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Does Porcine Reproductive and Respiratory Syndrome Virus Potentiate Classical Swine Fever Virus Infection in Weaner Pigs?

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With 1 figure and 2 tables

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Summary

Fifteen 6-week-old crossbred weaners weighing about 12 kg each were randomly divided into three groups of five animals each. One group of pigs was inoculated first with porcine reproductive and respiratory syndrome (PRRS) virus and then 3 days later with CSF virus. The second group received classical swine fever (CSF) virus, while the third group was inoculated with PRRS virus only. The aim of the experiment was to determine whether a primary PRRS virus infection influences the clinical outcome of experimentally induced CSF in young pigs.

The PRRS virus infected weaners developed mild respiratory symptoms and recovered completely. All five weaners which were inoculated with CSF virus only showed severe clinical signs typical of the acute form of CSF. One pig had to be killed 15 days post-inoculation (p.i.); the remaining four died between the 18th and 22nd day p.i. The clinical course of the animals inoculated with both viruses was slightly different from that of the pigs that received only CSF virus. Four out of five pigs from the PRRS/CSF group became febrile and viraemic earlier than the animals which received CSF virus only. These pigs had to be killed 15–17 days post CSF virus inoculation. One animal in this group survived the acute phase of CSF and recovered completely. It was concluded that the observed divergences of the clinical courses would not have been noticed under field conditions. Therefore these findings cast doubt on the relevance of PRRS virus infection potentiating significantly the clinical outcome of CSF in young pigs.

Introduction

During the outbreaks of classical swine fever (CSF) in Germany between 1993 and 1998, differing clinical courses were observed, ranging from mild to severe symptoms 'typical' of CSF, with low and high mortality rates, respectively. This diversity of clinical pictures and the different patterns of distribution and severity of morphological lesions were primarily attributed to host- and virus-related factors (Depner et al., 1996, 1997). However, veterinarians and epidemiologists also discussed the possibility of a dual infection of pigs with porcine reproductive and respiratory syndrome (PRRS) virus and CSF virus, which might lead to a changing clinical picture of CSF. Reports were vague, but since PRRS virus has become endemic in Germany, dual infections with PRRS virus and CSF virus could not be excluded. The occurrence of subclinical PRRS infections and the prolonged circulation of this virus within herds give ample opportunities for mixed PRRS/CSF infections during a CSF epidemic. This assumption

prompted us to perform a study, in order to determine whether a primary PRRS virus infection influences the clinical outcome of experimentally induced CSF in young pigs.

Materials and Methods

Animals and experimental design

Fifteen 6-week-old crossbred weaners weighing about 12 kg each and free of neutralizing antibodies against pestiviruses and PRRS virus were randomly divided into three groups of five animals each. One group of pigs (PRRS/CSF group) was inoculated with PRRS virus and infected 3 days later with CSF virus. The second group (CSF group) received CSF virus, while the third group (PRRS group) was inoculated with PRRS virus only. The pigs were inoculated intranasally with cell culture supernatant containing 10^6 $TCID_{50}$ of PRRS virus and/or $10^{4,7}$ $TCID_{50}$ of CSF virus, respectively.

The pigs were housed under identical conditions in two different isolation stables and monitored throughout the experiment. The PRRS/CSF group and the CSF group were housed in the same stable but in two different compartments without direct contact with each other. The PRRS group was kept in a separate stable. Blood samples for virological and serological investigations were collected twice weekly. Animals which died or had to be killed were submitted to a full necropsy.

Viruses

The CSF virus (Isolate 'Losten/Freese98') used in the experiment was isolated from a diseased pig from a CSF outbreak in northern Germany in January 1998. On the basis of sequence analysis of the 5′ non-translated region of the CSF virus genome, the isolate was grouped according to Fritzemeier et al. (1998) within subtype '2.3–Guestrow' (data not shown). The CSF virus isolate was passaged twice in PK15 cell cultures before the animals were inoculated.

The PRRS virus was isolated from a field case in the south-east of Germany in 1991 (Fichtner et al., 1993). The isolate was designated 'Cobbelsdorf' and caused, under experimental conditions, mild respiratory disease with increased body temperature during the first and second weeks of infection (Fichtner et al., 1993). The PRRS virus isolate was propagated first in porcine alveolar macrophages and then in MARC 145 cell cultures (Kim et al., 1993) over six passages.

