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VACCINATION OF PIGS AGAINST HOG CHOLERA (CLASSICAL SWINE FEVER) WITH A DETERGENT SPLIT VACCINE*

By

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DALSGAARD, K. and E. OVERBY: *Vaccination of pigs against hog cholera (classical swine fever) with a detergent split vaccine.* Acta vet. scand. 1976, *17*, 465—474. — Four pigs were inoculated subcutaneously with a detergent (triton X 100) split hog cholera virus in Freund's incomplete adjuvant. Four other pigs were in the same way inoculated with a detergent split bovine viral diarrhoea virus, also in Freund's incomplete adjuvant. In the experiment were used 3 control pigs. The vaccinations were repeated after 3 weeks. All pigs were challenged with highly virulent hog cholera virus (Tübingen) 12 weeks after primary inoculations. Signs of hog cholera were only noted in the control pigs.

This introductory experiment was succeeded by a larger experiment with subcutaneous inoculations of: 10 pigs with detergent split hog cholera virus in Freund's incomplete adjuvant, 10 pigs with detergent split hog cholera virus in a saponin (Quil A) solution, 10 pigs with detergent split bovine viral diarrhoea virus in Freund's incomplete adjuvant, 10 pigs with detergent split bovine viral diarrhoea virus in the Quil A solution plus 5 control pigs. The vaccinations were repeated after 3 weeks, and finally all pigs were challenged 9 weeks later with the highly virulent hog cholera virus strain.

With the exception of 1 animal which died accidentally, all animals survived in the groups inoculated with the Quil A vaccines and in the group inoculated with the detergent split hog cholera virus/oil adjuvant vaccine. In the group inoculated with the detergent split bovine viral diarrhoea virus/oil adjuvant vaccine, some of the pigs died of hog cholera.

hog cholera; detergent split vaccine; triton X 100; Quil A.

Although Denmark has been free of hog cholera (classical swine fever) since 1933, this disease nevertheless poses an ever present threat to the Danish pig population as the disease is still

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of a major nation-wide importance on the European continent including the 6 original EEC countries.

Hog cholera is usually controlled by strict veterinary safety measures including immediate stamping out of infected herds (*Overby & Schjerning-Thiesen* 1973). In some areas viz. Western Germany, the Netherlands and France, however, the spread of hog cholera, especially the more subclinical forms, has been so extensive that a vaccination policy has been included (*Overby* 1975).

A considerable number of vaccines against hog cholera has been reported and used for practical control. The 2 major types of vaccines are the inactivated and the live attenuated. Among the inactivated vaccines 2 have been used for control of the disease, e.g. the crystal violet vaccine (*Janowski et al.* 1959, *van Bekkum* 1966, *Jerabek et al.* 1970), and the formalin, aluminium hydroxide vaccine (*Gheorghiu et al.* 1961, *Janowski* 1965). None of these vaccines, however, seem to be of great value (*Pilchard* 1967, *Corthier et al.* 1975). Several live attenuated strains have been used successfully (*Bruckner et al.* 1962, *Aynaud & Asso* 1970, *Wachendörfer et al.* 1973) for the control of hog cholera, as such vaccines are easy to handle and they induce high levels of immunity. The major drawback of using attenuated live strains is the introduction of a replicating virus. Although virus replication is usually kept at a minimum, it may in some cases give rise to reproductive disorders and through disease in neonatal pigs or through passages in apparently healthy living pigs the vaccine strain may regain pathogenicity (*Young* 1952, *Young et al.* 1955, *Emerson & Delez* 1965, *Dunne & Clark* 1968, *Stewart et al.* 1973, *Overby & Eskildsen* 1976). However, the vaccines produced on virus cultivated at low temperature (*Launais et al.* 1972, 1974) or in tissue culture of different species (goat and lamb) (*Mihaita et al.* 1973, *Zuffa* 1976) are regarded as harmless in the countries where they are used.

In the present paper a new type of vaccine is presented. The vaccine is based on disruption of hog cholera virus infected cells with a detergent. It was previously reported (*Overby* 1973) that the Danish strain UG 59 of bovine viral diarrhoea virus is able to protect pigs against hog cholera. A detergent split vaccine on the basis of this virus was therefore included.

