Evaluation of a ceftiofur-washed whole cell *Streptococcus suis* bacterin in pigs

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Abstract

The efficacy of currently available washed whole cell *Streptococcus suis* bacterins is generally poor. We developed and tested the efficacy of a novel ceftiofur-washed whole cell bacterin. Sixty-six, 2-week-old specific pathogen free (SPF) pigs were randomly divided into 5 groups. Three groups were vaccinated 28 and 14 d prior to challenge. The 3 ceftiofur-washed whole cell bacterins each contained 1 of 3 different adjuvants (Montanide ISA 25, Montanide ISA 50, and Saponin). Pigs exhibiting severe central nervous system disease or severe joint swelling and lameness were euthanized immediately and necropsied. All remaining pigs were necropsied at 14 d post inoculation. The ceftiofur-washed whole cell *S. suis* bacterin with Montanide ISA 50 adjuvant significantly ($P < 0.05$) reduced bacteremia, meningitis, pneumonia, and mortality associated with *S. suis* challenge. Further work on this novel approach to bacterin production is warranted.

Résumé

Les bactérines à base de cellules entières lavées actuellement disponibles pour l'immunisation contre Streptococcus suis *sont généralement peu efficaces. Nous avons développé et testé l'efficacité d'une nouvelle bactérine constituée de cellules entières lavées avec du ceftiofur. Un total de 66 porcelets exempts d'agents pathogènes spécifiques (SPF) âgés de 2 semaines ont été divisés de manière aléatoire en cinq groupes. Trois groupes ont été vaccinés 28 et 14 j avant une infection défi. Les 3 bactérines à base de cellules entières lavées au ceftiofur étaient combinées à 1 de 3 adjuvants différents (Montanide ISA 25, Montanide ISA 50, et Saponin). Les animaux montrant des signes d'une atteinte sévère du système nerveux central ou d'inflammation articulaire et de boiterie furent euthanasiés immédiatement et une nécropsie effectuée. Tous les autres animaux furent soumis à une nécropsie 14 j post-inoculation. La bactérine constituée de cellules entières de* S. suis *lavées avec du ceftiofur combinées au Montanide ISA 50 a réduit de manière significative (*$P < 0,05$*) la bactériémie, la méningite, la pneumonie et la mortalité associées à l'infection défi avec* S. suis. *Des travaux supplémentaires basés sur cette nouvelle approche de production de bactérine sont justifiés.*

(Traduit par Docteur Serge Messier)

Streptococcus suis has been associated with meningitis, arthritis, bronchopneumonia, septicemia, and high mortality in nursery pigs (1,2). A protective immune response against *S. suis* serotype 2 has been experimentally induced by inoculation of pigs with live (3,4) and washed whole cell (5) cultures of *S. suis*. Efficacy of the currently available washed whole cell *S. suis* bacterins is highly variable and generally poor (6,7). For this reason, the use of autogenous washed whole cell bacterins for controlling *S. suis* infections is common but results are also generally poor and variable (2). The washed whole cell bacterins that are more commonly used for vaccinating against *S. suis* are inactivated by formalin (5–7) or heat (5). Subunit vaccines (7) and purified hemolysin vaccines (8) have also demonstrated variable efficacy against experimental challenge with virulent *S. suis* serotype 2 strains. Formalin inactivation acts on proteins and nucleic acids producing cross-links and structural rigidity (9), and heat inactivation causes protein denaturation. Ceftiofur is a cephalosporin that suppresses synthesis of the bacterial cell wall by competitive inhibition of the enzymes responsible for cross-linking the cell wall glycopeptide polymer units (10,11).

Different kinds of adjuvants, such as water in oil emulsion adjuvants (5,7), oil adjuvants (12), or aluminum hydroxide-based adjuvants (5–7,12) are commonly used for *S. suis* bacterins. Water in oil emulsion adjuvants have been shown to be superior to aluminum hydroxide-based adjuvants in their capacity to stimulate an immune response and in reducing mortality associated with *S. suis* serotype 2 strains (7). Oil adjuvants also induced a significantly higher serum antibody response against S. *suis* serotype 2 compared to *S. suis* vaccines with aluminum hydroxide-based adjuvants (12).

