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SCREENING FOR NEUTRALIZING ANTIBODIES AGAINST HOG CHOLERA- AND/ OR BOVINE VIRAL DIARRHEA VIRUS IN DANISH PIGS

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JENSEN, MERETHE HOLM: *Screening for neutralizing antibodies against Hog Cholera- and/or Bovine Viral Diarrhea virus in Danish pigs.* Acta vet. scand. 1985, *26*, 72—80. — Three thousand Danish porcine field sera were tested for the possible presence of neutralizing antibodies for Hog Cholera (HC) and Bovine viral Diarrhea (BVD).

The technique used was a microplate neutralization test read by peroxidase-linked antibody assay (NPLA) in the HC examination, and by cytopathogenic effect in the BVD examination. The initial dilution of the test sera was 1:2.

BVD-antibodies were demonstrated in 6.4 % of the sera, while all the sera were found to be free from antibodies to HC-virus.

HC/BVD antibodies; field sera; practicability; NPLA.

From Hog Cholera (HC)-free countries like Australia (*Snowdon & French* 1976), Ireland (*Lenihan & Collery* 1977) and Great Britain (*Roeder & Harkness* 1984) prevalences of Bovine Viral Diarrhea (BVD)-antibodies within the pig population have been described as varying from 1.6 to 43.5 % depending on the age of the animals and possibly to some extent on the degree of contact to cattle. In countries where HC is present the situation with regard to BVD-antibodies seems to be about the same (*Liess et al.* 1976, *Terpstra et al.* 1984).

Denmark has been free from HC for more than 50 years but does have BVD in the cattle population (*Meyling* 1984), and the occurrence of BVD-antibodies in porcine sera has been described (*Eskildsen* 1977). In a survey performed in 1978 using complement fixation (*Eskildsen* 1979) 3268 serum samples originating from 190 breeding centers were found negative as regards HC-

antibodies. BVD-antibodies were revealed in 4 of these herds. The aim of this survey was to determine the possible occurrence at present of neutralizing antibodies against HC- and/or BVD-virus in Danish pigs.

MATERIAL AND METHODS

Field sera

During the spring 1984 within a 2½ month period 3000 blood samples were selected from a total of 72,000 samples submitted from all parts of the country to this institute in connection with an eradication programme for Aujeszky's disease.

The blood samples were stored for a few days at 6—8°C while the Aujeszky-investigation was performed. Then the serum was removed by decanting, heat-inactivated at 56°C for 30 min and kept frozen at —20°C. The quality of the samples varied a great deal with respect to hemolysis and bacterial contamination.

Reference sera

HC-reference serum was collected from a pig 5 months after simultaneous intravenous and intramuscular inoculation of the Hannover strain of HC-virus.

The BVD-reference serum originated from a pig inoculated intravenously with BVD-virus, strain Ug59, and reinoculated 3 months later. The serum was collected 1 month after the re-inoculation.

Virus

The Japanese Ald strain of HC-virus and the Danish Ug59 strain of BVD-virus were used in the neutralization tests.

The mean infectivity titres of the HC-virus and the BVD-virus were 6.31 ± 0.32 $\log_{10}$ $TCID_{50}$ per 0.05 ml and 6.20 ± 0.37 $\log_{10}$ $TCID_{50}$ per 0.05 ml, respectively.

Cell cultures

The cell cultures and cell culture procedure used were described earlier (*Jensen* 1981), with the modification that fetal calf serum was replaced by a mixture of calf and adult cattle serum from a small, isolated stock of animals, free of BVD-virus and antibodies to BVD-virus. Furthermore, the PK 15 cells used in this investigation were the PK 15 A cells provided by the EEC reference laboratory for HC February 1984 (Professor B. Liess).

Neutralization test

The neutralization tests were performed in microplates as described earlier (*Jensen* 1981), except that 5 % calf/cattle serum was used instead of 2 % fetal calf serum.

For the initial screening a "mini-chessboard" was used, i.e., two serum dilutions, 1:2 and 1:4, against two dilutions, 10^{-4} and 10^{-5}, of each of the two viruses.

Plates with HC-virus were read by the peroxidase-linked assay as described earlier (*Jensen* 1981), with the washing procedure slightly modified to: physiological saline 2 min on the shaker and wash fluid 2×3 min on the shaker. The conjugate used was peroxidase-conjugated rabbit-anti-swine-immunoglobulins P 164 from DAKOPATTS.

Plates with BVD-virus were read mainly on the basis of the cytopathogenic effect of this virus. Only in case of doubt or difficulties by this reading were fixation and staining used.

All wells where virus infected cells were detected, were regarded as positive for virus multiplication. If any neutralization of the virus was observed the neutralization assay was repeated using serum dilutions from 1:2 to 1:16, or further, against virus dilutions 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}.

