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CLASSICAL SWINE FEVER VIRUS DETECTION IN FETAL SWINE TISSUES BY IMMUNOHISTOCHEMISTRY

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Abstract

The classical swine fever virus has the ability to cross the placental barrier, resulting in the infection of fetuses, which may consequently lead to persistent infection in piglets. The aim of this study was to report the lesions in fetuses naturally infected with CSFV during late gestation and clarify the nature of infected cells and the distribution of viral antigen in different tissues. A total of twenty-nine fetuses aged 82, 83 and 95 gestational days originating from three naturally CSFV infected sows were examined in this study. In all tested sows and their fetuses CSFV was detected using RT-PCR method. Immunohistochemistry method was used to detect viral antigen and monoclonal antibody WH303 was used on formalin fixed tissue samples of brain, spleen, heart, tonsils, kidney, ileocecal valve and umbilical cord. The most common lesions in the majority of fetuses were hyperemia, petechial haemorrhages in the skin, lymph nodes and kidneys. With the exception of myocardium, CSF viral antigen was detected in all the examined tissues. WH303 positive cells included endothelial cells, monocytes, macrophages and lymphocytes. The largest number of positive cells was found in kidneys in all of the examined fetuses. Reticular cells, macrophages, lymphocytes and endothelial cells in the spleen were also intensely and widely stained in most of the fetuses. These results showed that CSFV antigen can be detected in formalin-fixed, paraffin-embedded fetal tissue specimens originating from naturally CSFV infected sows by using monoclonal antibody WH303. Fetal kidneys proved to be a very useful organ for diag-

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nosis of the CSF virus. Having that in mind, it is assumed that persistently infected piglets may shed a high amount of viral particles through urine. However, further research is needed to confirm this hypothesis.

Key words: classical swine fever virus, fetuses, kidney, immunohistochemistry

DETEKCIJA VIRUSA KLASIČNE KUGE SVINJA U FETALNIM TKIVIMA PRASADI PRIMENOM IMUNOHISTOHEMIJSKE METODE

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Kratak sadržaj

Virus klasične kuge svinja poseduje mogućnost prelaska placentarne barijere, što može dovesti do infekcije fetusa i posledično do nastanka perzistentne infekcije kod prasadi. Cilj ovog istraživanja bio je utvrđivanje lezija koje nastaju kod fetusa prirodno inficiranih virusom klasične kuge svinja tokom kasne faze gestacije, kao i prirodu inficiranih ćelija i distribuciju virusnog antigena u različitim tkivima fetusa. Ukupno je ispitano dvadesetdevet fetusa starosti 82, 83 i 95 dana gestacije, poreklom od tri prirodno inficirane krmače virusom klasične kuge svinja. Prisustvo virusa potvrđeno je kod svih ispitanih krmača i njihovih fetusa upotrebom RT-PCR metode. Za imunohistohemijsku detekciju virusnog antigena u tkivnim isečcima mozga, slezine, srca, tonzila, bubrega, ileoceklane valvule i pupčane vrpce primenjeno je monoklonko antitelo WH303. Kod većine ispitanih fetusa ustanovljena je hiperemija i petehijlna krvavljenja na koži, limfnim čvorovima i bubrezima. Virusni antigen je detektovan u svim ispitanim tkivima fetusa, izuzev tkiva srca. Detektovane WH303 pozitivne ćelije obuhvatale su endotelne ćelije, monocite, makrofage i limfocite. Najveći procenat pozitivnih ćelija na virusni antigen utvrđen je u bubrezima kod svih ispitanih fetusa. Pored toga, veliki broj pozitivnih ćelija dokazan je u

retikularnim, limfoidnim i endotelnim ćelijama slezine kod većine fetusa. Rezultati dobijeni u ovom istraživanju pokazuju da se upotrebom monoklonskog antitela WH303 može detektovati antigen virusa klasične kuge svinja u parafinskim isečcima tkiva fetusa prasadi poreklom od prirodno inficiranih krmača. Pored toga, utvrđeno je da su fetalni bubrezi veoma pogodan materijal za dijagnostiku virusa klasične kuge svinja. Na osnovu ovih nalaza postavljena je hipoteza da perzistentno inficirana prasad mogu izlučivati velike količine virusnih čestica putem urina, međutim, potrebna su dodatna istraživanja kako bi se potvrdila ova hipoteza.