The objectives of this research were to test the efficacy and safety of a novel ceftiofur-washed whole cell *S. suis* bacterin adjuvanted with 3 different adjuvants (Montanide ISA 25, Montanide ISA 50, and Saponin).

The study was approved by the Iowa State University Committee on Animal Care and Use. Sixty-six specific pathogen free (SPF) pigs

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from a sow unit free of porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae*, and transmissible gastroenteritis virus based on periodic serologic monitoring of the breeding herd were used in the experiment. The pigs were weaned at 12 d of age and transported to the Iowa State University Livestock Infectious Disease Isolation Facility. The pigs were negative for *S. suis* type 2, *Bordetella bronchiseptica*, and *Haemophilus parasuis* by preinoculation culture of tonsil and nasal swabs.

For the homologous challenge, the *S. suis* serotype 2 isolate ISU-VDL #40634/94 was used. This isolate was obtained in 1994 from a field case of meningitis in a pig submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). The inoculum was prepared as previously described (6).

Three *S. suis* vaccines were prepared from the challenge isolate and inactivated with ceftiofur hydrochloride (0.5 mg/mL final concentration). A 500 mL culture of *S. suis* isolate ISU-VDL #40634/94 was prepared similar to that of the challenge inoculum (6). Briefly, 3 blood agar plates were streaked with 1 swabful each of *S. suis* brain homogenate and incubated for 17 h at 37°C and 5% CO_2. The colonies were then transferred via a sterile Dacron tipped swab to 6 mL of sterile, prewarmed Todd-Hewitt broth (THB), and incubated for 2 h at 37°C and 5% CO_2. After the incubation, 24 mL of sterile, prewarmed THB was added to the 6 mL culture. This was incubated for 2 h at 37°C and 5% CO_2. After the incubation, 470 mL of sterile, prewarmed THB was added to the 30 mL culture and incubated for 3 h at 37°C and 5% CO_2. A 1 mL aliquot was then taken to determine culture purity, viability, and concentration by the spread plate dilution method. The spread plate dilution method revealed a final concentration of 8×10^8 colony forming units (CFU)/mL. Then 5 mL of 50 mg/mL ceftiofur hydrochloride was added to the 500 mL of culture and incubated at 37°C and shaken (125 RPM) in an incubator for 24 h. The inactivated culture was checked for viability by washing a 1 mL aliquot of the product 3 times with an equal volume of phosphate buffered saline solution (PBSS), and then spread plated onto a sheep blood agar plate. No growth was observed after 48 h of incubation at 37°C and 5% CO_2. The inactivated culture was then concentrated by centrifugation at $3000 \times g$ for 30 min and half of the supernatant was discarded.

Three different adjuvants were used to prepare the final vaccine products: Montanide ISA 25 (oil in water emulsion; Seppic, Paris, France), 1:3 adjuvant to antigen ratio; Montanide ISA 50 (oil in water emulsion; Seppic) 6:5 adjuvant to antigen ratio; and Saponin (Quest International, Naarden, The Netherlands) 1:1 adjuvant to antigen ratio.

The 66 pigs were randomly divided into 5 groups in 2 different rooms in 1.2×2.4 m^2 pens. They were ear-tagged and fed ad libitum. Group 1 (n = 15), group 2 (n = 13), group 3 (n = 16), and group 4 (n = 14) were randomly assigned to 7 pens in 1 room. Group 5 (n = 8) was housed in 1 pen in a second room. The air flow into the rooms was 10 to 15 air changes per hour and the temperature was 26.5°C. Groups 2, 3, and 4 were given a 2 mL intramuscular dose of *S. suis* vaccine with either the Montanide ISA 25, Montanide ISA 50, or Saponin adjuvants, respectively, at 28 and 14 d prechallenge with *S. suis*. One hour before inoculation, all 66 pigs were given 5 mL of 1% acetic acid (AA) (pH 2.9) intranasally (IN) to enhance the severity of the *S. suis* challenge (13). Groups 1, 2, 3, and 4 were inoculated IN with 2 mL of 1.05×10^9 CFU/mL of the isolate ISU-VDL #40634/94 of *S. suis*. Group 5 remained as the uninoculated negative control group. After challenge, pigs from groups 1, 2, 3, and 4 were mixed so that 2 to 3 pigs from each group were placed in each of the 7 raised wire deck pens in room 1.

