500 in a concentration range between 100 mg. and 195 $\mu$g/ml., but no precipitation was seen in agar or in agarose.
The production of neutralizing antibodies against AVD in revaccinated pigs

The results of the neutralization test in the presence of guinea pig complement are listed in Figures 1, 2 and 3. Groups II, III and IV showed a strong booster effect within 1 week after revaccination, whereas with group I the booster effect was lower and a peak was not reached before 2 weeks post revacc. Up to 8 weeks post revacc. the mean titres of group IV were the highest and those of group I the lowest. The titres of group II and III were intermediate but II was nearer to I, and III was nearer to IV. After

Fig. 1. Neutralizing titres of pigs 1 and 2 weeks after revaccination with ADV vaccine. Curves indicate mean values. For comparison, the curve of mean values after a single vaccination is given (○ - · - · ○), calculated from former results (WITTMANN and JAKUBIK, 1978)

Fig. 2. Neutralizing titres of pigs 3 and 4 weeks after revaccination with ADV vaccine. Curves indicate mean values. For comparison the curve of mean values after a single vaccination is given (○ - · - · ○), calculated from former results (WITTMANN and JAKUBIK, 1978)
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10 weeks there was a tendency to equalization of the mean titres. After 18 and 20 weeks the titres abruptly fell to 1:4 and lower in all 4 groups. From Fig. 3 it is evident that the titre differences cited were not very clear since frequently there was an overlap of the standard deviations, but if one summarizes the results of the 4 groups from 1 to 18 weeks post revacc. no significant difference of the mean titres was obtained between the following groups: I/II in 90 %, I/III in 50 %, I/IV in 50 %, II/III in 50 %, II/IV in 70 % and III/IV in 90 % This confirms the assumption that antibody production with group I and II was apparently lower than with group III and IV. However, if the relative rise of antibody titres is considered in comparison to the initial titres at the time of revaccination, group II showed the best results after revaccination. The relative mean rise of the titres during the revaccination time tested was with group II 5.04-fold, whereas with group I the rise was only 1.94-fold, and with group III and IV 3.63-fold and 3.8-fold, respectively.

For demonstrating the effect of guinea pig complement (C) on ADV neutralization we tested 4 sera from each revaccination group in the presence and absence of C. In Table 1 the results are summarized. The highest enhancing effect of C occurred in the test with sera taken after the first vaccination, where practically no antibody could be detected in the absence of C. After revaccination neutralizing antibodies were found during the whole time of the revaccination period in the absence of C; however, their titres were considerably lower than those in the presence of C. The influence of C faded only after 18 and 20 weeks post revacc., when the titres were approaching the zero level. The mean enhancement by C was with all 4 groups after vaccination 59.6-fold, 1 week post revacc. 6.2-fold, 2 weeks post revacc. 6.2-fold, 6 weeks post revacc. 9.9-fold, 10 weeks post revacc. 6.5-fold, 14 weeks post revacc. 5.4-fold, 18 weeks post revacc. 3.2-fold and 20 weeks post revacc. 1.5-fold.

The influence of complement on the results of the neutralization test

Fig. 3. Standard deviation of the mean values of the neutralizing titres of the 4 revaccination groups of pigs

Table 1
Neutralizing titres in the presence and absence of guinea pig complement. a) — C: no com-
b) Group I 1 week, group II 2 weeks, group III 3 weeks, group IV 4 weeks after the first
d) Mean titre of the 4 animals. e) Enhancement

<table>
<thead>
<tr>
<th>Pig sera</th>
<th>Compl. added^</th>
<th>Post vaccination^</th>
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<tr>
<td>Group I</td>
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<tr>
<td>No. 6, 13, 19, 21</td>
<td>- C</td>
<td>Range&lt; 4</td>
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<td></td>
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<td></td>
<td>+ C</td>
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<td>El 114</td>
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<td>No. 2, 3, 24, 38</td>
<td>- C</td>
<td>Range&lt; 4</td>
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<td></td>
<td>+ C</td>
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<td>Mean 3</td>
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<tr>
<td></td>
<td>+ C</td>
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<td>El 19.0</td>
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<td></td>
<td>+ C</td>
<td>Range 24 - 128</td>
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<td>Mean 55</td>
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Discussion
Revaccination of pigs with inactivated ADV vaccine evoked a booster effect on the production of neutralizing antibodies. This booster effect reached its peak 1 week after revaccination if the revaccination was carried out between 2 and 4 weeks post vacc. However, if revaccination was done 1 week post vacc. the booster effect was significantly lower and reached its peak no earlier than 2 weeks post revacc. Besides, revaccination after 1 week post vacc. generally induced a lower antibody response for the following 8 weeks than later revaccination. Highest neutralizing titres were demonstrated in animals revaccinated 3 or 4 weeks post vacc. (group III and IV). The titres of the animals revaccinated 2 weeks post vacc. (group II) were frequently below the titres of group III and IV, but, the initial titres of group III and IV at the time of revaccination were generally higher than those of group II, and therefore the rise of antibody titres after revaccination was relatively higher with group II than with group III and IV. From 10 weeks post revacc. onward the titres of the 4 groups equalized somewhat and with all the groups the titres approached the zero level 18 to 20 weeks post revacc. In comparison, the titres after one vaccination had disappeared by 8 weeks post vacc. (WITTMANN and JAKUBIK, 1978).
From these results one can conclude that the optimal time for revaccination would be 3 or 4 weeks post vacc.; however, in a previous investigation we found that after a single vaccination the protection of the pigs against challenge infection had declined after 3 weeks (WITTMANN and JAKUBIK, 1977). Therefore, it is advisable to revaccinate 2 weeks post vacc. if the animals are immunized for the first time. But if it is only desired to produce
plement added; + C: 10% guinea pig complement added to the virus/serum mixture. 

