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STUDY OF ANTIBODIES SERO-PREVALENCE OF CLASSICAL SWINE FEVER VIRUS IN PIGS IN ALBANIA

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ABSTRACT

Classical swine fever (CSF) is one of the diseases that have caused major economic damages during the last decades. Although considerable progress has been made in the eradication and prevention of the disease, the threat for an epidemic still exists. Eradication measures in Albania are based on stamping-out in case disease is suspected and confirmed on pig holdings. Vaccination with "conventional" live attenuated vaccine is used as an additional tool to eradicate the disease. The aim of this study was to evaluate sero-prevalence against CSF virus in domestic pigs. To realize it 1000 blood samples were collected, from 16 regions. These sapmles were tested by ELISA Ab (Prio - Check CSFV 2.0) for detection of antibodies against CSF virus E2 glycoprotein in serum. Control aimed rural areas, without excluding concentrated swine growth complexes. Testing showed presence of specific antibodies against CSFV in 176 (17,6%) of serum samples.

To evaluate this positivity is necessary a thorough analysis, taking in consideration the vaccination used in different parts of our country, as a measure to control the disease. Detection of antibodies does not necessarily mean that the animal is infected. Virus Neutralization Test (VNT) for CSFV antibodies is considered Gold Standard, and is usually carried out in parallel with other pest viruses. To achieve this, 12 samples whith highest levels of antibodies against CSFV were sent for confirmation to EU Reference Laboratory for CSF, Hannover, Germany.

KEYWORDS: CSF, CSFV, ELISA Ab, VNT

INTRODUCTION

Classical swine fever is a highly contagious viral disease of worldwide importance. The World Organisation for Animal Health (OIE) lists CSF as a notifiable disease. It is a serious and highly infectious viral disease of domestic pigs and wild boar (Paton et al. 2003). Classical swine fever virus (CSFV) is an enveloped RNA virus belonging to the family of *Flaviviridae*, genus *pestivirus*, together with Bovine Viral Diarrhea Virus (BVDV) and Border Disease Virus (BDV). Its natural hosts are members of the *Suidae*, *i.e.* domestic swine and wild boar (Prodanov et al 2009). Worldwide, CSFV is considered to be one of the most important swine pathogens, which is systematically controlled in a majority of countries (Edwards et al 2000). The disease is important because the virus can become widespread and cause a high mortality, especially in young pig populations.

Until today, CSF remains a problem in many parts of the world where it has both, an economic impact on swine production and a constraining effect on trade. Since 1992, the control of CSF within the European Union (EU) is based on a policy of non-vaccination and eradication of the virus. There is only one serotype of CSF virus (CSFV).

Small virus, enveloped with pozitive-sens ARN, single strained, limited by 2 untranslated regions 3 'and 5' (Hess et al 1988). 12.5 - 16.5 kb genome, encodes only one polyprotein (Bouma et al.2001), from which after translation, as a result of a combination of virus and host cell enzymes, will form 12 mature protein (Meyers et al 1995). Virion is composed of four structural proteins (C, Ernst, E1 and E2), which are coded in the region 5' of the genome. So far remain still unknown the epidemiological situation regarding this disease. An important role in concealing the real situation plays partial vaccination, performed in different areas of the country. This vaccine not only creates a problem of diagnosis in areas where performed, also with uncontrolled movement of pigs it creates premium in other areas of the country. Likewise, little is known about the presence of this infection in wild boars. All these make it even more necessary to undertake this study, and we hope that through it, shed light on this infection in Albania.

