

**EVALUATION OF THE CLINICAL SIGNS AND
PATHOMORPHOLOGICAL CHANGES IN PIGLETS DERIVING
FROM VACCINATED SOWS (CHINA STRAIN) AFTER
EXPERIMENTAL INFECTION WITH CLASSICAL SWINE FEVER
VIRUS**

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Summary

An experimental study was conducted to investigate the clinical course of classical swine fever (CSF) infection in piglets deriving from sows vaccinated with China strain vaccine. The experiment was carried out in 24 clinically healthy weaner pigs, divided in four groups. Each experimental group consisted of 6 piglets, aged 28, 35, 44 and 54 days. Two piglets from each group were randomly selected and challenged by intramuscular injection of CSF virus. After that, challenged pigs were reintroduced into their respective pen, in order to detect the possibility of horizontal infection. All experimental animals were examined clinically. After death or sacrifice pathomorphological changes in all piglets were evaluated. The results of our experiment indicate that infection of piglets is possible despite the fact that they originate from vaccinated sows. In all animals clinical signs, which are distinctive for CSF were not detected. However, pathomorphological findings after death and / or sacrifice pointed on successful experimental infection. Certain variation in expression and distribution of pathomorphological changes in survived piglets were discovered.

Key words: classical swine fever, passive immunity, China strain

Classical swine fever (CSF) is an important viral disease caused by a RNA-virus, belonging to the family *Flaviviridae*, genus *Pestivirus* (Thiel et al., 1996). In countries in which CSF is endemic, prevention and control depend primarily on vaccination programs, using attenuated live-virus vaccines (Moennig, 2000). Clinical signs and pathomorphological changes in susceptible pigs established after infection with CSF are well known (Trautwein, 1988). However, in conditions when CSF occurs endemically, variability of clinical signs and necropsy findings can be noticed. The objective of this trail was to examine wheather the experimentaly infected piglets, aged 28, 35, 44 and 54 days, originating from sows vaccinated with China (C) - strain of classical swine fever virus (CSFV), will result in clinically evident or unapparent course of the disease. Also the influence of passive immunity on pathomorphological changes development after challenge was examined.

Materials and methods

Animals. The experiment was carried out on 24 clinically healthy, conventional weaned pigs, divided in four groups (A, B, C and D). The piglets were of mixed sex, originating from the same, known CSF-free herd. After selecting the piglets, ear tagging was carried out. Each experimental group consisted of 6 piglets, deriving from 6 different litters, aged 21, 28, 37, 47 days. The experimental animals originated from the sows that were several time vaccinated with C-strain of CSFV.

Virus. For challenge infection the CSFV (strain Baker) was used. The isolate was verified to be free from bovine viral diarrhoea virus (BVDV) by means of reverse transcriptase – polymerase chain reaction (RT-PCR). The pigs were challenged with a dose of 1 ml by intra-muscular (i/m) route. The titre was 2×10^5 median tissue-culture-infective doses (TCID₅₀/ml).

Experimental design. The experiment was divided in three subsequent periods: the acclimatization, the challenge and the post-challenge period. Upon arrival, the animals were allocated systematically (based on ear-tag number) to pens within 4 separate compartments. After acclimatization period of 7 days, when the piglets were 28, 35, 44 and 54 days old, two piglets from each group were randomly selected, separated and i/m inoculated with CSFV. To perform the inoculation the selected pigs were moved to a separate compartment where they remained 7 hours after challenge. After that, i/m challenged pigs were reintroduced into their respective pen, in order to detect the possibility of horizontal (contact) infection. All the groups of piglets were housed under identical conditions in 4 separate compartments. Each compartment consisted of a well isolated area of 35 m², with independent ventilation assigned for experimental work with the animals. Technical design and biosafety measures entirely prevented possibility of mechanic transmission of the virus from one compartment into the other. All materials necessary for cleaning of the compartments and pens, and feeding of the pigs were provided per compartment and were stored at the compartment.

Clinical examination. After the challenge, all the pigs were examined clinically. The following symptoms were recorded during the clinical examination: liveliness (apathy, lethargy), conjunctivitis, constipation, diarrhoea, coughing, ataxia, convulsions, posterior paresis, erythema and hemorrhages of the skin. Rectal temperature, feed intake and mortality were recorded daily, always at the same time. Fever was defined as rectal temperature ≥ 40.0 °C.

