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DETECTION OF THE CLASSICAL SWINE FEVER VIRUS ANTIGEN FOLLOWING EXPERIMENTAL INFECTION

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In this experiment, twelve pigs were infected by intramuscular inoculation of virulent virus strains of classical swine fever (Baker, Kansas, BAI, autochthonic isolate). Three animals survived infection without any clinical symptoms and were sacrificed 60 days post infection (p.i.). Nine animals developed an acute form of classical swine fever and died between 8 and 15 days p.i. Rectal temperature was monitored daily along with the clinical symptoms and usual hematological parameters. Viremia was determined by two immunoenzyme-ELISA tests (CHEKIT CSF VIRUS DR BOMMELI AG, Switzerland, and CSFV-IDEXX, USA). Pathomorphological changes were determined post mortem and tissues were examined for the presence of virus antigen by a fluorescent antibody - based test and immunoenzyme test.

In infected animals viremia occured between 3 and 5 days post infection. Virus antigens were detected in 73.03% of the examined blood samples using the Dr Bommeli test and in 56.18% using the IDEXX test. Viremia was not detected in animals that survived infection. Between day 3 and 5 p. i. leukopenia, lymphopenia and thrombocytopenia were observed in eight infected animals. Viral antigens were confirmed by the fluorescent antibody based test in 72.2 % of the examined tissues, by the IDEXX test in 84.4% and by the Dr Bommeli test in 100% samples.

Key words: classical swine fever, direct immunofluorescence test, ELISA, viremia.

INTRODUCTION

Fast and reliable diagnosis of classical swine fever is of great epizootiological and economic importance. Due to the variability of clinical symptoms of the disease and complex pathomorphological changes, as well as the frequent occurrence of atypical forms of the disease, definitive diagnosis of classical swine fever should be made only after laboratory examination (Manual of Standards for Diagnostic Test, 1996). Intra vitam diagnostics is based on the demonstration of virus presence in the peripheral blood leukocytes (Depner *et al.*

1996). Virus isolation and identification in the cell culture systems is highly sensitive and specific, but this procedure is laborious and time-consuming and therefore inadequate for fast diagnostics (Pearson, 992). Utilization of immunoenzymatic tests (ELISA), designed to detect the classical swine fever virus (CSFV), enables examination of a large number of samples, detection of animals in the incubation phase, detection of infected animals with atypical forms of the disease and detection of animals with persistent infection.

The aim of this study was to investigate the possibilities for early detection of viral antigen in blood using commercial ELISA sets and to compare them with a fluorescent antibody test for demonstrating the presence of virus in the tissues post mortem under experimental conditions.

MATERIAL AND METHODS

Experimental animals

The experiment was carried out on 12 pigs of the domestic white breed, 3-4 months old, weighing about 40 kg each. The animals were ear marked (numbers from 1-12) and placed in two separate rooms of the experimental block. Following infection with virulent strains of CSF virus, clinical symptoms and rectal temperature were checked and blood samples taken daily for haematological and virological examination.

Viruses

Infection was performed by intramuscular inoculation (2 ml) of virulent strains of CSF virus according to the following scheme:

Swine No. 1 and 2 - Baker's (USA) strain;

Swine No. 3 and No. 4 - Kansas' strain;

Swine No. 5 and No. 6 - BAI strain;

Swine No. 7 and No. 8 - CSF virus autochthonic isolate identified at the National Veterinary Research Institute, Pulawy - Poland;

Swine No.9 and No. 10 - passage BAI strain from 1996;

Swine No. 11 and No. 12 - passage BAI strain from 1997. (Passage strain BAI from 1996 and 1997: defibrinised blood of swine that were used in the production control of vaccine against swine fever at the Veterinary Institute, "Subotica".)

Antibody detection

Sera samples from all experimental animals were checked before infection for the presence of specific antibodies against classical swine fever virus by ELISA (Dr Bommeli AG CHEKIT CSF-SERO) according to the manufacturer's protocol. The presence of antibodies was monitored up to 20 days post infection.

Detection of viral antigens in blood

From the day of infection until death, 10 ml blood samples were taken daily in sterile tubes with EDTA. Blood was sampled until the end from the three animals that survived, while other pigs in the experimental group died. All samples were examined for the presence of virus by ELISA (Dr Bommeli, blood plasma) and IDEXX test (whole blood). A total of 126 samples were examined.