Ključne reči: virus klasične kuge svinja, fetusi, bubreg, imunohistohemija

INTRODUCTION

Classical swine fever (CSF) is one of the most important viral diseases in domestic pigs and wild boars (Blome et al., 2017). The causative agent, *Classical swine fever virus* (CSFV), belongs to the *Pestivirus* genus within the *Flaviviridae* family (Edwards et al., 2000). Other members of this genus are *Bovine viral diarrhea virus 1* and *2* (BVDV-1 and BVDV- 2), *Border disease virus* (BDV) and a growing number of unclassified and so-called atypical pestiviruses (Blome et al., 2017). The disease has a severe socio-economic impact on industrial pig production, small-scale pig keepers and tremendous impact on animal health and is therefore notifiable to the World Organization for Animal Health (OIE) (Edwards et al., 2000).

Different forms of the disease have been observed, ranging from subacute, acute or chronic forms, with lesions varying from mild to severe. In general, infections with more virulent strains typically result in acute hemorrhagic disease while infections with less virulent strains usually lead to chronic or subclinical forms (Muñoz-González et al., 2015). In countries with endemic outbreaks, like Serbia, the disease usually has an acute or subacute course, with classical clinical signs of infection in unvaccinated population (Prodanov-Radulović et al., 2014). When infection occurs during pregnancy, the virus can also infect the fetus due to its ability to pass the placental barrier which in turn might lead to persistent infection in piglets (Van Oirschot and Terpstra, 1977). The outcome of transplacental infection depends on many factors, including the stage of gestation and virulence of the virus. It is known that infection during early pregnancy leads to abortions, mummification and

stillbirth. However, when infection occurs in the second or third trimester of pregnancy, especially between 50th and 70th gestational day, an immunotolerance phenomenon can be induced and persistently infected offspring are born (Moennig et al., 2003). Those piglets seem to be healthy and could survive for several months but they eventually die due to the so-called „late onset" form of the CSF. During that period, they shed high viral loads which are sufficient for transmission (Cabezón et al., 2017). This may cause an uncontrolled spread of CSF and significant losses in large swine population. Due to uncharacteristic profiles of CSF clinical symptoms in pregnant sows, this may lead to delayed identification of potential new sources of infection. Therefore, very mild clinical symptoms might easily be overlooked (the so-called "carrier-sow syndrome"). This has an impact on the efficacy of vaccines and may complicate control in endemically affected countries (Blome et al., 2017). In the literature, there is little information about the pathogenesis of the transplacental infection detected on the field, during eradication campaign. Most data regarding transplacental virus transmission are obtained under experimental conditions (Muñoz-González et al., 2015; Dewulf et al., 2011; Van Der Molen and Van Oirsch, 1981; Von Benten et al., 1980).

Considering the significance of the transplacental infection and possible persistent infection in offspring regarding the disease control and eradication, the aims of this study were the following: reporting the lesions in naturally infected fetuses with CSFV; detecting CSFV in formalin-fixed, paraffin-embedded fetal tissues by immunohistochemistry, as well as to determining the tissue and cellular distribution of CSFV in pig fetuses to better understand the pathogenesis of naturally occurring disease.

