FINAL REPORT

STUDY 807-47041-00-99-09

PERFORMED AT ID-LELYSTAD
LELYSTAD, THE NETHERLANDS

TRANSMISSION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS THROUGH ORAL UPTAKE OF INFECTED PORCINE MUSCULAR TISSUE BY NAÏVE RECIPIENTS.

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SUMMARY

The Porcine Reproductive and Respiratory Syndrome virus (PRRSV) causes abortion and poor litter quality in third trimester pregnant sows. PRRSV is perceived to be highly infectious through the nasal and vaginal routes. Little information is available on the oral infectivity of PRRSV for pigs.

To assess more specifically whether European and or American strains of PRRS virus can be transmitted via infected pig meat, a feeding experiment was conducted. Twenty four, 8-weeks old, minimal disease pigs were infected with either a European (LV-Ter Huurne) or an American (SDSU#73) strain of PRRSV. Eleven days later the pigs were killed and the M. semimembranosus removed. Samples from the muscles were taken to examine for virus, and PRRSV was isolated from the muscle samples of 50% of the pigs .From each infected pig, the M. semimembranosus was fed to each of two 3-months old receiver pigs. Virus was isolated from the serum of all receiver pigs 6 days after the start of the feeding trial. The results of this study show that PRRSV, both the European and the American serotypes, is readily detectable in the meat of 8-weeks old pigs 11 days after infection. Furthermore, meat obtained from pigs recently infected with PRRSV is infectious when fed to receiver pigs.

1. INTRODUCTION

Porcine reproductive and respiratory syndrome virus (PRRSV) causes abortion in third trimester pregnant sows and poor litter quality. It may cause respiratory disease in young pigs (Terpstra, 1991). Infection of late term pregnant sows (80-95 days) with PRRSV can cause profound reproductive failure (Terpstra, 1991; Mengeling, 1996), especially due to a high level of mortality among the offspring of these sows at birth and the first week after birth. PRRSV is a ubiquitous pathogen. Two antigenic types can be distinguished, i.e. the European and the American types (Meulenberg, 1993).

Since PRRS is endemic in most of the pig producing countries (Wensvoort, 1991), and practitioners and farmers frequently complain that PRRS is causing economic losses in nursery and finishing pigs, the demand for disease control is increasing. Pigs are normally infected by PRRSV through the oral-nasal or the vaginal routes. The potential risk of transmission of PRRSV via fresh pig meat, offal and pig meat products is relevant to pig meat importing, PRRS negative countries. However, well documented experiments on the transmission of PRRSV through meat intake are not available, though, the literature does document quantitative data on several aspects of the chain of circumstances necessary for PRRSV to be infectious orally (Larochelle, 1997; Magar, 1995a+b). To assess more specifically whether meat obtained from pigs infected with either an European or American strain of PRRS virus is infectious orally, a feeding experiment was conducted.

2. AIMS OF THE STUDY

The aims of this study are

1.To determine if PRRSV (European and American serotypes) can be isolated from porcine muscular tissue in the period shortly after infection and to determine the levels of the virus in muscle.

2.To determine if muscular tissue containing detectable levels of PRRSV is infectious for susceptible pigs when fed orally.

3. TYPE OF STUDY

The type of the study was an open, randomized, controlled animal experiment.
It was performed at ID-Lelystad, Lelystad, The Netherlands, at the Edelhertweg (EHW) location.
Housing and husbandry of the pigs took place according to Standard Operating Procedures.

4. JUSTIFICATION OF THE TESTING SYSTEM

Pigs are the target species of Lelystad virus (PRRSV). Its most serious effects, in acute outbreaks in pig breeding herds, are on sows in the final third of gestation and on sucking piglets. PRRSV is capable of causing profound reproductive problems and a 50% reduction in average daily weight gain in pigs, associated with great economical loss. Two serotypes can be distinguished, i.e. the European (EU) and the American (US) types. The EU type is present in the European and some other markets. The US type, however, is present in most parts of the world where pigs are produced. Also in Europe, the US type has been isolated from the field since the introduction of a US-type based vaccine in 1996. In Denmark, the US type of PRRSV is readily isolated from field cases of PRRS. Review of the available data on the transmission of PRRSV via porcine meat show that there are practically no data on the topic. Therefore, the dissemination of PRRSV to muscles of naïve pigs after nasal infection is to be tested *in-vivo*. If PRRSV can be identified in muscle, its transmission after oral uptake to naïve pigs may be of importance for PRRS free countries importing pig meat from PRRS endemic regions. No validated *in-vitro* model is yet available to determine oral infectivity of PRRSV for receiver animals.

5. PROJECTED TIME SCHEDULE

Start of animal phase	:	October 1999
End of animal phase	:	December 1999
Draft report	:	February 2000
Second Draft	:	May 2000
Final report	:	August 2000

6. MATERIALS & METHODS

Virulent PRRSVs of known titre were selected to be used as inocula. SDSU#73 represents the American serotype, whereas LV –Ter Huurne (CDI-NL-2.91) represents the European serotype.

Table 1. PRRS Virus stocks

EU-type isolate (PRRSV Ter Huurne; code CDI-NL-2.91; vials 77-78-79)

Passage level	6, on porcine alveolar macrophages
Batch	22-03-99
Storage conditions	-70°C
Titres: Pre-inoculation Post-inoculation	 $10^{5.4}$ $TCID_{50}$ per mL $10^{5.3}$ $TCID_{50}$ per mL
Culturing system	Macrophages in RPMI 1640 in 5% FCS
Dose	1 ml intranasally per nostril
Additional info	The inoculum is tested for FMDV absence in a cow

US-type isolate (SDSU#73; batch 14-09-99; vials 16, 17, 18)

Passage level	5, on CL2621 cells
Storage conditions	-70°C
Titres: Pre-inoculation Post-inoculation	 $10^{5.6}$ $TCID_{50}$ per mL $10^{5.3}$ $TCID_{50}$ per mL
Culturing system	CL2621, HMEM + glutamine
Dose	1 ml intranasally per nostril
Additional info	The inoculum is tested for FMDV absence in a cow

Preparation and control of the viruses

The designated US type isolate was recovered from tissue harvest fluid obtained from a PRRS field case in the US. It was cultured at ID-Lelystad. Confirmation of the US type virus was done by genetic analysis and by monoclonal antibody typing. The EU type isolate was isolated during the PRRS epizootic in 1991 in the Netherlands. The challenge viruses were stored in 20 ml glass vials sealed with a rubber stopper. Just before administration they were thawed and transported on ice to the test facilities. Titres of the viruses were determined prior to and post-inoculation (back-titration) on porcine alveolar macrophages.

Documentation material

A translation in Dutch of the comprehensive protocol was provided to the animal caretakers and laboratory technicians involved in the experiment. Individual pig performance forms (Dutch version), pig registration forms and transmission forms were provided to the animal caretakers to record details of treatment and clinical performance. Stable reports, in a Dutch translation, were provided to record clinical observations, details of treatment and recording of body temperature during the study period.

