An experimental study with a vaccine strain of porcine reproductive and respiratory syndrome virus to determine effects on viremia assessed by reverse transcriptase-polymerase chain reaction in pigs fed rations medicated with tilmicosin or non-medicated

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Summary

Objectives: To determine if feed medicated with tilmicosin affects viremia (assessed using reverse transcriptase-polymerase chain reaction [RT-PCR]) in pigs exposed to a vaccine strain of porcine reproductive and respiratory syndrome virus (PRRSV), clinical signs associated with vaccination (body temperature), and average daily gain.

Materials and methods: Purebred Yorkshire pigs (N = 192) were each assigned to one of five treatment groups. Groups 1a and 1b remained PRRSV-negative (controls), while Groups 2, 3, and 4 were injected with a modified-live (MLV) PRRSV vaccine. Groups 1b and 2 were fed non-medicated feed. Rations contained tilmicosin at 400 mg per kg for Group 1a and Group 4 and 200 mg per kg for Group 3. Blood samples were collected to measure serum tilmicosin concentrations and assess PRRSV viremia. Bronchoalveolar lavage was performed and macrophages assessed for PRRSV viremia and tilmicosin concentrations.

Results: Groups 1a and 1b remained PRRSV-negative. Number of PRRSV copies per mL in serum was highest in inoculated pigs at 10 days post inoculation, but did not differ among the three inoculated groups. Average daily gain (ADG) was higher in groups fed rations containing 400 mg per kg tilmicosin than in groups on non-medicated rations. Clinical signs of disease were absent in all pigs.

Implications: Viremia associated with an MLV vaccine strain of PRRSV does not differ between pigs fed rations containing 200 or 400 mg per kg of tilmicosin. In the absence of clinical disease, pigs consuming tilmicosin-medicated feed have higher ADG than pigs consuming non-medicated feed.

Keywords: swine, porcine reproductive and respiratory syndrome, tilmicosin, viremia

Received: May 12, 2015
Accepted: October 21, 2015

Resumen - Un estudio experimental con una cepa vacunal del virus del síndrome reproductivo y respiratorio porcino para determinar los efectos en la viremia valorados mediante la reacción en cadena de la polimerasa de transcriptasa inversa en cerdos alimentados con raciones medicadas con tilmicosina o sin medicación

Objetivos: Determinar si el alimento medicado con tilmicosina afecta la viremia (valorada utilizando la reacción en cadena de la polimerasa de transcriptasa inversa [RT-PCR por sus siglas en inglés]) en cerdos expuestos a una cepa vacunal del virus del síndrome reproductivo y respiratorio porcino (PRRSV por sus siglas en inglés), signos clínicos relacionados con la vacuna (temperatura corporal), y la ganancia diaria promedio.

Materiales y métodos: Se asignaron cerdos de raza pura Yorkshire (N = 192) individualmente a uno de cinco grupos de tratamiento. Los grupos 1a y 1b permanecieron negativos al PRRSV (controles), mientras que los grupos 2, 3, y 4 fueron inyectados con una vacuna viva modificada contra el PRRSV. Los grupos 1b y 2 fueron alimentados con alimento no medicado. Las raciones contenían tilmicosina a 400 mg por kg para el Grupo 1a y Grupo 4 y 200 mg por kg para el Grupo 3. Se recolectaron muestras de sangre para medir las concentraciones de tilmicosina en suero y valorar la viremia de PRRSV. Se realizó lavado de bronquial y se valoraron las macrófagos en busca de la viremia de PRRSV y evaluar las concentraciones de tilmicosina.

Resultados: Los grupos 1a y 1b permanecieron negativos al PRRSV. El número de copias del PRRSV por mL en el suero fueron más altas en los cerdos inoculados a los 10 días post inoculación, pero no hubo diferencia entre los tres grupos inoculados. La ganancia diaria promedio "(ADG por sus siglas en inglés) fue más alta en los grupos alimentados con raciones que contenían 400 mg por kg de tilmicosina comparados con los grupos con raciones no medicadas. No se observaron signos clínicos de la enfermedad en ninguno de los cerdos.

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This article is available online at http://www.aasv.org/shap.html.

O'Sullivan TL, Johnson R, Poljak Z, et al. An experimental study with a vaccine strain of porcine reproductive and respiratory syndrome virus to determine effects on viremia assessed by reverse transcriptase-polymerase chain reaction in pigs fed rations medicated with tilmicosin or non-medicated. *J Swine Health Prod.* 2016;24(2):81–92.

Implicaciones: La viremia relacionada con una cepa de vacuna de MLV no difirió entre los cerdos alimentados con raciones que contenían de 200 ó 400 mg por kg de tilmicosina. En ausencia de enfermedad clínica, los cerdos que consumieron el alimento medicado con tilmicosina tuvieron una ADG más alta que los cerdos que consumieron el alimento no medicado.

Résumé - Étude expérimentale avec une souche vaccinale du virus du syndrome reproducteur et respiratoire porcin afin de déterminer les effets sur la virémie évaluer par réaction d'amplification en chaine par la polymérase à l'aide de la transcriptase réverse chez des porcs nourris avec des rations médicamentées avec du tilmicosin ou non-médicamentées

Objectifs: Déterminer si une ration médicamentée avec du tilmicosin affecte la virémie (évaluée en utilisant une réaction d'amplification en chaine par la polymérase avec la transcriptase réverse [RT-PCR]) chez des porcs exposés à une souche vaccinale du virus du syndrome reproducteur et respiratoire porcin (VSRRP), les signes cliniques associés à la vaccination (température corporelle), et le gain quotidien moyen.

Matériels et méthodes: Des porcs Yorkshire pur-sang (N = 192) ont été répartis dans un des cinq groupes de traitement. Les groupes 1a et 1b sont demeurés négatifs pour VSRRP (témoins), alors que les groupes 2, 3, et 4 ont été injectés avec un vaccin VSRRP vivant modifié. Les groupes 1b et 2 ont été nourris avec des rations non-médicamentées. Les rations contenaient du tilmicosin à un dosage de 400 mg par kg pour les groupes 1a et 4 et 200 mg par kg pour le Groupe 3. Des échantillons de sang ont été prélevés afin de mesurer les concentrations sériques de tilmicosin et vérifier la virémie par VSRRP. Un lavage bronchiolaire a été effectué et les macrophages évalués pour virémie par VSRRP et concentrations de tilmicosin.