Virological and serological examinations

Isolation of CSF virus from buffy coats was performed on PK15 cell cultures as described before (Dahle et al., 1993). Serum samples were tested for the presence of neutralizing antibodies against the homologous CSF virus using a direct neutralizing peroxidase-linked antibody assay (Hyera et al., 1987).

PRRS virus was isolated from blood samples over two cell culture passages in MARC 145 cells and assessed with an indirect immunofluorescence test (IIFT) using a polyclonal anti-PRRS serum from swine and a conjugated anti-swine IgG rabbit serum. PRRS serology was conducted using the IIFT with PRRS virus infected MARC 145 cells (Fichtner et al., 1994).

Results

Pigs inoculated with PRRS virus only (PRRS group)

Before inoculation with PRRS virus, body temperatures between 38.8 and 39.6 were measured. Post-inoculation (p.i.), two phases of increased temperature (39.7–39.9°C) were noted, between days 7 and 10 p.i. in two animals and between days 13 and 15 p.i. in three animals. The animals showed a mild dyspnea and tachypnea after day 7 p.i. These symptoms were mainly seen during stress situations, for example when the rectal temperatures were recorded. During the phases of increased body temperature the animals looked slightly apathetic, food uptake appeared to be disturbed and occasionally coughing was noticed. All five pigs recovered completely.

PRRS virus could be re-isolated from day 3 after PRRS virus inoculation onwards. The first antibodies against PRRS virus were detected 10 days after PRRS virus inoculation. High

antibody titres (> 320) were measured during the third and fourth weeks of infection. The antibody titres and the results of PRRS virus isolation are shown in Table 1.

Pigs inoculated with CSF virus only (CSF group)

After inoculation with CSF virus all five weaners fell ill and showed severe clinical signs typical of the acute form of CSF. One pig had to be killed 15 days p.i., and the remaining four died between the 18th and 22nd days p.i. The incubation period, the time before the febrile phase of CSF started (body temperature below 40°C), lasted 5 to 7 days (Table 2, Fig. 1). After onset of fever, progression of the disease was characterized by poor appetite, apathy, diarrhea, coughing, muscle tremor, weakness of the hind legs and swollen inguinal lymphnodes. The terminal stage was characterized by skin haemorrhages of various degrees.

CSF virus could be re-isolated regularly from the buffy coats obtained during the febrile phase. The first positive samples were obtained from four animals on day 7 p.i. and from one animal on day 12 p.i. (Table 2). A low titre (20) of neutralizing antibodies against the homologous CSF virus was detected in a serum sample taken 19 days p.i., 1 day before the pig died. No antibodies were found in serum samples of the remaining four animals. PRRS virus or antibodies against PRRS virus were not detected in this group.

Pigs inoculated first with PRRS virus and subsequently with CSF virus (PRRS/CSF group)

The clinical course of the animals inoculated with both viruses was slightly different from that of the pigs which received only CSF virus. The body temperatures ranged between 39.6 and 40.7°C 3 days after inoculation with PRRS virus, which was on average 0.5°C higher than before PRRS virus inoculation. Four to five days after CSF virus inoculation, a steep rise in body temperatures was noted (Fig. 1). Thereafter four pigs showed clinical symptoms of acute CSF and died or had to be euthanized 15–17 days after CSF virus inoculation. The average body temperatures of these four animals was higher compared to the pigs of the CSF group (Fig. 1). One animal developed a mild transient infection, being febrile during the second week of CSF virus infection. This animal recovered completely.