7. STUDY ANIMALS AND GROUPS

Origin of pigs

81 minimal disease (MD) pigs were used in the study, originating from a PRRSV free Danish farm.

Groups

The following groups were included in the study (see also Table 2):

Group 1 (Challenge pigs): 12 PRRSV naïve, MD pigs (8 weeks of age), challenged with a specific titre of the European virulent strain of PRRSV. After 11 days (estimated peak viraemia) the animals were killed and the M. semimembranosus extracted.

Group 2 (Challenge pigs): 12 PRRSV naïve, MD pigs (8 weeks of age), challenged with a specific titre of an American virulent strain of PRRSV (SDSU#73). After 11 days (estimated peak viraemia) the animals were killed and the M. semimembranosus extracted.

Group 3 (Control pigs): 4 PRRSV naïve, MD control pigs (8 weeks of age), remained in the facilities during the whole course of the experiment and acted as sentinels to monitor biosecurity.

Receiver pigs: 48 PRRSV naïve, MD pigs (3 months of age) were used for the feeding trial. Muscle obtained from each challenge pig was fed to each of 2 receiver pigs.

Pilot feeding study: 1 PRRSV naïve, MD pig (8 weeks of age) and 3 PRRSV naïve, MD pigs (3 months of age).

Spare pigs: 4 PRRSV naïve, MD pigs (3 months of age). Three of these pigs were obtained from the pilot feeding study.

Table 2: Numbers of pigs per age and group

GROUP	AGE*	NO./GROUP
1 EU Challenge	8 week	12
2 US challenge	8 week	12
3 Control	8 week	4
Receiver	3 month	48
Pilot study	8 week	1
Pilot study and then Spares	3 month	3
Spares	3 month	1
Total		81

** The age refers to the age at the start of the study*

Character of the study pigs

Specific requirements of the study pigs are listed in Table 3.

Table 3: Character of the study pigs

Specification	Test Animal
Species	Porcine
Breed	Danish LY-hybrid combinations
Age	8 weeks and 3 months
Sex	Female/male
Number	81 total
Identification	Ear tag number
Conditions	Non-vaccinated/healthy
Tested for sero-negativity	Brucellosis, PRRS

The pigs were randomly assigned to the test groups upon arrival at ID-Lelystad. The assignment of the pigs to the test groups was randomised using Microsoft Excel on a Digital computer. Proper identification took place upon arrival at the test facilities as described in the protocol. Animals were ear-tagged with a unique ID-Lelystad number.

8. GENERAL STUDY DESIGN AND EXECUTION

The study was conducted under strict laboratory conditions. The test substances (PRRSV) were intranasally inoculated on the same day to Groups 1 and 2 according to Standard Operating Procedures of ID-Lelystad. The experiment tested if PRRSV (European and American serotypes) can be isolated from porcine muscular tissue and determined the levels of the virus in muscle. Furthermore, it tested if muscular tissue containing detectable and undetectable levels of PRRSV is infectious for susceptible pigs when fed orally.

Feeding pilot

Muscular tissues extracted from the challenge pigs (Groups 1 and 2) were to be fed to receiver pigs. In order to ensure a satisfactory acceptance for, and a proper uptake of, raw pig meat by these animals, a pre-study pilot was performed.

Pigs 3 months old were used in a pilot feeding trial, as they were likely to consume 500 g of raw meat. Prior to the feeding, the pigs are deprived of food other than water for 2 days. For the pilot study, three receiver pigs were fed with a total of 500 grams of raw semimembranosus muscle of an 8-weeks old PRRSV naïve pig in a two days period (250 gram/d). Acceptance, uptake and behavior were recorded for the time of feeding. The 3 receiver pigs were subsequently kept as spare pigs for the transmission study.

Transmission experiment

The pigs in each group were housed together with direct contact occurring between pigs unless stated otherwise. Each group of pigs was housed separately in fully conditioned (pressure) stables. After five days of acclimatization, the pigs from Group 1 and 2 were challenged with an European strain and an American strain of PRRSV, respectively. Group 3 pigs served as controls. Eleven days after challenge, the pigs from Groups 1 and 2 were killed and the M. semimembranosus removed from both legs. After cooling for 24 hours at 4ºC, the muscles were frozen at -23ºC until used. Additionally, samples of each muscle were collected for virus titration before, and after, cooling and freezing. Simultaneously, from each challenge pig offal (lung, spleen, lymph nodes) was collected, and samples were prepared for virus titration. Offal was frozen until potential usage. Five hundred grams of the freshly frozen muscle from each challenge pig, was fed after thawing to each of two receiver pigs over a 2 day period (48 receiver pigs in total). Uptake of the raw meat was recorded for each individual pig. Each specified group of receiver pigs was maintained separately (*vide infra*). Virus isolation and serology performed on serum collected from the receiver animals established the incidence of transmission via the oral route. Three weeks after feeding the tissues, the receiver animals were slaughtered.

Pigs were monitored for fever from 2 days prior to inoculation till 4 days after inoculation and for clinical signs of disease daily until the end of the study. Initially, the pigs were tested on the source farm as negative for PRRSV antibodies by the immunoperoxidase monolayer assay (IPMA) and IDEXX ELISA and again by ELISA on introduction into the study facilities of ID-Lelystad.

Administration of the viruses

The appropriate inoculum was applied intranasally according to the Standard Operating Procedures. New equipment was used for each individual pig. Before and after the administration period, the

PRRSV- titres were determined (Table 1; *vide supra*). Transportation of the test substances (PRRSV) was on ice at all times.

Course of events

Prior to study

Blood collection and ELISA (PRRS) (n=81 blood collections)

Pilot study pigs (n = 3 x 3-months old pigs + 1 eight-weeks old pig = 4 pigs)

Transmission experiment pigs (n = 24 challenge pigs + 48 receiver pigs + 4 controls + 1 spare pig = 77 pigs)

Pilot study

Day 1	:	Introduce 3-months old and 8-weeks old pigs to facility (n = 4)
		Slaughter 8-weeks old pig and harvest M. semimembranosus.
Day 3-5	:	Feed 500gm of muscle tissue to 3-months old pigs (n = 3)

Transmission experiment

Day 1	:	Introduce challenge pigs (n = 24), receiver pigs (n = 48), control pigs (n =4) and spare pig to the facility (n = 77 total)
		Prepare virus stocks
Day 1-5	:	Continue to feed and acclimatise pigs for transmission expt (n = 77)
Day 5	:	Bleed all pigs for ELISA (n = 77 + 3 pilots = 80)
Day 6	:	**22-10-99**: Intranasally inoculate challenge pigs (n = 2 x 12 = 24)
Day 11	:	Bleed challenge pigs for ELISA and virus isolation (n = 2 x 12 = 24)
		Bleed control pigs for ELISA and virus isolation (n = 4)
Day 17		Euthanise (n = 24; *11 days Post inoculation)*
Day 17-19	:	Process challenge pigs (n=24)
Day 20-29	:	Determine PRRSV titre of muscles
		Spike muscle with virus if titre low
Day 30-31	:	Feed 500gm of muscle tissue to each receiver pig (n = 48)
Day 33	:	Bleed receiver pigs for ELISA and virus isolation (n = 48)
(Day 3 Post feeding)		Bleed control pigs for ELISA and virus isolation (n = 4)
Day 36	:	Bleed receiver pigs for virus isolation (n = 48)
(Day 6 Post feeding)		Bleed control pigs for virus isolation (n = 4)