The titres are given as $\log_{10}$ to the reciprocal value of the highest serum dilution neutralizing 100 $TCID_{50}$ per 0.05 ml.

Titration of virus for infectivity and of the reference serum for neutralization was included in all trials. The mean neutralizing titres of the HC-reference serum and the BVD-reference serum were 2.93 ± 0.15 and 3.76 ± 0.24, respectively.

RESULTS

Table 1 shows from which initial dilution of the test serum results could be obtained in neutralization tests against HC-virus. Because of toxicity or inadequate amounts of serum, 30 of the samples were tested from dilution 1:4 only, and 7 from 1:8 only.

Table 2 shows from which initial dilution of the test serum results could be obtained in neutralization tests against BVD-virus. In 4 of the 3000 samples the amount of serum was sufficient only for the HC-test. With 47 of the test samples there were problems concerning toxicity or inadequate amounts of serum; 30 were used from dilution 1:4, 15 from 1:8, and 2 from 1:16 only.

Table 1. The initial test serum dilution with which results were obtained in neutralization tests against HC-virus.

Initial dilution of the test serum	Number of sera
1:2	2963
1:4	30
1:8	7
Total number	3000

Table 2. The initial test serum dilution with which results were obtained in neutralization tests against BVD-virus.

Initial dilution of the test serum	Number of sera
1:2	2949
1:4	30
1:8	15
1:16	2
Total number	* 2996

* In 4 of the 3000 samples the amount of serum was sufficient only for the HC-test.

Table 3 shows the toxicity of the test samples to the cell systems. For 15 sera the toxic effect was the same to both cell systems; 7 sera were toxic to the porcine kidney/PK15 cells but not to calf kidney cells, while 12 sera showed toxicity to calf kidney cells but not to porcine kidney/PK15 cells. Three sera were more toxic to the porcine kidney/PK15 cells than to the calf kidney cells, and for 11 sera the situation was the opposite.

Table 3. Toxicity of test samples to the cell cultures used.

Toxicity to calf kidney cells in dilution of serum	Toxicity to porcine kidney cells (in dilution of serum)		
	1:2 non toxic	1:2 toxic	1:4 toxic
1:2 non toxic	2948	5	2
1:2 toxic	10	14	3
1:4 toxic	2	9	1
1:8 toxic	0	1	1

In total, 35 (1.2 %) of the sera were found to show HC-titres of $\geq$ 0.6. Of these, 17 had titres of $\geq$0.9 and only 9 had titres of 1.2—1.6.

A total of 192, i.e., 6.4 % of the sera, gave BVD titres of $\geq$ 0.6, 169 of these were $\geq$ 0.9, 149 were $\geq$ 1.2 and 40 from 2.1—3.0.

The neutralization results are summarized in Fig. 1, which illustrates the correlation between neutralization of HC- and BVD-virus by sera showing neuralizing titres of $\geq$ 0.3 to at least one of the viruses.

