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**Classical swine fever virus strain “C” protects the offspring by oral  
immunisation of pregnant sows**

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## 1 **Abstract**

2 The aim of this study was to evaluate if oral immunisation of wild sows protects  
3 the fetuses from transplacental infection. Two experiments were carried out with  
4 gilts vaccinated orally with C-strain virus approximately five weeks after  
5 insemination. They were challenged at mid-gestation with highly virulent classical  
6 swine fever virus (CSFV) or moderately virulent field virus. The results revealed that  
7 oral vaccination has no negative impact on the pregnancy, and all vaccinated sows  
8 developed neutralising antibodies. After infection no symptoms were detected in the  
9 six vaccinated-infected sows. Challenge virus could neither be found in blood, nasal  
10 and fecal swabs or saliva nor in organs sampled at necropsy. Likewise, all fetuses  
11 originating from vaccinated sows were virologically and serologically negative. In  
12 contrast, the controls developed a short viremia and as a result of the transplacental  
13 infection all fetuses were CSFV positive. In addition, 22 serologically positive wild  
14 sows of an endemically infected area, where oral vaccination had also been carried  
15 out, and their offspring were free from CSFV or viral RNA.

16 Our results confirm that oral immunisation of pregnant wild sows with C-strain  
17 vaccine may protect the fetuses against CSF.

18

19 *Keywords:* wild boar, classical swine fever, oral immunisation, C-strain vaccine,  
20 transplacental infection

21

## 22 **1. Introduction**

23 Classical swine fever (CSF) is one of the highly contagious, notifiable and  
24 economically important infectious viral diseases affecting domestic pigs and wild  
25 boar. During the past decades the disease has been present in Europe not only in  
26 domestic pig farms but also in wild boar populations. As CSF eradication in wild

1 boar is very problematical, oral immunisation has been studied as an additional  
2 control measure, first in Germany (Kaden et al., 2000, 2002, 2003a) and later on also  
3 in Luxembourg. For the past few years, oral vaccination has also been part of the  
4 CSF control measures in wild boar in France, in the Slovak Republic and in Bulgaria.

5 After the adoption of oral vaccination by Council Directive 2001/89/EC on  
6 Community measures for the control of CSF (Anonymous, 2001), vaccination may  
7 now be one element of the eradication measures in wild boar in the European Union  
8 (EU). Emergency vaccination of wild boar should be performed in situations, where  
9 an extensive spread of CSF virus (CSFV) is expected and the European Commission  
10 has acknowledged the submitted vaccination plan.

11 In the past, the efficacy of oral vaccination against CSF based on C-strain live  
12 vaccine (Riemser Schweinepestoralvakzine) was established in laboratory and field  
13 studies (Kaden et al., 2000, 2001, 2002, 2003a,b, 2005a,b; Kaden and Lange, 2001;  
14 Kern and Lahrmann, 2001). For evaluation of the efficacy of CSF vaccines it is very  
15 important to study not only the prevention of the horizontal transmission of CSFV  
16 from vaccinated pigs to contact animals but also from vaccinated pregnant sows to  
17 their offspring. As demonstrated by several groups (Trautwein et al., 1977; Van  
18 Oirschot and Terpstra, 1977; Van Oirschot, 1979; Frey et al., 1980; Meyer et al.,  
19 1980; Richter-Reichhelm et al., 1980; von Benten et al., 1980 and others),  
20 transplacental transmission of low virulent CSFV causes the development of  
21 persistently infected piglets. In principle, these animals are one of the causes for  
22 perpetuation of the disease in the population (Depner et al., 1995; Kaden 1998;  
23 Kaden et al., 2005b). If such a scenario also occurs in vaccinated pregnant wild sows,  
24 oral immunisation is an unsuitable tool for eradication of CSF in wild boar.  
25 Therefore, the goal of our studies was to investigate if oral vaccination with C-strain

1 vaccine protects the offspring from virus infection in utero when gilts are challenged  
2 with CSFV at mid-gestation.

