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**An update on safety studies on the attenuated
“RIEMSER[®] Schweinepestoralvakzine”
for vaccination of wild boar against classical swine fever**

Volker Kaden^{1*}, Elke Lange¹, Heike Küster², Thomas Müller³, Bodo Lange²

¹*Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Infectology, Südufer 10, 17493 Greifswald-Insel Riems, Germany;* ²*RIEMSER Arzneimittel AG, An der Wiek 7, 17493 Greifswald-Insel Riems, Germany;* ³*Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Epidemiology, Seestraße 55, 16868 Wusterhausen, Germany*

*Corresponding author: Tel.: +49 38351 7211; fax: +49 38351 7226.

E-mail address: volker.kaden@fli.bund.de

Abstract

The RIEMSER[®] Schweinepestoralvakzine is an attenuated vaccine for oral vaccination of wild boar against classical swine fever (CSF). The safety of this licensed bait vaccine which is based on the CSF virus (CSFV) strain “C” was investigated in 8 animal species, e.g. weaner pigs ($n = 111$), wild boar ($n = 11$), ruminants (cattle, goats and sheep, $n = 11$), foxes ($n = 5$), rabbits ($n = 12$), and mice ($n = 10$). Animals were vaccinated either with a single vaccine dose containing at least $10^{4.5}$ TCID₅₀, or with overdoses, i.e. the tenfold dose, or they were subjected to repeated application schemes. During the entire observation period none of the animals which were given the vaccine virus showed clinical signs, with the exception of rabbits. These reacted to the vaccination with fever. Orally vaccinated pigs did not transmit vaccine virus to susceptible contact animals (sentinels). In none of the species examined neither vaccine virus nor viral RNA could be detected in blood after vaccination. In one wild boar viral RNA could be established in the tonsil 21 days post-vaccination (dpv); all other organ samples tested virologically negative. Up to 77.5% of the pigs and wild boar developed virus neutralising antibodies (VNA) already 14 dpv. The mean VNA titres observed in the vaccination groups seemed to depend rather on individual factors than on the administered virus dose (virus titre per dose) or the vaccination scheme. These results are comparable with findings obtained during oral vaccination campaigns in wild boar and after parenteral vaccination with this C-strain virus. From the results presented here it can be concluded that RIEMSER[®] Schweinepestoralvakzine is safe for target and non-target species.

Keywords: classical swine fever, C-strain oral vaccine, safety, target and non-target species

1. Introduction

Classical swine fever (CSF) is a highly contagious viral disease of domestic pigs and wild boar which occurs especially in areas with a high population density. Independently of the currently auspicious epidemiological CSF situation in Central and Western Europe, the disease occurs worldwide. In the 1970s and 1980s, prophylactic and emergency vaccinations with safe and effective live vaccines played an essential role in the eradication of CSF in many parts of the world, including Europe. Since 1990, control and eradication of CSF in the European Union (EU) have been based on a non-vaccination and stamping-out strategy (Laddomada, 2000; Moennig, 2000). In compliance with Council Directive 2001/89/EC (Anonymous, 2001) routine vaccinations of domestic pigs against CSF are prohibited in the EU, however, emergency vaccination can be permitted if an extensive spread of CSF virus (CSFV) with subsequent high economic losses is to be expected. In contrast, vaccines against CSF are still widely used in domestic pigs in Asia and South America.

The control of CSF in wild boar populations is much more complicated than in domestic pigs, especially in regions with a high population density of wild boar. In such regions, the disease can persist over a relatively long period of time, as observed in Germany in the 1990s. Currently, several strategies for the control of CSF in wild boar are used. One of them includes the oral vaccination of wild boar in the frame of emergency vaccination combined with a reduction of the population density. This strategy was used in Germany at first (Kaden et al., 2000). According to Council Directive 2001/89/EC (Anonymous, 2001) oral emergency vaccination of wild boar is permitted if the above-mentioned conditions are fulfilled and the vaccination plan has been approved by the European Commission. Disease control via oral vaccination, however, is labour-intensive and requires safe and effective live vaccines (Chenut et al., 1999; Kaden et al., 2000, 2002) in a formulation suitable for wild boar of all

age classes (Kaden et al., 2002; Brauer et al., 2006). A better alternative to the currently existing modified live virus vaccines would be a safe and effective DIVA vaccine which permits to differentiate vaccinated from infected animals. Therefore, research on the development of live DIVA vaccines has been intensified in the past few years and several vaccine candidates for oral vaccination have been investigated (Koenig et al., 2007b; Wehrle et al., 2007; Kaden et al., 2008, Leifer et al., 2009). However, licensed CSF marker vaccines suitable for oral application in wildlife are not available yet.