Day 38 :	Bleed receiver pigs for ELISA and virus isolation (n = 48)
(Day 8 *Post feeding*)	Bleed control pigs for ELISA and virus isolation (n=4)
Day 40 :	Bleed receiver pigs for ELISA and virus isolation (n = 48)
(Day 10 *Post feeding*)	Bleed control pigs for ELISA and virus isolation (n = 4)
Day 45 :	Bleed receiver pigs for ELISA and virus isolation (if required) (n = 48)
(Day 15 *Post feeding*)	Bleed control pigs for ELISA (n=4)
Day 50 :	Bleed receiver pigs for ELISA and virus isolation (if required)(n = 48)
(Day 20 *Post feeding*)	Bleed control pigs for ELISA (n = 4)

Euthanise receiver pigs and control pigs and remove tonsils for virus isolation (n = 48 + 4 = 52) in case virus isolation proves to be negative and no seroconversion has taken place (excl. Controls).

9. HOUSING AND DAILY CARE

The pigs were housed under the same conditions at the following locations and buildings (Tables 4a+b+c).

Table 4a; Housing of receiver pigs fed muscle obtained from pigs infected with EU-PRRSV

Infected pig (number)	Building-Box for receivers	Designated receivers
3001	43-13	2798/2799
3002	43-13	2800/2801
3003	43-13	2802/2803
3004	43-13	2804/2805
3005	43-15	2810/2811
3006	43-15	2812/2813
3007	43-15	2814/2815
3008	43-13	2806/2807
3009	43-15	2816/2817
3010	43-15	2818/2819
3011	43-13	2808/2809
3012	43-15	2820/2821

Table 4b; Housing of receiver pigs fed muscle obtained from pigs infected with US-PRRSV

Infected pig (number)	Building-Box for receivers	Designated receivers
3013	43-19	2834/2835
3014	43-19	2836/2837
3015	43-19	2838/2839
3016	43-19	2840/2841
3017	43-17	2822/2823
3018	42-14	2847/2848
3019	42-14	2849/2850
3020	43-17	2824/2825
3021	43-17	2826/2827
3022	43-17	2828/2829
3023	43-17	2830/2831
3024	43-17	2832/2833

Table 4c; Housing of control pigs

Control pig (number)	Building-Box for receivers	Designated receivers
2794	EHW	Non
2795	EHW	Non
2796	EHW	Non
2797	EHW	Non

The pigs were housed in fully conditioned (pressure, humidity, and temperature) stables, completely separated per group. The facilities were cleaned daily. The pigs were fed pelleted feed and given unlimited access to fresh drinking water through a nipple. Instruments used to weigh the piglets, to measure body temperature, to draw blood and inoculate the animals are described in detail in appendix 10. In the event of injury, illness, or adverse reactions to the test materials, the pigs were provided the appropriate veterinary care, including clinical and laboratory diagnostics and the administration of necessary drugs.

Sequence of events

Prior to the feeding of the meat, and after PRRSV infection, the treatment sequence of the groups was as follows:

1. Controls
2. Receivers
3. Spare animals
4. EU-infected group
5. Shower procedure (or other staffing)
6. US-infected group

Meat-feeding sequence (indicated are building-box numbers where the receiver pigs were located)

1. 42-14
2. shower procedure (or other staffing)
3. 43-15
4. shower procedure (or other staffing)
5. 43-13
6. shower procedure (or other staffing)
7. 43-17
8. shower procedure (or other staffing)
9. 43-19

Table 5; Presence (+) or absence (-) of PRRSV before (*) and after freezing in muscles of challenge pigs 11 days after infection. For viral titres see Table11.

Location of receiver pigs: 42-14

Donor pig	US-PRRSV (*)	US-PRRSV	Receiver	Remarks
3018	-	-	2847/2848	
3019	-	-	2849/2850	

Location of receiver pigs: 43-15

Donor pig	EU-PRRSV (*)	EU-PRRSV	Receiver	Remarks
3005	-	-	2810/2811	
3006	-	-	2812/2813	
3007	-	-	2814/2815	
3009	-	-	2816/2817	
3010	-	-	2818/2819	
3012	+	-	2820/2821	Fed and kept separately from other pigs but in same room

Location of receiver pigs: 43-13

Donor pig	EU-PRRSV (*)	EU-PRRSV	Receiver	Remarks
3001	+	+	2798/2799	
3002	+	-	2800/2801	
3003	+	-	2802/2803	
3004	+	-	2804/2805	
3008	+	+	2806/2807	
3011	+	-	2808/2809	

Location of receiver pigs: 43-17

Donor pig	US-PRRSV (*)	US-PRRSV	Receiver	Remarks
3017	+	-	2822/2823	
3020	+	-	2824/2825	
3021	-	-	2826/2827	Fed and kept separately from other pigs but in same room
3022	+	+	2828/2829	
3023	+	-	2830/2831	
3024	+	-	2832/2833	

Location of receiver pigs: 43-19

Donor pig	US-PRRSV (*)	US-PRRSV	Receiver	Remarks
3013	-	+ (spike)	2834/2835	Fed separately from sentinel pigs
3014	-	+ (spike)	2836/2837	Fed separately from sentinel pigs
3015	-	+ (spike)	2838/2839	Fed separately from sentinel pigs
3016	-	+ (spike)	2840/2841	Fed separately from sentinel pigs
-	-		2842/2843	Sentinels
-	-		2844/2845	Sentinels

10. INSTRUMENTS

1)	Thermometer:	
	Type:	C402 two measurement modes
	Produced by:	Terumo
	Range of measurement:	32-42
2)	Syringes:	
	Type:	Disposable-Luer adapter
	Produced by:	Terumo
	Size:	2-30 ml
3)	Intranasal applications:	
	Type:	Disposable-Luer adapter
	Produced by:	Terumo
	Size:	2 ml
4)	Blood sampling equipment:	
	Needle	18Gx1½ Terumo
	Syringe	Terumo multi-sample incl. Adapter
	Tubes	10 ml disposable Terumo
		Terumo serum and heparin

All instruments used in this trial were calibrated by an authorized method and marked at the initiation of the study. Separate instruments were used for each group.

11. CLINICAL ASPECTS

11.1 Examination of the General Health Status

All pigs were observed for their general health status daily from day 3 before challenge until slaughter

Scoring system:	Appearance	:	0=normal,	1=abnormal
	Respiration	:	0=normal,	1=abnormal
	Appetite	:	0=normal,	1=decreased
	Death	:	0=no,	1=yes

All abnormalities were recorded and signed for.