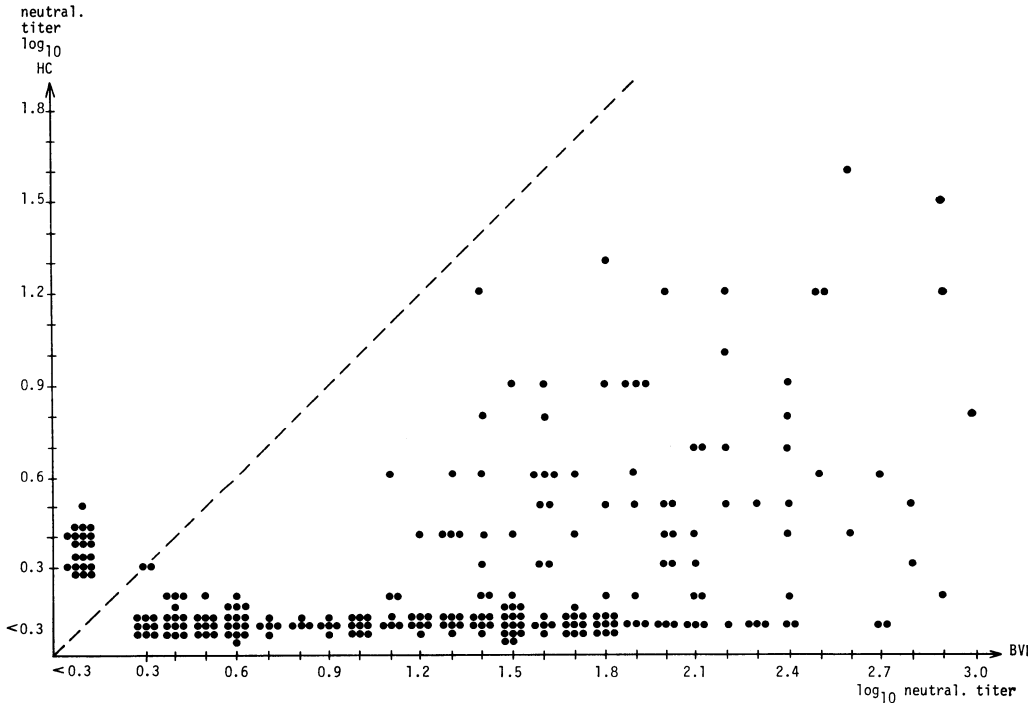

Figure 1. Correlation between neutralization of HC and BVD virus by field sera showing neutralizing titres $\geq$ 0.3 for at least one of the viruses.

All sera with HC-titres $\geq$ 0.6 were sera with higher titres against BVD-virus than against HC-virus. The antibodies were therefore considered not to be caused by a HC-virus (*Liess et al.* 1977, *Jensen* 1981). Even HC-reactions at the level of 0.5 seem to be caused by BVD-antibodies (the serum with titre 0.5 for HC-virus and no neutralization of BVD-virus was heavily contamintated and the reaction taken to be non-specific). Of the HC-reac-

tions at the very low titres of 0.4 and 0.3, which will normally be regarded as non-specific, 12 of 22, i.e., 55 %, and 7 of 19, i.e., 37 %, respectively, were connected with the presence of BVD-antibodies. Totally, 23, i.e., 0.8 % of the sera, gave a low level of non-specific neutralization of HC-virus.

The level of non-specific reactions to BVD-virus cannot be calculated from this material; some of the low-level reactions of 0.3—0.5 may be specific, but even if all reactions < 0.6 are taken as non-specific, they will not exceed 1.1 % of the sera.

There is no consistent correlation between titres against HC- and BVD-virus, the difference between the two ranging from 0.2—2.8 (i.e., 1.5—630 times higher BVD-than HC-titre). But the proportion of BVD-antibody positive sera giving HC-reaction seems to increase with higher BVD-titres. No HC-reaction was found at BVD-titres ≤ 1.0. Of sera with BVD-titres of 1.1—2.0, 34 % gave HC-reaction, while 63 % of sera with BVD-titres of 2.1—3.0 gave HC-reaction.

DISCUSSION AND CONCLUSIONS

As stated recently by *Terpstra et al.* (1984) the neutralization assay as such is still considered to be the most sensitive and reliable serological method for detection of and differentiation between HC- and BVD-antibodies in porcine sera. The practicability in large-scale survey of the neutralization peroxidase-linked antibody (NPLA) assay using 1:25 as the initial dilution of the test serum has been documented by *Terpstra et al.* (1984). In the present material the presence of BVD-virus-neutralizing antibodies (titres ≥ 0.6) were demonstrated in 6.4 % of test sera, while no antibodies caused by HC-virus were found. The BVD-antibody-containing sera were mainly from small and medium-sized herds, but large herds were represented, too. Part of the BVD-antibody-positive sera showed neutralization of HC-virus as well. The occurrence in a HC-free country of a few sera showing higher reaction to HC-virus than to BVD-virus has been described from Australia (*Snowdon & French* 1976) and Great Britain (*Roeder & Harkness* 1984). In the present investigation there was one serum sample showing BVD- and HC-reaction at about the same level. In a survey utilizing 1 strain of HC-virus and 1 strain of BVD-virus only, the possible lack of strain homology with the antibodies present may influence the titres obtained and thereby the differences between reactions to HC- and BVD-virus.

The lower BVD-titres (up to 2.0) found in this survey correspond to results from similar field surveys in Australia (*Snowdon & French* 1976) and Ireland (*Lenihan & Collery* 1977). As also observed by *Eskildsen* (1977, 1978) close contact with cattle with BVD may give rise to BVD-antibodies in pigs, but contact with cattle will not always explain the presence of BVD-antibodies in pigs. The high level of BVD-antibodies found in some of the sera in the present investigation is similar to findings in experimental BVD-virus infection (*Liess et al.* 1977).

Weak non-specific neutralization of HC-virus and BVD-virus (titres < 0.6) was seen in, respectively, 0.8 % and 1.1 % of the test samples.

Toxicity of test sera for the cell cultures used was seen, 1.2 % being toxic to porcine kidney/PK15 cells and 1.4 % to calf kidney cells.

It looks as if the HC-virus/porcine kidney/PK15 cell system and the BVD-virus/calf kidney cell system are sensitive to different interfering and toxic factors. In total, the use of the dilution 1:2 as initial dilution of test sera caused trouble in 1.8 % of tests against HC-virus and in 2.5 % of tests against BVD-virus. When 1:4 was used as initial dilution only 7 sera showed toxicity for porcine kidney/PK15 cells, while 14 were toxic to calf kidney cells.