3

## 4 **2. Materials and methods**

5

### 6 **2.1. Vaccine and vaccination**

7 For oral vaccination the conventional C-strain bait vaccine (Riemser  
8 Schweinepestoralvakzine) manufactured by the RIEMSER Arzneimittel AG  
9 (Greifswald-Insel Riems, Germany) was used (Kaden et al., 2000, 2004a). Two  
10 batches were administered, batch no. 101096 ( $10^{4.75}$  TCID<sub>50</sub>/ml) in experiment 1 and  
11 batch no. 540804 ( $10^{5.5}$  TCID<sub>50</sub>/ml) in experiment 2. To guarantee a complete uptake  
12 of the vaccine, the sows were vaccinated orally with the content of one vaccine bait  
13 (1.6-1.8 ml vaccine) by means of a syringe.

14

### 15 **2.2. Challenge virus**

16 In experiment 1, the pregnant sows were challenged intranasally with 200 TCID<sub>50</sub>  
17 whole blood (1 ml into each nostril) of the highly virulent CSFV strain “Koslov”. In  
18 experiment 2, the moderately virulent field isolate “11722-WIL” (subtype 2.3  
19 Rostock) derived from a wild boar shot in Rhineland-Palatinate was applied  
20 intranasally (1 ml,  $10^{5.5}$  TCID<sub>50</sub>). This challenge virus had undergone two passages  
21 in PK-15 cell cultures before use.

22

### 23 **2.3. Animals and housing**

24 For the animal experiments eight gilts (crossbreeding between Danish Landrace  
25 and German Edelschwein -F1- and Leicoma or German Landrace thoroughbred)  
26 obtained from a pig breeding farm in Mecklenburg-Western Pomerania were used.

1 Oestrus synchronisation and insemination were carried out at the pig holding. The  
2 animals were kept conventionally during the vaccination period. At the time of  
3 challenge, gilts were housed in separate pens and stables under isolated and high  
4 containment conditions.

5 Prior to oral vaccination, the animals were tested negative for antibodies to  
6 pestiviruses using virus neutralisation tests (VNT).

7

#### 8 ***2.4. Experimental design of the animal experiments***

9 In experiment 1, two gilts were vaccinated orally with the content of one vaccine  
10 dose (one bait) 35 days after insemination. On day 75 of pregnancy, 40 days after  
11 vaccination, the sows were challenged with the CSFV strain “Koslov”. Both sows  
12 farrowed between days 103 and 106 post inseminationem. In experiment 2, four gilts  
13 were immunised per os 36 days after insemination and were challenged 34 days later  
14 with the field isolate “11722-WIL”. On the same day of pregnancy two unvaccinated  
15 control sows were also infected. 35 days post infectionem (dpi), that is on day 105 of  
16 gestation, the fetuses from all sows were obtained by hysterectomy.

17 The sows of both experiments were observed for clinical signs post vaccinationem  
18 (pv) and pi. For serological investigations, blood samples were taken before  
19 vaccination, four weeks pv, on the day of farrowing and eight weeks later (day of  
20 necropsy) in experiment 1. Whole blood and nasal swabs were collected for detection  
21 of CSFV on the day of challenge as well as 5 and 7 days pi. Immediately after birth  
22 the piglets were tested for antibodies and virus. Subsequently, the offspring was used  
23 in a further study. In experiment 2, serum samples were collected on the days of  
24 vaccination and challenge (34 days pv) and at the time point of hysterectomy. For  
25 detection of CSFV pi all challenged gilts were bled on the day of challenge and 4, 7

1 as well as 9 days pi. At the same time nasal and fecal swabs as well as saliva were  
2 collected for detection of challenge virus.

3 Number and viability of fetuses were recorded and necropsy was carried out on all  
4 sows and their offspring. To detect CSFV in fetuses tonsils, spleen, kidney and  
5 mandibular as well as mesenteric lymph nodes were collected.