11.2 Measurement of Rectal Body Temperatures

All pigs were measured for rectal body temperatures daily from 2 days prior to until 3 days after challenge . On day 0 the rectal body temperature was measured at time of administration (or immediately before administration) and 4 hours after administration. Temperature changes in the control animals (group 3) helped in identifying stress-related temperature rises as compared to virus infection .

12. BLOOD SAMPLING

Blood was collected from the pigs according to the study design detailed in Section 8. Serum was produced according to standard laboratory techniques. Each blood sample from each animal was taken using a fresh, sterile needle and syringe. After clotting, the samples were placed on ice and transported to the lab. Each serum sample was divided into two aliquots. One sample to be used for the required investigations was stored at –20°C and the other was held at -70°C as a reserve sample. If the first aliquot was not used immediately for the required test it was also stored at -70°C until use.

13. PRRS ANTIBODY ELISA

Designated sera from pigs were tested for PRRS antibody using the ELISA of IDEXX (HerdCheck PRRS, IDEXX laboratories Inc, Westbroook, ME, USA). For description see *Appendix* 3.

14. PRRS VIRUS ISOLATION

An aliquot of each selected serum and muscle sample collected from the pigs was tested for PRRSV on porcine alveolar macrophages (PAMs) at ID-Lelystad. PAMs were used to isolate all PRRSV strains from serum and tissue suspensions. PAMs were seeded into 96-wells plastic plates about 16-24 hours prior to inoculation . Triturated, 10% tissue suspensions were prepared in RPMI1640 complete growth medium. For each sample, ten-fold serial dilutions were prepared in a 96-well plastic dummy plate and 50 µl of the dilutions was transferred to the corresponding well of the test-plate containing PAMs. Cells were examined daily for CPE. After two days, 50 µl of the growth medium was transferred to corresponding wells of two separate 96-wells plastic plates, containing fresh (16 hours) macrophages and were examined for CPE after two days of incubation. Additionally, the medium from the first plate was discarded and the cells were tested for PRRSV antigen. The plates were dried for one hour at 37^0C and placed in the freezer at -20^0C. Subsequently, cells were fixed for 10 minutes with a cold solution of paraformaldehyde (4%). A PRRSV specific monoclonal antibody (122.17; directed against the nucleocapsid protein of EU and US-serotype PRRSVs) was diluted (1:1000) in a buffer containing 0.5 M NaCl + 4% horse serum and 0.5% tween 80 (pH 7.2) and 50 µl added to the wells. Cells were incubated for one hour in a humidified incubator (37^0C) and subsequently washed three times in saline + 0.5% Tween 80. Rabbit anti-mouse HRPO conjugate (Dakopatts) was diluted (1:250) in 0.5 M NaCl + 4% horse serum and 0.5% tween 80, and 50 µl of the conjugate added to all wells of the plates. Plates were incubated one hour at 37^0C and rinsed with buffered saline containing Tween 80 before 50 µl of filtrated chromogen substrate (AEC) solution was added to all wells of the plates. Plates were next incubated at least 30 min at room temperature before the AEC was replaced by 50 µl of 0.05 M sodium-acetate, pH 5.0. For a test sample to be considered PRRSV positive, the cytoplasm of approximately 30 % of the cells in the wells inoculated with the sample are deeply red stained by the chromogen. A positive and negative control was included in the test.

15. STATISTICAL METHODS

Data were summarized using descriptive statistics. The distribution pattern of the data was assessed. Parametric data were grouped for time and variable, and overall group x time effects were assessed by analysis of variance (ANOVA) for repeated measures (body temperature). In the presence of significant time x group effects, multiple comparisons within a group were assessed by Scheffé technique. For all data, differences at matched time points between the test-substance-groups and the control group were assessed by analyzing the raw data using the two-tailed Student-*t*-test
For all tests, differences were considered statistically significant when $P < 0.05$. Statistical analysis was done using Instat-2®.

16. RESULTS

Pilot

The uptake of raw meat by the 3-month old recipients was satisfactory and allowed for the continuation of the trial.

Raw meat uptake by recipients

Table 6; Quality of the uptake of raw meat by “EU” receiver pigs” and “US receiver pigs”

EU receivers	16 Nov 1999	17 Nov 1999	US receivers	16 Nov 1999	17 Nov 1999
2798	Moderate	Good	2822	Good	Good
2799	Moderate	Good	2823	Good	Moderate
2800	Good	Good	2824	Good	Good
2801	Good	Good	2825	Moderate	Good
2802	Good	Good	2826	Good	Good
2803	Good	Good	2827	Moderate	Good
2804	Good	Good	2828	Good	Good
2805	Good	Good	2829	Good	Good
2806	Good	Good	2830	Good	Good
2807	Good	Good	2831	Moderate	Good
2808	Good	Moderate	2832	Good	Good
2809	Moderate	Good	2833	Good	Good
2810	Good	Good	2834	Good	Good
2811	Good	Good	2835	Moderate	Good
2812	Moderate	Moderate	2836	Good	Good
2813	Moderate	Good	2837	Moderate	Moderate
2814	Moderate	Good	2838	Good	Good
2815	Moderate	Good	2839	Good	Good
2816	Moderate	Moderate	2840	Good	Good
2817	Good	Good	2841	Good	Good
2818	Good	Good	2847	Good	Good
2819	Good	Good	2848	Good	Good
2820	Moderate	Moderate	2849	Good	Good
2821	Moderate	Moderate	2850	Good	Good

Good= Complete uptake of the raw meat (250 grams) within a short period of time (<2 min).
Moderate= Either incomplete uptake or delayed uptake of meat (>4 min).

All animals chewed the raw meat actively independent of the final uptake.

Serology

The ELISA (PRRS HerdCheck; IDEXX laboratories, Westbrook, ME, USA) was the chosen test to determine PRRSV specific antibodies in the sera of the pigs. Prior to challenge, all pigs were tested sero-negative in the ELISA (S/P ratio <0.4). By 11 days after infection, all but 5 pigs showed seroconversion and these were all animals exposed to the EU strain (Tables 7 and 8). At slaughter, the antibody levels in the US strain-infected pigs was significantly higher compared with the EU strain-infected pigs (P<0.01).

Table 7. PRRS ELISA results of sera sampled pre- and post-infection with an EU strain of PRRSV. PRRSV infection took place on October 22, 1999.

>0.4 = positive

Pig	21-10-99	27-10-99	2-11-99
	Day –1 PI	Day 5 PI	Day 11 PI
3001	<0,4	<0,4	0,471
3002	<0,4	<0,4	0,439
3003	<0,4	<0,4	0,195
3004	<0,4	<0,4	0,467
3005	<0,4	<0,4	0,552
3006	<0,4	<0,4	0,324
3007	<0,4	<0,4	0,053
3008	<0,4	<0,4	0,447
3009	<0,4		0,419
3010	<0,4	<0,4	1,166
3011	<0,4	<0,4	0.125
3012	<0,4	<0,4	0,233

Table 8. PRRS ELISA results of sera sampled pre- and post-infection with a US strain of PRRSV. PRRSV infection took place on October 22, 1999.