The possibility of large scale production of a new vaccine is discussed.

MATERIALS AND METHODS

Animals

Pigs of the Danish landrace were used throughout the experiments. At the beginning of each experiment the pigs were about 2 months of age and weighed about 30 kg each. A total of 56 pigs were used.

Preparation of antigens

Hog cholera virus (HCV) and bovine viral diarrhoea virus (BVDV) were grown in monolayer cultures in 1 l-roller bottles. HCV was grown in primary pig kidney cells, and BVDV in secondary calf testis cells. The details of virus propagation have been published previously (*Eskildsen & Overby* 1976). Two days after infection the cells were scraped off and pelleted by low speed centrifugation. The medium was discarded. The cell pellet was treated by an equal volume of triton X 100 (Room & Haas), 10 % in 0.01 M Tris/HCL pH 7.5. This mixture was sonicated at maximum output for 3×30 sec. in an ice bath. The mixture was left overnight at 4°C after which the sonication was repeated. This product was centrifuged at $50{,}000 \times g$ tip value for 1 hr. The supernatant was isolated and used for the preparation of vaccines. Innocuity was tested by inoculation of monolayers of the respective host cells.

Preparation of vaccines

Fifty µl of antigen (HCV or BVDV) were made up to 0.5 ml in phosphate buffered saline (PBS) pH 7.6 to constitute 1 antigen dose.

Two adjuvants were applied:

1. The saponin adjuvant Quil A (*Dalsgaard* 1974) was dissolved in PBS to a concentration of 2 mg/ml. Equal volumes of antigen and adjuvant solution were mixed to make a vaccine dose of 1 ml.
2. Oil adjuvant, Freund's incomplete, (*Freund* 1947) was prepared by mixing 9 parts of Bayol F (Esso Standard Oil Company, Linden, New Jersey) with 1 part of Arlacel A (Atlas Chemical Industries). This mixture was emulsified with equal volumes of antigen by suction through a syringe to make a vaccine dose of 1 ml.

Immunization

The pigs were vaccinated by subcutaneous injections of 1 ml of the respective vaccines. In the first experiment 4 pigs were vaccinated with the HCV/oil adjuvant vaccine, and 4 other pigs received the BVDV/oil adjuvant vaccine. The vaccinations were repeated after 3 weeks. Three control pigs were included.

In the second experiment 4 groups of 10 pigs each were vaccinated with the following vaccines respectively: HCV/oil adjuvant, BVDV/oil adjuvant, HCV/Quil A adjuvant and BVDV/Quil A adjuvant. Also in this case the vaccinations were repeated after 3 weeks, and 5 healthy pigs served as controls.

Neutralizing antibodies

Sera were collected from all pigs 1 week before challenge. Serum neutralizing antibodies against the tissue cell culture adapted ALD strain of HCV were estimated by immunofluorescence plaque technique published elsewhere (*Aynaud* 1968, *Overby* 1973).

Challenge

All the pigs were subjected to challenge 12 weeks after the primary vaccination. The challenge virus was a highly virulent strain of HCV (Tübingen). The virus had been passaged 5 times in pigs by inoculation of infectious blood. For challenge 2 ml of infectious blood was given (1 ml i.v. and 1 ml s.c.) to each animal. The body temperature of each animal was recorded daily in the morning.

RESULTS

The first experiment included 4 animals immunized with an HCV based vaccine and 4 animals immunized with a BVDV based vaccine, both with oil adjuvant. All animals were resistant to challenge with highly virulent HCV, whereas the 3 control animals all developed typical signs of hog cholera and eventually died after 3—7 days. The vaccinated animals showed no clinical