The pigs were monitored twice daily. Rectal temperatures, central nervous system (CNS) signs, joint swelling and lameness (0 = normal, 3 = severe), and clinical respiratory disease (0 = normal, 6 = severe) were scored, as previously described (14,15). Other clinical signs, such as inappetence and lethargy, were also noted. Pigs exhibiting severe CNS disease (ataxia, prostration, or opisthotonus) or severe joint swelling and lameness resulting in recumbency were euthanized immediately and necropsied. Complete necropsies were performed on all remaining pigs at 14 d post inoculation (pi). Brain; cerebellum; lung; joint tissue; heart; tonsil; spleen; inguinal; tracheobronchial; and mediastinal lymph nodes, liver, kidney, ileum, and turbinates were examined grossly, and samples were collected in 10% neutral buffered formalin for histopathology. Whole blood for bacteriology was collected from the jugular vein using a single-use blood collection system containing ethylenediaminetetraacetic acid (EDTA) (Vacutainer; Becton Dickinson, Franklin Lakes, New Jersey, USA), prior to inoculation; and at 0, 2, 4, and 7 d pi. Blood, meningeal swabs, serosa swabs, and joint swabs were collected for bacteriology at necropsy. All samples for bacteriology were immediately streaked onto sheep blood agar plates. All cultures were incubated at 37°C in 5% CO_2 for 24 to 48 h. Alpha-hemolytic *Streptococcus*-like colonies were tested for growth in 6.5% NaCl and production of amylase (16). Representative colonies that did not grow in NaCl and were positive for production of amylase were checked by coagglutination to determine if they were *S. suis* type 2 (17).

For statistical analysis, mortality, mortality delay, gross lesions, microscopic lesions, and isolation of *S. suis* from samples collected at necropsy were first analyzed using an overall χ^2 test to guard against type 1 error inflation for group differences at $\alpha = 0.05$. When the overall test was significant, indicating at least one group is different from the others, pairwise tests of group values were performed to determine which values differed from the others. For comparison of mortality, a Fisher's Exact test was performed and for the rest of the parameters the Wilcoxon non-parametric test was performed.

Clinical signs are summarized in Table I. The only clinical signs observed in the negative control group were mild transient respiratory signs between 3 to 10 d pi. In the positive control group, the first clinical signs of high temperature ($>$ 41°C) and lameness appeared by 2 d pi and high fevers persisted through 7 d pi. The highest respiratory scores were recorded in this group between 3 to 5 d pi. Central nervous system signs, characterized by ataxia, recumbency, and opisthotonus, were followed by mortality that occurred between 3 to 9 d pi with most of the deaths occurring between 4 to 6 d pi. Lameness associated with swollen joints started at 1 d pi and persisted through to the conclusion of the experiment at 14 d pi.

In the vaccinated groups (Montanide ISA 25, Montanide ISA 50, and Saponin) a delay in the appearance of the clinical signs and a decrease in their severity was observed. The length of time between inoculation and mortality was significantly ($P < 0.05$) longer in the Montanide ISA 50 and Saponin vaccinated groups. The number

Table I. Clinical parameters at each day post inoculation (pi) in pigs vaccinated (Montanide ISA 25, Montanide ISA 50, and Saponin groups) and unvaccinated (positive and negative control groups) against *Streptococcus suis*