>0.4 = positive

Pig	21-10-99	27-10-99	2-11-99
	Day –1 PI	Day 5 PI	Day 11 PI
3013	<0,4	<0,4	1,203
3014	<0,4	<0,4	1,471
3015	<0,4	<0,4	1,209
3016	<0,4	<0,4	0,875
3017	<0,4	<0,4	0,978
3018	<0,4	<0,4	0,97
3019	<0,4	<0,4	0,757
3020	<0,4	<0,4	1,73
3021	<0,4	<0,4	0,964
3022	<0,4	<0,4	0,959
3023	<0,4	<0,4	1,204
3024	<0,4	<0,4	1,647

Sera obtained from the receivers were tested for the presence of PRRSV specific antibodies at 5 time points after feeding the meat of challenge pigs on November 16/17, 1999. From the 24 pigs fed the potentially EU-PRRSV containing meat, 20 seroconverted (despite the presence of virus in the serum of all 24 pigs; *vide infra*), whereas in the US-group all animals seroconverted (Tables 9 and 10). No serology was performed on other samples collected from this study.

Table 9; Results of the IDEXX ELISA performed on the serum sample obtained from receivers fed the muscle tissue extracted from EU-serotype PRRSV infected pigs. Feeding took place on November 16/17, 1999. Numbers 2794-2797 represent the control pigs (group 3).

>0.4	= positive					
Location	Pig	19-11-99	24-11-99	26-11-99	1-12-99	6-12-99
		D3 PF	D8 PF	D10 PF	D15 PF	D20 PF at slaughter
EHW	2794	0.008	0.003	0.005	0.008	
EHW	2795	-0.070	-0.024	-0.075	-0.420	
EHW	2796	0.002	-0.001	0.000	0.016	
EHW	2797	-0.005	-0.001	-0.008	-0.006	
43-13	2798	0.023	0.218	0.655	1.090	1.368
43-13	2799	-0.085	-0.004	0.187	0.638	1.205
43-13	2800	-0.038	0.059	0.547	1.271	1.428
43-13	2801	0.025	0.025	0.223	0.626	0.743
43-13	2802	0.005	0.014	0.071	0.179	0.155
43-13	2803	0.012	0.261	1.019	1.551	1.606
43-13	2804	-0.015	0.059	0.265	1.135	1.255
43-13	2805	-0.033	0.094	0.187	0.171	0.145
43-13	2806	0.010	0.176	0.425	0.714	0.994
43-13	2807	-0.012	0.073	0.187	0.822	1.039
43-13	2808	-0.020	-0.025	-0.020	0.341	0.663
43-13	2809	0.000	0.018	0.095	0.561	0.873
43-15	2810	0.002	0.058	0.251	0.404	0.862
43-15	2811	0.012	0.000	0.106	0.450	0.718
43-15	2812	-0.074	-0.054	0.017	0.500	1.182
43-15	2813	-0.020	0.038	0.280	0.405	0.431
43-15	2814	-0.008	0.023	0.306	1.005	1.249
43-15	2815	-0.008	0.237	0.670	1.231	1.546
43-15	2816	-0.013	-0.009	0.006	0.051	0.169
43-15	2817	-0.028	0.194	0.548	1.877	1.831
43-15	2818	0.010	0.021	0.028	0.186	0.378
43-15	2819	0.374	0.402	0.746	1.429	1.567
43-15	2820[1]	-0.005	0.072	0.414	1.870	2.474
43-15	2821[1]	-0.003	0.075	0.171	1.199	1.694

PF – post feeding

[1]Receiver pigs 2820 and 2821 were fed and kept separately from other pigs but maintained within the same room as other pigs

Table 10; Results of the IDEXX ELISA performed on the serum samples obtained from receivers fed the muscle tissue extracted from US-serotype PRRSV-infected pigs. Feeding took place on November 16/17, 1999.

>0.4	= positive					
Location	Pig	19-11-99	24-11-99	26-11-99	1-12-99	6-12-99
		D3 PF	D8 PF	D10 PF	D15 PF	D20 PF at slaughter
43-17	2822	-0.015	0.117	0.258	0.651	0.981
43-17	2823	-0.002	0.665	0.598	2.284	2.524
43-17	2824	-0.013	0.392	0.854	1.669	1.796
43-17	2825	-0.026	0.452	0.686	1.656	1.902
43-17	2826[1]	-0.013	0.774	1.274	1.702	2.052
43-17	2827[1]	-0.069	0.236	0.714	1.066	1.561
43-17	2828	0.007	0.295	0.432	1.245	1.876
43-17	2829	-0.018	0.349	0.746	0.902	1.402
43-17	2830	-0.01	0.182	0.932	1.551	1.787
43-17	2831	0.007	0.671	0.991	1.59	1.908
43-17	2832	-0.02	0.703	0.971	1.545	1.879
43-17	2833	-0.018	0.144	0.553	0.929	1.601
43-19	2834[2]	-0.007	0.458	0.816	1.455	1.87
43-19	2835[2]	-0.002	0.285	0.732	1.499	2.077
43-19	2836[2]	-0.008	0.498	1.068	2.234	2.504
43-19	2837[2]	-0.01	0.028	0.487	1.575	2.311
43-19	2838[2]	-0.003	0.503	0.6	1.253	1.689
43-19	2839[2]	0.01	0.704	0.953	1.435	1.64
43-19	2840[2]	-0.04	0.24	0.469	0.83	1.31
43-19	2841[2]	0.01	0.589	0.838	1.251	1.588
43-19	2842[3]	0.113	0.23	0.487	0.853	1.491
43-19	2843[3]	0.014	0.047	0.117	1.105	1.527
43-19	2844[3]	-0.002	0.083	0.516	1.172	1.527
43-19	2845[3]	0.013	0.056	0.223	1.14	1.385
42-14	2847	0.019	0.254	0.558	0.904	1.179
42-14	2848	0.011	0.26	0.799	1.099	1.339
42-14	2849	0.045	0.279	0.969	1.485	
42-14	2850	0.018	0.03	0.506	0.998	1.513

PF - post feeding

[1]Receiver pigs 2826 and 2827 were fed and kept separately from other pigs but maintained within the same room as other pigs

[2]Fed spiked muscle

[3]Sentinels

Virology

Virus could be isolated from serum in all challenge animals by day 5 after intranasal inoculation. No significant differences were recorded with respect to viral titres in the serum of both groups 5 days after infection. However, eleven days after infection, 10 out of the 12 pigs from EU group were (still) viraemic whereas only 6 from the US group were viraemic. No virus could be isolated from the spleen taken from US strain-infected pigs, whereas 7 out of 12 pigs infected with the EU strain contained the virus in the spleen (*P*<0.05; Table 11).

Table 11; Results (expressed as $\log_{10}$ $TCID_{50}$/gm or ml) of virus levels in the serum, spleen and muscle after intranasal inoculation with the EU or US-serotype of PRRSV. *titres after spiking and before freezing.

