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HOG CHOLERA ANTIBODIES IN PIGS VACCINATED WITH AN AUJESZKY-VACCINE BASED ON ANTIGEN PRODUCED IN IB-RS-2 CELLS

By

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JENSEN, MERETHE HOLM: *Hog cholera antibodies in pigs vaccinated with an Aujeszky-vaccine based on antigen produced in IB-RS-2 cells.* Acta vet. scand. 1981, *22*, 517—523. — The cell line IB-RS-2 is confirmed to be persistently infected with hog cholera virus. Vaccination of pigs against Aujeszky's Disease with an inactivated vaccine based on antigen produced on this cell line induced neutralizing antibodies against hog cholera virus.

hog cholera antibodies; IB-RS-2 cells; Aujeszky-vaccination.

The porcine kidney cell line IB-RS-2 was originally described by *De Castro* in 1964 in connection with work on foot-and-mouth disease virus. The cell line is able to multiply not only in monolayer culture but also in suspension culture (*Chapman & Ramshaw* 1971). This circumstance makes this cell line attractive for production of viral antigen for vaccines (*Chapman & Ramshaw, Mowat et al.* 1974).

As early as in 1971 *Ribeiro et al.* described that a lysate of IB-RS-2 cells could protect pigs against hog cholera (HC). In 1973 a cytopathogenic agent belonging to the togavirus group was isolated from a clone of the IB-RS-2 cell line by *De Castro.* In addition it was described (*Ribeiro et al.* 1973) that lysates from this clone as well as from other IB-RS-2 cell clones could protect pigs against HC. In all observations the agent appeared to be avirulent in pigs. Since HC-virus and bovine viral diarrhea (BVD) virus are closely related and BVD-virus sometimes occurs as a contaminant in cell cultures it was for some years thought

that the agent found could be BVD-virus and not HC-virus. This was further supported by the finding that vaccination with BVD-virus can protect pigs against HC (*Beckenhauer et al.* 1961, *Sheffy et al.* 1961, *Overby* 1973). In 1978 *Laude* demonstrated that the cytopathogenic agent he isolated from his IB-RS-2 cells was HC-virus and not BVD-virus.

In connection with experiments on the use of IB-RS-2 cells for HC-virus work we too have studied the agent in IB-RS-2 cells.

The problem concerning the presence of HC-virus in the IB-RS-2 cell line was recently actualized when the Danish authorities were requested to permit the use of an Aujeszky-vaccine based on antigen produced on this cell line. In order to clarify whether this type of vaccine might give rise to the development of antibodies against HC-virus, sera from pigs vaccinated with this vaccine as well as sera from pigs vaccinated with two other Aujeszky-vaccines were tested for neutralizing antibodies against HC- and BVD-virus.

MATERIALS AND METHODS

Isolation of the IB-RS-2 agent

The procedure described by *Laude* (1978) was followed, i.e. freezing to —20°C, thawing and centrifugation of a 2—3 days old IB-RS-2* cell culture. The resulting supernatant fluid is designated IB-RS-2 agent in the following.

Virus titrations and neutralization assays

Cell cultures (secondary pig and calf kidney cell cultures), HC-virus (The Japanese ALD strain), BVD-virus (The Danish Ug 59 strain), HC and BVD immune sera, microplate assay for titration of virus infectivity and neutralizing antibodies (chessboard principle) read by peroxidase-linked antibody technique were as earlier described (*Jensen* 1981).

The IB-RS-2 agent as well as HC-virus and BVD-virus were inoculated simultaneously and in parallel in serial 10-fold dilutions into secondary pig kidney and secondary calf kidney cell cultures. The IB-RS-2 agent was used in parallel with HC-virus and BVD-virus as the viral antigen in neutralization assays with an HC immune serum and a BVD immune serum.

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* IB-RS-2 cells were kindly provided by The Animal Virus Research Institute, Pirbright, Woking, Surrey, England.

Vaccines and resulting sera

Three different commercially available Aujeszky-vaccines designated I, II and II were used. According to the specifications of the producers all the vaccines were of the inactivated type but based on Aujeszky-antigen produced by means of different cell cultures. BHK 21 clone CT cells were used for vaccine I, IB-RS-2 cells for vaccine II and primary pig kidney cells for vaccine III. Nine pigs of the Danish Landrace (weight 40—50 kg) were vaccinated i.m. with 2 ml of vaccine with intervals of 2 weeks, in total 5 vaccinations per animal. Blood samples were collected before vaccination and 2 weeks after each vaccination.

Pigs Nos. 1, 2 and 3 were vaccinated with vaccine I,
pigs Nos. 4, 5 and 6 were vaccinated with vaccine II and
pigs Nos. 7, 8 and 9 were vaccinated with vaccine III.

RESULTS

Fig. 1 shows the infectious titres of HC-virus, BVD-virus and IB-RS-2 agent obtained in secondary pig kidney and secondary calf kidney cells, respectively. The pig kidney cells were considerably more sensitive to the IB-RS-2 agent than were the calf kidney cells. This is in analogy to the HC-virus and in contrast to the BVD-virus, where calf kidney cells were the more sensitive.