Résultats: Les groupes 1a et 1b sont demeurés négatifs pour VSRRP. Le nombre de copies de VSRRP par mL de sérum était le plus élevé chez les porcs inoculés à 10 jours post-inoculation, mais ne différait pas parmi les trois groupes inoculés. Le gain quotidien moyen était plus élevé dans les groupes nourris avec la ration contenant 400 mg par kg de tilmicosin que dans les groupes recevant des rations non-médicamentées. Les signes cliniques de maladie étaient absents chez tous les porcs.

Implications: La virémie associée à une souche vivante modifiée de vaccin n'était pas différente entre des porcs nourris avec des rations contenant 200 ou 400 mg par kg de tilmicosin. En absence de maladie clinique, des porcs consommant une ration contenant du tilmicosin ont un gain quotidien moyen plus élevé que des porcs consommant une ration non-médicamentée.

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically important diseases in swine production worldwide and an extremely difficult disease to control and eliminate.[1] Recent estimates have placed annual economic losses attributed to PRRS at $664 million dollars in the United States alone.[2] The causative agent, PRRS virus (PRRSV), belongs to the family *Arteriviridae*, and the primary site of replication in the pig is in the alveolar macrophages.[3] The clinical presentation of PRRS varies greatly from farm to farm, but generally includes reproductive failure in breeding animals and interstitial pneumonia in all age groups, and this respiratory tract infection is often complicated by co-infections with other pathogens.[4,5] The production impact of PRRS is evident by fewer sows farrowing, and decreased growth, higher mortality rates, and reduced feed efficiency in growing pigs. The effect on production varies with the virulence of the strain of virus involved and the presence of other diseases or co-infections, as well as management factors. A variety of strategies have been used to help control PRRSV or eliminate it from a herd. One common practice is to attempt to create herd immunity by closing the breeding herd and ensuring exposure to PRRSV using a commercial vaccine or a field strain of the virus.[6,7] Because the purposeful exposure of the breeding herd to a field strain of PRRSV is unpredictable, some veterinary practitioners complement virus exposure with concurrent use of antimicrobials, specifically tilmicosin, at the time of inoculation, to minimize the clinical impact of PRRS during this period of strategic herd exposure.[8] The use of tilmicosin at the time of diagnosis of a new or ongoing PRRS outbreak in a herd is also practiced.[9]

The reason tilmicosin is commonly chosen as a medication during a PRRSV outbreak is that tilmicosin is considered an effective antibiotic for many swine respiratory bacterial pathogens and also because there are reports of tilmicosin having some antiviral efficacy, at least in vitro.[10] Tilmicosin, a semi-synthetic macrolide antibiotic, is primarily used in swine production as an in-feed antimicrobial indicated for treatment of respiratory diseases.[11,12] Tilmicosin has a broad spectrum of antibacterial activity and accumulates in the alveolar macrophages.[13] In addition, tilmicosin exhibits an anti-inflammatory potential, which appears to be clinically relevant but has not yet been fully characterized.[14] In vitro testing has demonstrated an anti-viral effect of tilmicosin on PRRSV,[15,16] which has prompted studies investigating the use of macrolides on-farm during PRRSV infection.[8,9]

The primary objectives of this study were to determine if feed medicated with tilmicosin would reduce viremia in pigs exposed to a vaccine strain of PRRSV, minimize clinical signs associated with vaccination (body temperature), and improve average daily gain (ADG). The secondary objectives were to determine the effect of tilmicosin on macrophage activity and lung pathology in pigs exposed to a modified live vaccine (MLV) strain of PRRSV.

Materials and methods

Animals and study design

The study protocol and animal procedures were reviewed and approved by the University of Guelph Animal Care Committee, which adheres to the policies and guidelines of the Canadian Council on Animal Care.

One hundred and ninety-two purebred Yorkshire pigs, each weighing approximately 20 kg, were obtained from the Arkell Swine Research Facility, University of Guelph (a PRRSV-negative facility) and enrolled in the study. The Arkell herd was created as a specific-pathogen-free herd and has maintained a high health status, hence pigs are free of important respiratory pathogens, including PRRSV and *Mycoplasma hyopneumoniae*. The pigs for this trial were individually identified with ear

tags, weighed, and systematically randomized into five treatment groups, balancing for sex and weight (Table 1). The control pigs (Group 1a and Group 1b) were housed at a separate location from the pigs in groups 2, 3, and 4 in order to maintain PRRSV-negative status. Half of Group 1 (Group 1a) was provided with tilmicosin (Pulmotil Premix; Elanco Animal Health, Guelph, Ontario), 400 mg per kg in the feed, and the other half (Group 1b) was provided with the identical feed without tilmicosin. Group 1 pigs were all housed in the same room at the Arkell Swine Research Facility in six pens, with eight to 10 pigs per pen. The PRRSV-challenged pigs (groups 2, 3, and 4) were housed at the Ponsonby General Animal Research Facility, University of Guelph. At this facility, each treatment group was housed in a separate room of nine pens, with five to six pigs per pen. All pigs were assigned to their groups and pens for an acclimatization period of 10 days prior to inoculation on Day 0.

All pigs at both housing locations were fed the same diet, except the feed given to pigs in groups 3 and 4 included tilmicosin at a concentration according to their group assignment for 10 days prior to PRRSV inoculation (Day 0) and during the entire trial period to 14 days post inoculation (dpi). All feed consisted of the same diet specifications (except for tilmicosin concentration) and was manufactured at the same time by one feed manufacturer according to their standard operating procedures. Two doses of tilmicosin (200 and 400 mg per kg) were used because these were the approved doses for the product at the time in Canada. Pigs in groups 2, 3, and 4 were inoculated by an intramuscular injection of 2 mL of Ingelvac PRRSV MLV vaccine (Boehringer Ingelheim [Canada] Ltd, Burlington Ontario, Canada).

Average daily gain

Each pig was weighed at the beginning of the trial (Day -10) and at the end of trial (Day 14). The average daily gain (ADG) for each pig was determined for the trial period of 24 days.

Body temperature measurements

A digital rectal thermometer (Vicks Speed Read; Proctor and Gamble, Hudson, New York) was used to measure daily individual pig body temperature on 0, 1, 2, 3, and 4 dpi. The same thermometer was used and cleaned with rubbing alcohol between pigs in groups 2, 3, and 4. A separate thermometer (same manufacturer) was used for the control pigs in groups 1a and 1b.