>1.8	= positive					
	27-10-99	2-11-99	2-11-99	2-11-99	2-11-99	
	5 d.p.i.	11 d.p.i.	11	11 d.p.i.	11 d.p.i.	
Pig.	Serum	Serum	spleen	muscle 1	muscle 2	Muscle titre
	Virus titre	virus titre	virus titre	virus titre	virus titre	After freezing
Controls						
2794	<1.8	n.t.	n.t.	n.t.	n.t.	n.t.
2795	<1.8	n.t.	n.t.	n.t.	n.t.	n.t.
2796	<1.8	n.t.	n.t.	n.t.	n.t.	n.t.
2797	<1.8	n.t.	n.t.	n.t.	n.t.	n.t.
EU infection						
3001	3.8	3.8	4.3	<1.8	4.3	3.3
3002	3.3	2.8	3.3	<1.8	3.3	<1.8
3003	3.3	2.8	3.3	3.3	3.3	<1.8
3004	4.8	3.8	3.3	<1.8	3.3	<1.8
3005	3.8	2.8	<1.8	<1.8	<1.8	<1.8
3006	2.8	<1.8	3.8	<1.8	<1.8	<1.8
3007	3.3	2.8	<1.8	<1.8	<1.8	<1.8
3008	2.3	2.8	<1.8	<1.8	3.8	3.8
3009	3.3	<1.8	3.3	<1.8	<1.8	<1.8
3010	3.3	3.8	<1.8	<1.8	<1.8	<1.8
3011	3.3	3.8	4.8	<1.8	3.8	<1.8
3012	3.8	2.8	<1.8	3.8	3.8	<1.8
US infection						
3013	2.8	<1.8	<1.8	<1.8	<1.8	4.3*
3014	3.3	<1.8	<1.8	<1.8	<1.8	3.8*
3015	3.3	<1.8	<1.8	<1.8	<1.8	4.3*
3016	2.8	2.8	<1.8	<1.8	<1.8	3.8*
3017	4.3	2.8	<1.8	3.3	<1.8	<1.8
3018	3.8	<1.8	<1.8	<1.8	<1.8	<1.8
3019	3.8	2.3	<1.8	<1.8	<1.8	<1.8
3020	3.8	2.3	<1.8	<1.8	3.8	<1.8
3021	2.8	2.8	<1.8	<1.8	<1.8	<1.8
3022	4.3	<1.8	<1.8	<1.8	3.3	3.8
3023	2.3	<1.8	<1.8	3.3	<1.8	<1.8
3024	3.8	2.3	<1.8	<1.8	3.3	<1.8

Results of the virus isolation from serum of the receiver pigs were comparable in the EU and US strain receivers. All pigs eventually became viraemic 6 days after feeding. By day 3 there was a significantly higher titre of PRRSV in the sera of pigs receiving EU virus isolation-positive meat as compared to the pigs receiving EU virus isolation-negative meat ($P<0.05$). No differences were recorded for the other animals and for other days (Tables 12 and 13). Transmission of PRRSV from receivers to sentinels occurred based upon the observation of viraemia in these sentinels. Despite undetectability of the viruses by virus isolation from the meat in some cases, it was apparently infectious to receivers, although it should be noted that aerosol transmission may have occurred within each room.

Table 12; Results of virus isolation and titration (expressed as $\log_{10}$ $TCID_{50}$/ml) from the sera of the receiver pigs that were fed meat extracted from EU strain infected pigs; virus isolation was performed on sera sampled from the receiver pigs four times at 2 to 3 day intervals after feeding. Feeding took place on November 16/17, 1999. Donor pigs are listed in Table 5.

>1.8	= positive						
Muscle virus isolation result before freezing	Muscle virus isolation result after freezing	Location	Pig	19-11-99 D3 PF	22-11-99 D6 PF	24-11-99 D8 PF	26-11-99 D10 PF
Controls							
		EHW	2794	<1.8	<1.8	<1.8	<1.8
		EHW	2795	<1.8	<1.8	<1.8	<1.8
		EHW	2796	<1.8	<1.8	<1.8	<1.8
		EHW	2797	<1.8	<1.8	<1.8	<1.8
"EU" receivers							
+	+	43-13	2798	3.3	3.8	2.8	3.8
+	+	43-13	2799	2.3	2.8	2.3	3.8
+	-	43-13	2800	2.3	3.8	2.8	2.3
+	-	43-13	2801	<1.8	2.8	2.3	2.8
+	-	43-13	2802	2.8	2.8	2.8	<1.8
+	-	43-13	2803	3.3	3.3	2.8	2.3
+	-	43-13	2804	<1.8	3.8	2.8	2.3
+	-	43-13	2805	2.3	3.8	3.8	4.3
+	+	43-13	2806	2.3	4.3	2.8	3.8
+	+	43-13	2807	2.3	3.8	3.3	3.3
+	-	43-13	2808	<1.8	2.8	2.8	3.3
+	-	43-13	2809	<1.8	3.8	2.8	2.8
-	-	43-15	2810	<1.8	3.8	3.3	4.8
-	-	43-15	2811	2.8	3.3	2.8	2.8
-	-	43-15	2812	<1.8	3.8	2.8	4.3
-	-	43-15	2813	<1.8	3.8	2.8	2.8
-	-	43-15	2814	<1.8	3.8	2.8	4.8
-	-	43-15	2815	<1.8	3.3	2.8	3.3
-	-	43-15	2816	<1.8	3.3	2.8	2.8
-	-	43-15	2817	<1.8	2.8	2.3	2.8
-	-	43-15	2818	<1.8	3.3	2.3	2.8
-	-	43-15	2819	<1.8	2.8	2.8	3.8
+	-	43-15	2820[1]	2.3	3.8	3.8	4.8
+	-	43-15	2821[1]	2.8	3.8	2.8	3.8

PF – post feeding of muscle

[1]Receiver pigs were fed and kept separately from other pigs but maintained within the same room as other pigs

Table 13; Results of virus isolation and titration (expressed as $\log_{10}$) from the sera of the receiver pigs that were fed meat extracted from US strain infected pigs; virus isolation was performed on sera sampled from the receiver pigs four times at 2 day intervals after feeding. Feeding took place on November 16/17, 1999. Donor pigs are listed in Table 5.