The efficacy of the RIEMSER[®] Schweinepestoralvakzine, a conventional CSF live vaccine based on the “C” strain was demonstrated in various studies (Kaden et al., 2000, 2002, 2003; Kern and Lahrmann, 2000; Von Rüden et al., 2008) and resulted in the licensing of this bait vaccine in Germany in 2005. So far, there are no reports on experimental safety studies with this vaccine. Therefore, this paper intends to provide information on safety studies of the German CSF oral vaccine (RIEMSER[®] Schweinepestoralvakzine) including virological and serological investigations in line with selected safety experiments carried out in past years. As there are no guidelines for the licensing of CSF oral vaccines in the European Union, safety studies were carried out according to the European Pharmacopoeia 5.0 “Swine-fever vaccine (live), classical, freeze-dried” (Anonymous, 2005), which was in force during the study period, and the national guideline for licensing and testing of batches of CSF live vaccines (edited by the former Federal Research Centre for Virus Diseases of Animals, Tübingen, 1985), both destined for parenteral administration. Furthermore, the specific conditions of oral vaccination in the field were taken into account.

2. Materials and methods

2.1. Vaccine

The CSF oral vaccine used (RIEMSER® Schweinepestoralvakzine, RIEMSER Arzneimittel AG, Greifswald-Insel Riems, Germany) is based on the lapinised and attenuated C (*Chinese*) strain and was provided by the All-Russian State Centre for Quality and Standardisation of Veterinary Drugs and Food (Moscow, Russia) in 1977. After several hundred serial passages in rabbits, the vaccine strain was adapted to primary porcine foetal kidney cells first and subsequently to permanent porcine kidney cells, which later on were also used for propagation of the commercial oral vaccine virus. This vaccine is filled into blisters (about 1.6 ml vaccine per blister), which are covered with a cereal-based bait matrix (size of the bait: 4 x 4 x 1.6 cm). A total of 12 different vaccine batches were used for the individual experiments. The vaccine dose contained at least $10^{4.5}$ tissue culture infection doses₅₀ (TCID₅₀).

2.2. Animals

For the safety studies 111 weaner pigs (87 vaccinated and 24 sentinel animals) aged 6-7 weeks, 11 wild boars aged 3.5-11 months, three bovines aged 1-2 years (breed German Simmental, Holstein-Friesian), three goats of mixed sex aged 6-30 months (German Improved White breed), 5 six-month-old sheep of mixed sex (German Improved Land breed), 12 rabbits (breed: Russians, mixed sex, 6 months old), 10 white mice (3 months old), and 5 young red foxes (*Lupus lupus*) at the age of 8 months were used. Whereas wild boar, goats, rabbits and mice were derived from our own breeding unit, domestic pigs, cattle, sheep and foxes were purchased from commercial farms in the federal states of Mecklenburg-Western

Pomerania and Saxony. Prior to the experiments the animals were tested negative for antibodies to pestiviruses using virus neutralisation test (VNT). All animals were monitored daily for clinical signs. Rectal temperature was also taken daily in all animals except for wild boar, foxes and mice. Body weight was partially monitored (see Table 1). At the end of the experiments the animals were either euthanised for necropsy or re-used for other studies, e.g. for efficacy testing of vaccine batches. Generally, animals were randomized into the individual groups. All handling and invasive procedures were conducted in compliance with the German Animal Welfare Act.

2.3. Experimental design

2.3.1. Safety studies in domestic pigs and wild boar

2.3.1.1 Safety of one vaccine dose

Eight wild boars and 3 groups of 5 weaner pigs each were held in separate pens and vaccinated orally with one vaccine dose (equivalent to 1 vaccine bait) using three different batches (nos. 220600, 230600 and 240600). One day post-vaccination (dpv), 5 unvaccinated control animals were brought into contact with domestic pigs of each vaccination group to investigate the shedding of vaccine virus by vaccinated animals. Seroconversion after vaccination was examined 14 (pigs, wild boar) and 28 dpv (wild boar and contact controls) by VNT.