Blood sample collection and serum PRRSV RT-PCR

Blood samples were collected from the orbital sinus on Day 0 (prior to inoculation), and on 2, 5, 7, 10, and 14 dpi from all animals. After collection, blood samples were stored at 4°C and allowed to clot, at which time the samples were centrifuged for 20 minutes and serum was removed. Quantitative PRRSV reverse transcriptase-polymerase chain reaction (RT-PCR) was conducted on all serum samples by the Animal Health Laboratory, University of Guelph, to assess PRRSV copies per mL. This was performed using an EZ-PRRSV kit (Tetracore Inc, Rockville, Maryland) and following the manufacturer's recommendations. Serum samples were subsequently stored at -80°C.

Bronchoalveolar lavage and post mortem examinations

Bronchoalveolar lavage (BAL) was performed on 20 pigs at 2 dpi and on 20 different pigs at 14 dpi (40 pigs total) to collect pulmonary alveolar macrophages (Table 1). In choosing these 40 pigs for BAL, five pigs per group assignment were randomly selected from groups 2, 3, and 4 at 2 dpi using a random number generator. Similarly, three pigs were randomly chosen from Group 1a and two pigs from Group 1b at 2 dpi to represent five pigs total from the PRRSV-negative groups and to balance with the numbers selected from Groups 2, 3, and 4 (PRRSV-inoculated). Subsequently, three different pigs were chosen from Group 1a and two different pigs from Group 1b at 14 dpi (totaling five pigs from PRRSV-negative groups at 14 dpi). At 14 dpi, 15 different pigs (five per group) were randomly chosen from groups 2, 3, and 4 in the same manner as at 2 dpi.

Pigs selected for BAL were pre-medicated with atropine (0.04 mg per kg) intramuscularly (IM). Fifteen to 20 minutes later pigs were given 3 to 4 mL IM of an anesthetic containing 1mg per kg butorphanol, 50 mg per mL ketamine, and 10 mg per mL xylazine. Pigs were placed in lateral recumbency, and palpebral reflexes and

Table 1: Treatment groups in a study to determine the effect of treatment with in-feed tilmicosin on viremia, clinical signs associated with vaccination (body temperature), average daily gain, macrophage activity, and lung pathology in pigs inoculated with a MLV PRRSV vaccine*

Group	n	No. of pigs euthanized for BAL		Inoculated with PRRSV vaccine	Tilmicosin in feed (mg per kg)
		at 2 dpi	at 14 dpi		
1a	29	3	3	No	400
1b	29	2	2	No	0
2	46	5	5	Yes	0
3	42	5	5	Yes	200
4	46	5	5	Yes	400

* Yorkshire pigs (N = 192 at start of trial), approximately 20 kg in body weight, were each randomly assigned to one of five treatment groups and, according to the group assignment, were inoculated with a MLV PRRSV vaccine at the label dose (Inglevac, Boehringer Ingelheim [Canada] Ltd, Burlington Ontario, Canada) or not inoculated (Day 0), and fed a ration medicated or not medicated with tilmicosin (Pulmotil Premix; Elanco Animal Health, Guelph, Ontario, Canada). Group 1a and Group 1b (not inoculated) were housed separately from groups 2, 3, and 4 (inoculated).

n = number of pigs per group at start of trial; MLV = modified live virus; PRRSV = porcine reproductive and respiratory syndrome virus; BAL = bronchoalveolar lavage; dpi = days post inoculation.

jaw tone were assessed. Pigs exhibiting jaw tone and a lateral palpebral reflex after 15 to 20 minutes post IM injection received the same anesthetic intravenously (IV), via the ear vein, titrated to effect. Pigs were then placed in dorsal recumbency, the mouth was positioned open with a speculum, and the larynx was sprayed once with lidocaine, 10 mg per spray (Odan Laboratories Ltd, Montreal, Quebec, Canada). A pediatric bronchoscope was passed into the trachea and inserted into the right caudal lung lobe. Sixty mL of sterile phosphate-buffered saline (PBS) was gently flushed into the lung. Typically, 30 to 40 mL of bronchoalveolar lavage fluid (BALF) was recovered from each BAL. The BALF was immediately placed on ice and submitted to the Animal Health Laboratory, University of Guelph, for cytological and quality-control assessment. Immediately after the BAL, each pig was euthanized with a lethal IV injection, via the ear vein, of 5 mL of pentobarbital (240 mg per mL). The bronchoscope was aseptically prepared between pigs with glutaraldehyde (ASEPT-sterile 28; Ecolab Co, Mississauga, Ontario, Canada) and isopropyl alcohol and allowed to dry. After euthanasia, post mortem examinations were performed on all 40 pigs (20 pigs at 2 dpi and 20 pigs at 14 dpi), which included sampling at three sites of the right and left cranial and caudal lung lobes for histopathology and immunohistochemistry for PRRSV. Alveolar macrophages were isolated from the lavage fluid for in vitro studies designed to evaluate the effects of tilmicosin on macrophage activity, and for tilmicosin concentration determination using high performance liquid chromatography (HPLC).

Determination of tilmicosin concentration in BALF and serum

Ten animals per group were selected from the study population, using simple random sampling, to have serum tilmicosin concentration levels determined using HPLC. Additionally, for animals selected to have BAL performed, tilmicosin serum concentrations were determined using HPLC. The HPLC analysis was performed on a Waters Alliance 2695 HPLC system (Mississauga, Ontario, Canada) with a Waters 2996 photodiode array detector. A gradient separation was carried out on an XTerra Phenyl Column (5 µm, 4.6 mm × 100 mm, Waters, Dublin, Ireland) using a mobile phase containing (A) water-acetic acid (1% volume by volume [v/v]) and (B) acetonitrile-acetic acid (1% v/v). The gradient started at 8 minutes with 85% A and reached 70% A at 20 minutes. The flow rate was 1 mL per minute and the eluent was monitored at 290 nm. The retention times were 19.7 minutes for tilmicosin and 23.2 minutes for tylosin (internal standard). Tilmicosin and tylosin standards were purchased from Sigma-Aldrich, Oakville, Ontario, Canada. Calibration standards and quality controls were prepared in blank swine serum. A modified solid-phase extraction (SPE) technique was used for tilmicosin sample extraction.[17] Briefly, the Sep-Pak C18 SPE cartridge (Waters, Milford, Maryland) was conditioned with methanol and water, then 1 mL of serum or BALF sample spiked with tylosin internal standard was applied to the cartridge. The cartridge was washed with water followed by 5% methanol, and tilmicosin was eluted with acetonitrile-methanol-0.5% phosphoric acid. Serum calibration curves were prepared on 14 separate days. Five points of the calibration curves were linear and reproducible in the concentration range from 0.05 µg per mL to 0.5 µg per mL, with the correlation coefficient (r^2) > 0.99 for all curves. The limit of detection (LOD) was 0.03 µg per mL (based on three times the signal-to-noise ratio) and the limit of quantitation (LOQ) was 0.05 µg per mL. The intra-day and inter-day assay precisions were 12.28% and 8.97%. The accuracy for each calibration standard was within 15%, except at LOQ (0.05 µg per mL), where it deviated by less than 20%. Average recovery was 91.2%, with 90.1% at LOQ (0.05 µg per mL).