MATERIAL AND METHODS

Material

Twenty-nine fetuses originating from three sows naturally infected with CSFV were morphologically and immunohistochemically examined in this study. All fetal samples were collected during big CSF outbreaks in Serbia, in 2006. Veterinary regulatory measures included stamping out policy of all affected pigs on farms and backyard productions. All sows were of landrace breed, non-vaccinated, reared in rural backyard holdings and were randomly chosen. Blood samples were taken from each sow for viral detection. All sows were virologically negative for African swine fever, porcine reproductive respiratory syndrome, and Aujeszky's disease. Fetuses were obtained by removing the uterus from each sow after euthanasia. The first sow was in the 83rd day

of gestation and had 6 fetuses; the second sow was in 82nd day of gestation and had 11 fetuses, and finally the third sow was in 95th day of gestation with 12 fetuses. Necropsies of all fetuses were performed and gross lesions were recorded. From each swine fetus, the following organs were sampled: brain, kidney, spleen, umbilical cord, intestine, tonsils, ileocecal valve and heart. Tissue samples of kidney, spleen and tonsils were pooled for molecular detection of CSF viral RNA. All tissue samples were fixed in 10% neutral buffered formaldehyde for 48 hours for immunohistochemistry, and embedded in paraffin according to standard laboratory procedures. The samples from four CSF negative pig fetuses were used as control. Tissue samples from two 45-days-old pigs naturally infected with CSFV were used as positive controls.

Methods

Detection of CSFV RNA genome

Conventional, gel based reverse transcriptase-polymerase chain reaction (RT-PCR) test was applied to detect genomic RNA of CSFV in unclotted blood of sows and fetal tissue samples. Total RNA was extracted by TRIzol® reagent (ThermoFisher Scientific, USA) according to manufacturer's instruction. Briefly, 750 µl of TRIzol® reagent was mixed with 250 µL of sample. After 10 min, 200 µL of chloroform was added, mixed and the suspension was centrifuged for 15 min at 14,000 g at 4°C. The RNA containing aqueous phase was removed and precipitated with 500 µL of isopropanol, maintained at room temperature for 10 min, and centrifuged for 10 min at 14,000 g. The RNA pellet was washed with 500 µL of 75% cold ethanol, centrifuged for 5 min at 14,000 g, then dried and resuspended in 50 µL of PCR clean water, and stored at -70°C until examination or was immediately included in RT-PCR. The obtained RNA extracts were further amplified by using primers for E2 region of the CSFV genome gp55-U: 5'-ATA TAT GCT CAA GGG CGA GT-3' (sense, position in genome of the Alfort strain is 3378-3397) and gp55-L: 5'-ACA GCA GTA GTA TCC ATT TCT TTA-3' (antisense, position in genome of the Alfort strain is 3685-3662) described by Katz et al. (1993). One-step RT-PCR amplification was done using commercial kit Qiagen OneStep RT-PCR kit chemistry (QIAGEN, Germany) according to manufacturer's instruction, with small modifications. Briefly, the amplification reaction was carried out at a volume of 25 µL containing 13.5 µL of nuclease-free water, 5 µL of 5 x PCR buffer, 1 µL of dNTP mix (containing 10 mM of each dNTP), 0.25 µL of stock solution of 100 µM of each primer, 1 µL of one step RT-PCR enzyme mix and 4 µL of RNA template. As a positive control in reaction, Alfort 187 strain of

CSFV was used. The amplification conditions (Thermocycler Gradient, Eppendorf, Germany) were as follows: reverse transcription stage at 50°C for 30 min, followed by an initial PCR activation step at 95°C for 15 min, 40 cycles of 95°C for 30 s, 55°C for 60 s, 72°C for 60 s, and a final extension at 72°C for 10 minutes. Amplified products were detected and visualized by electrophoresis on 1.5 agarose gel stained with ethidium bromide.