2.3.1.2. Safety of one overdose of the vaccine

Forty-two weaner pigs and 3 wild boars were vaccinated orally with one overdose, i.e. 10 vaccine doses. Three groups of 4 pigs each were vaccinated with the three batches mentioned above (2.3.1.1) and brought into contact with two unvaccinated animals on day 2 after vaccination. Additionally, groups of 3 pigs each were vaccinated with 8 different batches (370203, 380203, 390303, 0560105, 0570105, 731206, 741206 and 751206), whereas three wild boars were immunised with one overdose of another batch (640206). Virus neutralising antibody titres were determined on the day of vaccination, 14 and/or 21 dpv as well as 28 dpv (only contact controls). At necropsy, EDTA blood and organs (tonsil, spleen, kidney, mandibular and mesenteric lymph nodes, lung, bone marrow) were collected from all wild boar and selected domestic pigs for detection of CSFV or viral RNA.

2.3.1.3. Safety after repeated vaccination

In one experiment 5 weaner pigs were vaccinated twice with one oral vaccine dose at an interval of 14 or 28 days using three different batches (220600, 230600 and 240600). Blood samples were taken on days 0, 14, 28, 42, 56, 84 and 112 days after the first vaccination (dpv₁) for detection of antibodies.

In another experiment, six weaner pigs each received one overdose of vaccine batch no. 741206 at an interval of four days. The vaccinated animals were kept together with three sentinels beginning one day after the second vaccination. Blood samples were taken 21 dpv₁.

2.3.2. Safety studies in cattle, goats, sheep, rabbits, foxes and mice

Cattle, goats and sheep were vaccinated orally with the 2.5-fold vaccine dose. Furthermore, one bait was offered in a feeding trough for free uptake. Blood was collected 0, 26 and 40 dpv for determination of VNA titres.

The rabbits ($n = 8$) were inoculated orally with 1.0 ml RIEMSER[®] Schweinepestoralvakzine by direct administration into the mouth cavity using a syringe. On day 1 after oral vaccination, animals were brought into contact with 4 unvaccinated conspecifics (sentinels). Blood samples were collected 0, 14, 20 and 35 dpv.

For vaccination of mice, animals were kept in separate cages and offered ad libitum 1 vaccine dose (i.e. 1.6 ml) diluted in 10 ml drinking water. The animals were bled for detection of VNA at necropsy, 21 dpv. Foxes, kept individually in catches, were offered one vaccine bait and successful bait uptake was recorded. Blood samples were collected 21 dpv for serological investigations.

2.4. Serological and virological investigation of samples

2.4.1. Antibody detection

Antibodies were detected by means of VNT as described by Kaden et al. (2001). In order to detect homologous VNA titres the C-strain vaccine virus was used instead of the CSFV strain Alfort 187.

2.4.2 Detection of vaccine virus or viral RNA

Re-isolation of the vaccine virus from blood and organ tissues was carried out on confluent permanent EFN-R cells (CCLV, RIE 86) (Kaden et al., 2004b), whereas for detection of viral RNA a real-time RT-PCR (rtRT-PCR) protocol established for routine diagnosis and validated by the National Reference Laboratory for CSF was used (Hoffmann et al., 2005). The presence of antigen of the vaccine virus in bone marrow smears was detected by an indirect immunofluorescence test (Ahrens et al., 2000) using a mouse-anti-CSFV-E2 monoclonal antibody and a polyclonal FITC-labelled goat anti-mouse antibody (DAKO Cytomation, Gilestrup, Denmark) as conjugate.

2.5. Statistical analysis

The statistical analysis was carried out with the statistics program SigmaStat 3.0 (SPSS Science Software gmb, Erkrath, Germany). The results were considered statistically significant when $p < 0.05$.

3. Results

3.1. Safety in domestic pigs and wild boar

Domestic pigs and wild boar inoculated with one vaccine dose, one overdose (10-fold vaccine dose) or/and repeated vaccination showed no clinical signs during the entire observation period. All animals showed an increase in body weight as recorded in Table 1. The increase of the mean body weight of pigs vaccinated with 10 doses (one overdose) was slightly lower than in the control group on day 28 post- vaccination, however,

these differences were not significant. In contrast, the animals vaccinated with one dose showed a significant higher increase of the mean body weight 28 dpv.

In general, the rectal temperatures of pigs vaccinated with a single dose or one overdose remained within the normal range after vaccination as shown in Fig. 1 for one overdose (three batches). A small number of piglets reacted with a slight and temporary increase in body temperature independently of the time of vaccination, but did not develop fever. No gross lesions as a result of the vaccine application were found at necropsy, neither in domestic pigs nor in wild boar. However, catarrhal pneumonia in apical lobes was detected in individual vaccinated and control piglets.