Macrophage and cell culture preparation

Alveolar macrophages were isolated from the BALF according to Brumbaugh et al[18] and Cao et al,[19] with minor modifications. Briefly, filtered raw BALF was centrifuged at 400*g* for 5 minutes at 4°C. Cell pellets were washed three times with PBS containing 3% penicillin-streptomycin (Invitrogen, Camarillo, California), and then re-suspended in PBS-Ross Park Memorial Institute (RPMI) solution containing 10% fetal bovine serum, 3% penicillin-streptomycin, and 0.2% gentamicin (Walk-Chemie Medical GmbH, Steinbach, Germany). For each animal, viable macrophages were counted using 25 µL trypan blue as a vital stain. Samples were then diluted to the concentration of 1×10^6 macrophage cells per mL with RPMI solution containing 10% heat-inactivated fetal bovine serum. Cells were plated into 24-well tissue culture plates and incubated at 37°C with 5% CO_2 and 95% O_2. Cells were allowed to adhere for 2 hours, and non-adherent cells and media were removed by gentle aspiration. After cell adherence, lipopolysaccharide (LPS) was added to make the final concentrations of individual wells equivalent to either 10 ng per mL or 100 ng per mL (LPS in 50 mL RPMI containing 10% fetal bovine serum) in triplicates. After 16 hours incubation, the medium was harvested and frozen for enzyme immunoassay analysis (EIA; Prostaglandin E2-EIA Monoclonal Kit, Cayman Chemical Co, Ann Arbor, Michigan). To each well, 0.5 mL of water free of PBS and ribonucleic acid was then added, and the plates were stored at -80°C until assayed by RT-PCR.

Measurement of cytokines (PGE_2, IL-10, and TNF-α) in alveolar macrophages

Frozen samples were thawed on ice and centrifuged at 13,000*g* and 4°C. Alveolar macrophages from untreated animals and animals treated with tilmicosin were examined for PGE_2, IL-10, and TNF-α production using commercial EIA kits: Prostaglandin E2-EIA Monoclonal Kit, Cayman Chemical Co; IL-10 Swine ELISA Kit, and TNF-α Swine ELISA Kit (Invitrogen), respectively. The concentration of each cytokine was determined according to the manufacturers' protocols. The LOD for PGE_2 was 15 pg per mL, and LODs for IL-10 and TNF-α were 6.2 pg per mL and 23.4 pg per mL, respectively.

Macrophage PRRSV titre determination using RT-PCR

Alveolar macrophages adhered to the tissue culture plates were detached by scrubbing and suspended in RNase-free water (Walk-Chemie Medical GmbH). The samples were frozen at -80°C for subsequent RT-PCR analysis. The number of PRRSV virus copies per mL was determined in the recovered macrophages using quantitative PRRSV RT-PCR at the Animal Health Laboratory, University of Guelph.

Histopathology

Cranial and caudal lung samples from the 40 animals on which BALs were performed were fixed in 10% formalin. The samples were processed for histologic examination, stained with hematoxylin and eosin, and examined by light microscopy. For each animal, lung sections were evaluated for the presence or absence of predetermined lesions indicative of respiratory disease in pigs.[20] Immunohistochemistry (IHC) was

performed on sequential sections of all lung samples using an automated stainer (Dako, Burlington, Ontario, Canada) and an anti-PRRSV mouse monoclonal antibody (SDOW17; RTI, Brookings, South Dakota) with horseradish peroxidase-labelled streptavidin-biotin detection (LSAB2, Dako) and Nova Red chromogen (Vector Laboratories, Burlington, Ontario, Canada). Lung sections were assessed for immunostaining. Histologic sections and IHC slides were evaluated by the same veterinary pathologist (JDL), who was blinded to treatment group of individual animals.

Statistical analysis

The association between PRRSV viremia and group assignment was modeled using a mixed linear regression model (PROC MIXED procedure SAS 9.3; SAS Institute Inc, Cary, North Carolina). In this model, housing location (barn) was considered a fixed effect and pen was modeled as a random effect. The quantitative PRRSV PCR values (PRRSV copies per mL) were transformed to base 10 logarithms for optimum model fit and presentation. Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to identify the best-fitting correlation structure for repeated measures conducted on the same animal over time. The association between body temperature and group assignment was also modeled using mixed linear regression in the same manner. Temperature was back-transformed for presentation. Mixed linear regression was used to determine any effect of group assignment with ADG. In this model, body weight at the start of the trial, sex, and barn were modeled as fixed effects and pen was modeled as a random effect. Model diagnostics were performed on all models. Univariable analyses were conducted using exact logistic regression models to determine if there were statistically significant associations between histologic lesions identified and group assignment. Cytokine concentrations in alveolar macrophages were analysed using the Wilcoxon rank test to test for significant differences between cytokine control wells and LPS-induced wells, and additionally, to test for differences between treatment groups. For presentation purposes, mean concentration values were also analyzed using a *t* test (with unequal variance). The results were presented as mean concentrations for ease of interpretation.

Results

Pig health and performance

No clinical signs of disease were noted in any of the pigs throughout the entire length of the trial, including signs of respiratory disease in the pigs inoculated with MLV-PRRSV vaccine. Nine pigs were euthanized at various points in the trial in accordance with the animal use protocol set by the University of Guelph for reasons unrelated to the trial. The least squares means of average daily gains (ADGs) by group over the entire trial period are presented in Table 2. The mean ADG was 79 g per day greater for Group 4 versus Group 2 ($P < .001$). Mean ADG was lower in Group 1a and Group 1b than in groups 2, 3, and 4 ($P < .001$). The overall mean of rectal temperature for Group 2 was 0.09°C lower ($P < .05$) than overall mean rectal temperature for Group 4 over the 4 days of measurement. No other associations between body temperature and treatment or by day were found, and inoculation with a vaccine strain of PRRSV did not result in a rise in rectal temperature.