Immunohistochemistry

To demonstrate CSFV envelope glycoprotein E2 in fetal tissues, the commercially available monoclonal mouse anti-CSFV antibody WH303 (APHA Scientific, UK; catalogue number RAE0826) was used on fixed samples. IHC staining kit applied in this study was Novolink Polymer Detection Systems, Novocastra (Leica Biosystems, USA). Tissue sections were dewaxed and rehydrated in xylene and graded series of alcohol. Antigen retrieval was achieved by heating the sections in a microwave oven (560W) for 21 minutes in a citric buffer (pH 6.0), as previously described by Polaček et al., (2007). Endogenous peroxidase activity was abolished by incubation of the sections with "Peroxidase block" for 5 minutes at room temperature. After two washes in PBS for 5 minutes, all slides were incubated with "Protein block" for 5 minutes. After this step, all slides were washed two times in PBS for 5 minutes and incubated overnight at 4°C in a humidified chamber with primary antibody diluted 1:50 in PBS. After incubation with primary antibody overnight, the slides were given two 5-minute rinses with PBS. "Post-primary antibody" was applied like the secondary antibody for 30 minutes at room temperature. After two washes in PBS for 5 minutes, all slides were incubated with "Novolink™ Polymer" for 30 minutes. Then, all tissue sections were rinsed two times with PBS, and gently rocked. The final reaction was produced by incubating the sections with DAB working solution (50µL of DAB Chromogen was added to 1ml of Novolink™ DAB Substrate Buffer) for 5 minutes. After that, all slides were rinsed in tap water, and counterstained with Mayer's haematoxylin. PBS was used in place of specific primary antibodies as negative controls.

RESULTS

Results of molecular testing

The results of RT-PCR test confirm that the RNA of the CSFV genome was detected in all blood samples of sows and in all tested fetal tissue samples.

Gross lesions

Some of the detected gross lesions were characteristic of CSF, but not present in all examined fetuses. The most common finding in the majority of fetuses was hyperemia, and it was seen in the skin, submandibular lymph nodes, spleen, tonsils, and the brain (Figure 1a). Petechiae were observed in the skin of the dorso-lateral aspect of neck and abdomen in 19 fetuses (Figure 1b). Some fetuses had petechial haemorrhages in the renal cortex and urinary bladder. In few cases, lymph nodes were swollen and hemorrhagic. There were no macroscopic pathological distinctions in the severity of lesions between fetuses originating from different sows.

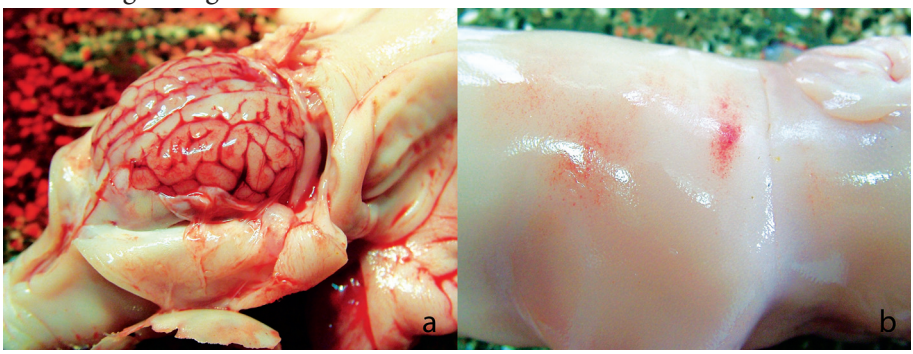

Figure 1. (a) Hyperemia in fetal brain; (b) Petechial hemorrhages in the skin

Distribution of CSF viral antigen

The results of immunohistochemical staining are summarized in Table 1. The monoclonal antibody WH303 specific for CSFV glycoprotein E2 gave a positive staining reaction in tonsils, spleen, kidneys, ileocecal valve, brain, umbilical cord, but no immunoreactivity was detected in the myocardium. Positive cells typically exhibited a dark brown reaction on the membrane. With the exception of myocardium, in all the examined tissues, WH303 positive cells included endothelial cells, monocytes, macrophages and lymphocytes. The most immunopositive tissues were observed in fetuses originating from the first sow (83 gestational days). No differences in the amount or distribution of immunoreactive products were observed between fetuses from the second and third sow (82 and 95 gestational days, respectively).