Virus isolation or detection of viral RNA in EDTA blood of vaccinated pigs showed a negative result. Only the tonsil of one wild boar turned out to be positive in rtRT-PCR 21 dpv with a Ct value of 38.4, whereas all other organ and blood samples of vaccinated pigs and wild boars scored virologically negative.

Most of the domestic pigs and wild boars showed moderate development of VNA 14 days after administration of one vaccine dose with mean VNA titres ranging between 1:24 and 1:55. In the wild boar group, VNA titres drastically increased 14 days later. Repeated vaccination at intervals of 14 and 28 days resulted in high mean VNA titres 28 dpv but there were no significant differences between both groups (Table 2). After vaccination with one overdose relatively low mean VNA titres in wild boars and in three groups of weaner pigs were detected 21 dpv, however, no correlation was observed between the virus titre of the individual vaccine batches and the induced mean antibody titres (Table 3).

All sentinels kept in contact with pigs vaccinated orally with C-strain vaccine remained healthy and did not develop antibodies against the vaccine virus.

3.2. Safety in other species

Whereas cattle accepted the vaccine baits very well, goats and sheep showed a delayed bait uptake. The bait vaccine neither induced clinical signs nor an increase in body temperature in these species (data not shown). Whereas goats and sheep did not develop antibodies against the vaccine virus, two of three bovines developed an immune response resulting in low VNA titres of 1:8 and 1:11 at 26 dpv and 1:32 and 1:181 at 40 dpv, respectively.

Vaccinated rabbits reacted with moderate fever ($40.2 \pm 0.32^\circ\text{C}$) and slight apathy as well as short anorexia during the febrile state. In contrast, sentinel rabbits neither showed clinical symptoms and fever (mean rectal temperature: $38.5 \pm 0.28^\circ\text{C}$) nor seroconversion (data not shown). Table 4 shows the seroconversion of vaccinated rabbits.

Foxes and mice remained healthy after oral administration of C-strain vaccine and did not develop neutralising antibodies (data not shown).

4. Discussion

The high safety of live-attenuated C-strain vaccines for domestic pigs has been demonstrated worldwide in many cases (Bognár and Mészáros, 1963; Bran et al., 1966; Tielen et al., 1974; Précausta et al., 1977; Beer et al., 1978; Lin and Lee, 1981; Glaner et al., 1984; Terpstra et al., 1990; Dahle and Liess, 1995 and others). These vaccines are also known to have a high efficacy in CSF control in domestic pig populations (summarised by Aynaud, 1988; Van Oirschot, 2003). Although oral vaccination of wild boar with the C-strain vaccine (RIEMSER[®] Schweinepestoralvakzine)

has been carried out for almost 15 years without any problems in Germany (Kaden et al., 2000, 2002, 2006) and other European countries there were concerns with regard to the safety of this vaccine for wild boar and other wildlife species.

Safety studies described here clearly demonstrate that this vaccine is safe for domestic pigs and wild boar as no effect on the general health status or the growth of vaccinated animals was seen. However, as expected pigs with a higher initial body weight showed a more considerable weight gain 28 dpv than animals with a lower initial body weight. Also, no batch-related influence on the development of the body weight was observed. The absence of virus shedding after parenteral vaccination of the C-strain vaccine had already been proven (Glaner et al., 1984; Dahle and Liess, 1995; Koenig et al., 2007). Our study provides further evidence that the vaccine virus is not shed in secretions and excretions of vaccinated animals since none of the sentinel pigs kept in contact with vaccinated animals developed antibodies in any of the experiments conducted. These results confirm findings by Chenut et al. (1999) and previous own experiences (Kaden et al., 2004b), according to which neither virus nor viral RNA was detected in faeces and nasal swabs after oral application. Interestingly, in our study vaccine virus was only found 21 dpv by means of rtRT-PCR in the tonsil of one orally vaccinated animal but not in blood. This may indicate that the C-strain virus only replicates at a low level after oral vaccination. However, in previous studies vaccine virus could be detected in different organs, mainly in tonsils, spleen and Ln. mandibularis (Kaden et al., 2004b). A recent parenteral vaccination study suggests that the C-strain virus may persist for relatively long periods in individual domestic pigs, as proven by the detection of viral RNA in blood and tonsils up to 14 dpv and 42 dpv, respectively, whereas virus isolation succeeded only up to 6 dpv (Koenig et al., 2007a). In contrast, C-strain virus and viral RNA were only detectable for 8 or 9 days after oral vaccine intake (Kaden et al., 2004b). However, viral RNA can also be detected for a relatively long time in individual orally vaccinated pigs, in experimentally vaccinated animals up to

35 dpv and in field samples derived from wild boar for up to 32 days after distribution of vaccine baits (Kaden et al., unpublished). However, the virus strain does not replicate in bone marrow as the recent study and previous investigations (Fischer et al., 1991) demonstrate. These findings indicate that attenuated C-strain virus may take a different pathway than virulent CSFV.