vaccination. c) Reciprocal of the lowest and highest neutralizing titre of the 4 animals. 

index by C: titre with C / titre without C

Table 1

<table>
<thead>
<tr>
<th>Weeks post revaccination</th>
<th>Neutralizing titres</th>
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<tr>
<td></td>
<td>1</td>
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<tr>
<td>2 - 56</td>
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<td>26</td>
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<tr>
<td>427</td>
<td>245</td>
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<td>14.2</td>
<td>9.4</td>
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24 - 72                   | 18 - 128            | 8 - 32*            | 5 - 32             | 5 - 18             | <4 - 8             | <4 - 6             |                    |
| 55                       | 57                  | 20*                | 9                  | 5                  | 3                  |                    |
| 97 - 389                 | 56 - 128            | 128 - 169*         | 56 - 128           | 24 - 42            | 18 - 32            | <4 - 8             |                    |
| 292                      | 102                 | 149*               | 95                 | 31                 | 27                 | 3                  |                    |
| 5.3                      | 1.8                 | 7.5                | 5.9                | 3.4                | 5.4                | 1.0                |                    |

18 - 72                   | 18 - 97             | 5 - 97             | 3 - 97             | 5 - 72             | 5 - 24             |                    | n. d.              |
| 49                       | 59                  | 32                 | 31                 | 25                 | 11                 |                    |
| 223 - 512                | 128 - 389           | 128 - 223          | 128 - 223          | 32 - 223           | <4 - 32            |                    |
| 481                      | 362                 | 238                | 162                | 124                | 18                 |                    |
| 98                       | 6.1                 | 7.4                | 5.2                | 5.0                | 1.6                |                    |

32 - 72                   | 32 - 97             | 8 - 42             | <4 - 97            | 5 - 32             | <4 - 14            |                    |
| 51                       | 51                  | 19                 | 28                 | 12                 | 6                  |                    |
| 389 - 512                | 223 - 512           | 128 - 389          | 32 - 128           | 56 - 128           | <4 - 32            |                    |
| 481                      | 385                 | 259                | 65                 | 84                 | 6                  |                    |
| 8.8                      | 7.6                 | 13.6               | 2.3                | 7.0                | 1.0                |                    |

* Only serum of pig no. 2 and no. 3 examined

high antibody levels, e.g. in vaccinated pregnant sows for transfer of maternal antibodies with the colostrum to their piglets, the last revaccination may be optimal at 3 or 4 weeks before the end of pregnancy.

After the first vaccination only complement (C)-dependent antibodies could be demonstrated for the test period from 1 to 4 weeks. After revaccination C-dependent antibodies and, also, small amounts of C-independent antibodies were present. This latter finding corresponds with the situation in ADV infected pigs (WITTMANN et al., 1976). Towards the end of the efficacy of vaccination (after 18 to 20 weeks), the C-dependency of antibodies was reduced. We are now testing whether the C-dependency is connected with the Ig class.

Pig complement evokes no enhancing effect on neutralization (WITTMANN et al., 1976). Therefore, one might assume that in vivo the activity of neutralizing antibodies is low, especially after only one vaccination. However, experimental data show that protection against challenge infection is well developed from the 5th day post vacc. onward (WITTMANN and JAKUBIK, 1977, and unpublished results). This may indicate that the antibodies find other activating substances in the pig in vivo. On the other hand, we do not know yet what role is played by the cell-mediated immunity after the application of a DEAE dextran-containing inactivated vaccine, since DEAE dextran acts on lymphocyte reactions which are typical for cell-mediated immunity: it enhances ADV-specific stimulation of ADV-sensitized lymphocytes (WITTMANN and BARTENBACH, 1977), it enhances stimulation of mouse T cells by concanavalin A (BARTENBACH and WITTMANN, 1976), and
it enhances the mixed lymphocyte reaction of pig lymphocytes (in preparation).