Presence of PRRSV antigen in serum and lung tissue, and lung histologic lesions

The prevalence of pigs with PRRSV viremia following inoculation and the number of serum PRRSV copies per mL (transformed to base 10 logarithms) per group by day are presented in Table 3. The controls (groups 1a and 1b) did not develop PRRSV viremia over the entire trial period. Number of PRRSV copies per mL serum did not differ among groups 2, 3, and 4 over the entire study period or on any particular day measured.

All 20 lung samples from the pigs subjected to BAL at 2 dpi were immunohistochemically negative for PRRSV antigen in lung. Similarly, all 20 lung samples from pigs subjected to BAL at 14 dpi were immunohistochemically negative for PRRSV antigen. There were no significant differences in the histologic lesions identified among the treatment groups; the lesions identified are summarized in tables 4a and 4b.

Tilmicosin concentrations and BAL results

Serum tilmicosin concentration levels of the 10 animals randomly selected per group at 2, 7, and 14 dpi are presented in Table 5. The groups receiving non-medicated feed had no detectable serum tilmicosin concentrations. The groups receiving tilmicosin-medicated feed had detectable serum concentrations of tilmicosin by 7 dpi.

None of the 20 animals that had a BAL performed at 2 dpi had detectable levels of tilmicosin in their serum or alveolar macrophages. Similarly, PRRSV nucleic acid was not detected in alveolar macrophages of any animal at 2 dpi. The results of the cytokine concentrations for PGE-2, IL-10,

Table 2: Least squares means of average daily gain (kg) of Yorkshire pigs over the entire study period (24 days) by group*

Group	n	Mean (kg)	SD	Minimum	Maximum
1a	24	0.698[a]	0.111	0.472	0.856
1b	26	0.637[b]	0.112	0.392	0.856
2	38	0.765[c]	0.161	0.438	1.324
3	36	0.796[c]	0.165	0.484	1.394
4	39	0.844[d]	0.150	0.502	1.102

* Study and group assignments described in Table 1. Mixed linear regression model was performed with initial weight, sex, and barn modeled as fixed effects and pen modeled as random effect (SAS 9.3; SAS Institute Inc, Cary, North Carolina). Differences were considered statistically significant at $P < .05$. n = number of pigs per group at end of trial.

[abcd] Within a column, different superscripts indicate statistical differences between groups ($P < .001$).

SD = standard deviation.

Table 3: Prevalence of PRRSV and least squares means [95% CI] of number of PRRSV copies per mL of serum (expressed as base 10 logarithms) in the five study groups 0, 2, 4, 7, 10, and 14 days post inoculation (dpi) with a MLV PRRSV vaccine*

Group	Day 0	2 dpi	4 dpi	7 dpi	10 dpi	14 dpi
1a	0/29	0/29	0/26	0/25	0/25	0/24
1b	0/29	0/29	0/27	0/27	0/26	0/26
2	0/46	19/44	32/39	35/38	35/38	34/38
		2.99	4.36	4.89	4.72	4.51
		[2.73-3.26]	[3.99-4.71]	[4.51-5.26]	[4.37-5.07]	[4.13-4.89]
3	0/42	16/41	28/36	31/36	32/36	28/36
		3.05	4.24	4.64	4.56	4.33
		[2.78-3.23]	[3.87-4.62]	[4.25 -5.03]	[4.20-4.92]	[3.94-4.72]
4	0/46	23/46	34/41	36/41	40/40	31/39
		3.25	4.43	5.01	4.95	4.32
		[2.99-3.50]	[4.08-4.78]	[4.64-5.37]	[4.60-5.29]	[3.95-4.69]

* Study and group assignments described in Table 1. No significant differences were measured between treatment groups over the entire trial period or on any day using a mixed linear regression model, with pen as a random effect and accounting for repeated measures in individual pigs using Toeplitz correlation structure (SAS 9.3; SAS Institute Inc, Cary, North Carolina).

PRRSV = porcine reproductive and respiratory syndrome virus; CI = confidence interval; MLV = modified live virus.

and TNF-∝ in alveolar macrophages from BALF of randomly selected pigs at 2 and 14 dpi are shown in Table 6a and Table 6b, respectively. In summary, the mean concentrations (at 2 dpi) of TNF-∝ differed from the control well in Group 1a; Il-10 and TNF-∝ differed from the control well in Group 2; PGE-2 and TNF-∝ differed from the control well in Group 3; and PGE-2 and TNF-∝ differed from the control well in Group 4. There was also a difference in the TNF-∝ between Group 3 and Group 4. Similarly, at 14 dpi, the mean concentrations of PGE-2, IL-10, and TNF-∝ differed from the control well in Group 2; and PGE-2 and TNF-∝ differed from the control well in Group 3 and Group 4. No between-group differences were found in cytokine concentrations at 14 dpi. The descriptive results for tilmicosin serum and macrophage concentrations, macrophage cytokine concentration, and macrophage PRRSV titres on the 20 animals randomly selected for BAL at 14 dpi are presented in Table 7.

Discussion

Pigs medicated with tilmicosin in the feed at concentrations of 200 mg per kg or 400 mg per kg and treated for 10 days prior to inoculation with a vaccine strain of PRRSV showed no reduction in viremia compared to untreated controls. The MLV vaccine used in the study to infect pigs with virus proved to be effective in creating a viremia, with the mean viral titre being highest at 7 dpi. The prevalence of PCR-positive animals did not differ between groups at each day tested post-inoculation (highest at 10 dpi) and likewise the amount of virus as measured by $\log_{10}$ PRRSV copies per mL did not differ between groups. Others have found less lung damage in tilmicosin-treated pigs challenged by a field strain of PRRSV, compared to non-treated pigs.[21] In the present trial, there was very little lung pathology because the vaccine strain of PRRSV used in this trial is relatively non-pathogenic. The results reflect in part that the sensitivity of IHC is low when antigen load in tissue is low, and that only two sections of lung per pig were examined.[22] In addition, the pigs used in this trial were from a high-health herd, and there was no evidence of secondary respiratory pathogens present. It is quite possible that if a highly pathogenic field strain of PRRSV had been used to inoculate the pigs, the results may have been different. Likewise, tilmicosin is an effective treatment for many of the common secondary bacterial swine pathogens,[23] and therefore one would expect the use of tilmicosin to greatly reduce lung pathology if bacterial pathogens were also present, which is often the case in outbreaks of PRRS involving field strains. The fact that tilmicosin did not affect the level of viremia in the present trial does suggest that the positive results observed in clinical cases[8,16] might be due to the effect on secondary bacterial pathogens or through other indirect means and not because of anti-viral effects, particularly prevention of viral replication, which has been suggested.[9] However, since the vaccine strain of PRRSV used in this study is attenuated, it would be necessary to repeat the trial with a field strain to compare results.