GROUP	Parameter	1[a]	2	3	4	5	6	7	8	9	10	11	12	13
Positive control	Temperature[b]	6.6%	53.3%	57.1%	27.3%	22.2%	40%	0%	0%	0%	0%	0%	0%	0%
n = 15	Respiratory[c]	0.27	0.27	0.92	1.1	0.9	0.2	0.25	0.75	0	0.33	0	0	0.67
	CNS[d]	0	0.13	0.64	0.9	1	1	0	0	0	0	0	0	0
	Lameness[e]	0.13	0.73	0.57	0.82	0.89	0.6	0.75	1	1	1.33	1.33	1	0.67
	Cumulative mortality	0%	0%	6.7%	26.7%	40%	66.7%	73.3%	73.3%	80%	80%	80%	80%	80%
Montanide ISA 25	Temperature	15.4%	0%	15.4%	15.4%	27.3%	12.5%	0%	0%	0%	0%	0%	0%	0%
n = 13	Respiratory	0.23	0.23	0.84	0.38	0.9	0.57	0.33	0.66	0.33	0.33	0.17	0.17	0.33
	CNS	0	0	0	0.85	0.81	0.43	0	0	0	0	0	0	0
	Lameness	0.23	0.53	0.23	0.54	0.54	0	0	0.17	0	0	0.17	0.17	0
	Cumulative mortality	0%	0%	0%	0%	15.4%	38.5%	53.8%	53.8%	53.8%	53.8%	53.8%	53.8%	53.8%
Montanide ISA 50	Temperature	12.5%	18.7%	12.5%	20%	7.7%	0%	8.3%	0%	0%	0%	0%	0%	0%
n = 16	Respiratory	0.37	0.62	0.75	0.8	0.45	0.5	0.5	0.36	0.1	0.27	0.18	0.18	0.36
	CNS	0	0.31	0.37	0.53	0.23	0	0.25	0	0	0	0	0	0
	Lameness	0.12	0.37	0.31	0.33	0.23	0	0.1	0.27	0	0	0.1	0	0
	Cumulative mortality	0%	0%	0%	6.2%	18.7%	25%	31.2%	31.2%	31.2%	31.2%	31.2%	31.2%	31.2%
Saponin	Temperature	0%	14.3%	7.1%	0%	15.4%	10%	0%	0%	11.1%	0%	0%	0%	0%
n = 14	Respiratory	0.14	0.14	0.14	0.46	0.38	0.55	0	0.22	0.11	0.12	0	0.12	0.12
	CNS	0	0	0.21	0	0.69	0	0	0	0.33	0	0	0	0
	Lameness	0	0.14	0.28	0.31	0.61	0.11	0.22	0.11	0.11	0.12	0.37	0.5	0.57
	Cumulative mortality	0%	0%	0%	7.1%	7.1%	35.7%	35.7%	35.7%	35.7%	42.8%	42.8%	42.8%	42.8%
Negative control	Temperature	12.5%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
n = 8	Respiratory	0	0	0.25	0.25	0.25	0.37	0.25	0.25	0.25	0.37	0	0	0
	CNS	0	0	0	0	0	0	0	0	0	0	0	0	0
	Lameness	0	0	0	0	0	0	0	0	0	0	0	0	0
	Cumulative mortality	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%

CNS — central nervous system

[a] Days post inoculation

[b] Percentage of pigs with greater than 41°C rectal temperature

[c] Mean respiratory score (0 = absence, 6 = severe)

[d] Mean central nervous system score (0 = absence, 3 = severe)

[e] Mean lameness score (0 = absence, 3 = severe)

of pigs with high temperatures (> 41°C) was significantly less ($P < 0.05$) in all vaccinated groups compared to the positive controls, and fevers disappeared sooner in the vaccinated pigs (by 7 to 8 d pi). Central nervous system signs and the period of time that mortality occurred were shorter in duration (4 to 7 d pi) in the vaccinated pigs. Lameness was less severe in vaccinated groups than in the positive controls. The Montanide ISA 50 group had the lowest lameness scores among the vaccinated groups. The Saponin adjuvanted vaccine caused injection site reactions characterized by red and swollen areas 3 to 5 cm in diameter for 3 to 4 d duration following both doses at 28 and 14 d preinoculation. No injection site reactions were observed with the other 2 vaccines. Overall mortality was significantly lower ($P < 0.05$) in the Montanide ISA 50 group (Table I).

