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Brief Communication

DETECTION OF ANTIBODIES AGAINST SWINE FEVER VIRUS BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

The significance of a serological diagnosis in the eradication and control of subclinical swine fever (SF) is well recognized since it may be the only means of showing the presence of the infection. Accordingly, the capacity for large scale serological testing will be an important factor in the eradication of such infections.

Several methods are available for the detection of antibodies against swine fever virus but so far, only the neutralization test has been shown to differentiate unambiguously between antibodies against BVD (bovine virus diarrhea) and SF virus (*Liess et al.* 1977, *Holm Jensen* 1981).

Owing to the non-cytopathogenic character of SF virus the neutralization test is rather laborious and therefore less useful for large scale testing than enzyme-linked immunosorbent assays (ELISA). This report describes such an assay for antibodies against SF virus.

The Alfort strain of SF virus was used for production of antigen in PK 15 cells. Cells were scraped off with a rubber policeman and pelleted by centrifugation. The cell pellet was extracted in 2 volumes of 20 mmol/l Tris, pH 7.2, 1 % Triton X-100, 0.5 mmol/l EDTA by sonication (modified from *Dalsgaard & Overby* 1976). The extract was clarified at 5000 G for 20 min, precipitated by ammonium sulphate at 40 % saturation and resuspended in 20 mmol/l Tris, pH 7.2, 0.1 % Triton X-100, 0.5 mmol/l EDTA. This preparation was used as antigen in ELISA. The antigen was diluted 1/2000 in carbonate buffer, pH 9.6, and coated onto NUNC Immunoplate II overnight at room temperature. After washing of the plates in PBS-0.05 % Tween 20 (PBS-T) test sera were applied at a dilution of 1/2 in PBS-T (or serially diluted in PBS-T containing 10 % normal swine serum) and left in the plates overnight at 4°C. The ability of the test sera to inhibit the reaction between antigen and a rabbit antiserum to SF virus was assessed by reaction with rabbit antiserum and peroxidase-labelled anti-rabbit IgG (DAKO, P 217, Copenhagen), both diluted

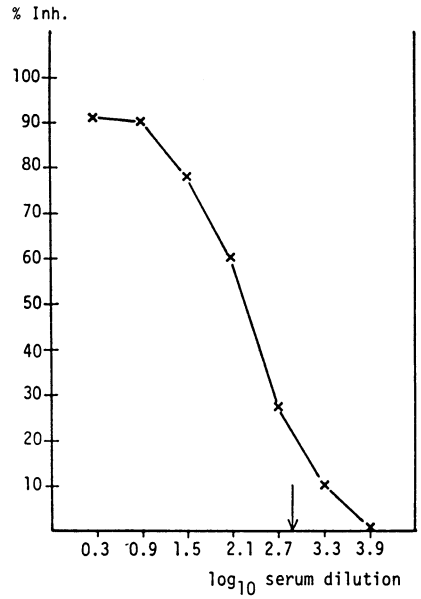

Figure 1. Titration in ELISA of a porcine reference serum produced by inoculation with the Alfort strain of SF virus. Arrow indicates neutralizing titre.

1/200 in PBS-T containing 10 % normal swine serum. The plates were incubated at 37°C for 30 min with each serum. The rabbit antiserum was prepared by 2 i.v. injections of lapinized virus (Vadimun®). The antibody-reactivity of test sera was calculated as percentage inhibition, in that percentage inhibition =

$$\frac{(\text{O.D. negative ref.} - \text{O.D. test serum}) \times 100}{\text{O.D. negative ref.} - \text{O.D. background}}$$

The optical density (OD) of background is the value obtained with a strong positive serum.