In vitro studies have reported that PRRSV replication in porcine pulmonary alveolar macrophages that were exposed to 0.1 and 1.0 µg per mL tilmicosin was reduced by 3 to 4 logs of virus.[24] It has been suggested that the antiviral activity of tilmicosin might be related to the drug's ability to enter macrophages and accumulate intracellularly, causing endosomal pH to rise. Tilmicosin is highly lipophilic and is efficiently taken up by macrophages through lipid cell membranes. Efflux is slow, and researchers report 37% of tilmicosin is still cell-associated after 24 hours, mainly in lysosomes.[25] Kreutz and Ackermann[26] have shown that PRRSV requires a low-pH-dependent pathway for cell entry, and this work was confirmed by Nauwynck et al.[27] In vitro studies have shown that another macrolide, tylvalosin, accumulates in macrophages more readily than tilmicosin and may have more potential for PRRSV

Table 4a: Summary of the frequency of histologic lesions identified in pig lung tissue, by group, following inoculation with MLV PRRSV vaccine, on formalin-fixed samples collected 2 days post inoculation (dpi)*

Lesion	Cell type	Percentage (count) of lungs with histologic lesions				
		Group 1a n = 3	Group 1b n = 2	Group 2 n = 5	Group 3 n = 5	Group 4 n = 5
Alveolar septal infiltrates	Macrophages	100.0 (3)	100.0 (2)	100.0 (5)	100.0 (5)	100.0 (5)
	Lymphocytes	66.7 (2)	100.0 (2)	80.0 (4)	100.0 (5)	100.0 (5)
	Neutrophils	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Alveolar infiltrates	Macrophages	100.0 (3)	100.0 (2)	80.0 (4)	60.0 (3)	80.0 (4)
	Lymphocytes	33.3 (1)	100.0 (2)	60.0 (3)	60.0 (3)	60.0 (3)
	Neutrophils	33.3 (1)	0.0 (0)	20.0 (1)	20.0 (1)	0.0 (0)
Perivascular cuffing	Lymphocytes	66.7 (2)	0.0 (0)	60.0 (3)	60.0 (3)	80.0 (4)
	Plasma cells	33.3 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	Macrophages	33.3 (1)	100.0 (2)	60.0 (3)	40.0 (2)	0.0 (0)
Peribronchial infiltrates	Macrophages	100.0 (3)	100.0 (2)	40.0 (2)	80.0 (4)	20.0 (1)
	Lymphocytes	66.7 (2)	0.0 (0)	20.0 (1)	40.0 (2)	60.0 (3)
Interlobular septal lesions	Stromal fibrosis	66.7 (2)	50.0 (1)	80.0 (4)	80.0 (4)	80.0 (4)
	Macrophage infiltration	66.7 (2)	50.0 (1)	60.0 (3)	60.0 (3)	80.0 (4)
	Lymphocyte infiltration	33.3 (1)	50.0 (1)	20.0 (1)	20.0 (1)	40.0 (2)

* Study and group assignments described in Table 1. No significant differences in the probability of lesion identification were measured between groups using exact logistic regression (Stata 12; StataCorp LP, College Station, Texas). Lesions were all evaluated by the same veterinary pathologist (JDL), who was blinded to group assignment. Type II pneumocyte hyperplasia was not identified in any of the samples.

MLV = modified live virus; PRRSV = porcine reproductive and respiratory syndrome virus; n = number in group examined.

control.[15] In addition, inhibition of PRRSV replication in vitro depends to some extent on the type of virus. The relatively low serum concentrations of tilmicosin found in this study were expected, as pharmacokinetic studies in the literature have noted that tilmicosin quickly disappears from serum but accumulates in phagocytes. Shen et al[11] found peak serum concentrations, after a single individual oral dose of 20 or 40 mg of tilmicosin, were 1.19 ± 0.30 μg per mL and 2.03 ± 0.28 μg per mL, respectively. These concentrations were achieved after fasting the animal and then feeding the medicated feed a single time. The peak levels surpassed the concentrations observed in the present study where pigs were fed free-choice. In the present study, tilmicosin was detected in alveolar macrophages, but PRRSV was detected in macrophages as well.

In addition to antibacterial effects, macrolides have immune-modulatory activities.[28] There has been speculation that a reduction in inflammatory response to PRRSV might explain some of the benefits observed when pigs are fed tilmicosin during a PRRS outbreak. In the present study, the inoculation of pigs with a vaccine strain of PRRSV did not result in a rise in rectal temperature, so it was not possible to determine if tilmicosin helped prevent pyrexia. Similarly, the mean concentrations of PGE-2, IL-10, and TNF-∝ in alveolar macrophages collected from BALF did not demonstrate less inflammatory response in the treatment group. The pigs housed at the Ponsonby facility (groups 2, 3, and 4) had higher ADGs than the pigs housed at the Arkell facility. There is no biological reason why injecting healthy PRRS-negative pigs with PRRSV vaccine would stimulate better growth rate. It must be assumed that the housing conditions at the Ponsonby facility were superior to those at the Arkell facility and that housing and environmental factors were the most likely reason for the differences in performance between the two sites. In both facilities, the pigs receiving 400 mg per kg of tilmicosin in the feed had higher ADGs than the pigs not receiving tilmicosin. It should be noted that there were no clinical signs of disease in any of the pigs during the trial and that this growth-promoting effect occurred in pigs with a high-health status. This phenomenon of feeding antibiotics to healthy pigs and achieving improved performance has been well documented and used widely in the industry for decades. Presumably, if there had been a bacterial respiratory disease challenge, the differences in the groups might have been even greater. Positive benefits from feeding tilmicosin to pigs during a PRRS outbreak might be explained on the basis of this growth-promoting effect and on the control of secondary bacterial diseases. This present study does not support the theory that the benefits of feeding tilmicosin are related to an antiviral effect. However, a non-pathogenic vaccine strain of PRRSV was used in this study, and this association should be further investigated using different field strains under similar experimental design.