MATERIAL AND METHODS

Antibody Detection with ELISA Ab Test

During Fall-Winter 2013 were analised a total of 1000 blood samples, from 16 regions. Samples were collected in sloughterhouses, from domestic pigs of age 3-6 months. Target in this study were unvaccinated pigs. Blood samples were collected in vacutainer tubes, without anticoagulants. Serum was preleved and stored at -300 until testing. All samples were tested by ELISA Ab (Prio - Check CSFV 2.0) for detection of antibodies against the E2 glycoprotein of CSF virus in serum. The key reagent is a mAb directed against an epitope located on the A domain of the envelope protein E2 of CSFV, making the test more specific for CSFV. The mAb is labeled with an enzyme that generates a color signal. This signal is measured and when no color is formed then the sample, containing anti-CSFV antibodies that competed for the A domain of the E2 protein, is positive for CSFV. ELISA plates are coated with E2 protein. At each well of ELISA plate were added 50 µl of tested sera together with positive and negative standard sera, and incubated for 1 hour in 37°C. During this time anti-CSFV antibody present in the sample relates with CSFV E2 antigen that coats the ELISA plate. After thorough washing, anti CSFV E2 (A domain) HRPO conjugate was added, and again put in incubation for 30 minutes at 37°C. After another set of washing, was added chromogene substrate (TMB) and incubated for 15 minutes. At the end reaction was blocked by adding stop solution. Calculation of optical density is measured using given formula, when positivity is expressed in percentage correlated with corrected reference values.

Virus Neutralization Assay Nd50

Serum samples that expressed highest levels of antiboidies from ELISA Ab, were then tested with VNT for differenciation of antibodies. Neutralisation test is performed in cell cultures using a constant virus/varying-serum method. As CSFV is noncytopathic, any non-neutralised virus must be detected, after multiplication, by an indicator system (OIE Manual 2008). Seras are first inactivated for 30 minutes in 56o C. Initial serum dilution is 1/5 (final dilution 1/10). Test is carried out in a flat-bottomed microtitre plate. Dilute 50 µl from each serums in 50µl growth medium (Eagle MEM plus 5% Fetal serum) into dublicate wells of microtitre plate. Also a third well is included for each samples as a negative controle were virus is not added. Then add 50µl os virus suspension in each well ad incubate for 1 hour in 37oC. After incubation is added growth medium with 2x105 cells/ml, and incubate for 3-4 days at 37oC. After this time, take the plate, discard medium, wash with 0,15M NaCl and then fixed in Acetone/PBS for 10 minutes. After that at each well is added 50µl monoclonal anti-CSF antibodies. Again a thorough washing, and IgG HRPO Conjugate is added. The plate was in incubation for 10 minutes. After another set of washing was added chromogene substrate (TMB) in each well and

incubated for 15 minutes. The test is read visually. Monolayer is examined microscopycally to determine the end-point of the titration. The following controls are included in the test: cell control, positive serum and back titration of test virus. The back-titration should confirm that virus has been used ata concentration of between 30 and 300 TCID50/50 µl.

RESULTS AND DISCUSSIONS

Serology is the method of choice for surveillance of CSF in an apparently disease-free area or for insuring that there are no residual foci of infection during an eradication program (Peters et al 1986). Antibodies are first detectable 2 to 3 weeks after infection, persist in surviving animals for the duration of their life and are a good indicator that infection with CSFV has been present in a pig herd. The most commonly used tests for antibody detection are virus neutralization tests (VNT) and ELISAs. The VNT is regarded as the "gold standard" but it is labor intensive and time consuming, as it relies on cell culture technology. From 1000 blood samples, collected in 16 regions of the country, 17.6% of them resulted positive for presence of antibodies against CSF. Regions with highest percentage of antibodies levels, were Kurbin 89%, Mirdita 65%, Kruje 50%, Elbasan 41% (as show in Table 2). These region also have a big population of domestic pigs.

Table 1: Test Results for Antibody Detection against CSFV

Regions	Serum	No Samples w/Ab against CSFV	%
Durres	34	-	
Elbasan	17	7	41%
Fier	45	-	
Gjirokaster	47	-	
Korce	18	1	6%
Kruje	12	6	50%
Kurbin	28	25	89%
Lezhe	339	89	26%
Lushnje	24	-	
Mat	25	-	
Mirdite	17	11	65%
Puke	26	3	12%
Sarande	104	-	
Shkoder	175	34	19%
Tirane	54	-	
Vlore	35	-	
Total	**1000**	**176**	**17,6%**

We used numerous techniques, mostly those that have gained international acceptance and have been integrated into different international Classical swine fever control programs.

12 samples resulted strong positive with ELISA Ab, were delivered for confirmation to EU Reference Laboratory for CSF, Hannover Germany, and were tested with VNT, among other methods, and were fully confirmatory.