With the exception of the mildly affected pig which survived the disease, CSF viraemia was detected in all pigs of this group during the febrile phase of the disease. The first positive samples were obtained from two animals during the CSF incubation period on day 4 p.i. No

Table 1. PRRS virus isolation and antibody detection in pigs infected first with PRRS virus and then 3 days later with CSF virus and in pigs infected only with PRRS virus

		Days post-inoculation with PRRS virus							
Group	Animal no.	0	3	7	10	15	22	29	36
PRRS	1128	n*	**n**	n	80**	320	320	320	
	1129	n	n	**n**	320	320	>1280	>1280	
	1130	n	**n**	n	**80**	**320**	**1280**	**1280**	
	1131	n	n	**n**	80	**320**	**1280**	>1280	
	1132	n	**n**	n	80	**320**	**>1280**	>1280	
PRRS/CSF	967	n	**n**	n	n	80			
	968	n	**n**	**n**	**80**	**80**			
	969	n	n	**n**	80	**80**			
	970	n	n	**n**	**80**	80			
	971	n		n	**80**	80	80		>320

*n: no antibodies detected in the initial serum dilution of 1:80.
**80: antibody titre expressed as reciprocal value of the serum dilution of 1:80.
Bold text: PRRS virus isolation positive.

Table 2. CSF virus isolation and fever in pigs infected first with PRRS virus and then 3 days later with CSF virus and in pigs infected only with CSF virus

Group	Animal no.	Days post-inoculation with CSF virus (d.p.i.)																						Date of death (d.p.i.)
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
CSF	1133	n				n			P					P		P					P				20
	1134	n				n			P					P		P					P				22
	1135	n				n			P					P		P					P				20
	1136	n				n			P					P			P								15
	1137	n				n			n					P											18
CSF/PRRS	967	n				n			P					P											15
	968	n				P			P					P											17
	969	n				P			P					P		P									17
	970	n				n			P					P		P									17
	971	n				n			n					n							n				convalescent

n: CSF virus isolation negative.
P: CSF virus isolation positive.
Boxes indicate the period of fever.

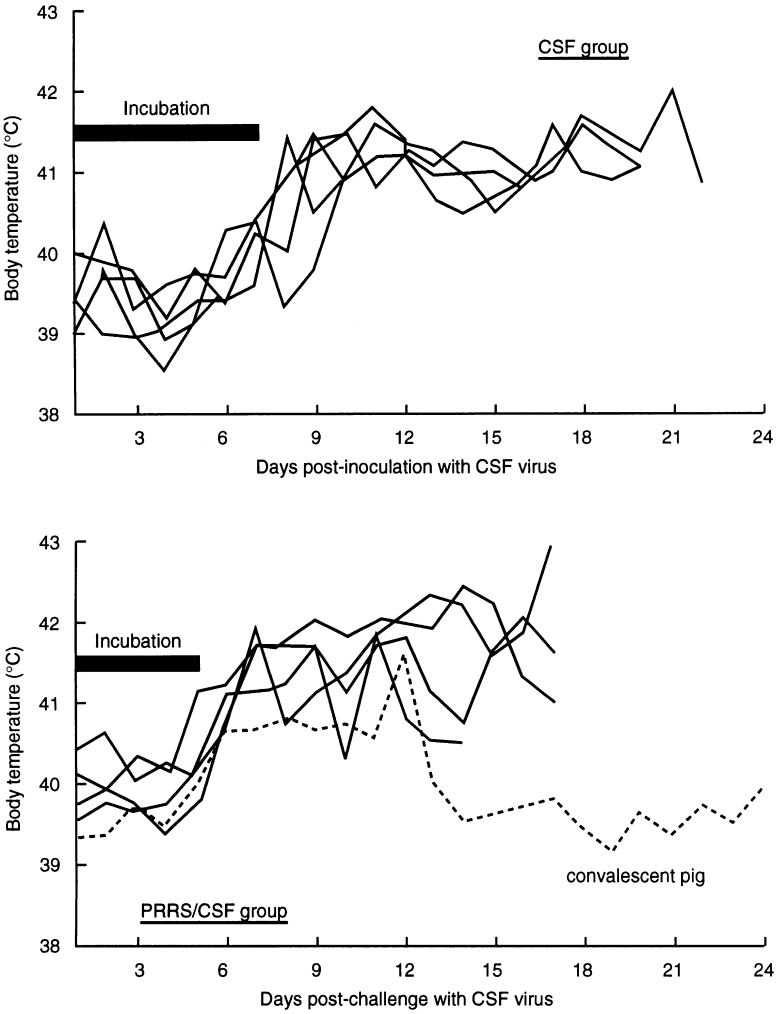

Fig. 1. Body temperatures in pigs infected first with PRRS virus and then 3 days later with CSF virus (PRRS/CSF group) and in pigs infected only with CSF virus (CSF group).

antibodies against CSF virus were found in the serum samples of the pigs which died. The surviving pig developed neutralizing antibodies with titres of 320 on day 19 p.i. and 240 on day 33 p.i.