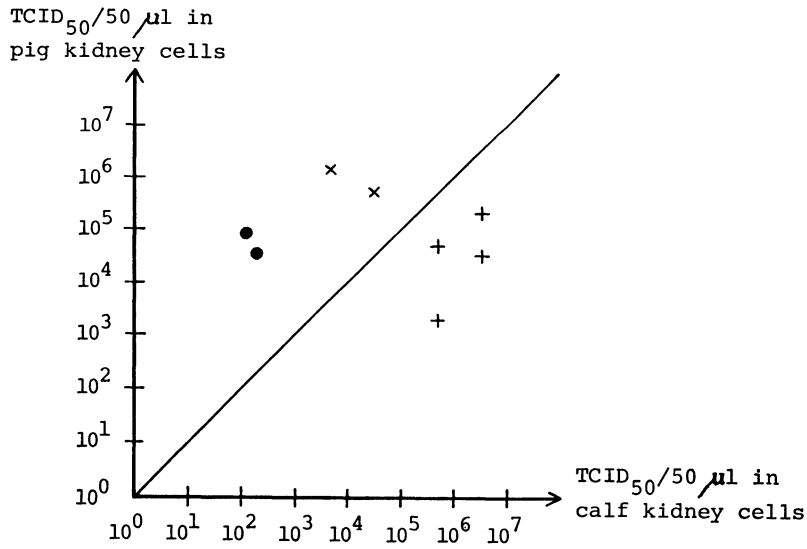

Figure 1. Infectious titers of the HC-virus (×), BVD-virus (+) and IB-RS-2 (●) agent in secondary pig and calf kidney cell cultures, respectively.

Fig. 2 illustrates the results of neutralization experiments using an HC immune serum and a BVD immune serum against HC-virus, BVD-virus and IB-RS-2 agent, respectively. The HC-virus and the IB-RS-2 agent were neutralized at similar level by the HC immune serum. The BVD-virus was neutralized by the BVD immune serum. The HC-virus and the IB-RS-2 agent were not neutralized by the BVD-serum, while the BVD-virus to some extent was neutralized by the HC-serum. These results classify our IB-RS-2 agent as an HC-virus similarly to the findings of *Laude* (1978).

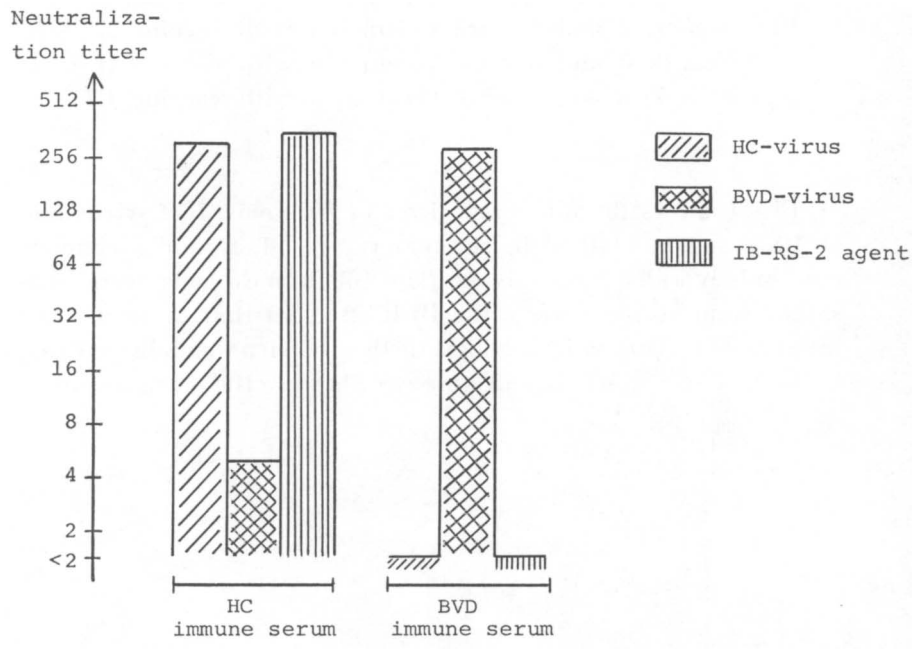

Figure 2. Results of neutralization assays with an HC immune serum and a BVD immune serum using HC-virus, BVD-virus and IB-RS-2 agent as the viral antigens. Titers are given as the reciprocal value of the highest serum dilution which in a 50 µl amount neutralizes 100 $TCID_{50}$.