Table 2: Test Results for Virus Neutralization Assays (EU Reference Laboratory for CSF)

No Sample	CSF 902 (Alfort) gt1.1	CSF0940 Cstrain gt1.1	Pestivac Pasteur Vaccine gt1.1	CSF 0104 (Diepholz) gt2.3	CSF 1055 (Lit 2011) gt2.1	CSF 0573 (Italy/Parma) gt2.2	BVDV NADL	BDV Moredun
1	240	80	320	320	40			
2	160	80	120	160	30			
3	1920	480	2560	480	80	240	<5	20

Table 2: Contd.,

4	1280	240	960	640	160	240	<5	30
5	1280	240	1280	320	80	320	<5	20
6	1920	160	1920	480	80	160	<5	60
7	2560	160	1920	960	120	160	<5	30
8	1280	160	1920	160	120	160	7.5	20
9	1920	240	1920	480	80	240	<5	60
10	640	240	480	960	160	240	40	80
11	640	320	480	960	80	480	10	60
12	3840	960	3840	960	240	320	15	80

They were tested in VNT with CSFV0902, (Alfort 187, gt 1.1), CSF0104, (Diepholz, gt 2.3) and CSF0940 (Cstrain gt 1.1), CSF1055 (Lithuania, gt 2.1), CSF0573 (Italy, 2.2) and with Pestivac Pasteur vaccine (provided by our laboratory). Pestivac Pasteur vaccine is based on strain Thiverval IP-77 (gt 1.1). In addition, samples 3-12 were tested in VNT with BVDV strain NADL and BDV strain Moredun. All tested serum samples were positive in VNT with CSF0902, CSF0104, CSF0940, CSF1055, CSF0573 and with Pestivac Pasteur vaccine. For samples 3 to 9 and 12 highest titers (ND50) were obtained in VNT with CSF0902 (gt 1.1) and with the Pestivac Pasteur vaccine (gt 1.1). For samples 1, 10 and 11 the highest titers (ND50) were obtained in VNT with CSF0104 (gt 2.3). For sample 2 titers were equally high in VNT with CSF0902 (gt 1.1) and CSF0104 (gt 2.3). CSF0902 (gt 1.1) and Pestivac Pasteur vaccine (gt 1.1) are antigenetically (E2 sequence) closely related, thus similar antibody titers are in line with expectations.

With regard to ruminant pestiviruses, low titres were detected for samples 10,11 and 12, remaining sera were tested negative (see table 2).

CONCLUSIONS

In total were analyzed 1000 and were registered 17,6% seropozitive in domestic pigs. Target for sampling were unvaccinated pigs, at age 3-6 months. Resultes were confirmed in EU Reference Laboratory for CSF, Hannover, by different test. Detection of antibodies does not necessarily mean that the animal is infectious. On the contrary, in most cases where antibodies are present, infectious virus will no longer be detectable. This figures correlated with the situation in Albania. From 1996 and now on, no measures were taken to eradicate the infection apart from vaccination.

The disease is controlled by vaccination with the live C-strain vaccine, and pigs can be protected against infection for at least 10 months Vaccination scheme cover almost 40 % of pig population, and it is applied in sows and weaning piglets.

However information taken from the field is still poor. Within the European Union, the focus of the control of CSFV in the domestic pig population is primarily to avoid new introduction of CSFV infections. The importance of education and disease awareness needs to be emphasized, and all involved in combating disease, hunters, farmers, field and state veterinarians, needs to be part of this education.

According to EU Reference Laboratory results, testing pigs for antibodies, when vaccination against CSF with a modified life vaccine is performed, there’s no chance to exclude that the pigs had contact with a CSF field strain. We have test results that are indicating towards a vaccination titer, as in sample no. 3, but even in that case we cannot actually exclude that the pigs had for example contact with a CSF field virus of e.g. gt 1.1. We always have to include background information in the interpretation of test results. In addition, it is important to know what kind of CSF filed

virus could be expected to circulate in our country, or in case there is no CSF at the moment, what are the strains that are circulating in neighboring countries or in countries that are related in terms of relevant trade (exchange pigs, pig products etc. but also movement of people).

This finding gives an economical value to our study, because Albania doesn't present a potential risk for the transportation of this infection in other countries of community. In the mean time help to have a right orientation for national strategies in order to decrease or eleminate unneeded costs, also accredited founds might be used in other problematic. This study will be a good help for Sector of Veterinary and Public Health, WA, as a basis to prepare national plans and strategies for the disease control.

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