Anaphylactic reaction of the immediate type developed in the majority of vaccinated pigs when they were revaccinated 1 week post vacc., whereas, apart from one case 2 weeks post vacc., no anaphylactic reaction occurred with the other animals revaccinated from 2 to 4 weeks post vacc. Precipitating antibodies against the vaccine constituents CT antigen and calf serum could be detected in the postvaccinal sera but not in the prevaccinal sera. The number and the titres of sera reacting with these two antigens was lowest at 1 week post vacc. and increased 2 weeks post vacc.; thereafter the response was maximal, with especially high titres against calf serum. Antibodies against DEAE dextran were demonstrated in prevaccinal and postvaccinal sera. It is unlikely that the animals had been in contact with DEAE dextran before vaccination, but it is very likely that these animals had had contact with dextran, e.g. by injection of iron dextran after birth. Therefore, we assume that dextran antibodies rather than DEAE dextran-specific antibodies were involved in the reaction. The negative results with dextran and dextran sulfate in immunodiffusion can be caused by non-antigenic properties of these compounds. Spontaneous dextran anaphylaxis is also known in humans without detectable reason. It is assumed that this anaphylaxis is evoked by the intestinal uptake of sugars with the food (PATTON, 1958; BLOCH, 1967).

On account of the antibodies present, the anaphylactic reaction in our experiment could have been evoked by DEAE dextran and/or by the other vaccine constituents. Two findings are important: a) no anaphylactic reactions occurred with the 39 animals after primary vaccination, in spite of the presence of dextran antibodies, and b) anaphylactic reactions occurred nearly exclusively 1 week post vacc., in spite of the presence of antibodies against the various vaccine constituents during all the time tested (4 weeks). This does not speak in favour of an exclusive dextran anaphylaxis and it is possible that reactions between early antibodies and CT antigen and calf serum were enhanced in the presence of DEAE dextran. Furthermore, one must consider that the second vaccine dose was injected into the same site as the first dose, where large amounts of local antibodies might have been present after 1 week which reacted with the antigens. At later times the local antibody concentration might have been reduced. In any case it must be avoided that a) revaccination is done 1 week post vacc., and b) that the second vaccine injection is carried out at the same site as the first injection. As far as serum and cell constituents are concerned, the danger of anaphylaxis can be reduced if porcine serum and porcine cells are used for virus production. Attempts to remove heterologous serum before virus inoculation by repeated washing from the cell cultures was ineffective, since serum traces sufficient to initiate a serum specific immunologic response remained in the cell cultures (unpublished results).

We thank Dr. J. Cox for correcting the English text.

Summary

Four groups of 8 week old pigs were intramuscularly vaccinated with an Aujeszky virus disease vaccine, consisting of ethyleneimine-inactivated virus and DEAE dextran as an adjuvant, revaccination of vaccinated animal groups at the same site of the first inoculation was done after 1, 2, 3, and 4 weeks. Revaccination evoked a rise in neutralizing antibody titres of the
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Neutralizing antibody titres in serum. The booster effect was least marked after 1 week revaccination. The highest neutralization titres appeared during a revaccination period of 8 weeks with those animals which were revaccinated at 3 and 4 weeks post vacc. From 10 weeks onward an equalization of the mean titres to a certain degree took place and the titres approached the zero level after 18 to 20 weeks.

After the first vaccination only complement-(C)-dependent antibodies could be demonstrated. After revaccination C-dependent antibodies and also smaller amounts of C-independent antibodies were present. Towards the end of the efficacy of vaccination the proportion of C-independent antibodies rose.

When pigs were revaccinated 1 week post vacc. severe anaphylactic reactions occurred with the majority of the animals. This was not the case after 2-week, 3-week and 4-week revaccination. Antibodies against non-viral vaccine constituents (cellular antigen, calf serum, and DEAE dextran) could be demonstrated by the double immunodiffusion test in the sera of vaccinated pigs. Antibodies against DEAE dextran (dextran?) were already present in prevaccinal sera.

The results are discussed with regard to optimal revaccination time, the complement-dependency of antibodies, and the reason and the prevention of anaphylactic reactions.

Zusammenfassung
Neutralisierende Antikörper in Seren vom Schwein nach Revakzination mit einer Vaccine aus inaktivierendem Aujeszkyvirus


Wurden die Schweine eine Woche nach der ersten Impfung revakziniert, so traten bei der Mehrzahl der Tiere anaphylaktische Reaktionen auf. Dies war nicht der Fall, wenn die Revakzination 2, 3 und 4 Wochen post vacc. vorgenommen wurde. Antikörper gegen die nicht-viralen Vakzinebestandteile (Zellantigen, Kälberserum und DEAE Dextran) konnten mit Hilfe der doppelten Immunodiffusionstests in den Seren vakzinerter Tiere nachgewiesen werden. Antikörper gegen DEAE Dextran (Dextran?) waren auch schon in den prävakzinalen Seren vorhanden.