Necropsy. All the animals were submitted to a complete necropsy immediately after death. Surviving 4 pigs were euthanized on 35 dpi (T61[®], Intervet International).

Results and discussions

Clinical signs of the disease. The results of clinical investigations for i/m and contactly infected piglets are summarised in table 1 and 2.

In i/m infected piglets from all the groups starting from 3 dpi clinical signs compatible with CSF were detected. The established body temperatures and clinical signs of disease correspond to characteristic signs of the acute disease (Dahle and Liess, 1992; van Oirschot and Terpstra, 1989), but characteristic skin haemorrhages were not visible in all the piglets.

Table 1

Clinical signs of the disease in i/m challenged piglets

Clinical signs of disease	No.	i/m challenged piglets							
		Group A		Group B		Group C		Group D	
		I/1	I/6	II/2	II/3	III/3	III/4	IV/3	IV/5
First day of elevated body temperature	3 *	3	3	3	3	2	2	2	3
Liveliness (apathy, lethargy)	3	3	3	3	3	3	3	3	3
Conjunctivitis	4	4	5	5		5	4	-	
Constipation	4	4	-	-	-	5	4	-	
Diarrhoea	5	5	5	7	6	6	5	-	
Coughing	-	-	-	-	-	-	-	-	
Dispnea	-	-	-	-	-	-	-	-	
Ataxia	6	6	5	5	5	5	6	-	
Convulsions	-	5	-	6	5	5	6	-	
Posterior paresis	-	-	6	6	7	7	7	-	
Erythema of the skin	7	-	-	-	-	7	-	-	
Haemorrhages of the skin	7	-	-	-	-	9	-	-	
Death of experimental animal	8	7	7	11	10	12	8	S	

*- The numbers express the day when the clinical symptom was for the first time noticed i. e. days post infection (dpi); S- survived piglet

As skin changes are considered to be one of the characteristic signs of CSF (Dahle and Lies, 1992), certainly that their absence in the case of CSF in piglets of this age could represent a great problem in clinical diagnosis. This could especially be a problem on large swine farms, where immunoprophylaxis against CSF is regularly conducted. Also constipation was not recorded only in piglets aged 35 days and in one piglet aged 44 days (table 1). However, two inoculated piglets in the group D (aged 54 days) showed completely different clinical course of the disease. In piglet No. IV/5, after initial signs of the disease from 3 to 6 dpi, later on the health conditions improved and by the end of the experiment it did not show any clinical signs of CSF. This piglet survived experimental infection and was euthanised on day 35 dpi. Depner et al. (1997) report about experimental conditions that may cause several forms of CSF with identical virus isolate. Since these two piglets were the same age, nutritive status, from the herd that continually carried out immunoprophylaxis program against CSF, it can be concluded that the

consequence that influenced the different outcome of the infection was associated with individual characteristics of an animal (passive immunity).

It is assumed that the principal means of CSFV transmission is direct contact between the infected and susceptible pigs (Terpstra, 1988) and under natural conditions, the mode of entry of CSFV in the pig is the oronasal route (Biront and Leunen, 1987). Infected pigs may shed the virus before the onset of a disease and continue to do so during the entire disease period (Terpstra, 1988). It is assumed that on average it takes 4 days for a challenged pig to become infected (Dewulf, 2002). As a evidence that i/m challenged piglets had excreted the sufficient amount of CSFV for contact infection are clinical signs of the disease and death of piglets of the same age that originate from vaccinated sows.

