The Veterinary Journal 187 (2011) 310–319

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Respiratory function and pulmonary lesions in pigs infected with porcine reproductive and respiratory syndrome virus

Judith Wagner [a], Annette Kneucker [a], Elisabeth Liebler-Tenorio [a], Vicky Fachinger [b], Melanie Glaser [a], Stefan Pesch [c], Michael P. Murtaugh [d], Petra Reinhold [a,*]

[a] Institute of Molecular Pathogenesis in the 'Friedrich-Loeffler-Institut' (Federal Research Institute for Animal Health), Naumburger Str. 96a, 07743 Jena, Germany
[b] Boehringer Ingelheim Vetmedica GmbH, Corporate R&D, Binger Str. 173, 55216 Ingelheim, Germany
[c] Bioscreen EVDMC GmbH, Mendelstr. 11, 48149 Münster, Germany
[d] Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN 55108, USA

ARTICLE INFO

Article history:
Accepted 22 December 2009

Keywords:
Porcine reproductive and respiratory syndrome virus
Pulmonary function testing
Pathology
Pigs

ABSTRACT

Pulmonary dysfunction was evaluated in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV, isolate VR-2332) and compared to clinical and pathological findings. Infected pigs developed fever, reduced appetite, respiratory distress and dullness at 9 days post-inoculation (dpi). Non-invasive pulmonary function tests using impulse oscillometry and rebreathing of test gases (He, CO) revealed peripheral airway obstruction, reduced lung compliance and reduced lung CO-transfer factor. PRRSV-induced pulmonary dysfunction was most marked at 9–18 dpi and was accompanied by a significantly increased respiratory rate and decreased tidal volume. Expiration was affected more than inspiration. On histopathological examination, multifocal areas of interstitial pneumonia (more severe and extensive at 10 dpi than 21 dpi) were identified as a possible structural basis for reduced lung compliance and gas exchange disturbances.

© 2009 Elsevier Ltd. All rights reserved.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV), a member of the family *Arteriviridae*, genus *Arterivirus*, causes porcine reproductive and respiratory syndrome (PRRS), an important cause of production losses in pigs (Rossow, 1998). The consequences of PRRSV infection have been well documented for the reproductive system and for systemic infection (Christianson et al., 1992; Botner et al., 1994; Mengeling et al., 1996; Lager et al., 1997; Kranker et al., 1998). Numerous studies have evaluated the clinical, pathological and immunological features of the respiratory form of PRRS (Labarque et al., 2000; Samsom et al., 2000; Opriessnig et al., 2002), but the effects on pulmonary function have not been investigated, even though respiratory dysfunction may have a significant impact on the clinical outcome of the disease.

Typical clinical signs of respiratory infection with PRRSV are tachypnoea or dyspnoea that can be accompanied by an increased respiratory effort, lethargy, fever and occasionally coughing, sneezing and chemosis (Rossow et al., 1994; Opriessnig et al., 2002). Gross lung lesions include failure of the lungs to collapse, as well as moderately well demarcated, mottled, brown areas of pneumonia (Opriessnig et al., 2002). Microscopic lung lesions are characterised by type II pneumocytic hypertrophy and hyperplasia, septal infiltration with mononuclear cells and alveolar exudates (Opriessnig et al., 2002). No data are currently available on the pathophysiological features and derangements of pulmonary function induced by PRRSV. Since the lungs are important for oxygen supply to all tissues, pulmonary dysfunction might have significant clinical and subclinical systemic consequences.

Pulmonary function testing has been applied to pigs with bacterial respiratory infections by our group (Reinhold et al., 2005, 2008). A combination of impulse oscillometry and rebreathing of test gases can be used to evaluate lung ventilation, respiratory mechanics and pulmonary gas exchange in spontaneously breathing pigs. Since both methods are non-invasive and applicable to conscious animals, changes in pulmonary function can be evaluated over time. In this study, pulmonary dysfunction was characterised in relation to clinical signs and pathological changes in pigs with experimental PRRSV infection.