6

### 7 ***2.5. Field analysis on the transplacental infection in a vaccination zone***

8 Twenty-two serologically positive wild sows of different age and stage of  
9 pregnancy as well as their offspring derived from an endemically infected area  
10 (northern part of Mecklenburg-Western Pomerania), where oral immunisation had  
11 also been carried out, were investigated for CSFV over a period of three and a half  
12 years. Eighteen wild sows were  $\geq 1$  year old, three  $\leq 1$  year old. In total, 104 embryos  
13 and fetuses were collected. The adults and their offspring were investigated for  
14 CSFV and viral RNA.

15

### 16 ***2.6. Virus isolation***

17 Re-isolation of challenge virus from buffy coat, nasal and fecal swabs, from saliva  
18 and organ samples was carried out on confluent PK-15 cell cultures as described by  
19 Kaden et al. (2004b). For detection of CSFV in cell cultures, a mouse anti-CSFV-E2  
20 monoclonal antibody (mAb  $\alpha$  18, kindly provided by E. Weiland, Tübingen, or mAb  
21 HCTC 50/2/1, kindly provided by B. Köllner, Insel Riems) was used in combination  
22 with a horseradish peroxidase-conjugated anti-mouse antibody (Pierce, Rockford,  
23 USA).

24

### 25 ***2.7. Reverse transcription polymerase chain reaction (RT-PCR)***



1 RT-PCR was carried out directly from organ samples, whole blood, squeezed  
2 nasal, fecal and saliva swabs as well as from organ suspensions obtained from  
3 embryos. RNA was extracted from organs and organ suspensions by means of  
4 QIAamp Tissue Kit, from whole blood using QIAamp RNA Blood Mini Kit  
5 according to the manufacturer's instructions (QIAGEN GmbH, Hilden, Germany).  
6 RT-PCR was performed with commercially synthesised primers (MWG BIOTECH  
7 AG, Ebersberg, Germany) from the E<sup>rns</sup> region of CSFV followed by a nested PCR  
8 described by Liu et al. (1991) in experiment 1, and later with the primers NTR 51  
9 (anti-sense) and NTR 53 (sense) corresponding to the 5' non-translated region as  
10 described by Kaden et al. (2004a). The PCR amplified products were analysed by  
11 agarose gel electrophoresis with visualisation by UV transillumination using the gel  
12 documentation system "ImageDoc" (Herolab, Wiesloch, Germany). Each RT-PCR  
13 run included two negative controls (DEPC-treated H<sub>2</sub>O, negative tissue sample) and  
14 a positive control (CSFV positive tissue sample).

15

## 16 **2.8. Antibody detection**

17 Sera of the field samples were tested for antibodies using the commercial ELISA  
18 "CHEKIT CSF-Sero" according to the manufacturer's instructions (Dr. Bommeli  
19 AG, Liebefeld-Bern, Switzerland, Intervet BV, The Netherlands). In contrast, sera  
20 derived from animal experiments were checked by means of VNT as described by  
21 Kaden et al. (2001).

22

## 23 **2.9. Determination of the leukocyte counts**

24 Heparinised blood was collected 0, 5 and 7 dpi (experiment 1) or 0, 4, 7, 9 and 36  
25 dpi (experiment 2) for determination of the leukocyte counts using the automatic

1 analysers Technicon H1, Bayer Diagnostics, Munich, or Cell Dyn 3700 (Abbot  
2 GmbH, Wiesbaden, Germany).

3

### 4 **3. Results**

5

#### 6 ***3.1. Animal experiments***

7

##### 8 *Clinical, serological and virological findings in sows after vaccination*

9 No clinical signs were observed in the vaccinated gilts of both experiments except  
10 for one animal of the second group, which showed lameness and partial anorexia.  
11 The pregnancy was not disturbed in any of the sows. The body temperatures  
12 remained normal in all vaccinated animals as well as in the controls.

13 Neither CSFV-specific antibodies nor virus were detected in the blood of the  
14 animals on the day of vaccination. On the day of challenge, neutralising antibodies  
15 were diagnosed in all orally vaccinated gilts (Table 1).

16

##### 17 *Clinical and haematological findings after infection*

18 Whereas none of the vaccinated gilts reacted with an increased body temperature  
19 ( $>40^{\circ}\text{C}$ ) pi, a transient slight increase of the body temperatures was already recorded  
20 between days 5 and 11 pi in the control sows (data not shown). None of the  
21 vaccinated gilts of both experiments developed clinical signs as result of CSFV  
22 challenge.