Table 4b: Summary of the frequency of histologic lesions identified in pig lung tissue, by group, following inoculation with MLV PRRSV vaccine, on formalin fixed samples collected 14 days post inoculation (dpi)*

Lesion	Cell type	Percentage (count) of lungs with histologic lesions				
		Group 1a n = 3	Group 1b n = 2	Group 2 n = 5	Group 3 n = 5	Group 4 n = 5
Alveolar septal infiltrates	Macrophages	100.0 (3)	100.0 (2)	100.0 (5)	100.0 (5)	100.0 (5)
	Lymphocytes	100.0 (3)	100.0 (2)	100.0 (5)	100.0 (5)	100.0 (5)
	Neutrophils	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Alveolar infiltrates	Macrophages	66.7 (2)	50.0 (1)	100.0 (5)	40.0 (2)	60.0 (3)
	Lymphocytes	66.7 (2)	50.0 (1)	60.0 (3)	40.0 (2)	40.0 (2)
	Neutrophils	66.7 (2)	50.0 (1)	0.0 (0)	0.0 (0)	40.0 (2)
Perivascular cuffing	Lymphocytes	66.7 (2)	100.0 (2)	100.0 (5)	100.0 (5)	100.0 (5)
	Plasma cells	33.3 (1)	0.0 (0)	0.0 (0)	20.0 (1)	0.0 (0)
	Macrophages	0.0 (0)	0.0 (0)	40.0 (2)	20.0 (1)	0.0 (0)
Peribronchial infiltrates	Macrophages	0.0 (0)	0.0 (0)	60.0 (3)	40.0 (2)	20.0 (1)
	Lymphocytes	66.7 (2)	0.0 (0)	80.0 (4)	60.0 (3)	60.0 (3)
Interlobular septal lesions	Stromal fibrosis	0.0 (0)	0.0 (0)	60.0 (3)	60.0 (3)	20.0 (1)
	Macrophage infiltration	0.0 (0)	0.0 (0)	20.0 (1)	40.0 (2)	0.0 (0)
	Lymphocyte infiltration	0.0 (0)	0.0 (0)	40.0 (2)	20.0 (1)	20.0 (1)

* Study and group assignments described in Table 1. No significant differences in the probability of lesion identification were measured between groups using exact logistic regression (Stata 12; StataCorp LP, College Station, Texas). Lesions were all evaluated by the same pathologist (JDL), who was blinded to group assignment. Type II pneumocyte hyperplasia was not identified in any of the samples.
MLV = modified live virus; PRRSV = porcine reproductive and respiratory syndrome virus; n = number in group examined.

Implications

- Under the conditions of this study, PRRSV viremia associated with a MLV vaccine strain is not significantly different in pigs fed a ration containing 200 mg per kg or 400 mg per kg tilmicosin, compared to pigs fed a ration containing no tilmicosin.
- Pigs consuming tilmicosin-medicated feed have faster growth rate, indicated by a higher ADG, than pigs fed non-medicated feed in the absence of clinical signs of disease.

Acknowledgements

This research was supported by the University of Guelph-Ontario Ministry of Agriculture, Food and Rural Affairs Research Partnership and the Canadian Swine Health Board. The authors thank the staff of the Ponsonby and Arkell research facilities for their assistance.

Conflict of interest

None reported.

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Table 5: Serum tilmicosin concentration in 10 pigs randomly selected per group at 2, 7, and 14 days post inoculation (dpi) with a MLV PRRSV vaccine*

Group	Animal ID	Serum tilmicosin (μg/mL)		
		2 dpi	7 dpi	14 dpi
1a	104	ND	0.073	0.075
	133	ND	0.064	0.073
	138	ND	0.071	0.080
	154	0.061	0.063	0.065
	157	0.055	0.072	ND
	159	0.056	0.078	0.061
	166	ND	0.075	0.084
	169	ND	0.064	0.076
	182	ND	0.088	0.065
	188	ND	0.075	0.063
3	14	ND	0.056	ND
	15	ND	ND	ND
	31	ND	ND	ND
	33	ND	0.054	ND
	37	ND	ND	ND
	62	ND	ND	ND
	63	ND	ND	0.060
	72	ND	ND	ND
	89	ND	ND	0.064
	97	ND	ND	ND
4	26	ND	0.059	0.056
	29	ND	0.057	0.065
	56	ND	0.058	0.070
	58	ND	0.059	0.074
	68	ND	0.054	0.058
	81	ND	0.051	0.052
	90	ND	0.056	0.062
	121	ND	0.060	0.067
	131	ND	0.050	0.063
	140	ND	0.053	0.061

* Study and group assignments described in Table 1. Serum tilmicosin determined by high performance liquid chromatography. Tilmicosin was not detected in samples from groups 1b and 2, where pigs were not fed tilmicosin.

MLV = modified live virus; PRRSV = porcine reproductive and respiratory syndrome virus; ND = not detected.

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Table 6a: Mean concentrations of the cytokines PGE-2, IL-10, and TNF-∝ in alveolar macrophages collected from BALF of pigs randomly selected at 2 days post inoculation (dpi) with a MLV PRRSV vaccine*

	Mean alveolar macrophage cytokine concentration (pg/mL) (no. of successful well cultures)					
	PGE-2		IL-10		TNF-∝	
	Control well	LPS induced	Control well	LPS induced	Control well	LPS induced
Group	[95% CI] (n)	[95% CI] (n)	[95% CI] (n)	[95% CI] (n)	[95% CI] (n)	[95% CI] (n)
1a	125.70 [-20.10-271.50] (3)	314.23 [-50.35-678.82] (3)	0.33 [-0.54-1.21] (3)	3.43 [-1.50-8.37] (3)	372.17† [-224.31-968.64] (3)	16,149.57† [-2307.41-34,606.54] (3)
1b	392.55 [-1741.46-2526.56] (2)	981.30 [-4352.77-6315.37] (2)	1.35 [-11.99-14.69] (2)	3.85 [0.67-7.03] (2)	703.00 [-1225.81-2631.81] (2)	27483.0 [-77,640.21-132,606.80] (2)
2	415.25 [217.32-613.18] (4)	1038.18 [543.43-1532.92] (4)	0.28† [-0.60-1.15] (4)	3.75† [0.33-7.17] (4)	466.53† [115.95-817.10] (4)	17224.05† [12902.18-21545.91] (4)
3	324.88† [275.76-373.99] (4)	812.15† [689.43-934.87] (4)	0.73 [0.33-1.12] (4)	2.65 [1.99-3.31] (4)	728.15† [91.12-1365.18] (4)	25894.55†‡ [14197.1-37592.0] (4)
4	280.65† [132.80-428.50] (4)	701.55† [332.06-1071.04] (4)	0.63 [-0.44-1.69] (4)	6.05 [2.36-9.74] (4)	725.08† [-32.26-1482.41] (4)	16004.40†‡ [8037.05-23971.75] (4)

* Study and group treatment assignments described in Table 1. Difference of means determined by two-sample *t* test with unequal variance of group means. Statistical significance also confirmed with non-parametric Wilcoxon rank sum test at $P < .05$.
† Difference between control and LPS-induced concentrations within cytokine and within group is statistically significant ($P < .05$).
‡ Difference in LPS-induced cytokine level between groups is statistically significant ($P < .05$).
BALF = bronchoalveolar lavage fluid; MLV = modified live virus; LPS = lipopolysaccharide.