Table 2

Clinical signs of the disease in piglets exposed to contact infection

Clinical signs of disease	Group A				Group B				Group C				Group D	
	I/2	I/3	I/4	I/5	II/1	II/4	II/5	II/6	III/1	III/2	III/5	III/6	IV/1	IV/6
First day of elevated body temperature	9 *	12	5	10	10	10	8	10	11	9	10	10	11	10
Liveliness (apathy, lethargy)	9	13	8	9	10	10	9	10	11	10	10	9	11	11
Conjunctivitis	10	13	11	10	11	11	10	11	11	10	11	9	11	11
Constipation	10	-	10	10	11	11	9	11	12	10	12	11	-	12
Diarrhea	11	18	11	12	12	12	10	12	13	-	13	12	-	13
Coughing	-	20	-	-	-	-	-	-	-	-	-	-	-	-
Dispnea	-	20	-	-	-	-	-	-	-	-	-	-	-	-
Ataxia	12	-	12	12	12	12	10	12	11	11	14	11	14	13
Convulsions	12	-	-	-	13	13	-	13	11	11	15	11	-	-
Posterior paresis	13	-	13	-	14	-	-	-	12	12	17	12	-	14
Erythema of the skin	-	23	-	-	-	-	-	-	-	10	13	-	12	12
Hemorrhages of the skin	14	-	12	-	14	-	-	-	-	11	16	-	13	14
Death of experimental animal	15	S	15	13	15	13	11	13	13	11	23	13	21	19

*- The numbers express the day when the clinical symptom was for the first time noticed i. e. days post infection (dpi); S- survived piglet

In horizontally infected piglets aged 28, all the piglets succumbed until 23 dpi, except one (No. I/3) who showed different clinical course of the disease. This piglet, starting from 23 dpi, exhibited distinct exanthema on the skin of inside the pelvic limbs, but clinical signs were generally of weak intensity, whereby the symptoms of respiratory disease dominated (dispnea, coughing, purulent nasal discharge) followed by intermittent diarrhoea. This piglet survived contact infection and on 35 dpi was euthanised. In the group of piglets aged 44 days, which were exposed to contact infection, the course of the disease was relatively short (3 to 4

days), except for one piglet (No.III/5). In this piglet clinical signs indicated the development of subacute form of the disease (blotching of the ears, intermittent diarrhoea) combined with the symptoms of respiratory disease (dispnea, coughing, purulent nasal discharge). The clinical course of the disease in this piglet clearly suggested on development of secondary bacterial infections (Trautwein, 1988). In the group D (aged 54 days), two piglets (No. IV/2 and IV /4) did not show signs of clinical disease, or increase of body temperature and on 35 dpi they were euthanised. The reason for this completely diverging course remains questionable, possibly suggesting significant variations of the passive immunity. Kleiboeker (2002) assume that in the cases when CSF occurs in vaccinated herds, the disease has chronic course with non-characteristic clinical picture. Depner et al. (2000) concluded that the presence of maternal antibodies influences the clinical course of CSF in such a way that the disease becomes obscure and the outcome is rather transient than lethal.

Gross pathological findings. The results of gross patomorphological findings for all the experimental groups are summarized in tables 3, 4, 5 and 6.

Table 3.
Gross pathological findings in piglets from the group A (aged 28 days)

Pathomorphological changes	No.	i/m challenge		Contact infection			
		I/1	I/6	I/2	I/3	I/4	I/5
Icterus	-*	-	-	++	-	-	-
Petechiae epiglottidis	-	-	-	-	-	-	++
Tonsillitis diphtheroides necroticans	++	++	+++	-	+++	+++	+++
Lymphadenitis haemorrhagica acuta	+++	+++	+++	+	+	+	+
Petechiae thymi	+++	+++	+++	-	++	+++	+++
Infarctus haemorrhagicus lienis	+++	+++	+	++	+++	+++	+++
Ecchymosis pulmonalis	-	+++	-	-	-	++	++
Petechiae epicardii	-	+++	-	-	+++	++	++
Petechiae endocardii	-	-	-	-	+++	-	-
Petechiae corticis renalis	+++	+++	+++	+	+++	+	+
Petechiae vesicae urinariae	+	+	+	+++	+++	+	+
Haemorrhagiae hepatis	+	++	-	-	-	+	+
Petechiae et ecchymoses cholecistae	-	-	-	-	+++	-	-
Petechiae tunicae serosae ventriculi	-	-	-	-	+++	-	-
Petechiae tunicae mucosae ventriculi	+++	+++	+++	-	+++	+	+
Petechiae tunicae serosae intestini	-	-	-	-	+++	-	-
Petechiae tunicae mucosae intestini	+++	+++	++	-	++	-	-
Petechiae tunicae mucosae caeci	+++	+++	-	-	-	+++	+++
Typhlitis diptheroidea necrotica	-	-	-	++	-	-	-
Petechiae tunicae serosae coli	-	-	+++	-	-	-	-
Petechiae tunicae mucosae coli	+++	+++	-	-	+++	+	+
Colitis diphtheroides diffusa et follicularis	-	-	+++	+++	-	-	-
Haemorrhagiae mucosae recti	+++	+++	-	-	+++	+++	+++