Gross and microscopic lesions are summarized in Table II. The main gross lesions observed in the inoculated groups were meningitis; lung lesions, characterized by well demarcated red-purple consolidation involving 2% to 20% of the lung, particularly in the cranioventral lobes; polyserositis; enlargement of the lymph nodes, between 2 to 4 times the normal size; presence of fibrin tags in the abdominal cavity; and increased fluid in the joints. There were significantly less ($P < 0.05$) pigs with meningitis in the Montanide ISA 50 and Saponin vaccinated groups, and there were significantly more ($P < 0.05$) pigs with enlarged lymph nodes in the Saponin vaccinated group. No gross lesions were observed in pigs in the negative control group.

Microscopically, the lesions observed in the *S. suis* inoculated groups were fibrinosuppurative meningitis, multifocal suppurative interstitial pneumonia, fibrinosuppurative and lymphohistiocytic synovitis, and multifocal suppurative lymphadenitis. There were significantly less ($P < 0.05$) pigs with meningitis and the lung lesions were significantly less ($P < 0.05$) severe in the Montanide ISA 50

Table II. Gross and microscopic lesions in vaccinated and unvaccinated pigs inoculated with *Streptococcus suis*

	Gross lesions				Microscopic lesions			
Group	Meningitis	Pneumonia	Polyserositis	Enlarged lymph nodes	Meningitis	Pneumonia	Synovitis	Lymphadenitis
Positive control	10/15[a]	7/15[a]	0/11[a]	1/15[a]	11/15[a]	12/15[a]	2/15[a]	6/15[a]
Montanide ISA 25	5/13[ac]	2/13[a]	0/13[a]	0/13[a]	7/13[ac]	8/13[ac]	1/13[a]	2/13[ac]
Montanide ISA 50	3/16[bc]	4/16[a]	0/16[a]	1/16[a]	4/16[bc]	6/16[bcd]	1/16[a]	3/16[ac]
Saponin	3/14[bc]	3/14[a]	2/14[a]	9/14[b]	3/14[bc]	6/14[bc]	3/14[a]	11/14[d]
Negative control	0/8[bc]	0/8[a]	0/8[a]	0/8[a]	0/8[b]	0/8[d]	0/8[a]	0/8[bc]

Values in each column with different superscripts are significantly different ($P < 0.05$)

Table III. Isolation of *Streptococcus suis* type 2 in blood at 0, 2, 4, and 7 d post inoculation, and in blood, brain, serosal surfaces, and joints at the time of the necropsy in vaccinated and unvaccinated pigs inoculated with *S. suis*

	Blood collected prior to necropsy from live pigs				Samples collected at necropsy			
Group	0[a]	2	4	7	Blood	Brain	Serosa[b]	Joints
Positive control	0/15	2/15	4/11	1/4	9/15[c]	7/15[c]	1/15[c]	1/15[c]
Montanide ISA 25	0/13	0/13	1/13	0/6	4/13[ce]	5/13[c]	0/13[c]	0/13[c]
Montanide ISA 50	0/16	1/16	2/15	0/12	1/16[de]	4/16[c]	2/16[c]	0/16[c]
Saponin	0/14	1/14	2/13	1/9	2/14[de]	4/14[c]	1/14[c]	1/14[c]
Negative control	0/8	0/8	0/8	0/8	0/8[de]	0/8[c]	0/8[c]	0/8[c]

[a] Days post inoculation

[b] Swab from pleura, pericardium and peritoneum

[cde] Values in each column with different superscripts are significantly different ($P < 0.05$)

and Saponin vaccinated groups. No microscopic lesions were observed in pigs in the negative control group.

Table III summarizes the isolation of *S. suis* from blood at 0, 2, 4, and 7 d pi; and from the tissues and blood of the animals from each group at the time of necropsy. *Streptococcus suis* serotype 2 was isolated from the blood of 0 out of 58 pigs at 0 d pi, from 4 out of 58 pigs at 2 d pi, from 9 out of 58 at 4 d pi, and from 2 out of 58 pigs at 7 d pi. *Streptococcus suis* serotype 2 was isolated at the time of necropsy from blood of 16 out of 58 pigs, meninges of 20 out of 58 pigs, joints of 2 out of 58 pigs, and serosal surfaces of 4 out of 58 pigs. There were significantly fewer ($P < 0.05$) pigs with *S. suis* bacteremia in the Montanide ISA 50 and Saponin vaccinated groups. No growth of *S. suis* was detected from any of the pigs in the negative control group.