In all the examined fetuses, the largest number of positive cells was found in kidneys, and a numerous positive duct epithelial, endothelial and mononuclear cells were detected (Figure 2a and 2b). In the spleen, specific immu-

noreactivity was observed in reticular cells, macrophages, lymphocytes and endothelial cells (Figure 2c). In tonsils, specific immunopositivity was detected in the crypt-epithelial cells, macrophages and lymphocytes (Figure 2d). Immunoreactive endothelial cells were observed in umbilical arteries of the umbilical cord (Figure 3a). In the ileocecal valve, viral antigen was detected in crypt epithelial cells and a small number of intraepithelial lymphocytes (Figure 3b). In the brain, virus antigen was found in glial cells, endothelial cells, and in the cells of the mononuclear inflammatory perivascular and meningeal infiltrates (Figure 3c and 3d). No immunoreactivity was observed in neurons.

Table 1. Immunohistochemistry (IHC) results for 29 pig fetuses naturally infected with CSFV

Fetus number	Age (days of gestation)	Brain	Kidney	Spleen	Umbilical cord	Tonsils	Heart	Ileocecal valve
		IHC	IHC	IHC	IHC	IHC	IHC	IHC
1	83	+	+	/	+	+	-	+
2	83	+	+	+	-	-	-	-
3	83	+	+	+	+	+	-	+
4	83	+	+	+	+	+	-	+
5	83	+	+	+	+	+	-	+
6	83	-	+	+	+	+	-	+
7	82	+	+	+	+	+	-	+
8	82	-	+	-	-	-	-	-
9	82	+	+	+	-	+	-	+
10	82	-	+	+	/	-	-	-
11	82	-	+	+	-	-	-	-
12	82	-	+	-	-	+	-	+
13	82	-	+	+	+	-	-	+
14	82	+	+	+	-	-	-	+
15	82	-	+	+	-	+	-	-
16	82	-	+	-	+	+	-	+
17	82	+	+	-	-	-	-	-
18	95	-	+	+	-	+	-	-
19	95	+	+	+	-	-	-	+
20	95	-	+	-	-	-	-	-
21	95	-	+	+	-	-	-	-
22	95	-	+	-	+	+	-	+
23	95	+	+	-	-	-	-	-
24	95	-	+	+	-	-	-	-
25	95	-	+	+	-	-	-	+
26	95	+	+	-	-	+	-	+
27	95	-	+	+	+	-	-	-
28	95	-	+	-	-	-	-	-
29	95	-	+	+	-	-	-	+
Total	29	12	29	19	10	13	0	16

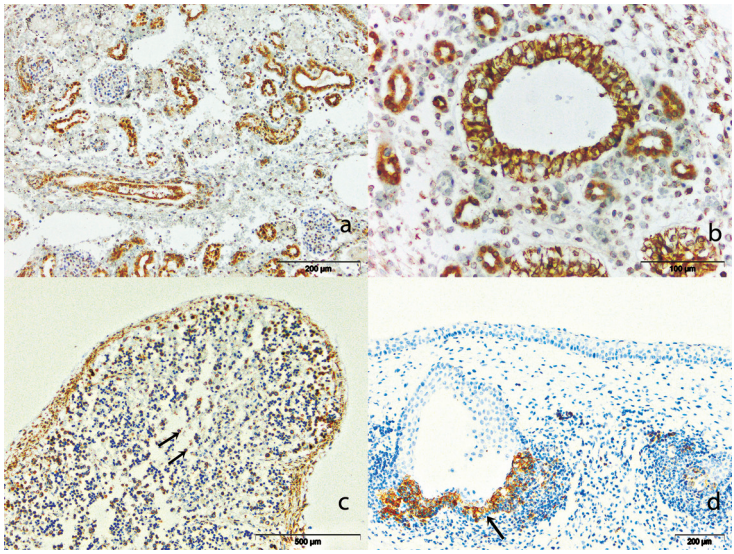

Figure 2. WH303 monoclonal antibody immunoreactivity in fetal tissues: (a, b) Kidney. CSFV antigen positive cells are detected in endothelial cells, tubulocytes and duct epithelial cells; (c) Spleen. CSFV antigen positive cells distributed mostly in the cortex. Scattered positive macrofages detected in the red pulp (arrow); (d) Tonsil. CSFV antigen positive cells detected in the crypt epithelium (arrow)