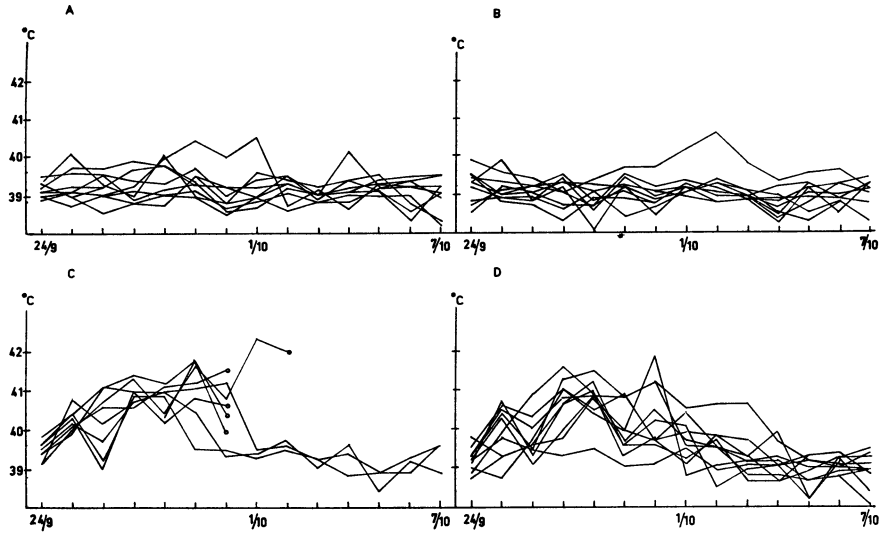

Figure 1. Temperature curves for 4 groups of pigs after challenge. The groups were vaccinated with the following vaccines:
A. Hog cholera antigen + oil
B. Hog cholera antigen + Quil A
C. Bovine viral diarrhoea antigen + oil
D. Bovine viral diarrhoea antigen + Quil A

Table 1. The reciprocal values of serum neutralizing antibody titres, estimated by immunofluorescence plaque technique.

Vaccine	1 week before challenge	2 weeks after challenge
HCV/oil adj. vac.	8	>2048
HCV/Quil A adj. vac.	8—16	>2048
BVDV/oil adj. vac.	<2	1024*
BVDV/Quil A adj. vac.	4	512

* surviving pigs.

symptoms at all. The reciprocal values of serum neutralizing antibody titres 1 week before challenge were in the range of 2—8.

The titres found in the second experiment are listed in Table 1. The body temperatures of the animals are shown in Fig. 1. It can be seen that all the animals receiving a vaccine based on HCV antigen resisted the challenge irrespective of the adjuvant used. The pigs showed no temperature rise and no clinical symptoms at all. The group of pigs immunized with the BVD/oil adjuvant vaccine showed clinical symptoms of hog cholera. Three pigs recovered completely after having shown a high temperature rise for 3 days, but 5 pigs eventually died. Post-mortem examination showed typical hog cholera lesions. The group of pigs immunized with the BVDV/Quil A adjuvant vaccine showed initial fever, but all the animals recovered completely. The 5 control pigs died after 4—7 days and showed typical hog cholera lesions.

In the group of pigs receiving HCV/oil adjuvant vaccine 1 animal had to be sacrificed before challenge. In the group of pigs receiving BVDV/oil adjuvant vaccine 2 of the pigs were lost during the immunization period. For one of these post-mortem examination indicated a thrombosis to be the cause of death, and on the basis of the pathological findings death from hog cholera could be completely excluded. In the case of the other one a leg was seriously damaged in the fence so the animal had to be killed before challenge.

All the different vaccine preparations were well tolerated by the pigs, no systemic effects or local reactions were observed.

DISCUSSION

The possibility of preparing effective vaccines against a number of enveloped viruses by treatment with detergents has been

demonstrated and reported in the literature. *Appleyard* (1976) used this approach for the preparation of an influenza subunit vaccine. Hog cholera virus has been classified as an animal togavirus (*Horzinek* 1973), and detergent split vaccines against other members of this group have also been reported. Good results in the protection against Semliki forest virus (*Kääriäinen et al.* 1969) have been observed, and a promising vaccine against rubella virus has recently been presented (*Cappel & Cuyper* 1976).

When rabies was inactivated (*Atanasiu et al.* 1974) with triton X 100 a glycoprotein, with a high degree of purity, was released. It induced neutralizing antibodies upon injection of rabbits with as small amounts as 10—50 µg.

In the present paper evidence has been presented that the treatment of HCV infected cells with the detergent may be used also for the preparation of a vaccine against hog cholera.