The high mortality and high incidence of clinical disease observed in the positive control group validates the ability of the intranasal acetic acid and *S. suis* inoculation model (13) to reproduce *S. suis*-associated disease. Since transmission of *S. suis* is thought to be by inhalation, our model adequately reproduces *S. suis*-associated pneumonia and meningitis, which are the most common signs associated with *S. suis* in nursery pigs in the field. However, the high level of mortality observed (80%) in our challenge model is considerably higher than reported in *S. suis* outbreaks in the field and would certainly represent the extreme in field case exposure scenarios.

The ceftiofur-washed whole cell Montanide ISA 50 adjuvanted *S. suis* bacterin significantly reduced mortality associated with challenge by a homologous strain of *S. suis*. The Montanide ISA 50 and Saponin adjuvanted vaccines appeared to be more efficacious in delaying onset of mortality and decreasing clinical signs and lesions associated with *S. suis* than the Montanide ISA 25 adjuvanted product. The significant delay in onset of mortality in the Montanide ISA 50 and Saponin vaccinated groups may be enough to allow time for producers to detect the sick animals and begin appropriate antimicrobial treatment. However, the Saponin vaccine induced unacceptable injection site reactions and significantly more severe gross and microscopic lesions in lymph nodes making it an unacceptable choice for commercialization.

Inactivation with ceftiofur was chosen in hopes that protective antigens would be better preserved. Damage to the epitopes by fixation with formalin (cross-links and structural rigidity of proteins and nucleic acids) or heat treatment (protein denaturation) may decrease the effectiveness of the bacterins in providing protection against *S. suis* and may explain the inconsistent results with these types of inactivated vaccines. Mortality rates of 0% to 80% in vaccinated pigs compared to 75% to 80% in unvaccinated pigs (7), 25% to 75% in vaccinated versus 100% in unvaccinated pigs (5), and 40% in vaccinated compared to 63% in unvaccinated pigs (6) have been reported after using different formalin and heat inactivated vaccines prior to *S. suis* challenge. It is difficult to compare results from these studies with those obtained in this experiment since different *S. suis* strains, challenge models, inactivation methods, and adjuvants were used.

In a previous experiment (6), we used the same *S. suis* challenge strain as in the current experiment. In the previous experiment the pigs were coinfected with *S. suis* and PRRSV and were not treated with acetic acid prior to challenge. We observed 40% mortality in coinfected pigs vaccinated with the formalin inactivated aluminum hydroxide adjuvanted bacterin prior to the homologous challenge. In comparison, mortality rates were 13.8% lower than that obtained using our Montanide ISA 25 vaccine, similar to mortality rates obtained using the Saponin vaccine, but almost 9% higher than that

obtained using Montanide ISA 50 vaccine in our study. We also had considerably higher mortality rates (80% compared to 63%) in the unvaccinated positive controls in the current study.

The mechanism of bacterial inactivation of ceftiofur is by suppression of synthesis of the bacterial cell wall by competitive inhibition of the enzymes responsible for cross-linking of the cell wall glycopeptide polymer units. Several studies have confirmed that some bacterial protein fractions present in different virulent strains of *S. suis*, such as the 44-kDa protein (18), 110-kDa protein (19), or 128-kDa protein (20), induce protective immunity. Unlike other forms of inactivation, ceftiofur treatment may better preserve the antigenicity of the glycopeptide polymer units and the ceftiofur-washed whole cell vaccines may contain both external cell wall surface proteins and internal cell wall surface proteins. Ceftiofur-washed whole cell vaccines may be at least as good, or better, at inducing homologous and heterologous protection against *S. suis*, as well as other pathogenic bacteria. Further studies comparing this product to current commercial products with homologous and heterologous challenge are warranted.