Hematological parameters

From day 3 after infection, when the majority of the experimental animals were febrile, 3 ml of blood was sampled. The samples were examined on a

semiautomatic counter for blood elements (AVL, type AL 816) by the method of impedance. A total of 96 samples were examined.

Detection of virus in tissues

Material for investigation was taken immediately after death or after sacrificing the animals. From every animal the following organs were examined: tonsils, mandibular and mesenterial lymph nodes, kidney, parts of lungs, spleen, liver and ileum. The presence of antigen CSF virus was sought in 54 samples by TFA (direct immunofluorescence) with polyclonal antisera using conjugated FITC (Mevak, Nitra). A total of 45 tissue samples were examined by ELISA (CHEKIT SCF VIRUS-II, DR BOMEMELI AG) and by a classical Swine Fever Virus Antigen Test (IDEXX).

RESULTS AND DISCUSSION

Detection of antibodies

Before infection, positive results were obtained for specific antibodies for gp 55 CSF virus with the Dr Bommeli test, in three animals (No. 5, No. 7 and No. 11). These findings indicate that there was previous contact with CSF virus of moderate virulence or pestivirus of ruminants (BVDV). Two animals (No. 7 and No. 11) survived the infection without any clinical symptoms, but one (No. 5) became ill and died. One more animal survived the infection (No. 2) and it was serologically negative. This indicates the relative importance of proving seroconversion in estimating protection from the disease, because the final result is influenced by numerous factors that are connected to features of the virus and of the host (Depner *et al.,* 1996; Leforban *et al.,* 1992; Liess, 1994). In animal No. 2 seroconversion was detected on day 11 post infection, which coincides with the findings of other authors (Muller *et al.,* 1996; Pejsak, 1993; Wensvoort *et al.,* 1988). They consider that minimal concentrations of antibodies occur in sera already on day 7 after infection, but in most cases it is possible to detect them only in the second week. The results of serological investigation by ELISA closely coincide with those from the neutralization test for early detection of antibodies after infection (Have, 1984).

Clinical signs of the disease and necropsy findings

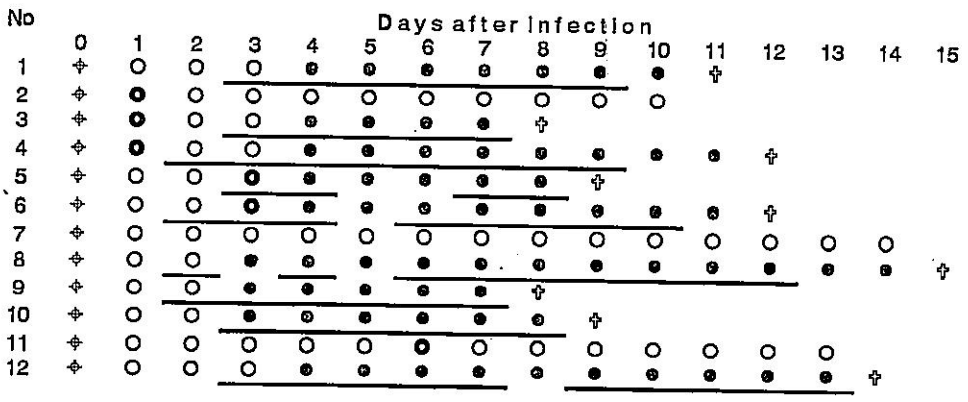
In all sick animals the disease had an acute course. Body temperature greater than 40 ^{o}C was recorded between day 3 and 5 post infection. All the animals had seral or purulent conjunctivitis (3 to 5 days), clinical symptoms in the respiratory system (5 to 11 days) and neurological symptoms (5 to 7 days). Other clinical symptoms were usual for the acute course, such as intermittent diarrhea and hyperemia and haemorrhagic skin changes. This was noticed in seven pigs (7 to 10 days). There were no differences in clinical manifestations of the disease depending on the viral strain used. At necropsy, bleeding was present in most or all systemic organs and also on serous membranes (haemorrhagic diathesis), inflammation was observed in the digestive and respiratory systems (haemorrhage-dphtheroid gastroenteritis and lobular bronchopneumonia). Bleeding in the epiglottis and kidney parenchyma was found in seven animals, in the mucous membrane of gallbladder in five animals andin the urinary bladder serous layer in three pigs. Haemorrhagic impacts on the margins of the spleen, pathognomic for an acute course of classical swine fever, were found in five animals.