>1.8	= positive						
Muscle virus isolation before freezing	Muscle virus isolation after freezing	Location	Pig	19-11-99 D3 PF	22-11-99 D6 PF	24-11-99 D8 PF	26-11-99 D10 PF
			“US” receivers				
+	-	43-17	2822	<1.8	3.8	3.3	2.8
+	-	43-17	2823	<1.8	3.3	2.3	3.3
+	-	43-17	2824	2.3	4.3	2.8	3.3
+	-	43-17	2825	3.3	3.3	2.3	3.3
-	-	43-17	2826[1]	3.8	3.8	4.3	3.8
-	-	43-17	2827[1]	2.8	3.3	2.3	2.8
+	+	43-17	2828	2.8	3.3	2.3	2.3
+	+	43-17	2829	2.3	3.8	2.8	3.3
+	-	43-17	2830	<1.8	3.3	<1.8	2.8
+	-	43-17	2831	<1.8	4.3	3.3	2.8
+	-	43-17	2832	3.3	3.3	<1.8	3.3
+	-	43-17	2833	2.3	2.8	2.3	<1.8
-	+	43-19	2834[2]	<1.8	3.8	<1.8	2.3
-	+	43-19	2835[2]	<1.8	2.8	<1.8	2.8
-	+	43-19	2836[2]	3.3	4.3	2.8	3.3
-	+	43-19	2837[2]	<1.8	3.8	3.8	<1.8
-	+	43-19	2838[2]	3.3	3.8	2.8	<1.8
-	+	43-19	2839[2]	4.3	3.3	<1.8	3.3
-	+	43-19	2840[2]	4.8	3.8	3.8	3.8
-	+	43-19	2841[2]	<1.8	3.8	2.8	2.8
		43-19	2842[3]	<1.8	3.3	2.3	3.3
		43-19	2843[3]	<1.8	2.8	3.3	2.8
		43-19	2844[3]	<1.8	2.8	3.3	2.3
		43-19	2845[3]	<1.8	3.3	3.3	3.8
			2846	Pilot	pilot	pilot	Pilot
-	-	42-14	2847	2.8	3.3	3.3	3.8
-	-	42-14	2848	<1.8	4.3	2.8	3.3
-	-	42-14	2849	<1.8	4.8	2.8	3.8
-	-	42-14	2850	nt	3.8	4.3	3.3

PF - post feeding of muscle
[1]Receiver pigs were fed and kept separately from other pigs but maintained in the same room with other pigs
[2]Fed spiked muscle
[3]Sentinels
nt; not tested

Table 14; Actual PRRSV titres in the muscle after spiking 500 grams of muscular tissue with SDSU#73 to an anticipated virus titre of $10^{3.5}$ TCID50/gm.

Pig	Log_{10} Titre
3013	3.3
3014	2.8
3015	3.3
3016	2.8

Post challenge rectal temperatures

Post-infection rectal body temperatures of challenge pigs were higher as compared to pre-infection body temperatures and body temperatures of control pigs. For both groups this was shown to be significant only on day 2 after infection (*P*<0.05; Tables 15,16, and 17).

Table 15. Rectal temperature °C: Group 1; EU-PRRSV-infected (infection day=0)

Pig	Day=-1	Day=0	Day=1	Day=2	Day=3
3001	39.2	39.1	40.1	41.2	40.2
3002	39.3	39.2	39.9	40.2	40.7
3003	39.1	39	40.0	40.4	39.2
3004	39	39.1	40.1	40.3	40.4
3005	38.9	39.1	40.4	40.2	40.7
3006	39.2	39.1	40.5	40.6	40.2
3007	39.1	39	40.7	42	39.8
3008	38.9	39.3	39.8	40.3	39.8
3009	39	39.2	39.9	40.5	40.3
3010	39.2	39.3	39.8	40	39.4
3011	39.1	39.1	40.2	39.8	40.4
3012	39	39.2	40.1	40.1	39.8

Table 16. Rectal temperature °C: Group 2; US-PRRSV-infected (infection day=0)

Pig	Day=-1	Day=0	Day=1	Day=2	Day=3
3013	39.1	39.3	39.6	41.4	40.1
3014	39.3	39.4	39.7	40.7	40.1
3015	39.2	39.2	40.1	40.6	39.9
3016	39.2	39.3	39.8	40.8	41.1
3017	38.9	39	39.7	41.1	41.3
3018	39.4	39	40	41.3	40.5
3019	39.3	39.2	39.5	41.3	41.2
3020	38.7	39	39.9	40.8	40.5
3021	38.9	39.1	40	39.9	40.8
3022	39.4	39.2	39.8	40.1	39.5
3023	39.2	39	39.7	40.5	40.2
3024	39.2	39.2	39.9	41.5	41.3

Table 17. Rectal temperature °C: Group 3; Control pigs

Pig	Day=-1	Day=0	Day=1	Day=2	Day=3
2794	39.2	39.3	39.3	39.4	39.5
2795	39.3	39.1	39.3	39.2	39.5
2796	39.2	39.3	39.4	39.5	39.2
2797	39.2	39.3	39.3	39.6	39.4

Disease and use of medication

Post-transportation diarrhoea without other clinical signs occurred in most of the pigs and lasted for two days. None of the experimental animals exhibited severe signs of disease of any kind. Post-inoculation, in both the EU and the US groups, 3 animals became lethargic and showed inappetence for a short period of time. No fulminant respiratory distress was recorded. The use of medication was limited to 3 animals (Table 18). This reflects the low incidence of disease during the experiment.

Table 18. Use of medication during the course of the experiment.

Pig	Date	Disease	Drug	Remarks
2804	Oct 26, 1999	Arthritis	Streptomycin-Penicillin/Finadyne	Cured after 6 days
2805	Oct 27, 1999	Arthritis	Streptomycin-Penicillin/Finadyne	Cured after 7 days
2827	Nov 30, 1999	Arthritis	Streptomycin-Penicillin/Finadyne	Cured after 4 days

17. DISCUSSION

When exposed to either the European or US strains of PRRSV by intranasal inoculation, all pigs became viraemic 5 days later and most had seroconverted 11 days post-exposure. In this study, muscle of pigs infected with either strain of virus infected animals fed the muscle, including instances where virus was undetectable in PAM culture, indicating that the oral infectious dose of the two PRRSV strains studied is smaller than $10^{1.8}$ $TCID_{50}$/gram tissue the detection limit of the assay. It should be noted that due to the housing arrangements some pigs may have become infected with PRRSV via aerosol transmission and not by eating muscle of challenge pigs.

The IDEXX ELISA is only a semi-quantitative test for the determination of PRRS-specific antibody titres. Nonetheless, the sera of US strain infected pigs 1 day after infection showed a significantly higher S/P ratio compared to the EU group when sera of all challenge pigs were tested together. This difference might be related to pathogenesis differences of the two virus strains studied, as exemplified by an obvious difference between the two groups in the distribution of the virus through the body. No virus could be isolated from the spleen taken from US-infected pigs, whereas 7 out of 12 pigs infected with the EU strain contained the virus in the spleen. No differences were recorded with respect to viral titres in the sera of both groups 5 days after infection; all animals were viraemic. However, eleven days after infection, 10 of the 12 pigs from the EU group remained viraemic whereas only 6 from the US-infected group were so. Pathogenesis differences may relate to the manner and the efficacy of viral antigen presentation to B-cells, with concomitant differences in antibody levels. Finally, the recorded difference in antibody levels might be related to the test format of the IDEXX ELISA. In this indirect test, only the nucleocapsid protein of an EU strain is present, whereas all viral antigens of a US reference strain are present.

Mild clinical signs were seen in the infected animals after exposure to the PRRSV strains. Most predominant were mild respiratory distress and fever (body temp > 40ºC). In some cases lethargy and inappetence were recorded. These signs resemble the clinical signs seen in the field. The SDSU#73 strain can be highly virulent and able to cause severe pneumonia in slaughter pigs with lung involvement exceeding 80%. We were not able to fully reproduce these reported signs in our relatively brief study. It is difficult to explain this difference, but it could be related to genetically determined susceptibility differences, or environmental or management factors.