The seroconversion of animals vaccinated orally with one dose shows a similar course as after parenteral vaccination (Dahle and Liess, 1995), however, only 77.5% of pigs and wild boar developed antibodies 14 dpv. About 87% (34 out of 38) of the vaccinated animals turned seropositive on day 28 pv. The kinetics of VNA provides evidence that the onset of the immune response after oral vaccination of pigs with one vaccine dose is delayed compared to parenteral vaccination. The differences in the mean VNA titres observed in individual groups of pigs after oral administration of overdoses cannot be explained yet. We suppose that these differences in VNA titres are based on the individual immunological reactivity of the animals and/or incomplete vaccine uptake. The large standard deviations of VNA titres obtained in individual groups may underline this presumption. Oral vaccination of pigs at intervals of 14 or 28 days did not lead to significant differences in VNA titres. These findings support previous investigations obtained with a small wild boar group (Kaden et al., 2004a). Cattle, sheep, goats, as well as foxes and mice were used as model species for wildlife species which under field conditions might be bait competitors. None of these animals developed clinical signs and fever (rectal temperature only checked in ruminants) as a result of vaccination and bait uptake. Therefore, C-strain live vaccine also seems to be safe for these non-target species, albeit this assessment is only based on a limited number of investigated animals. As expected, orally vaccinated rabbits developed a significant increase in rectal body temperature on days 3 and/or 4 and a reduced food intake after administration of lapinised C-strain

virus; slightly later than after intravenous inoculation (Lange, unpublished). Chenut et al. (1999) also observed fever in rabbits after oral administration of C-strain virus, occurring slightly later than in our study. Most likely, the lower antigen content (10^3 pig PD₅₀) of the vaccine virus used by Chenut et al. (1999) is the reason for the delayed increase in rectal body temperature. Regarding seroconversion in non-target species, only cattle and rabbits developed neutralising antibodies after oral intake of C-strain vaccine. Foxes remained seronegative after intake of vaccine baits. This result was expected and agrees with findings of Dewulf et al. (2001) after infection of dogs, cats and rats with CSFV as these species as well as foxes are not the natural host for CSFV.

In conclusion, results presented here and experiences during oral vaccination of wild boar against CSF in the field suggest that the CSF oral vaccine (RIEMSER® Schweinepestoralvakzine) based on the CSFV strain “C” is safe for the target species, i.e. wild boar and domestic pigs, as well as for relevant non-target wildlife. As the vaccine virus is genetically very stable, there is no risk of reversion to virulence (Aynaud, 1988) and development of new virus types caused by recombination of vaccine and field virus is considered very low.

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References

- Ahrens, U., Kaden, V., Drexler, Ch., Visser, N., 2000. Efficacy of the classical swine fever (CSF) marker vaccine Porcilis[®] Pesti in pregnant sows. *Vet. Microbiol.* 77, 83-97.
- Anonymous, 2001. Council Directive 2001/89/EC on Community measures for control of classical swine fever, 23 October. *Off. J. Eur. Communities* L 316, 5-35.
- Anonymous, 2005. Klassische-Schweinepest-Lebend-Impfstoff (gefriergetrocknet), *Vaccinum pestis classicae suillae vivum cryodesticcatum*. In: *Ph. Eur.*, 5. Ausgabe, Grundwerk. Deutscher Apotheker Verlag Stuttgart, Govi-Verlag – Pharmazeutischer Verlag GmbH Eschborn, pp. 1118-1120.
- Aynaud, J.M., 1988. Principles of vaccination. In : Liess, B. (Ed.), *Classical Swine Fever and Related Viral Infections*. Martinus Nijhoff, Boston, pp. 165-180.
- Beer, J., Wittmann, J., Lehnert, E., Tesmer, S., Glaner, M., Kaden, V., Karge, E., 1978. Die Immunprophylaxe und –metaphylaxe gegen Schweinepest in der Deutschen Demokratischen Republik mit Schweinepest-Lebendvirusvakzine „Riems“. *Mh. Vet.-Med.* 33, 543-548.
- Bognár, K., Mészáros, J., 1963. Experiences with a “Chinese” strain of lapinized swine fever virus. *Mag. Állatorv. Lapja.* 18, 69-74.
- Bran, L., Mihaita, S., Popa, M., Totorcea, N., Albu, T., 1966. Sur la stabilité de quelques caractères biologiques de la souche C de virus lapinisé de la peste porcine. *Bull. Off. Int. Epiz.* 66, 681-693.