In a survey with several samples from each herd it will, from a practical point of view, be sufficient to use 1:4 as the initial dilution of serum. It is true that this will lead to some low but specific reactions (titres < 0.6) not being recognized, but unless an infection is quite recently introduced in a herd, samples giving a more pronounced reaction are to be expected.

In connection with experimental work and/or control measures, where bacterial contamination and toxicity of the test sera usually present no problem, the 1:2 dilution can be used without difficulties (own observations).

The practical performance of screening for HC-virus neutralizing antibodies is very much depending on the purpose of such a screening, which may be different for HC-free countries and countries in which HC is present.

Liess & Prager (1976) prefer to use 1:5 as the intial dilution in neutralization tests for HC-virus and perform the necessary differentiation afterwards. If the purpose is detection of HC-infected herds in a country with HC present, a screening system

as described by *Terpstra et al.* (1984) may be utilized. When 1:25 is used as the initial dilution of test sera, HC-reacting BVD-antibodies will in most cases cause no trouble at all, and the need for differentiation between HC- and BVD-antibodies will be negligible. There will of course be a certain risk of false negatives, since lower HC-titres will not be detected.

If the purpose of the survey is to document the absence of HC from a country, the use of 1:4 or even 1:2 as initial dilution of serum will ensure a high sensitivity of the screening. Using this procedure the present work has documented the absence from Danish pigs of antibodies caused by HC-virus.

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REFERENCES

Eskildsen, M.: Use of modified direct complement fixation test in the demonstration of antibodies against classical swine fever and bovine viral diarrhoea virus in swine serum. CEC seminar on Hog Cholera/Classical Swine Fever and African Swine Fever. EUR 5904. 1977, p. 333—337.

Eskildsen, M.: Personal communication, 1979.

Jensen, M. H.: Detection of antibodies against hog cholera virus and bovine diarrhea virus in porcine serum. A comparative examination using CF, PLA and NPLA assays. Acta vet. scand. 1981, *22*, 85—98.

Lenihan, P. & P. Collery: Bovine viral diarrhoea infection in pigs in Ireland: A serological survey and an epidemiological study. CEC seminar on Hog Cholera/Classical Swine Fever and African Swine Fever EUR 5904. 1977, p. 314—332.

Liess, B., H. R. Frey, D. Prager, S. M. Hafez & B. Roeder: The course of the natural swine fever virus infection in individual swine and investigations on the development of inapparent SF infections. CEC Coordination of agricultural research. Diagnosis and Epizootiology of Classical Swine Fever. EUR 5486. 1976, p. 99—113.

Liess, B. & D. Prager: Detection of neutralizing antibodies (NIF test). Use of new technical equipment (CCSC system) for laboratory swine fever diagnosis. CEC Coordination of agricultural research. Diagnosis and Epizootiology of Classical Swine Fever. EUR 5486. 1976, p. 187—197.

Liess, B., H. R. Frey & D. Prager: Antibody response of pigs following experimental infections with strains of hog cholera, bovine viral diarrhoea virus. CEC seminar on Hog Cholera/Classical Swine Fever and African Swine Fever. EUR 5904. 1977, p. 200—213.

Meyling, A.: Detection of BVD virus in viremic cattle by an indirect immunoperoxidase technique. CEC programme of Co-ordination of Research on Animal Pathology. Recent Advances in Virus Diagnosis. 1984, p. 37—46.

Roeder, P. L. & J. W. Harkness: Personal communication, 1984.

Snowdon, W. A. & E. L. French: The role of mucosal disease virus in the epizootiology and control of swine fever. CEC coordination of agricultural research. Diagnosis and Epizootiology of Classical Swine Fever. EUR 5486. 1976, p. 159—183.

Terpstra, C., M. Bloemraad & A. L. J. Gielkens: The neutralizing peroxidase-linked assay for detection of antibody against swine fever virus. Vet. Microbiol. 1984, *9*, 113—120.

SAMMENDRAG

Undersøgelse af danske svinesera for neutraliserende antistoffer mod svinepestvirus og bovin virus diarrhe virus.

3000 danske svinesera er undersøgt for muligt indhold af neutraliserende antistoffer overfor svinepest (SP)-virus og bovin virus diarrhe (BVD)-virus. BVD-antistoffer blev påvist i 6,4 % af materialet, medens antistoffer forårsaget af SP-virus ikke blev påvist.

Neutralisationstitreringerne blev udført i mikroplader og for SP-undersøgelsens vedkommende aflæst under anvendelse af „peroxidase-linked antibody (NPLA) assay". BVD-undersøgelsen blev aflæst ved hjælp af den cytopatogene effekt af det anvendte BVD-virus.

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