Apparently, the normal procedure of post-slaughter handling of the meat allowed sufficient virus in the muscle, obtained from viraemic pigs, to persist. Despite the series of procedures to which infected muscle was subjected electrocution, exsanguination, M. semimembranosus extraction, cooling for 24 hours, freezing for 10 days at –23ºC and thawing and feeding, complete inactivation of the virus in the muscle did not appear to always occur. Virus was sometimes detected in PAMs after these events and there were adequate levels of virus to be infectious orally. PRRSV stability depends, in part, on temperature and pH. As the low pH of the porcine stomach destroys the virus rapidly, oral infectivity is considered to be via the mucosal surfaces of the mouth or the tonsils.

The levels of PRRSV in the meat at slaughter observed in this study resemble those reported by others. Bloemraad et al (1994) recovered the European strain of PRRSV from the M. semimembranosus of experimentally infected 6months-old pigs at low levels ($10^{2.7}$ -$10^{3.5}$ $TCID_{50}$/gm) 5 days post infection at slaughter, whereas no virus was recovered after storing for 48 hours at 4ºC. Other researchers have also studied the recovery of PRRSV from muscle tissues of experimentally infected pigs of different ages, concluding that PRRSV might be isolated from the muscle at low levels resulting from infected residual blood (Mengeling, 1995; Frey, 1995; Magar, 1995). No data were presented in these studies on whether the virus could be transmitted through the oral uptake of infected meat.

From commercial pork, low levels of PRRSV have been isolated occasionally only, mainly because pigs are infected well before slaughter at 95 kg (Frey, 1995; Magar, 1995). In the present study storage of the meat for 10 days at –23ºC had some negative effects on the virus titre in the samples as compared to pre-storage titres, but in many cases the muscle remained infectious for the receiver pigs after oral uptake. Spiking of the meat, in order to be certain that US-PRRSV was administered to the receivers, led to the onset of viraemia in the receivers.

The present study demonstrates that PRRSV can be infectious through the oral route. As stated above, PRRSV-containing muscle was shown to be orally infectious to pigs even on at least four occasions when the virus was undetectable by virus isolation on porcine alveolar macrophages prior to and post freezing (box 42-14, receiver pig 2847, box 43-15, receiver pig 2811, box 43-17 receiver pigs 2826, 2827).

This indicates that the oral infectious dose of the two PRRSV strains studied is less than $10^{1.8}$ $TCID_{50}$/gram tissue, the detection limit of the virus isolation assay. Others have reported a high titre (10^7 $TCID_{50}$/ml) laboratory stock of PRRSV to be orally infectious (Magar, 1995b). In our study, however, the pigs had to ingest 250 grams of raw meat twice, requiring proper chewing before swallowing. Chewing apparently allows for enhanced contact of the virus with the mucosal surfaces of the mouth and attachment of the virus to cells with subsequent uptake into these cells (e.g. tonsillar cells). We observed a positive association between the virus titre in the meat before freezing and the time to which virus could be isolated from the sera of receivers after oral uptake of the meat. Sentinel pigs housed with receiver pigs were viraemic 6 days post-feeding (Table 13), so some receiver pigs may have been infected by horizontal transmission from orally infected pen mates, and not by eating muscle of challenge pigs. These animals are more likely to be those first viraemic 6 days after feeding.

From this experiment the following conclusions can be drawn:

- PRRSV, both the EU and the US-serotypes, is readily detectable in the meat of 8 weeks-old pigs 11 days after intranasal infection.

- Meat obtained from pigs recently infected with PRRSV is orally infectious to receiver pigs.

- Post-slaughter handling of meat allowed sufficient PRRSV to survive to be orally infectious.

- PRRSV titres in the meat may vary from ($<10^{1.8}$ – $10^{4.3}$ $TCID_{50}$/gram tissue) at 11 days post-infection.

- Freezing meat prepared under commercial conditions appears to have some effect on reducing PRRSV titres in meat.

- Despite undetectability by virus isolation, the level of PRRSV in the meat may still be sufficient to orally infect 3 month-old receiver pigs.

- There seems to be a positive association between the PRRSV virus titre in the meat before freezing and the time of onset of viraemia in receivers after oral uptake of the meat, alternatively those pigs viraemic 6 days post feeding were infected by horizontal transmission from orally infected pen mates.

- Pigs orally infected with PRRSV are infectious to sentinel pigs (the virus spreads horizontally).

18. REFERENCES

Bloemraad M, de Kluijver EP, Petersen A, et al., 1994. Porcine reproductive and respiratory syndrome virus: temperature and pH stability of Lelystad virus and survival in tissue specimens from viraemic pigs. Vet Microbiology; 42: 361-371.

Frey ML, Landgraf JG, Schmitt BJ, et al., 1995. Recovery of porcine reproductive and respiratory syndrome virus from tissues of slaughter weight pigs. Proceedings of the Second International Symposium on Porcine Reproductive and Respiratory Syndrome (PRRS), Aug. 9-10, Copenhagen, p28.

Larochelle R and Magar R., 1997. Evaluation of the presence of porcine reproductive and respiratory syndrome virus in packaged meat using virus isolation and polymerase chain reaction (PCR) method.
Vet. Microbiology; 58:1-8.

Magar R, Robinson Y, Dubuc C, and Rochelle R., 1995. Evaluation of the persistence of porcine reproductive and respiratory syndrome virus in pig carcasses. Vet. Rec.; 137:559-561.

Magar R, Robinson Y, Dubuc C, and Rochelle R., 1995b. Isolation and experimental oral transmission in pigs of a porcine reproductive and respiratory syndrome virus isolate. In Corona- and related viruses. Talbot, PJ and Levy, GA, eds. Plenum press, NY; pp139-144.

Mengeling WL, Lager, and KM Vorwald AC., 1995. Diagnosis of porcine reproductive and respiratory syndrome. J. Vet. Diagn. Invest.; 7:3-16.

Mengeling WL., Vorwald AC, Lager, KM, Brockmeier SL., 1996. Comparison among strains of Porcine Reproductive and Respiratory Syndrome Virus for their ability to cause reproductive failure. AJVR; 57:6:834-839.

Meulenberg JJM, Hulst MM, de Meijer EJ, et al., 1993. Lelystad virus, the causative agent of Porcine Epidemic Abortion and Respiratory Syndrome (PEARS) is related to LDV and EAV. Virology; 192:62-74.

Terpstra C, Wensvoort G, and Pol, JMA., 1991. Experimental reproduction of porcine epidemic abortion and respiratory syndrome (mystery swine disease) by infection with Lelystad virus: Koch’s postulates fulfilled. Vet Quart.; 13:131-136.

LOCATION OF RAW DATA

All raw data will be stored at ID-Lelystad, Research Branch Houtribweg, NL-8200 AB, Lelystad, The Netherlands, during a minimum of 5 years after approval of the final report.