23 Both vaccinated sows of experiment 1 as well as three out of four vaccinated gilts  
24 of experiment 2 showed constant leukocyte counts after infection. A slight  
25 leukopenia ( $<10.000$  leukocytes/ $\mu\text{l}$ ) occurred in one gilt (no. 9318) of the second  
26 experiment on day 4 pi as a result of partially clotted blood. Three days later the

1 leukocyte count was on the normal level again. On different days pi a moderate  
2 leukopenia was detected in the control gilts (data not shown).

3 Neither abortions nor other disturbances of pregnancy were observed after  
4 infection. In experiment 1, one sow (no. 2) farrowed 11 normally developed viable  
5 piglets, one dead and one macerated fetus 39 dpi. The second sow (no. 5) farrowed  
6 42 dpi. Her litter consisted of 15 animals, 11 normally developed viable piglets, two  
7 dead fetuses and one mummified fetus. The following litters were obtained by  
8 hysterectomy from the four vaccinated gilts of the second experiment: 15 fetuses  
9 (sow no. 9318), 13 fetuses (sow no. 9347), 18 fetuses (sow no. 9441), 8 fetuses (sow  
10 no. 9492). The control sows of this experiment contained 10 fetuses (sow no. 9330)  
11 and 13 fetuses (sow no. 9427). Further details are described in Table 2.

12

### 13 *Serological and virological findings pi*

14 Both gilts of experiment 1 as well as the vaccinated-infected sows of experiment 2  
15 showed increased neutralising antibody titres beginning 4 or 9 dpi. The controls also  
16 became seropositive (Table 1).

17 Whereas none of the vaccinated gilts developed viremia after infection, the  
18 controls were diagnosed viremic for at least two days (Table 3). Blood samples and  
19 all swabs taken from vaccinated animals pi as well as the tissues sampled at necropsy  
20 were negative for CSFV and viral RNA (Tables 2 and 3). The controls were also  
21 virologically negative at the end of the study. In contrast, all fetuses of both controls  
22 were positive for CSFV or CSFV-RNA (Table 2), whereas the fetuses derived from  
23 the vaccinated gilts were virologically negative.

24

### 25 *3.2 Analysis of field samples*

1 The investigated embryos and fetuses of the wild sows were diagnosed with a  
2 crown-rump length of 1.8 to 27 cm (Table 4). All 22 sows and their fetuses (104)  
3 were virologically negative independently of the age of the wild sows and the stage  
4 of pregnancy.

5

#### 6 **4. Discussion**

7 After CSF has been confirmed, the main goal of all control measures in wild boar  
8 is to interrupt the chain of infection by a reduction of the reproduction rate ( $R_0$ ) <1  
9 (Guberti et al., 1998; Artois et al., 2002; Kaden et al., 2002). Oral immunisation of  
10 wild boar against CSF may support the eradication (Kaden et al., 2000, 2002,  
11 2003a,b; Kern and Lahrmann, 2000). Despite oral vaccination, the fading out of the  
12 disease took a long time in Mecklenburg-Western Pomerania and in Brandenburg  
13 (Kaden et al., 2002). The causes may be numerous and diverse. Besides the  
14 ineffectiveness of oral immunisation persistent infections may also be the cause for a  
15 prolonged eradication period. One source for the persistence of CSF in wild boar  
16 populations might be persistently infected piglets as a result of transplacental  
17 transmission of CSFV from susceptible pregnant wild sows to their offspring  
18 (Depner et al., 1995; Kaden, 1998; Artois et al., 2002). Although it can be assumed  
19 that this transmission route does not play a key role in the spread of virus among  
20 wild boar (Kaden et al., 2005b), it is possible that individual pregnant wild sows  
21 transmit CSFV to their progeny by this route. As it is difficult to obtain gravidity in  
22 wild sows by artificial insemination or to determine the stage of gestation in wild  
23 sows after natural covering we studied the transplacental transmission of CSFV in  
24 pregnant domestic gilts which had been vaccinated orally before, and also evaluated  
25 field samples from pregnant wild sows and their offspring. These wild sows were  
26 shot in an endemically infected area, where vaccination had also been carried out.