Materials and methods

Animals

Twenty-four German hybrid pigs from a closed specific-pathogen-free herd known to be free of PRRSV were transported to our institute at 24–27 days of age and were enrolled in the study after a quarantine period of 21 days. Pigs were housed according to the guidelines for animal welfare and fed twice daily with a

* Corresponding author. Tel.: +49 3641 804269; fax: +49 3641 804228.
E-mail address: petra.reinhold@fli.bund.de (P. Reinhold).

1090-0233/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tvjl.2009.12.022

commercial grower diet without antibiotics. Water was supplied ad libitum. Pigs were housed in two groups of 12 animals each, with pens of six pigs being separated from each other.

Virus

VR-2332, the prototype type II PRRSV isolated in 1989 in Minnesota, USA, has been shown to be virulent for sows and piglets (Rossow et al., 1994; Opriessnig et al., 2002; Nielsen et al., 2003). Sixth passage VR-2332, propagated and titrated on MA-104 cells, was prepared at a concentration of 593 $\log_{10}$ 50% tissue culture infectious doses/mL for use as the inoculum.

Experimental design

The study had a randomised, negatively controlled design (Table 1) and was performed at biosafety level 2. Ethical approval was obtained from the Commission for the Protection of Animals of the State of Thuringia, Germany (registration number 04-001/07).

Twelve pigs were exposed to PRRSV and 12 pigs served as controls. On the day of challenge, pigs exposed to PRRSV had an age of 62.2 ± 1.0 days (mean ± standard deviation, SD) and a body weight of 22 ± 2 kg, while controls were 61.2 ± 0.7 days old and weighed 22 ± 2 kg. Pigs were inoculated intranasally (1 mL per nostril) and intramuscularly (1 mL per pig) with either PRRSV VR-2332 or 0.9% saline (controls). For intranasal inoculation, a 10 mL syringe was connected to a tube (40 cm × 1.7 mm inner diameter feeding tube, Rüsch sterile, Ref 224000, size No. 3. Willy Rüsch GmbH) which had been inserted approximately 3 cm into the nose. One millilitre of virus solution, along with 9 mL air, per syringe was administered into each nostril with manual pressure, followed by IM injection of 1 mL into the gluteal muscle.

Clinical observations were recorded twice daily and included general behaviour, feed intake, appetite, rectal temperature, respiratory rate (RR) and the presence or absence of clinical signs of respiratory disease or diarrhoea. To monitor for the presence of PRRSV by PCR and for seroconversion, blood samples were collected three times before challenge (−11, −5 days and −1 h) and six times after challenge (3, 7, 10, 14, 17 and 21 days post-inoculation, dpi). Blood samples, nasal swabs, rectal swabs and tracheal swabs were also collected to exclude concurrent infections (Table 1).

Pulmonary function tests (PFTs) were performed twice before challenge (−7 and −3 days) and seven times after challenge (2, 4, 6, 9, 12, 15, 18 and 21 dpi) in eight pigs exposed to PRRSV and in eight controls (Table 1). Body weight was determined 1 day before each PFT and at postmortem examination. Four pigs per group (without PFT) were sacrificed at 10 dpi and eight pigs per group (with PFT) were sacrificed at 21 dpi for gross pathological, histopathological and immunohistochemical examination (Table 1).

Quantitative real-time PCR

To quantify the PRRSV load in blood, serum samples were analysed by real-time PCR for PRRSV strain VR-2332 and its attenuated form, Ingelvac MLV. RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen). Reverse transcription was carried out using the Multiscribe RT Enzyme Kit (Applied Biosystems). Reverse transcription conditions were 25 °C for 10 min, 37 °C for 120 min and 85 °C for 5 s. Amplification was carried out in triplicate using the TaqMan Universal PCR Master Kit (Applied Biosystems) and the PRRS VR-2332-specific primers MLV F1 (5′-GCAGCTCCCATCTACAGCTGATT-3′) and MLV R1 (5′-AGACAATGTGAGTCA AAACGGGAAAGAT-3′). The probe was TET-5′-TTGGCTAGCTAACAAATTTGATTGGGC AGTGGAGAGTTT-3′-TAMRA. Thermal cycling conditions were 50 °C for 2 min, 95 °C for 10 min, then 50 cycles of 94 °C for 15 s and 60 °C for 1 min. PRRSV cDNA quantification was achieved by comparison of the unknown sample with a standard curve derived from known amounts of plasmid DNA.