The use of infected cell material for the preparation of a detergent split vaccine has to our knowledge not been published before. Usually infected and/or precipitated medium or purified virus has been used. In case of hog cholera, however, the yield of virus in the tissue culture medium is very low (10^{5-6} PFU) compared to other viruses. During our studies on the isolation of a common glycoprotein of the HCV/BVDV viruses (*Dalsgaard* 1976) it was discovered that large quantities of immunoprecipitating antigens could be isolated from infected cells by triton X 100. The infected cells were harvested only 48 hrs. after infection. At this time of virus replication most of the virus is still within the cells. The release of virus to the medium usually occurs within 4—5 days.

From a practical point of view the most interesting result of this investigation is that the application of only 100 µl of HCV antigen given as 2 injections resulted in complete protection of the pigs.

An attractive approach would be to make the vaccine on the basis of BVDV, but in the second experiment of the present investigation the protection was only partial. This may be a quantitative phenomenon, but previous results (*Volenec et al.* 1972, *Dalsgaard & Overby* 1976) have indicated that BVDV may well prime the immune system for a recognition of HCV antigen, but a true anamnestic response is not obtained. This mechanism is in good agreement with the finding that the saponin adjuvant Quil A was more effective than oil. It has recently been demonstrated

that Quil A possibly has an enhancing effect on B-lymphocyte stimulation (*Ebbesen et al.* 1976).

In this paper the innocuity of the HCV based vaccine was tested by inoculation of tissue cultures. The innocuity was furthermore confirmed by the fact that none of the pigs reacted to the vaccination. As an extra safety measure the detergent treatment could be followed by use of acetylethyleneimine (AEI) (*Brown* 1963). Such experiments using HCV grown in PK 15 cells are planned. The serum neutralizing antibodies obtained 1 week before challenge are all at such low values that the possibility of cell mediated immunity may have to be considered.

REFERENCES

Appleyard, G.: Influenza subunit vaccines. Report 8th Meeting of NWEMG, Helsinki, June 1976.

Atanasiu, P., H. Tsiang, P. Perrin, S. Favre & J. Sisman: Extraction d'un antigène soluble (glycoprotéine) par le Triton X 100 à partir d'un vaccin antirabique de culture tissulaire de premier explant. Résultats d'immunisation et pouvoir protecteur. (Extraction of a soluble antigen (a glycoprotein) from rabies tissue culture vaccines using Triton X 100. Immunization and protection experiments). Ann. Microbiol. (Milano) 1974, *125* B, 539—557.

Aynaud, J. M.: Étude de la multiplication in vitro d'un clone du virus de la peste porcine. (Multiplication in vitro study of a virus strain of hog cholera). Rech. vét. 1968, *1*, 25—36.

Aynaud, J. M. & J. Asso: La souche lapinisé dite „chinoise" du virus de la peste porcine classique. (The chinese strain of classical swine fever virus). Rec. Méd. vét. 1970, *146*, 119—137.

Bekkum, J. G. van: Serological aspects of the vaccination against hog cholera with crystal violet vaccine. T. Diergeneesk. 1966, *91*, 149—158.

Brown, F.: The use of acetylethyleneimine in the production of inactivated FMD vaccines. J. Hyg. (Lond.) 1963, *61*, 337—344.

Bruckner, A. H., R. M. Scott & R. W. Swart: Two year stability of modified hog cholera vaccines, Swivax and Swivine. Allied Vet. 1962, *33*, 100—102.

Cappel, R. & F. De Cuyper: Efficacy and immune response to rubella subunits vaccines. Arch. Virol. 1976, *50*, 207—213.

Corthier, G., C. Galicher & J. Gelfi: Peste porcine: étude comparée du pouvoir immunogéne des vaccins a virus vivant et a virus inactivé par la cinétique des anticorps neutralisants dans le sérum. (Swine fever: a comparative study of the immunogenicity of live-virus and inactivated virus vaccines using the kinetics of serum neutralizing antibodies). Ann. Rech. vét. 1975, *6*, 93—101.

Dalsgaard, K.: Saponin adjuvants. III. Isolation of a substance from Quillaja saponaria Molina with adjuvant activity in foot-and-mouth disease vaccines. Arch. ges. Virusforsch. 1974, *44*, 243—254.

Dalsgaard, K.: The technique of quantitative immunoelectrophoresis (crossed immunoelectrophoresis) and its application in the study of viral antigens. EUR 5451, 1976, 320—329.