1 Our laboratory experiments revealed that orally vaccinated gilts neither showed an  
2 increase of body temperature nor other clinical signs and that the pregnancy was not  
3 disturbed by C-strain virus. This demonstrates the safety of the C-strain oral vaccine  
4 for gilts. This result complies with the findings established in the field after oral  
5 vaccination of wild boar (Kaden et al., 2000, 2002, 2003b) or after parenteral  
6 immunisation (Tesmer et al., 1973) or after oral immunisation of non-pregnant pigs  
7 and wild boar with C-strain vaccine (Chenut et al., 1999; Kaden et al., 2001). As  
8 expected, all vaccinated animals seroconverted pv. Whereas one gilt developed only  
9 a low antibody titre 28 dpv, all others had antibody titres between 90 and 641 ND<sub>50</sub>.  
10 Similar antibody titres were also detected after vaccination of weaner pigs and wild  
11 boar (Kaden and Lange, 2001; Kaden et al., 2004b).

12 The infection of the vaccinated pregnant gilts with highly (experiment 1) or  
13 moderately virulent (experiment 2) CSFV at mid-gestation did not result in clinical  
14 signs in any of the animals. Whereas both controls showed distinct leukopenia the  
15 leukocyte counts of the vaccinated and infected gilts were inconspicuous. Challenge  
16 virus could neither be detected in blood, nasal and fecal swabs or saliva nor in organ  
17 samples of vaccinated-infected sows at necropsy. In contrast, the control gilts  
18 became viremic. However, neither CSFV nor viral RNA was detected pi in the  
19 swabs. This result was not expected. It is possibly related with the time points of  
20 sampling, as sampling was not carried out daily. At the end of the experiment, the  
21 control sows were also investigated virologically negative, i.e. the controls survived  
22 the infection without virus persistence and developed neutralising antibody titres  
23 (>100 ND<sub>50</sub>). The vaccinated gilts showed a distinct booster reaction pi except for  
24 two animals which had relatively high antibody titres (641 ND<sub>50</sub>) on the day of  
25 challenge.

1 The oral vaccination of pregnant gilts with C-strain vaccine successfully protected  
2 all fetuses of these sows from infections with the highly virulent CSFV strain  
3 “Koslov” or the moderately virulent field isolate. Whereas in the litters of the control  
4 gilts infected with the field isolate mummies and dead fetuses were found at  
5 necropsy (Fig. 1) in addition to alive piglets which all were diagnosed CSFV  
6 positive, neither virus nor RNA was found in any of the samples of the fetuses of the  
7 vaccinated-infected sows taken at the time of hysterectomy. Dead fetuses were  
8 detected in three vaccinated sows which had litters consisting of 12 to 18 fetuses.  
9 One fetus derived from a sow with 15 animals was mummified. This death rate  
10 represents a physiological mortality rate in large litters. Normally, one to two dead  
11 fetuses can be expected in such litters (Rommel, 1987). Our results in vaccinated-  
12 infected gilts indicate that a single dose of the C-strain bait vaccine prevents  
13 transplacental transmission of CSFV in mid-gestation. Vertical transmission of  
14 challenge virus in pregnant sows vaccinated with C-strain has not been reported yet.  
15 Tesmer et al. (1973) neither found challenge virus in the blood of piglets derived  
16 from pregnant sows which were vaccinated parenterally with the vaccine strain “C”,  
17 which also was the basis for the oral vaccine used in our animal studies. In contrast,  
18 E2 subunit vaccines did not prevent virus transmission in utero. They were only able  
19 to reduce transplacental CSFV transmission (Ahrens et al., 2000; De Smit et al.,  
20 2000; Depner et al., 2001).