PRRS virus could be re-isolated from all pigs of the group. On day 3 post PRRS virus inoculation, the day when the animals were challenged with CSF virus, two weaners were positive for PRRS virus. Low titres (80) of antibodies against PRRS virus were detected in all serum samples taken from day 10 post PRRS virus inoculation onwards. The antibody titres and the results of PRRS virus isolation are shown in Table 1.

Discussion

Several studies have been conducted to evaluate the effect of PRRS virus infection on a subsequent challenge with different bacterial pathogens in pigs without observing potentiation of the symptoms (Cooper et al., 1995). However, in a few studies the interaction of PRRS virus

infection and bacterial infections (e.g. *Streptococcus suis*) has been proven (Galina et al., 1994). PRRS virus has also been found in association with other viral diseases, including swine influenza, porcine respiratory coronavirus, paramyxovirus and encephalomyocarditis virus. It is not yet clear to what extent these viruses act synergistically. Brun et al. (1994) could not find any difference between groups of pigs infected with PRRS virus and swine influenza virus or swine influenza virus alone, while Van Reeth et al. (1994) observed potentiation of illness caused by PRRS virus in pigs experimentally challenged 4 days p.i. with both swine influenza and porcine respiratory coronavirus. This apparent increased susceptibility to secondary diseases suggests that PRRS virus infection causes some degree of immunosuppression. Immunosuppression is also a common finding in pigs infected with CSF virus (Trautwein, 1988). Therefore, an infection with both viruses might lead to a clinical course which differs from that of a solitary CSF infection. So far there are no reports about PRRS/CSF interactions in the pig. However, it is understandable that, due to the variety of clinical pictures in field cases of CSF, a synergistic activity of CSF virus and other pathogens, including the PRRS virus, was often used as an explanation for the diversity of symptoms.

The PRRS virus infected weaners in our experiment developed mild symptoms, similar to those seen in previously conducted experiments with this PRRS virus isolate (Fichtner et al., 1993). These pigs would probably not have attracted any attention under field conditions. However, the subsequent inoculation with CSF virus somehow accelerated the disease process. Four out of five pigs from the PRRS/CSF group became febrile and viraemic earlier than the animals which received CSF virus only. The slight difference between the incubation periods in the two groups indicates that there was some difference in the pathogenesis. In terms of clinical signs in the weaners (e.g. fever development), particularly at the beginning of the disease, a homogeneous clinical picture was seen in the group of pigs which received only CSF virus, while the pigs infected first with PRRS virus and 3 days later with CSF virus reacted less uniformly. However, the clinical differences disappeared towards the terminal stage of the CSF infection in all pigs that died. Nevertheless, it has to be pointed out that the above-mentioned divergence of clinical courses would not have been noticed under field conditions. Therefore these findings cast doubts on the relevance of PRRS virus infection potentiating the clinical outcome of CSF in young pigs. On the contrary, one pig infected with both viruses survived the infections while none of the pigs of the CSF group recovered.

The results of CSF virus isolation and antibody detection as well as the clinical course of CSF were in line with data from animal experiments conducted with other CSF field virus isolates from Germany (Depner et al., 1994, 1997). The group of weaners subsequently inoculated with CSF virus showed a less pronounced antibody response against PRRS compared to the pigs which received only PRRS virus. This could be explained by the severe leukopenia induced by CSF virus infection (data not shown).

So far we can conclude that the challenge of weaner pigs with CSF virus during the early phase of PRRS does not significantly potentiate the disease. The variety of clinical pictures of CSF, as observed during recent CSF outbreaks in Germany, thus appears related more to host and CSF virus factors than to mixed PRRS/CSF virus infections. However, the question remains whether a CSF virus challenge at a more advanced stage of PRRS (e.g. 14 days after PRRS virus infection) leads to a potentiation of the clinical course of CSF. A synergistic activity of these two pathogens cannot be completely excluded yet.