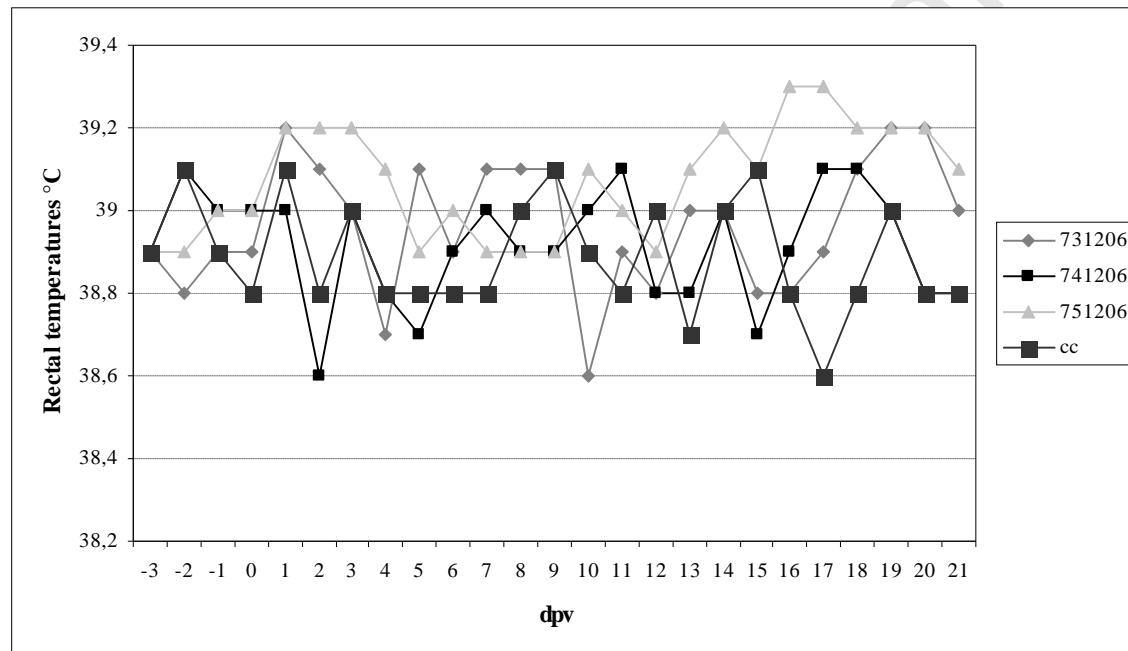
- Brauer, A., Lange, E., Kaden, V., 2006. Oral immunisation of wild boar against classical swine fever: uptake studies of new baits and investigations on the stability of lyophilised C-strain vaccine. *Eur. J. Wildl. Res.* 52, 271-276.
- Chenut, G., Saintilan, A.-F., Burger, C., Rosenthal, F., Cruciére, C., Picard, M., Bruyère, V., Albina, E., 1999. Oral vaccination of swine with a classical swine fever vaccine (Chinese strain) and transmission studies in rabbits and sheep. *Vet. Microbiol.*, 64, 265-276.
- Dahle, J., Liess, B., 1995. Assessment of safety and protective value of a cell culture modified strain „C“ vaccine of hog cholera/classical swine fever virus. *Berl. Münch. Tierärztl. Wschr.* 108, 20-25.
- Dewulf, J., Laevens, H., Koenen, F., Mintiens, K., de Kruif, A., 2001. Evaluation of the potential of dogs, cats and rats to spread classical swine fever virus. *Vet. Rec.* 149, 212-213.
- Fischer, U., Kaden V., Beyer, J., 1991. Zur Nachweissicherheit von Schweinepestantigen und zur Differenzierung der Feld- und Vakzinevirusinfektion mit dem direkten Immunofluoreszenztest an Organmaterial. *Mh. Vet.-Med.* 46, 133-136.
- Glaner, M., Kaden V., Tesmer, S., Hahnefeld, H., 1984. The vaccine against porcine cholera („Riemsers Schweinepest-Vakzine“) – a cell culture vaccine of a high immunobiological valency. *Medicamentum, special issues for veterinary science*, 21-25.
- Hoffmann, B., Beer, M., Schelp, C., Schirmmeier, H., Depner, K., 2005. Validation of a real-time RT-PCR assay for sensitive and specific detection of classical swine fever. *J. Virol. Methods* 130, 36-44.