21 Our investigations of pregnant wild sows and their unborn offspring derived from  
22 an endemically infected area, where oral immunisation had also been carried out,  
23 demonstrated that all tested animals (22 wild sows and 104 fetuses) were  
24 virologically negative. These antibody-bearing sows may have seroconverted as a  
25 result of vaccination or after survival of an infection or both. As the pregnant animals  
26 were hunted within a population infected endemically with CSFV we assume that

1 also vaccinated and infected sows were involved in our investigations. These field  
2 data confirm the laboratory findings regarding the absence of transplacental  
3 transmission of CSFV in sows vaccinated before.

4

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6

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9

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1 Table 1

2 Neutralising antibody titres after vaccination and infection

Experi- ment	Number of the sow	Neutralising antibody titres (ND <sub>50</sub> )							
		dpv		dpi					
		0	28	0	4	7	9	36	39/42
1	2	0	90	90	nt	nt	256	nt	512
	5	0	128	128	nt	nt	326	nt	512
2	9318	0	113	113	226	226	906	906	
	9347	0	641	641	453	641	906	641	
	9441	0	641	641	641	641	906	906	
	9462	0	20	20	320	453	1283	641	
	9330*	0	0	0	0	0	0	226	
	9427*	0	0	0	0	0	0	113	

3

4 Comments: \* – non-vaccinated controls

5 nt – not tested

1 Table 2

2 Virological findings in blood and organ samples of fetuses

Sample	Number of CSFV/RNA positive fetuses per sow							
	Vaccinated sows (offspring)						Control sows	
	Sow 2 (11 alive, 1 dead)	Sow 5 (12 alive, 2 dead, 1 mummified)	Sow 9318 (15 alive)	Sow 9347 (13 alive)	Sow 9441 (17 alive, 1 dead)	Sow 9462 (8 alive)	Sow 9330 (8 alive, 2 dead)	Sow 9427 (8 alive, 4 dead, 1 mummified)
Buffy coat	0/11	0/12	0/15	0/13	0/17	0/8	8/8	8/8
Tonsils	0/12	0/14	0/15	0/13	0/18	0/8	10/10	13/13
Spleen	0/12	0/14	0/15	0/13	0/18	0/8	10/10	13/13
Mandibular lymph nodes	0/12	0/14	0/15	0/13	0/18	0/8	10/10	13/13
Mesenteric lymph nodes	0/12	0/14	0/15	0/13	0/18	0/8	10/10	13/13
Kidney	0/12	0/14	0/15	0/13	0/18	0/8	10/10	13/13

3

1 Table 3

2 Virological findings in two control sows after challenge

d pi	CSFV or RNA positive sample per animal (sow no. 9339 / sow no. 9427)					
	Buffy coat (VI)	Whole blood (RT-PCR)	Nasal excretion	Saliva	Feces <sup>1</sup>	Organs <sup>2</sup>
0	-/-	-/-	-/-	-/-	-/-	
4	-/-	+/+	-/-	-/-	-/-	
7	+/-	+/-	-/-	-/-	-/-	
9	-/+	-/-	-/-	-/-	-/-	
36	-/-	-/-	-/-	-/-	-/-	-/-

3

4 Comments: <sup>1</sup>only virus isolation

5 <sup>2</sup>virus isolation and RT-PCR

6

7

## 1 Table 4

## 2 Results of the investigated wild sows and their progeny from the field study

Wild sows		Progeny (embryos/fetuses)		Number of virologically positive animals per group	
Age	Number	Number of individuals	Crown-rump length (cm, from...to)	Wild sows	Embryos or fetuses
>1 year old	18	87	1.8 – 27	0/18	0/87
≤1 year old	3	12	1.9 – 27	0/3	0/12
Without data	1	5	2.8 – 25	0/1	0/5
Absolute	22	104	1.8 – 27	0/22	0/104

- 1 Figure 1
- 2 Offspring of control-sows (a: no. 9427; b, c: no. 9330)
- 3 a: One mummified fetus and 4 dead fetuses with subcutaneous edema and
- 4 progressive autolysis within a litter of 13 relatively equal piglets
- 5 b: Note cutaneous petechiation of the upper fetus
- 6 c: Diffuse subcutaneous edema and autolysis
- 7

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1 Figure 1



2

3



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