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References

1. Touil F, Higgins R, Nadeu M. Isolation of *Streptococcus suis* from diseased pigs in Canada. Vet Microbiol 1988;17:171–177.
2. Torremorell M, Pijoan C, Trigo E. Vaccination against *Streptococcus suis:* effect on nursery mortality. Swine Health Prod 1997;5:139–143.
3. Holt ME, Enright MR, Alexander TJL. Immunization of pigs with live cultures of *Streptococcus suis* type 2. Res Vet Sci 1988;45: 349–352.
4. Busque P, Higgins F, Caya F, Quessy S. Immunization of pigs against *Streptococcus suis* serotype 2 infection using a live avirulent strain. Can J Vet Res 1997;61:275–279.
5. Holt ME, Enright MR, Alexander TJL. Immunization of pigs with killed cultures of *Streptococcus suis* type 2. Res Vet Sci 1990;48: 23–27.
6. Halbur PG, Thanawongnuwech R, Brown G, et al. Efficacy of antimicrobial treatments and vaccination regimens for control of porcine reproductive and respiratory syndrome virus and *Streptococcus suis* coinfection of nursery pigs. J Clin Microbiol 2000;38:1156–1160.
7. Wisselink HJ, Vecht U, Stockhofe-Zurwieden N, Smith HE. Protection of pigs against challenge with virulent *Streptococcus suis* serotype 2 strains by a muramidase-released protein and extracellular factor vaccine. Vet Rec 2001;148:473–477.
8. Jacobs AAC, Van Den Berg AJG, Loeffen PLW. Protection of experimentally infected pigs by suilysin, the thiol-activated haemolysin of *Streptococcus suis*. Vet Rec 1996;139:225–228.
9. Tizard IR. Vaccination and vaccines. In: Tizard IR, ed. Veterinary immunology: an introduction. 6th ed. Philadelphia: WB Saunders Company, 2000:235–252.
10. Tipper DJ, Strominger JL. Biosynthesis of the peptidoglycan of bacterial cell walls. XII. Inhibition of cross-linking by penicillins and cephalosporins: studies in *Staphylococcus aureus in vivo*. J Biol Chem 1968;243:3169–3179.
11. Hornish RE, Kotarski SF. Cephalosporins in veterinary medicine — Ceftiofur use in food animals. Curr Top Med Chem 2002;2:717–731.
12. Ripley PH. Vaccines against streptococcal meningitis. Pig Vet Soc Proc 1983;10:25–39.
13. Pallarés FJ, Halbur PG, Schmitt CS, et al. Comparison of experimental models for *Streptococcus suis* infection of conventional pigs. Can J Vet Res 2003;67:225–228.
14. Halbur PG, Paul PS, Frey ML, et al. Comparison of the pathogenicity of two U.S. porcine reproductive and respiratory syndrome virus isolates with that of the Lelystad virus. Vet Pathol 1995;32: 648–660.
15. Halbur PG, Paul PS, Meng X-J, Lum MA, Andrews JJ, Rathje JA. Comparative pathogenicity of nine US porcine reproductive and respiratory syndrome virus (PRRSV) isolates in a five-week-old cesarean-derived, colostrum-deprived pig model. J Vet Diagn Invest 1996;8:11–20.
16. Devriese LA, Ceyssens K, Hommez J, Kilpper-Balz R, Schleifer KH. Characteristics of different *Streptococcus suis* ecovars and description of a simplified identification method. Vet Microbiol 1991;26:141–150.
17. Higgins R, Gottschalk M. An update on *Streptococcus suis* identification. J Vet Diagn Invest 1990;2:249–252.
18. Gottschalk M, Higgins R, Jacques M, Dubreuil D. Production and characterization of two *Streptococcus suis* capsular type 2 mutants. Vet Microbiol 1992;30:59–71.
19. Quessy S, Dubreuil JD, Caya M, Higgins R. Discrimination of virulent and avirulent *Streptococcus suis* capsular type 2 isolates from different geographical origins. Infect Immun 1995;63: 1975–1979.
20. Quessy S, Dubreuil JD, Caya M, Létourneau R, Higgins R. Comparison of pig, rabbit, and mouse IgG response to *Streptococcus suis* serotype 2 proteins and active immunization of mice against the infection. Can J Vet Res 1994;58:220–223.