* - not found; + mild; ++ moderate; +++ severe

In directly infected piglets that succumbed (total of 7), pathomorphological examination has indicated lesions characteristic for CSF (haemorrhagic infarcts of spleen, petechial haemorrhages in kidneys, urinary bladder and lymph nodes, serous membranes). Also, in one piglet (No. IV/5) that survived i/m challenge and was euthanized on 35 dpi, pathomorphologic examination detected changes that suggest for CSF infection (infarction of spleen, swelling of body lymph nodes and petechial haemorrhages of urinary bladder). However, the lack of prominent macroscopic lesions does not rule out the infection with CSFV.

Table 4.
Gross pathological findings in piglets from the group B (aged 35 days)

Pathomorphological changes	No.	i/m challenge		Contact infection			
		II/2	II/3	II/1	II/4	II/5	II/6
Icterus		++	.*	-	-	-	-
Haemorrhagiae subcutaneae		-	++	-	-	++	-
Petechiae epiglottidis		++	-	-	+++	-	-
Tonsillitis diphtheroides necroticans		++	++	+++	+++	+	+++
Lymphadenitis haemorrhagica acuta		+++	+++	+++	+++	++	+
Petechiae thymi		+++	+++	++	+	+	++
Infarctus haemorrhagicus lienis		+++	+++	+++	+	+++	+++
Ecchymosis pulmonalis		-	+++	+++	-	-	-
Petechiae et ecchymoses epicardii		+++	-	-	-	+++	+++
Petechiae endocardii		+++	-	++	-	-	-
Petechiae corticis renalis		+++	+++	+++	+	+	+
Petechiae vesicae urinariae		+	+	++	++	+	+
Petechiae tunicae serosae ventriculi		+++	-	-	-	-	-
Petechiae tunicae mucosae ventriculi		+++	+++	+++	+++	+++	+++
Petechiae tunicae mucosae intestini		-	+++	-	+++	-	+++
Petechiae tunicae mucosae caeci		+++	+++	-	+++	++	+++
Petechiae tunicae mucosae coli		+++	+++	+++	+++	++	+++
Haemorrhagiae mucosae recti		+++	+++	+++	+++	++	-

Table 5.
Gross pathological findings in piglets from the group C (aged 44 days)

Pathomorphological changes	No.	i/m challenge		Contact infection			
		III/3	III/4	III/1	III/2	III/5	III/6
Haemorrhagiae subcutaneae		+++	.*	-	-	-	-
Petechiae epiglottidis		+++	-	-	-	-	-
Tonsillitis diphtheroides necroticans		+	+++	+++	+++	++	+++
Lymphadenitis haemorrhagica acuta		+++	+++	++	+++	+	++
Petechiae thymi		+++	+++	++	+++	+++	+++
Infarctus haemorrhagicus lienis		+++	+++	+++	+++	+++	++
Ecchymosis pulmonalis		+++	-	-	-	++	-
Petechiae epicardii		+++	-	++	-	-	-
Petechiae endocardii		+++	-	-	-	-	-
Petechiae corticis renalis		+++	+++	+	+	++	+
Petechiae vesicae urinariae		+++	+	++	+++	++	+

Haemorrhagiae hepatis	-	++	+	-	++	-
Petechiae et ecchymoses cholecistae	+++	+++	-	-	+	-
Petechiae tunicae mucosae ventriculi	+++	-	+++	+++	+++	-
Petechiae tunicae mucosae intestini	+++	+++	-	-	-	+++
Petechiae tunicae mucosae caeci	+++	+++	+++	+++	++	+++
Typhlitis diptheroidea necrotica	-	-	-	-	+++	+
Petechiae tunicae mucosae coli	+++	-	+++	+++	-	+++
Colitis diptheroides diffusa et follicularis	-	++	-	-	+++	-
Proctitis necrotica et follicularis	-	-	-	-	+++	-
Haemorrhagiae mucosae recti	+++	+++	+++	+++	++	-