Detection of viral antigen in blood

According to data in the literature, it is possible to confirm the presence of CSF virus in blood between days 3 and 7 post infection (following intranasal inoculation of virus) or from day 1 to 3 after body temperature increases (Dahle *et al.*, 1993; Liess et al, 1995; Laevens et al., 1998; Lipowski *et al.*, 1996; Shannon *et al.*, 1993). Viremia was discovered between the first and third day from the beginning of the febrile condition in all sick animals using both ELISA tests,. It was possible to monitor high body temperature, inappetance, apathy and serosal conjunctivitis. These findings confirm that viremia can be detected even before clear symptoms of classical swine fever appear (Liess *et al*, 1994). Virus was detected in all the blood samples until the death of the animals using both tests.

Table 1. a and 1. b show the time when CSFV was detected in blood and the days when the body temperature was higher than 40°C. Cumulative data from the comparative investigation of viremia are displayed in Table 2.

Table 1a: Detection of CSF virus in the blood plasma of experimentally infected pigs by Dr Bommeli ELISA

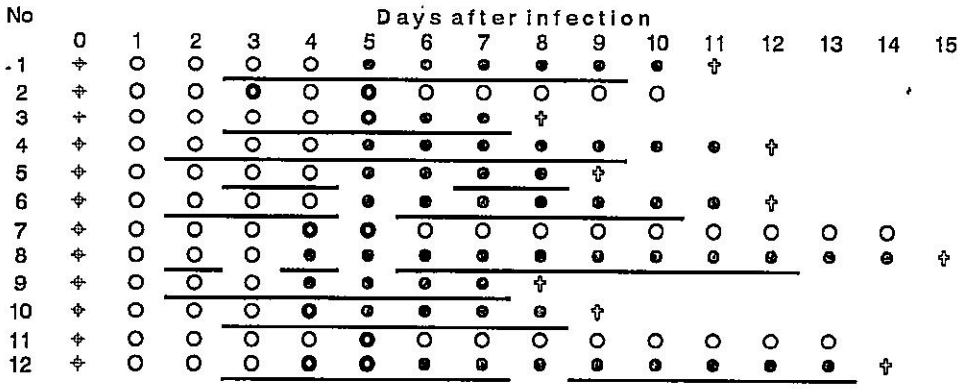


⌖ - CSF virus inoculation
○ - negative ELISA result
◐ - doubtful ELISA result
● - positive ELISA result
___ fever (>40,0°C)
✞ - death

Viremia index was determined one to two days later using the IDEXX test and compared to results with the Dr Bommeli test. The difference in results obtained for 15 samples (17%) (65 positive samples using Dr Bommeli, 50 positive with IDEXX) indicates the higher sensitivity of the Dr Bommeli test (Depner. *et al.*, 1996).

Viremia was not detected in animals that survived Some samples were examined by both ELISA tests, without any suspicion of its presence. Data in the literature show that virus may be transiently detected in the blood of all pigs that

Table 1b Detection of CSF virus in the blood plasma of experimentally infected pigs by IDEXX ELISA



⊕ - CSF virus inoculation
O - negative ELISA result
O - doubtful ELISA result
● - positive ELISA result
___ fever (>40,0°C)
✝ - death

Table 2 : Detection of CSF virus in blood from sick pigs by Dr Bommeli and IDEXX ELISA

Total n = 89	BOMMELI			IDEXX		
	+	±	–	+	±	–
n	65	4	20	50	6	33
%	73,03	4,49	22,47	56,18	6,74	37,08

+ positive, ± - doubtful, - negative

survive infection without clinical symptoms on different days after infection. This has mainly been demonstrated for series of blood samples from the same animal (Dahle *et al.*, 1993; Depner *et al.*, 2000). Our results are presented in Table 3.