References

Brun, A., C. Charreyre, A. Vaganay, and G. Reynaud, 1994: Porcine reproductive and respiratory syndrome. Role of Togavirus and other infectious agents in the respiratory disease of swine In: Proceedings of the 13th International Pig Veterinary Congress, Bangkok, Thailand, 26–30 June, p. 52.

Cooper, V. L., A. R. Doster, R. A. Hesse, and N. B. Harris, 1995: Porcine reproductive and respiratory syndrome: NEB × 1 PRRSV infection did not potentiate bacterial pathogens J. Vet. Diagn. Invest. **7,** 313–320.

Dahle, J., G. Schagemann, V. Moennig, and B. Liess, 1993: Clinical, virological and serological findings

after intranasal inoculation of pigs with bovine viral diarrhoea virus and subsequent intranasal challenge with hog cholera virus J. Vet. Med. B **40,** 46–54.

Depner, K., A. Gruber, and B. Liess, 1994: Experimental infection of weaner pigs with a field isolate of Hog Cholera/Classical Swine Fever Virus derived from a recent outbreak in Lower Saxony. I: Clinical, virological and serological findings. Wien. Tierärztl. Mschr. **81,** 370–373.

Depner, K. R., U. Hinrichs, K. Bickhardt, I. Greiser-Wilke, J. Pohlenz, V. Moennig, and B. Liess, 1997: Influence of breed-related factors on the course of classical swine fever virus infection. Vet. Record **140,** 506–507.

Depner, K. R., Rodriguez, A., Pohlenz, J., and B. Liess, 1996: Persistent classical swine fever virus infection in pigs infected after weaning with a virus isolated during the 1995 epidemic in Germany: Clinical, virological, serological and pathological findings. Eur. J. Vet. Path. **2,** 61.

Fichtner, D., S. Bergmann, and H. Schirrmeier, 1994: Einsatz des indirekten Immunfluoreszenztests zum Nachweis von Antikörpern gegen das Virus des Porcine Reproductive and Respiratory Syndrome (PRRS) Monatshefte. Vet.-Med. **49,** 223–227.

Fichtner, D., J. Beyer, D. Leopoldt, H. Schirrmeier, S. Bergmann, and U. Fischer, 1993: Experimentelle Reproduktion der respiratorischen Verlaufsform der Infektion mit dem Erreger des Seuchenhaften Spätabortes der Schweine. Berl Munch. Tierärztl. Wschr. **106,** 145–149.

Fritzemeier, J., I. Greiser-Wilke, K. R. Depner, and V. Moennig, 1998: Molecular epidemiology of CSF in Germany. In: Proceedings of the OIE Symposium on Classical Swine Fever (Hog Cholera). Birmingham. 9–10 July, 1998.

Galina, L., C. Piljoan, M. Sitjar, W. T. Christianson, K. Rossow, and J. E. Collins, 1994: Interaction between *Streptococcus suis* serotype-2 and porcine reproductive and respiratory syndrome virus in specific pathogen-free piglets. Vet. Record **134,** 60–64.

Hyera, J. M. K., J. Dahle, B. Liess, V. Moennig, and H.-R. Frey, 1987: Gewinnung hochtitriger Antiseren gegen BVD-Virus aus Schweinen und ihre Verwendung fur direkte Immunofluoreszenz- und Immunoperoxidase-Techniken Dtsch. Tierärztl. Wschr. **94,** 541–612.

Kim, H. S., J. Kwang, J. J. Yoon, H. S. Joo, and M. L. Frey, 1993: Enhanced replication of porcine reproductive and respiratory syndrome (PRRS) virus in a homologeneous subpopulation of MA-104 cell line. Arch. Virol. **133,** 477–413.

Trautwein, G., 1988: Pathology and pathogenesis of the disease. In: Liess, B. (ed.), Classical Swine Fever and Related Viral Infections, p.27. Martinus Nijhoff Publishing, Dordrecht, NL.

Van Reeth, K., A. Koyen, and M. Pensaert, 1994: Clinical effects of dual infections with porcine epidemic abortion and respiratory syndrome virus, porcine respiratory coronavirus and swine influenza virus. In: Proceedings of the 13th International Pig Veterinary Congress, Bangkok, Thailand, 26–30 June, p. 51.