Dalsgaard, K. & E. Overby: Immunity against challenge with swine fever induced by a virus-specified glycopeptide isolated from infected cells. Report EEC seminar on classical swine fever virus. Hannover, September 1976. Proc. to be published by the Directorate-General Scientific and Technical Information and Information Management, Luxembourg.

Dunne, H. W. & C. D. Clark: Embryonic death, foetal mummification, stillbirth, and neonatal death in pigs of gilts vaccinated with attenuated live-virus hog cholera vaccine. Amer. J. vet. Res. 1968, *29*, 787—796.

Ebbesen, P., K. Dalsgaard & M. Høier-Madsen: Prolonged survival of AKR mice treated with the saponin adjuvant Quil A. Acta path. microbiol. scand., Sect. A 1976, *84*, 358—360.

Emerson, J. L. & A. L. Delez: Cerebellar hypoplasia, hypomyelinogenesis and congenital tremors of pigs associated with prenatal hog cholera vaccination of sows. J. Amer. vet. med. Ass. 1965, *147*, 1346—1349.

Eskildsen, M. & E. Overby: Serological diagnosis of classical swine fever. A comparison of a modified direct complement fixation test with an immunofluorescence plaque neutralization test in the diagnosis of experimental subclinical infection. Acta vet. scand. 1976, *17*, 131—141.

Freund, J.: Some aspects of active immunization. Ann. Rev. Microbiol. 1947, *1*, 291—308.

Gheorghiu, I., T. Albu, C. Surdan, N. Carp & M. Popa: La valeur immunogène du vaccin antipesteux porcin formolé-adsorbé, preparé avec l'antigène bacteriologique stérile et infecté par la salmonella in vivo et in vitro. (The immunological value of formalin inactivated hog cholera vaccine, prepared with the antigen bacteriological sterile and infected with salmonella in vivo and in vitro). Lucr. Inst. Pasteur (Buc.) 1961, *5*, 129—138.

Horzinek, M. C.: The structure of togaviruses. Progr. med. Virol. 1973, *16*, 109—156.

Janowski, H. J.: Hog cholera: immunity-inactivated vaccines. Report FAO/OIE int. meeting on hog cholera and African swine fever. Rome 1965.

Janowski, H., S. Majdan & M. Mierzejewska: Recherches concernant la résistance chez les porcs vaccinés contre la peste porcine avec le vaccin au cristal violet. (Investigations concerning the resistence of pigs vaccinated against hog cholera with a crystal violet vaccine). Med. weteryn. 1959, *2*, 71—85.

Jerabek, J., R. Hrabak & O. Sima: Crystal violet inactivation of hog cholera virus grown in tissue culture. Acta veter. (Brno) 1970, *39*, 467—477.

Kääriäinen, L., K. Simons & C. H. von Bonsdorf: Studies in subviral components of Semliki Forest virus. Ann. Med. exp. Fenn. 1969, *47*, 235—245.

Launais, M., J. M. Aynaud & G. Corthier: Peste porcine classique: Propriétés d'un clone (souche „Thiverval") isolé en culture cellulaire à basse température. Application dans la vaccination. (Classical swine fever: The properties of the "Thiverval" strain of virus isolated in tissue culture at low temperature. The use for vaccination). Rev. Méd. vét. 1972, *123*, 1537—1554.

Launais, M., J. M. Aynaud, G. Corthier & H. Laude: Peste porcine classique: Caractères de l'immunité, innocuité vis-à-vis des truies en gestation et stabilité génétique de la souche „Thiverval". (Swine fever. Characteristics of immunity, innocuity for pregnant sows and genetic stability of the "Thiverval" strain). Rev. Méd. vét. 1974, *125*, 175—194.

Mihaita, S., N. Totorcea, T. Albu, N. Tigaeru & S. Tibrea: Attempts to adapt the "C" lapinized swine fever virus strain into goats. Lucr.Inst. Pasteur (Buc.) 1973, *9*, 129—134.

Overby, E.: Immunization against swine fever with a Danish bovine viral diarrhoea virus strain, UG 59. Nord. Vet.-Med. 1973, *25*, 497—503.