Haematological parameters

According to some authors (Susa *et al.*, 1992; Summerfield *et al.*, 1998; Hoffmann *et al.*, 1971) the number of leukocytes, lymphocytes and trombocytes was extremely reduced in pigs infected by highly virulent CSF virus. This effect was noticed in eight sick animals between days 3 and 5 post infection. In 61 blood samples (85.92%) of leukocyte count were below $10x10^9$/l (leukopenia). Lower values were detected immediately before death (0.8-3.4 $x10^9$/l). Lymphopenia

Table 3. Detection of CSF virus in blood samples of surviving pigs by Dr Bommeli and IDEXX ELISA

Total n = 37	BOMMELI			IDEXX		
	+	±	–	+	±	–
n	0	3	34	0	5	32
%	0	8.11	91.89	0	13.52	86.48

+ positive, ± - doubtful, - negative

was detected in 63 samples (88.73%). Values for thrombocyte counts lower than $200x10^9$/l were detected in 40 blood samples (56.33%) between days 4 and 9 after inspection. There were no important changes in number of erythrocytes and concentration of haemoglobin in ill animals.

Changes in blood cell count, characteristic for infection with CSF virus were not observed in animals that survived

Detection of classical swine fever virus antigen in tissues

The results obtained by the different methods for CSF virus in various tissues, are presented in Table 4.

Table 4. Detection of CSF virus in tissues of dead pigs by ELISA and direct immunofluoresence

Tissue	DR BOMMELI			IDEXX			Immunoflorescence	
	+	±	–	+	±	–	+	–
Tonsils	**9**	0	0	**9**	0	0	**6**	3
Lungs	**9**	0	0	**5**	3	1	nt	nt
Mesenterial ln.	**9**	0	0	**7**	2	0	**7**	2
Spleen	**9**	0	0	**8**	1	0	**5**	4
Ileum	**9**	0	0	**9**	0	0	nt	nt
Mandibular ln.	nt	nt	nt	nt	nt	nt	**3**	6
Kidney	nt	nt	nt	nt	nt	nt	**9**	0
Liver	nt	nt	nt	nt	nt	nt	**9**	0
Total	45	0	0	38	6	1	39	15

n.t. = not tested + positive, ± - doubtful, - negative

The ELISA test showed higher sensitivity for detecting virus antigen in different organs using compared to the test of fluorescent antibodies (TFA). The results show that it is necessary to confirm a negative finding with TFA by isolation of virus in cell culture (Terpstra, 1991). Virus was detected by direct immunofluorescence in examining all the samples of liver and kidney tissue and, compared to ELISA in a smaller number of examined tonsils, spleen and lymph nodes.

The virus was not detected in clinically healthy animals (No. 2, No. 7 and No. 11) that were sacrificed on 60 day post infection by either or TFA. Experimentally it was shown that the test was not suitable for detecting virus in the tissues of animals that survived infection without any symptoms for 30 days after infection (Dahle *et al.*, 1993; Dahle and Liess, 1995; Lipowsky, 1996).

It can be concluded that ELISA tests are promising and sensitive tools in the rapid detection of infection in live pigs. The sensitivity of the ELISA kits tested was about 80% compared to virus isolation (Table 5). However, this method requires less specialized facilities and can be performed more rapidly. Compared to other procedures for detection of specific antibodies for the virus, this method allows simultaneous testing of a large number of samples, which is of great importance in practice and gives an insight about the spread of the disease.

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DOKAZIVANJE PRISUSTVA ANTIGENA VIRUSA KLASIČNE KUGE SVINJA POSLE EKSPERIMENTALNE INFEKCIJE

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SADRŽAJ

U ovom radu su prikazani rezultati dobijeni ispitivanjem krvi svinja veštački inficiranih virusom klasične svinjske kuge primenom dva različita ELISA testa a u tkivima i testa direktne imunofluorescence. Eksperimentalne životinje su bile inficirane intramuskularnom inokulacijom virulentnih sojeva CSF (Classical swine fever virusa, Baker, Kansas, BAI, autohtoni izolat) . Viremija je kod obolelih jedinki prvi put utvrđena između trećeg i petog dana nakon infekcije. Virusni antigeni su potvrđeni u 73,03% ispitanih uzoraka krvi upotrebom testa dr Bommeli, a u 56,18% IDEXX-testom. Viremija nije ustanovljena kod preživelih jedinki. Leukopenija, limfopenija i trombocitopenija potvrđene su kod osam obolelih životinja između trećeg i petog dana nakon infekcije. Virusni antigeni dokazani su testom fluorescentnih antitela u 72,22% pregledanih tkiva, testom IDEXX u 84,44% i testom dr Bommeli u 100% uzoraka.