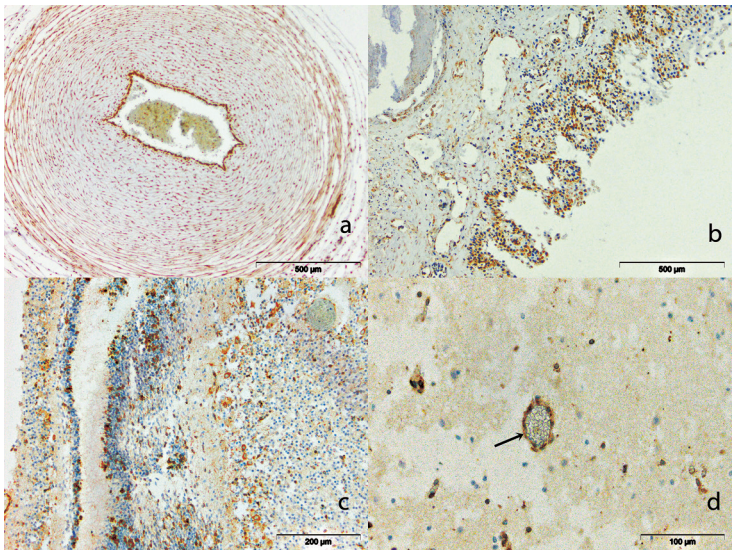

Figure 3. WH303 monoclonal antibody immunoreactivity in fetal tissues: (a) Umbilical cord. CSFV antigen positive cells distributed in vascular endothelium; (b) Ileocecal valve. CSFV antigen positive cells detected within crypt epithelium; (c,d) Brain**.** CSFV antigen positive cells detected in glial cells, meningeal infiltrates and endothelial cells (arrow)

DISCUSSION

Although other research groups reported analyses of CSFV in different swine tissues by immunohistochemistry, to our best knowledge none of the previous examinations have provided analysis of fetal swine tissues under natural conditions, like the present study. These data are important from the point of controlling CSF disease, considering that transplacentally infected piglets can play a role especially for constant virus shedding and circulation in the swine population for a long period of time. The literature data on immunopathogenesis and tissue tropism of CSFV in infected fetuses is scarce, so it is difficult to compare this type of study with our results. The main reason for that may be strict eradication strategies implemented in most European countries in the past. Although many countries are officially free from this devastating disease, in many countries outside Europe the disease is still epidemic or endemic. According to the official OIE reports for the last 5 years (2016-2020), CSF was detected in countries of South America (Brazil, Colombia, Bolivia, Ecuador, Peru, Haiti), and Asia (China, Nepal, Thailand, Singapore, Philippines, Malaysia, Japan, Russia) (https://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseasedistributionmap). In the Republic of Serbia, the last outbreak was reported in 2010 and the control of CSF was carried out for decades by C strain mass vaccination until the end of 2019. All CSF viruses isolated in the Republic of Serbia in 2006 when this study was performed were members of subgroup 2.3 (Milićević et al., 2013).

The results of this retroactive study showed that CSFV antigen can be detected in formalin-fixed, paraffin-embedded fetal tissue samples originating from naturally CSFV exposed sows by using monoclonal antibody WH303. A murine monoclonal antibody WH303 detects the CSF virus specific antigenic domain on the major envelope glycoprotein (E2), and binds to all strains of CSF virus, but not to other pestiviruses. The availability of reliable diagnostic tests is crucial for effective CSF control, and different diagnostic laboratory test procedures for confirmation of the viral antigen presence are available. Among them, immunohistochemistry proved to be a very reliable and suitable method for viral antigen detection in tissue samples, as well as for studying the pathogenesis of both natural and experimental CSFV infections (Belák et al., 2008; Polaček et al., 2007; 2014; 2016; Sánchez-Cordon et al., 2003; Choi and Chae, 2003a; De las Mulas et al., 1997). Besides that, the importance of diagnostic IHC is further emphasized by the observation of Belák et al., (2008) where this test gave positive results long (even seven days) before the appearance of the first clinical symptoms. As mentioned above, the delayed diagnosis may cause

an uncontrolled spread of CSF and consequently lead to heavy losses in swine population. Considering that CSFV infected pregnant sows have atypical and discrete clinical symptoms and that their offspring are a potential reservoir of the disease, we found it very important to perform this study of the tissue distribution of CSFV in pig fetuses in order to study virus biology and possibly confirm some answers regarding pathogenesis.