- Kaden, V., Schurig, U., Steyer, H., 2001. Oral immunization of pigs against classical swine fever. Course of the disease and virus transmission after simultaneous vaccination and infection. *Acta Virol.*, 45, 23-29.
- Kaden, V., Lange, E., Fischer, U., Strebelow, G., 2000. Oral immunisation of wild boar against classical swine fever: evaluation of the first field study in Germany. *Vet. Microbiol.*, 73, 239-252.
- Kaden, V., Heyne, H., Kiupel, H., Letz, W., Kern, B., Lemmer, U., Gossger, K., Rothe, A., Böhme, H., Tyrpe, P., 2002. Oral immunisation of wild boar against classical swine fever: concluding analysis of the recent field trials in Germany. *Berl. Münch. Tierärztl. Wschr.* 115, 179-185.
- Kaden, V., Renner, C., Rothe, A., Lange, E., Hänel, A., Gossger, K., 2003. Evaluation of the oral immunisation of wild boar against classical swine fever in Baden-Württemberg. *Berl. Münch. Tierärztl. Wschr.* 116, 362-367.
- Kaden, V., Lange, E., Steyer, H., 2004a. Does multiple oral vaccination of wild boar against classical swine fever (CSF) have a positive influence on the immunity? *Dtsch tierärztl. Wschr.* 111, 63-67.
- Kaden, V., Lange, E., Riebe, R., B. Lange, 2004b. Classical swine fever virus strain „C“. How long is it detectable after oral vaccination? *J. Vet. Med. B* 51, 260-262.
- Kaden, V., Kramer, M., Kern, B., Hlinak, A., Mewes, L., Hänel, A., Renner, C., Dedek, J., Bruer, W., 2006. Diagnostic procedures after completion of oral vaccination against classical swine fever in wild boar. *Rev. sci. tech. Off. int. Epiz.* 25, 989-997.

- Kaden, V., Lange, E., Faust, A., 2008. Oral vaccination against classical swine fever with a chimeric *Pestivirus*: comparative investigations of liquid and lyophilised virus. *Eur. J. Wildl. Res.* 54, 237-244.
- Kern, B., Lahrmann, K.H., 2000. Oral Immunisierung gegen klassische Schweinepest (KSP) beim Schwarzwild in Brandenburg 1995 bis 1997. *Dtsch. tierärztl. Wschr.* 107, 490-495.
- Koenig, P., Hoffmann, B., Depner, K.R., Reimann, I., Teifke, J.P., Beer, M., 2007a. Detection of classical swine fever vaccine in blood and tissue samples of pigs vaccinated either with a conventional C-strain vaccine or a modified live marker vaccine. *Vet. Microbiol.* 120, 343-351.
- Koenig, P., Lange, E., Reimann, I., Beer, M., 2007b. CP7_E2alf: A safe and efficient marker vaccine strain for oral immunisation of wild boar against classical swine fever virus (CSFV). *Vaccine* 25, 3391-3399.
- Laddomada, A., 2000. Incidence and control of CSF in Europe. *Vet. Microbiol.* 73, 121-130.
- Leifer, I., Lange, E., Reimann, I., Blome, S., Juanola, S., Duran, J.P., Beer, M., 2009. Modified live marker vaccine candidate CP7_E2alf provides early onset of protection against lethal challenge infection with classical swine fever virus after intramuscular and oral immunisation. *Vaccine* (in press).
- Lin, T.T.C., Lee, R.C.T., 1981. An overall report on the development of a highly safe and potent lapinized hog cholera virus strain for hog cholera control in Taiwan. *Natl. Sci. Coun. Spec. Publ.* 5, 1-5.
- Moennig, V., 2000. Introduction to classical swine fever virus, disease and control policy. *Vet. Microbiol.* 73, 93-102.