Fig. 1 shows the titration in ELISA of a reference antiserum prepared against the Alfort strain of SF virus*. This serum shows a plateau around 90 % inhibition and a sigmoid titration curve. The end-point in the neutralization test corresponds to approximately 20 % inhibition in ELISA. The results in Figs. 2 a-b were obtained with experimental SF and BVD porcine sera. The origin and the neutralizing titres of these sera have been described in detail by *Holm Jensen* (1981). Fig. 2a shows the

* Provided by professor B. Liess, Hannover.

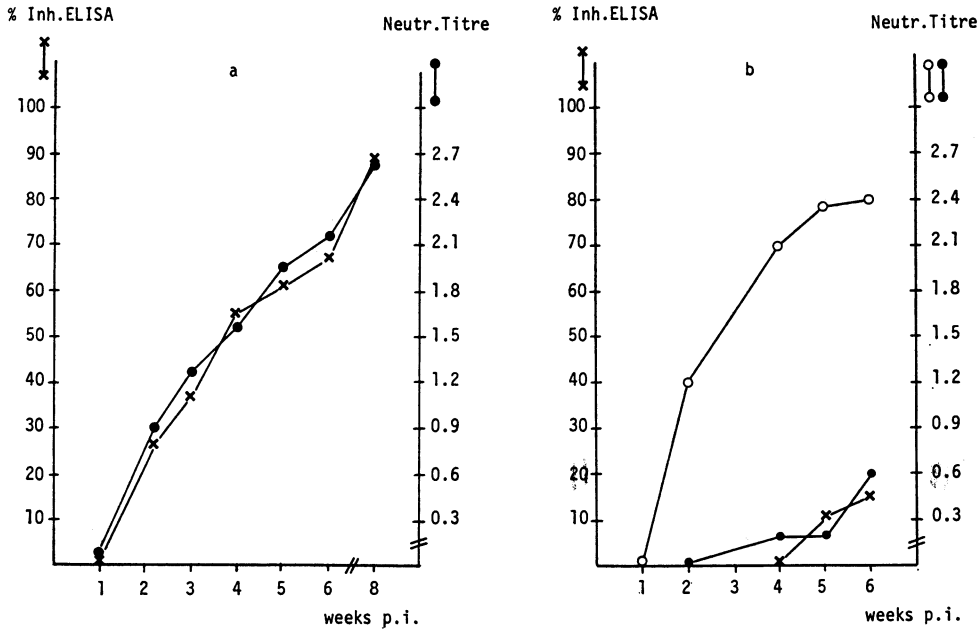

Figure 2 a-b. Development of antibodies in pigs after inoculation with the Hannover strain of SF virus (a) and BVD virus, strain UG 59, (b). Results shown are the average titres of 4 pigs.
●——● neutralizing titre against SF virus, ×——× per cent inhibition in ELISA at a dilution of 1:2, O——O neutralizing titre against BVD virus.

development of SF antibodies after inoculation of the Hannover strain of SF virus (average titres of 4 pigs). It appears that the ELISA closely parallels the neutralization test in detecting an early antibody response after infection. Similar results have been obtained after infection with other strains of SF virus (data not shown). Inoculation of pigs with BVD virus (Fig. 2b) leads to a neutralizing antibody response against the homologous virus comparable to that seen after inoculation of SF virus. Part of this response is a late and weak response against SF virus, manifested both in ELISA and neutralization test. The sensitivity of the ELISA will be dependent on the cut-off level of inhibition, that is, the level above which sera are considered to be positive for antibodies. The cut-off level should reflect the balance between the required sensitivity and the number of heterologous reactions that can be tolerated.

In a screening of 176 randomly selected porcine field sera for SF antibodies only 2 sera gave more than 20 % inhibition in the ELISA, namely 25 and 50 %, respectively. These sera showed high BVD virus neutralizing titres and low to moderate SF virus neutralizing titres. This indicates that BVD reactions may reach as high as 50 % inhibition in the ELISA. On the other hand, BVD reactions above 50 % inhibition have not been seen with these or other sera (not shown), indicating that, above this level, the ELISA may be expected to show absolute specificity for SF virus antibodies.

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