The pathological lesions in the acute and chronic form of CSF are well-documented (Moennig et al., 2003; Choi and Chae, 2003b; Knoetig et al., 1999; Belák et al., 2008). In the acute form, lesions are usually accompanied by secondary pathogens, but in general they included widespread petechial haemorrhages and ecchymosis, especially on skin, lymph nodes, epiglottis, bladder and kidneys, as well as infarctions of the margin of the spleen. In piglets with a congenital form of CSF infection, pathological changes were less typical and usually included cerebellar hypoplasia, microcephaly, pulmonary hypoplasia, hydrops and other malformations. This underlines that pathological mechanism involved are different from those responsible for postnatal infections (Liess 1987). In the present study, these findings were not detected in fetuses; however, the most common findings were hyperaemia in most organs, as well as petechial haemorrhages in the skin, lymph nodes and kidneys which coincide with the acute form of the disease. Although persistently infected offspring may be clinically normal at birth, it may take several months before they develop lesions and other disturbances that lead to death (Van Oirschot and Terpstra, 1977).

CSF viral distribution in different tissues depends on strains of the virus (highly, moderately or low virulent strain), age of pigs, duration of infection, and susceptibility of the breed (Choi and Chae, 2003a). The immunohistochemical detection of CSFV antigen in tissues of both naturally and experimentally infected pigs over time is well documented (Polaček et al., 2014; Liu et al., 2011; Gómez-Villamandos et al., 2006; Risatti et al., 2005; Choi and Chae, 2003a; De las Mulas et al., 1997). Previous studies have demonstrated that CSFV has a particular affinity for cells of the immune system, phagocytes of the macrophage and monocyte lineage (reticulo-endothelial cells), epithelial and vascular endothelial cells (Feng et al., 2012; Ressang, 1973; Cheville and Mengeling, 1969). In pig fetuses, CSF viral antigen was found predominantly in vascular endothelium, mononuclear cells and epithelial cells and these data are in accordance with those from a previous study. However, immunohistochemical staining of tissues in previous studies showed viral antigen distribution mainly in the cytoplasm of infected cells, while membranous expression was specific in fetal tissues. The reason for different cellular locations of

WH303 in fetuses compared to mature pigs is not known. However, it may be likely associated with some morphogenetic events in fetal development.

In our study, the general course of tissue tropism for the 7 examined tissue samples (from high to low) was as follows: kidney, spleen, ileocecal valve, tonsil, brain, umbilical cord and heart. The heart was the only organ without immunoreactivity in this study, and this is in agreement with previous studies where it was found that the myocardium is not considered as replication site for CSF virus in pigs (Belák et al., 2008; De las Mulas et al., 1997). In general, muscle cells are not considered the sites of CSFV replication (Liess 1987). However, Liu et al., (2011) had shown that low viral content could be detected in the heart of CSF infected pigs using real-time PCR method. This could be explained by the fact that real-time PCR is believed to be more sensitive than IHC method.

Recent work by our group demonstrated that the fetal kidneys were very useful organ for IHC diagnosis of the CSF virus, since out of all examined fetuses the highest level of viral antigen was detected in this organ. Previous studies also indicate that CSFV antigen positive cells in kidneys are a common finding, although the number of positive cells could be low (De las Mulas et al., 1997; Belák et al., 2008). In contrast, Choi and Chae (2003b) have shown that CSFV nucleic acid could be detected in kidneys by in situ hybridization, but they failed to detect viral antigen by immunohistochemistry. These authors explained this discrepancy by the fact that CSF disease process associated with glomerulonephritis is related in some way to the accumulation of viral nucleic acid/antibody complexes, and not complete CSFV particles. Another explanation was that CSFV infected cells may express small amount or no viral antigen on the cellular surface and this could result in failure to detect viral antigen by immunohistochemistry. Nevertheless, some authors preferred kidney tissue samples for diagnosis of CSF by direct immunofluorescence using frozen sections (Pearson, 1992; Terpstra, 1991).