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Table 6b: Mean concentrations of the cytokines PGE-2, IL-10, and TNF-∝ in alveolar macrophages collected from BALF of pigs randomly selected at14 days post inoculation (dpi) with a MLV PRRSV vaccine*

	Mean alveolar macrophage cytokine concentration (pg/mL) 14 dpi (no. of successful well cultures)					
	PGE-2		IL-10		TNF-∝	
Group†	Control well [95%CI] (n)	LPS induced [95%CI] (n)	Control well [95%CI] (n)	LPS induced [95%CI] (n)	Control well [95%CI] (n)	LPS induced [95%CI] (n)
1a	77.23 [-152.44-306.90] (3)	273.37 [-408.35-955.09] (3)	6.63 [-3.00-16.27] (3)	7.17 [4.62-9.72] (3)	146.10 [-188.05-480.25] (3)	8134.10 [-8128.67-24,396.87] (3)
1b	97.3 [-977.64-1172.25] (2)	298.25 [-2111.48-2707.98] (2)	8.2 [-9.59-25.99] (2)	6.3 [-3.86-16.46] (2)	505.95 [-5118.45-6130.35] (2)	12,178.55 [-5142.49-19,214.61] (2)
2	248.02† [135.58-360.46] (5)	674.42† [407.68-941.16] (5)	9.6† [8.17-11.03] (5)	20.76† [7.28-34.24] (5)	747.52† [516.42-978.62] (5)	13790.98† [6672.53-20,909.43] (5)
3	155.13† [95.51-214.76] (4)	537.13† [288.55-785.72] (4)	9.65 [4.97-14.33] (4)	15.7 [1.99-3.31] (4)	465.88† [168.78-762.97] (4)	10471.93† [1435.15-19,508.70] (4)
4	281.12† [206.77-355.47] (4)	761.18† [540.69-981.67] (4)	13.08 [7.31-18.85] (5)	21.78 [8.31-35.25] (5)	1037.86† [738.22-1337.50] (5)	12276.08† [7706.77-16,845.39] (5)

* Study and group assignments described in Table 1. Difference of means determined by two sample *t* test with unequal variance of group means. Significance also confirmed with non-parametric Wilcoxon rank sum test at $P < .05$.

† Difference between control and LPS-induced wells within cytokine and within group is statistically significant ($P < .05$).

BALF = bronchoalveolar lavage fluid; MLV = modified live virus; PRRSV = porcine reproductive and respiratory syndrome virus; LPS = lipopolysaccharide; CI = confidence interval.

Table 7: Tilmicosin concentrations in serum and alveolar macrophages, macrophage cytokines PGE-2, IL-10 and TNF-∝ concentrations, and macrophage PRRSV copies/mL in 20 pigs randomly selected for bronchoalveolar lavage 14 dpi with MLV PRRSV vaccine*

Group	Animal ID	Tilmicosin concentration (μg/mL) 14 dpi		Cytokine tilmicosin concentration (pg/mL) 14 dpi						Alveolar macrophage PRRSV copies/mL 14 dpi
		Serum	Alveolar macrophage	PGE-2		IL-10		TNF-∝		
				Control well	LPS induced	Control well	LPS induced	Control well	LPS induced	
1a	78	0.074	ND	1.6	31.3	4.7	6.3	8.5	759.9	ND
	109	0.086	ND	1.6	31.3	11.1	8.3	277.3	10380.9	ND
	176	0.091	ND	180.3	571.5	4.1	6.9	152.5	13261.5	ND
1b	165	ND	ND	181.9	487.9	9.6	7.1	948.6	12732.3	ND
	186	ND	ND	12.7	108.6	6.8	5.5	63.3	11624.8	ND
2	21	ND	ND	384.7	907.4	11.1	18.8	1047.3	9364.6	2.17E + 03
	41	ND	ND	233.5	488.5	9.4	12.1	709.5	11803.9	1.82E + 05
	69	ND	ND	249.0	904.6	9.6	30.6	787.6	11942.5	4.37E + 03
	101	ND	ND	242.7	489.2	7.9	9.0	603.4	11989.8	3.67E + 05
	117	ND	ND	130.2	582.4	10.0	33.3	589.8	23854.1	1.12E + 06
3	50	0.053	ND	179.3	483.2	6.9	8.8	669.6	9317.0	2.80E + 03
	67	ND	ND	ND	ND	10.9	27.9	217.1	7878.8	9.50E + 05
	80	ND	ND	ND	ND	ND	ND	ND	ND	5.73E + 03
	98	ND	ND	131.3	475.6	7.6	10.0	497.7	18736.4	3.86E + 05
	142	ND	ND	154.8	652.6	13.2	16.1	479.1	5955.2	6.14E + 05
4	47	0.071	ND	251.8	579.0	14.0	33.0	1045.1	12562.3	1.08E + 06
	60	0.087	ND	198.3	661.1	9.3	14.2	697.2	8846.4	1.25E + 04
	70	0.072	ND	326.0	883.1	8.0	8.7	1338.5	16331.3	ND
	87	0.061	ND	349.0	1007.5	19.7	32.5	326.0	883.1	4.31E + 04
	91	0.068	ND	280.5	675.2	14.4	20.5	1168.1	15388.4	1.28E + 04

* Study and group assignments described in Table 1. Pigs vaccinated with Ingelvac PRRSV MLV (Boehringer [Canada] Ltd, Burlington, Ontario, Canada).

PRRSV = porcine reproductive and respiratory syndrome virus; MLV = modified live virus; dpi = days post inoculation; ND = not detected; LPS = lipopolysaccharide.