- Précausta, P., Brun, A., Kato, F., 1977. Pest porcine classique: Quelques notions complémentaires sur l'innocuité de la souche chinoise CL. *Revue Méd. Vét.* 128, 191-200.
- Terpstra, C., Woortmeyer, R., Barteling, S.J., 1990. Development and properties of a cell culture produced vaccine for hog cholera based on the Chinese strain. *Dtsch. tierärztl. Wschr.* 97, 77-79.
- Tesmer, S., Urbaneck, D., Kaden, V., Wittmann, W., Hahnefeld, H., 1973. Zur Wirkung von Schweinepest-Lebendvirusvakzine aus dem Impfstamm „C“ bei tragenden Sauen und deren Nachzucht. *Mh. Vet.-Med.* 28, 251-254.
- Tielen, M.J.M., Van Bekkum, J.G., Robijns, K., Brus, D.H.J., 1974. Een onderzoek naar eventuele schadelijke neveneffecten van de enting van varkens met een levend vaccin (C-stam) tegen varkenspest. *Tijdschr. Diergeneesk.* 99, 205-205.
- Van Oirschot, J.T., 2003. Vaccinology of classical swine fever: from lab to field. *Vet. Microbiol.* 96, 367-384.
- Von Rüden, S., Staubach, C., Kaden, V., Hess, R.G., Blicke, J., Kühne, S., Sonneburg, J., Fröhlich, A., Teuffert, J., Moennig, V., 2008. Retrospective analysis of the oral immunisation of wild boar populations against classical swine fever virus (CSFV) in region Eifel of Rhineland-Palatinate. *Vet. Microbiol.* 132, 29-38.
- Wehrle, F., Renzullo, S., Faust, A., Beer, M., Kaden, V., Hofmann, M.A., 2007. Chimeric pestiviruses: candidates for live-attenuated classical swine fever marker vaccines. *J. Gen. Virol.* 88, 2247-2258.

Fig. 1 Mean rectal temperatures of pigs vaccinated with one overdose



cc – contact controls

Table 1. Development of body weight in domestic pigs after oral application of different vaccine doses

Vaccine dose	Vaccination scheme/interval	Mean body weight (kg)							Remark
		0 dpv	14 dpv	28 dpv	42 dpv	56 dpv	84 dpv	112 dpv	
Overdose (tenfold dose)	Once	12.7±1.0	14.7±1.5	18.7±2.0					
One dose	Twice/bi- weekly	15.1±1.6	17.2±1.9	22.9±2.2	26.5±2.7	35.0±4.1			
	Twice/at 4 weeks	14.6±1.5		24.7±2.0		35.6±4.4	44.6±5.6	57.2±6.8	
Control group (unvaccinated)	–	13.3±1.2	16.4±1.3	19.4±2.2					used also as contact controls

Table 2. Seroconversion after application of one vaccine dose

Application scheme	Species	Mean VNA titres (ND ₅₀)						
		0 dpv	14 dpv	28 dpv	42 dpv	56 dpv	84 dpv	112 dpv
Once	Pigs	neg	51.2±85.8					
	Wild boar	neg	24.0±4.0	118.6±40.2				
Twice at intervals of 14 days	Pigs	neg	15.8±12.5	269.7±150.7	329.1±73.2	641.3±140.4		
Twice at intervals of 28 days	Pigs	neg		146.6±82.4		430.9±192.6	637.7±319.2	1204.8±853.0

Table 3. Seroconversion after application of the tenfold dose (one overdose)

Application scheme	Species	Batch no.	Virus content per dose (bait) (log ₁₀ TCID ₅₀)	Mean VNA titres (ND ₅₀)		
				0 dpv	14 dpv	21 dpv
Once	Pigs	370203	5.25	neg	nt	286 ₊ 212.6
	Pigs	380203	5.5	neg	nt	298.7 _± 125.5
	Pigs	390203	5.5	neg	nt	188.3 _± 64.3
	Pigs	560105	6.0	neg	nt	178.3 _± 164.4
	Pigs	570105	6.3	neg	nt	100.3 _± 47.9
	Pigs	220600	4.5	neg	111.4 _± 132.1	nt
	Pigs	230600	4.8	neg	116.0 _± 128.3	nt
	Pigs	240600	4.8	neg	112.4 _± 131.2	nt
	Pigs	731206	6.7	neg	nt	44.3 _± 20.5
	Pigs	741206	6.5	neg	nt	35.2 _± 28.0
	Pigs	751206	6.3	neg	nt	40.3 _± 43.7
	Wild boar	640206	6.3	neg	33.0 _± 17.9	66.0 _± 35.3
Twice (interval of 4 days)	Pigs	741206	6.5	neg	nt	33.3 _± 11.1

nt = not tested

1
2 Table 4. Seroconversion in rabbits after application of CSF oral vaccine
3

dpv	Number of seropositive animals per group	Mean VNA titres (ND ₅₀) per vaccination group ²
0	0/8	negative
14	4/6 ¹	30.5±16.7
20	5/8	256.0±0
35	5/8	256,0±0

4
5 ¹no serum from two animals
6 ²seropositive animals
7