It is shown that in transplacentally, persistently infected piglets, the infection severely affects tissues comprising the immune system, including severe depletion of lymphocytes in thymus and secondary lymphoid organs (Van Der Molen and Van Oirsch, 1981) and viral antigen was widespread in lymphoid tissues (Polaček et al, 2007). In our study, the viral antigen was widely detected in spleen followed by tonsils, which is in accordance with these studies. However, the main route of entry of CSFV in postnatal infection is oronasal and the tonsils are the primary replication site of the virus. In the present study, viral antigen was also detected in tonsils in a certain number of examined fetuses, which might mean that the virus gains access from the bloodstream to tonsils as the secondary replication site.

As mentioned above, CSFV is found to be highly susceptible to infecting vascular endothelial cells (Bensaude et al., 2004) and they are primary target cells for the virus. Dysfunction of blood vessels plays an important role in the pathogenesis of CSF and it was proved that CSFV plays a pathophysiological role in vascular dysfunction through its effect on oxidative stress (He et al., 2014; Bensaude et al., 2004). In the recent study, viral antigen was detected in blood vessels of the brain, spleen, kidneys, as well as in umbilical cord, due to transplacental passage. Some other swine viruses also replicate in umbilical cord due to their ability to cross the placenta of the pregnant sows, as previously described in influenza infection (Khatri and Chattha, 2015) and PRRS virus (Lager and Halbur, 1996).

CSFV, as well as *Bovine viral diarrhea virus* (both viruses of the same genus - *Pestivirus*) are characterized by severe clinical and histopathologic changes in the intestine. The CSFV also has a pathogenic effect on gut-associated lymphoid tissue (GALT) and infection takes place initially in monocytes-macrophages and lymphocytes in lymphoid tissue (Sánchez-Cordón et al., 2003). In the present study, epithelial cells of ileocecal valve crypts and cells in the lumen of these crypts revealed a slightly positive reaction, and immunopositive signal was detected in half of the examined fetuses.

Nervous tissue is considered as one of the target tissues for CSFV, and histological lesions in the brains of pigs with chronic CSFV infection are similar to those in pigs experimentally inoculated with a low-virulence CSFV strain (Choi and Chae, 2003). Previous studies reported viral antigen detection in neurons, glial cells, endothelial cells, and cell infiltrate during CSF infection (Pan et al., 1993; De las Mulas et al., 1997; Gómez-Villamandos et al., 2006; Polaček et al., 2008). A similar viral antigen distribution has been detected in our study, with the main exception of IHC positive neurons, although infection of neurons is reported in natural outbreaks of CSF (Pan et al., 1993; Trautwein, 1988). This difference may be due to a more prolonged course of the natural infection studied in mature pigs.

CONCLUSION

Regarding the control of this viral disease, the results presented herein indicate the importance of the kidneys in the pathogenesis of transplacental CSFV infection, considering that the highest amount of viral antigen was detected in this fetal organ and all examined fetal kidneys were immunopositive for the antigen of the CSFV. Having that in mind, it is assumed that persistently infected piglets may shed high amount of viral particles through urine. However, further research is needed to confirm this hypothesis.

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Author's contributions:

VP and BĐ drafted the manuscript, carried out literature research and immunohistochemical examination; TP carried out the virological examination; JPR participated in the design of the study; MS did the reviewing and editing; IV participated in the immunohistochemical examination and in manuscript revision. SKA was involved in the study design, she revised manuscript critically and gave the final approval of the version to be published.

Competing Interests

Authors declared no conflict of interests regarding the present paper.

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