Overby, E.: Subklinisk klassisk svinepest. Et aktuelt diagnostisk og immunologisk problem. (Subclinical classical swine fever. A current diagnostic and immunological problem). Dansk Vet.-T. 1975, *58*, 917—923.

Overby, E. & K. Schjerning-Thiesen: Klassisk svinepest — et aktuelt problem for fællesmarkedslandene. (Classical swine fever — a current problem for the common market countries). Medlemsbl. danske Dyrlægeforen. 1973, *56*, 553—555.

Overby, E. & M. Eskildsen: Transplacental infection in susceptible gilts after inoculations with: I. lapinised swine fever vaccine, II. bovine viral diarrhoea virus strains. Report EEC seminar on classical swine fever virus. Hannover, September 1976. Proc. to be published by the Directorate-General Scientific and Technical Information and Information Management, Luxembourg.

Pilchard, I.: Experimental inactivated-virus hog cholera vaccines: induction period of immunity. Amer. J. vet. Res. 1967, *28*, 915—923.

Stewart, W. C., E. A. Carbrey & J. I. Kresse: Transplacental hog cholera infection in susceptible sows. Amer. J. vet. Res. 1973, *34*, 637—640.

Volenec, F. J., B. E. Sheffy & J. A. Baker: Immunobiologic heterotypic activity associated with viral and soluble components of bovine virus diarrhea virus. Arch. ges. Virusforsch. 1972, *36*, 275—283.

Wachendörfer, G., D. Manz, U. Förster, W. Dingeldein & H. Gemmer: Erfahrungen mit dem Einsatz von Suiferin C in Schweinepestbeständen. (Experience with Suiferin C in hog cholera infected herds). Dtsch. tierärztl. Wschr. 1973, *80*, 59—62.

Young, G. A.: A preliminary report on the etiology of edema of newborn pigs. J. Amer. vet. med. Ass. 1952, *121*, 394—396.

Young, G. A., R. L. Kitchell, A. J. Luedke & J. H. Sautter: The effect of viral and other infections of the dam on fetal development in swine. I. Modified live hog cholera viruses — immunological, virological, and gross pathological studies. J. Amer. vet. med. Ass. 1955, *126*, 165—171.

Zuffa, A.: Acquirement of an avirulent swine fever virus suited for preparation of live vaccine in cell cultures. Report FAO/EEC consultation on eradication of classical and African swine fever in Europe, Hannover, September 1976.

SAMMENDRAG

Vaccination mod klassisk svinepest med en detergent-behandlet vaccine.

Fire grise blev injiceret subkutant med detergent (triton X 100)-behandlet svinepest-virus i Freunds inkomplette adjuvant, og 4 grise blev tilsvarende injiceret med detergent-behandlet bovint virusdiarrhoe-virus, også i Freunds inkomplette adjuvant. Der indgik 3 kontrolgrise i forsøget. Vaccinationerne blev gentaget efter 3 ugers forløb. Alle grise blev udsat for højvirulent svinepest-virus (Tübingen) 12 uger efter første vaccination. Kun kontrolgrisene udviklede symptomer på svinepest.

Dette introduktionsforsøg blev efterfulgt af et større forsøg med subkutan injektion af: 10 grise med et detergent-behandlet svinepest-virus i Freunds inkomplette adjuvant, 10 grise med et detergent-behandlet svinepest-virus i en saponin (Quil A)-opløsning, 10 grise med et detergent-behandlet bovint virusdiarrhoe-virus i olie-adjuvanten, 10 grise med et detergent-behandlet bovint virusdiarrhoe-virus i Quil A-opløsningen; der var 5 kontrolgrise. Vaccinationerne blev gentaget efter 3 ugers forløb, og alle grise blev udsat for højvirulent svinepest-virus 9 uger senere.

Med undtagelse af 1 dyr, som døde accidentelt, overlevede samtlige dyr i de grupper, der var injiceret med Quil A-vaccinerne, og i gruppen, der var injiceret med detergent-behandlet svinepest-virus/olie-adjuvant vaccine. I gruppen, der var injiceret med detergent-behandlet bovint virusdiarrhoe-virus/olie-adjuvant vaccine, var der dødsfald, som skyldtes svinepest.

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