Die Ergebnisse werden im Hinblick auf optimale Revakzinationszeit, Komplementabhängigkeit der Antikörper und die Ursache und Vermeidung der anaphylaktischen Reaktionen diskutiert.
Résumé

Anticorps neutralisants dans des sérums de porcs revaccinés avec un vaccin à virus d’Aujeszky inactivé

4 groupes de porcs âgés de 8 semaines ont reçu par voie intramusculaire un vaccin de virus d’Aujeszky inactivé à l’éthylénimine avec du DEAE-dextran comme adjuvant. Une partie de chaque groupe a été revaccinée de la même manière après 2, 3 et 4 semaines. La revaccination a provoqué une augmentation des anticorps neutralisants dans le sérum. L’effet de Booster fut le plus faible lorsque la revaccination a eu lieu une semaine après la vaccination. Les titres de neutralisation les plus élevés sont intervenus durant une période de revaccination de huit jours chez les animaux revaccinés 3 et 4 semaines après la vaccination. Une égalisation de la valeur moyenne de titres fut observée à partir de la dixième semaine dans tous les groupes vaccinés. Les titres ont atteint une valeur nulle après 18—20 semaines.

Des anticorps dépendant du complément (C) seulement ont été mis en évidence après une seule vaccination. Une petite quantité d’anticorps indépendants de C est apparue en plus après la revaccination. La quantité d’anticorps indépendants de C a augmenté à la fin de la période d’immunité (18—20 semaines post revacc.).

Des réactions anaphylactiques ont été observées chez la plupart des animaux lorsque les porcs durent revaccinés une semaine après la première vaccination. Ce ne fut plus le cas après 2, 3 et 4 semaines. Des anticorps anti-éléments non viraux (antigène cellulaire, sérum du veau, DEAE-dextran) ont été trouvés dans les sérums des animaux vaccinés à l’aide du double test d’immunodiffusion. Des anticorps anti DEAE-dextran (dextran?) furent déjà trouvés dans les sérums d’animaux prévaccinés.

Les résultats sont discutés en fonction du moment optimal de revaccination, de la dépendance du complément des anticorps, de l’origine et l’événement des réactions anaphylactiques.

Resumen

Anticuerpos neutralizantes en sueros sanguíneos de cerdos tras revacunación con una vacuna de virus inactivado de Aujeszky

Se vacunaron por vía intramuscular cuatro grupos de cerdos, de 8 semanas de edad, con una vacuna que consistía en virus de Aujeszky inactivado por etilenimina y dextrano DEAE como adyuvante, revacunándose cada vez un grupo de animales tras una, 2, 3 y 4 semanas en el mismo sitio en que se aplicó la vacuna primera. La revacunación conducía a un aumento de los anticuerpos neutralizantes en el suero sanguíneo. El efecto impelente era más débil cuando la revacunación se llevaba a cabo una semana post vacc. Los títulos de neutralización más elevados aparecían durante un periodo de revacunación de ocho semanas en aquellos animales que fueron revacunados 3 y 4 semanas post vacc. A partir de la semana 10º se produjo en todos los grupos vacunados cierta asimilación de los valores medios de los títulos y al cabo de 18 a 20 semanas se aproximaban los títulos al valor cero.

Tras la vacunación única se pudieron identificar nada más anticuerpos dependientes del complemento (C). Después de la revacunación se hallaban presentes, al lado de los anticuerpos dependientes del C, otros no dependientes del C en cantidad escasa. Hacia el final del periodo de inmunidad (18—20 semanas post revacc.) aumentaba el contingente de anticuerpos que no dependían del C.
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Al revacunar los cerdos una semana después de la vacunación primera aparecían en la mayoría de los animales reacciones anafilácticas. Este hecho no acontecía cuando se llevaba a cabo la revacunación 2, 3 y 4 semanas post vacc. Se pudieron identificar anticuerpos frente a los componentes no virales (antígeno celular, suero sanguíneo de ternero y dextrano DEAE) en los sueros de los animales vacunados con ayuda de la prueba de inmunodifusión doble. En los sueros prevacunales se hallaban ya presentes anticuerpos frente a dextrano DEAE (¿dextrano?).

Los resultados se discuten con respecto al tiempo óptimo de revacunación, dependencia de los anticuerpos del C y la causa y evitación de las reacciones anafilácticas.

References


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