ELISA

A commercial PRRSV ELISA (HerdCheck PRRS ELISA, IDEXX) was used to detect anti-PRRS antibodies in the blood serum of pigs before and after challenge. Samples with sample-to-positive (S/P) ratio ⩾ 0.4 were considered to be positive for antibodies against PRRSV, as recommended by the manufacturer.

Differential microbiological examinations

Routine bacteriological culture was performed on nasal, tracheal and faecal swabs for *Bordetella* spp., *Pasteurella* spp., *Haemophilus* spp., *Actinobacillus pleuropneumoniae* (APP) and *Salmonella* spp. (Table 1). Samples were tested for *Mycoplasma* spp. by indirect immunofluorescence and for *Chlamydia* spp. by PCR.

Paired serum samples from each pig (blood collected at the beginning of the study and at postmortem examination) were used for serology (Table 1). Commercial ELISA test kits were used to detect antibodies against *Mycoplasma hyopneumoniae* (DAKO M. hyo ELISA, Oxoid), APP (Cypress Diagnostics), swine influenza virus (SIV; IDEXX), transmissible gastroenteritis virus (TGEV; SVANOVA) and porcine

Table 1
Study design for PRRSV monitoring, additional microbiological analysis, pulmonary function testing and postmortem examination.

		Period before challenge					Days post-inoculation											
		−11 d	−7 d	−5 d	−3 d	−1 h	2	3	4	6	7	9	10	12	14	15	17	18	21
Blood collection	PRRSV PCR	X[a]		X[a]		X[a]		X[a]			X[a]		X[a]		X[b]		X[b]		X[b]
	PRRSV serology	X[a]		X[a]		X[a]		X[a]			X[a]		X[a]		X[b]		X[b]		X[b]
Blood collection	*M. hyopneumoniae*	X[a]											X[c]						X[b]
	APP																		
	PCV-2																		
	PRCV																		
	SIV																		
	TGEV																		
Nasal swabs	*Bordetella* spp.					X[a]							X[a]						X[b]
	Pasteurella spp.																		
	Haemophilus spp.																		
	APP																		
	Mycoplasma spp.																		
	Chlamydia spp.																		
Tracheal swabs	*Bordetella* spp.												X[c]						X[b]
	Pasteurella spp.																		
	Haemophilus spp.																		
	APP																		
	Mycoplasma spp.																		
	Chlamydia spp.																		
Rectal swabs	*Salmonella* spp.			X[a]		X[a]							X[a]						X[b]
	Chlamydia spp.																		
PFT			X[b]		X[b]		X[b]		X[b]	X[b]		X[b]		X[b]		X[b]		X[b]	
Postmortem examination													X[c]						X[b]

M. hyopneumoniae, *Mycoplasma hyopneumoniae*; APP, *Actinobacillus pleuropneumoniae*; PCV-2, porcine circovirus type 2; PRCV, porcine respiratory coronavirus; SIV, swine influenza virus; TGEV, transmissible gastroenteritis virus; PFT, pulmonary function tests (8 pigs per group examined postmortem at 21 dpi).
[a] n = 12 Pigs per group.
[b] n = 8 Pigs per group (with PFT).
[c] n = 4 Pigs per group (without PFT).

Download English Version:

https://daneshyari.com/en/article/2464779

Download Persian Version:

https://daneshyari.com/article/2464779

Daneshyari.com