*- not found; + mild; ++ moderate; +++ severe

In contactly infected piglets that succumbed (total of 13), gross examination of the organs showed multiple petechial haemorrhages to a variable extent on the serosal membranes, thymus, parenchyma of kidneys, urinary bladder and throughout the whole gut. The body lymph nodes in all the animals were severely swollen with petechial haemorrhages, predominantly in the cortex. Pathomorphological examination indicated petechial haemorrhages in most organ systems (hemorrhagic diathesis), that are considered to be important characteristics of CSF (Trautwein, 1988; Dahle and Liess 1992). The post mortem findings were consistent to the classical lesions of the acute to subacute course of the disease (Trautwein, 1988).

Table 6.

Gross pathological findings in piglets from the D group (aged 54 days)

Pathomorphological changes	No.	i/m challenge		Contact infection			
		IV/3	IV/5	IV/1	IV/2	IV/4	IV/6
Petechiae epiglottidis	+++	-	+++	-	-	-	-
Tonsillitis diptheroides necroticans	+++	-	+++	-	-	-	-
Lymphadenitis simplex acuta	-	++	-	++	++	-	-
Lymphadenitis haemorrhagica acuta	+++	-	+++	-	-	+++	-
Petechiae thymi	+++	-	+++	-	-	+++	-
Infarctus haemorrhagicus lienis	+++	++	+++	++	++	+++	-
Ecchymosis pulmonalis	+++	-	+	-	-	+++	-
Petechiae epicardii	+	-	+++	-	-	+++	-
Petechiae endocardii	-	-	+++	+	-	-	-
Petechiae corticis renalis	++	-	+++	-	+	++	-
Petechiae vesicae urinariae	+	+++	+++	++	-	+++	-
Petechiae cholecistae	-	-	-	-	-	+++	-
Petechiae tunicae mucosae ventriculi	+++	-	+++	-	-	+++	-
Gastritis diptheroides circumscripta	-	-	+++	-	-	-	-
Petechiae tunicae serosae intestini	-	-	+++	-	-	-	-
Petechiae tunicae mucosae intestini	+++	-	+++	-	-	+++	-
Petechiae tunicae mucosae caeci	-	-	-	-	-	+++	-
Typhlitis diptheroidea necrotica	-	-	+++	-	-	-	-
Petechiae tunicae mucosae coli	+++	-	+++	-	-	+++	-
Colitis diptheroides diffusa et follicularis	-	-	++	-	-	-	-
Haemorrhagiae mucosae recti	+++	-	+++	-	-	+++	-

- not found; + mild; ++ moderate; +++ severe

Although, before euthanising in 3 survived horizontally infected piglets no clinical signs typical for CSF were observed, pathomorphological lesions (infarction of spleen, oedema of lymph nodes, petechial haemorrhages on urinary bladder and gall bladder) suggest on CSF infection. In addition, in piglet No. 1/3 severe fibrinous and diphteroid inflammatory reaction were present in the digestive tract. It should be mentioned that with additional laboratory investigations by RT-PCR the CSFV was detected in tissues of survived piglets. The clinical protection in pigs from CSF is not the primary aim, and it might even be contraindicated since it can mask a present infection in an animal that may be a source of secondary outbreak of the disease (Dewulf, 2002). According to Terpstra (1988), offspring from vaccinated sows may experience a subclinical infection even with a strain of high virulence. Because subclinically infected pigs may live for months and shed the virus, they represent the major impediment to control of epizootics (Kleiboeker, 2002).

Conclusions

The results of our experiment indicate that infection of piglets is possible despite the fact that they originate from sows vaccinated with C-strain of CSFV. The passive immunity did not protected the piglets from experimental infection with virulent CSFV, but it did influenced on the severity of clinical signs and the frequency of some pathomorphological changes. The observed differences were mostly connected to the survived piglets.

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