Fig. 3 shows the results of the examination for neutralizing antibodies against HC- and BVD-virus in the sera from pigs vaccinated with the 3 different Aujeszky-vaccines. In the serum from 3 pigs vaccinated with vaccine II HC-virus neutralizing antibodies were detected 2 weeks after the 3rd and the 4th vaccination respectively, while no antibodies against BVD-virus

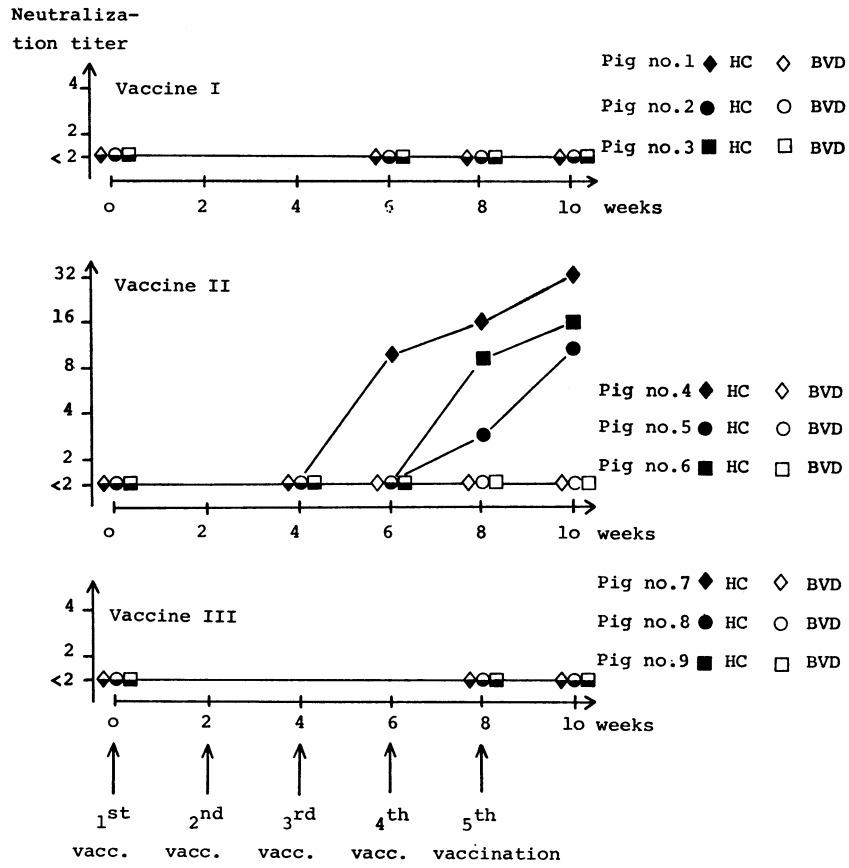

Figure 3. Examination for neutralizing antibodies against HC- and BVD-virus in sera from pigs after vaccination with 3 different Aujeszky-vaccines. Titers are given as the reciprocal value of the highest serum dilution which in a 50 µl amount neutralizes 100 $TCID_{50}$.

could be demonstrated. Neither vaccine I nor vaccine II gave rise to detectable antibodies against HC-virus or BVD-virus.

None of the pigs revealed clinical symptoms in connection with the vaccinations.

DISCUSSION AND CONCLUSION

Already in 1973 *Ribeiro et al.* concluded that the pig from which the IB-RS-2 cell line was derived must have been infected with the agent demonstrated later on. This conclusion is supported by the isolation of an avirulent HC-virus from IB-RS-2 cells originating from a French laboratory (*Laude* 1978) and by

the results presented here. The infection is persistent and it seems to be present in all cells and to spread directly from mother cells to daughter cells (*De Castro* 1973). The infection can not be eliminated by cultivation of the cells using HC-antibody containing serum (*De Castro* 1973). Both *De Castro* (1973) and *Laude* (1978) describe their IB-RS-2 agent as cytopathogenic. *Laude & Gelfi* (1980) used the cytopathogenic effect in 2 pig kidney cell lines as criterion when reading the neutralization tests for HC-antibody detection. The IB-RS-2 agent isolated at our institute has shown only some cytopathogenic effect in secondary pig kidney cell cultures.

Vaccine II is an inactivated vaccine based on Aujeszky-virus propagated in IB-RS-2 cells. The manufacturer of this vaccine recommends the use of 2 vaccinations with an interval of 1—2 months followed by a booster vaccination every 6th month. In the present work we have used 5 vaccinations with 2-weeks-intervals. However, 1 of the 3 vaccinated pigs developed HC-antibodies already after the 3rd vaccination and the other 2 after the 4th vaccination. Although the level of HC-antibodies was low it was sufficiently high to be recorded.

It is thus concluded that this vaccine under certain circumstances can give rise to neutralizing antibodies against HC-virus. Therefore, although the HC-virus in IB-RS-2 cells is avirulent in pigs and probably inactivated in the vaccine the use of this type of vaccine may cause problems in HC-free areas.

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SAMMENDRAG

Svinepestantistoffer i grise efter vaccination med en Aujeszky-vaccine fremstillet på antigen produceret i IB-RS-2 celler.

Cellelinien IB-RS-2 er persistent inficeret med svinepestvirus. Vaccination af grise mod Morbus Aujeszky ved hjælp af en inaktiveret vaccine baseret på antigen produceret i denne cellelinie kan foranledige fremkomst af neutraliserende antistoffer mod svinepestvirus.

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