ONTARIO PORK

Ontario Pork Research Final Report (project # 19-006) Executive Summary

Reporting Date: 28-02-2022

Introduction: *Streptococcus suis* disease management is wearisome due to restrictions in antibiotic use and lack of proven effective vaccines. Currently, field immunization efforts focus on **bacterins** (autogenous vaccines) with contradictory results and, in many cases, vaccinations are initiated without detailed knowledge of immunological and protective responses induced by these vaccines. In addition, there is lack of a standardized protocol for vaccine formulation and, more importantly, of scientific evaluation of the effect of formulation on vaccine efficacy. Adjuvants are key components in vaccine preparations and can dramatically influence the immune response to the vaccine antigen(s). However, the effect of adjuvants on *S. suis* bacterin protective capacity has never been scientifically evaluated. We propose to study and compare, **for the first time**, the immune response and protection generated by a bacterin formulated with different adjuvants under controlled conditions using experimental challenge of piglets.

Objectives: (*original objectives from project proposal*)

General aim: To test improvement strategies to increase the immunogenic capacity of autogenous vaccines.
Objective: To test different adjuvants that may significantly improve the protective capacity of a bacterin against *S. suis.*

Materials and Methods: To respond to this objective the immunogenicity and protective capacity of different vaccine formulations were evaluated in weaned piglets under controlled experimental conditions.

Results and Discussion: The goal of our study was to experimentally evaluate the immunogenicity and protective efficacy against homologous challenge in weaned piglets of a *S. suis* bacterin-based vaccine formulated with six different commercial adjuvants. The vaccine formulated with Montanide™ ISA 61 VG induced a significant increase in anti-*S. suis* antibodies, protected against mortality and significantly reduced morbidity and severity of clinical signs. Vaccines formulated with Montanide ISA 206 VG or Montanide ISA 201 VG also induced a significant increase in anti-*S. suis* antibodies, showed partial protection and reduction of clinical signs severity. Vaccines formulated with Alhydrogel®, Emulsigen®-D, or Quil-A® induced a low antibody response and failed to protect vaccinated piglets against a homologous challenge.

Conclusions: This is the first controlled experimental study conducted to compare the effect of six different commercial adjuvants, widely used in animal vaccine production, on *S. suis* vaccine efficacy. Taken together the results from our study indicate that the type of adjuvant formulation has paramount importance on the efficacy and protection of bacterin-based vaccines against *S. suis*.

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Introduction:

Streptococcus suis is a Gram-positive bacterium with 29 serotypes described based on the immunogenicity of its capsular polysaccharide (CPS) [1]. It causes disease in weaned and, occasionally, in suckling and grower piglets, with clinical signs of meningitis, arthritis, endocarditis, septicemia, and sudden death [2]. *S. suis* has a worldwide prevalence and it is estimated that 100% of pig farms are positive, since it is normal inhabitant of the upper respiratory tract [2]. Antimicrobials are not only used to treat disease but also as prophylaxis/metaphylaxis to control *S. suis* in pig herds, although their use has been limited in some countries due to the increasing occurrence of antimicrobial resistance [3]. Prevention of clinical disease caused by *S. suis* is mainly based on the control of predisposal factors and the use of vaccines [4]. Since there are no universal efficacious commercial vaccines against *S. suis* infection, the use of autogenous vaccines ("bacterins") is widespread [5]. The production of the *S. suis* autogenous vaccine starts with the isolation of a specific bacterial strain(s) causing the problem in a particular farm; this bacterial isolate is then killed (generally with formalin) and formulated with a specific adjuvant [6]. The plethora of *S. suis* serotypes and strains, and a wide variety of adjuvants and vaccine production methods, make the evaluation of the efficacy of autogenous vaccines difficult, and often ambiguous data are acquired from field trials [5]. Adjuvants are key components of a vaccine formulation and have the capacity to not only increase the vaccine-induced immune response but also modulate the type of this response and consequently the protection level obtained. Despite the importance of adjuvants, few studies have compared the effect of different adjuvants in the same experimental trial or at least under the same conditions [7, 8].

The goal of this study was to evaluate the influence of different commercial adjuvants included in a *S. suis* serotype 2 bacterin-vaccine formulation on the immunogenicity and protection against homologous challenge. This is the first controlled experimental study conducted to compare the effect of six different commercial adjuvants, widely used in animal vaccine production, on *S. suis* vaccine efficacy.

Objectives: (original objectives from project proposal)

General aim: To test improvement strategies to increase the immunogenic capacity of autogenous vaccines.

Specific Question: What can be done to improve the immunogenicity of autogenous vaccines, and thus their cost-benefit?

Objective: To test different adjuvants that may significantly improve the protective capacity of a bacterin against *S. suis*.

Materials and Methods:

Preparation of the bacterin: *Streptococcus suis* serotype 2 strain P1/7, a well-characterized virulent reference strain [9], was used to prepare the vaccine. To inactivate the bacteria, formaldehyde was added to the final concentration of 0.5%. The bacterin pellet was re-suspended in 250 mL of sterile PBS and thiomersal was added to a final concentration of 0.01% v/v. Each immunization dose contained the equivalent to 10^9 CFU/mL.

Formulation of *S. suis* bacterin with different adjuvants: Six commercial adjuvants were used to make different formulations of the vaccine in this study. Vaccine formulations consisted of formalin-inactivated 10^9 CFU of *S. suis* serotype 2 strain P1/7 formulated with Alhydrogel®-2% (Croda, formerly known as Brenntag Biosector A/S, InvivoGen, USA) at a final concentration of 50% v/v (Group 1), Emulsigen®-D (MVP Adjuvants®, Phibro Animal Health Corporation, USA) at a final concentration of 20% v/v (Group 2), Quil-A® (Croda) at a final concentration of 0.3 mg/mL (Group 3), Montanide™ ISA 206 VG (Group 4), Montanide™ ISA 61 VG (Group 5), and Montanide™ ISA 201 ISA VG (Group 6) (SEPPIC, USA). All procedures for vaccine formulations with tested adjuvants were done according to the manufacturer's protocols. Placebo controls (corresponding adjuvant only) were included in each group.

Animals: The protocols and procedures were approved by the Animal Welfare Committee of the University of Montreal (protocol number Rech-2014). Recently weaned, three-week-old, Landrace/white mixed breed piglets were acquired from a commercial farm in Quebec, with no history of clinical problems caused by *S. suis*, no vaccination program against this pathogen and free of Porcine Reproductive and Respiratory Syndrome virus. Upon arrival, piglets were weighed, individually tagged, assigned to two groups (placebo or vaccinated; n = 10 per group) with equal average weight (approximately 5-6 kg), and placed in the Level II experimental animal facility of the Faculty of Veterinary Medicine, University of Montreal. Piglets were fed commercial, pelleted non-medicated food, with an addition of dry veggie supplements. The same procedure was performed for all 6 adjuvant groups of piglets for a total of 120 piglets.

Immunization and challenge of pigs: Two days upon arrival, piglets were immunized intramuscularly (IM) in the neck muscle, with 1 mL of formalin-killed *S. suis* serotype 2 strain P1/7 with selected adjuvant (vaccine group) or adjuvant only in PBS solution (placebo control group). The second dose of vaccine and placebo were administered IM two weeks after the first dose (see Figure A below). Twelve days after the second injection, the immunized and control animals were challenged with an intraperitoneal (IP) injection of 5 mL (5×10^9 CFU) of *S. suis* serotype 2 strain P1/7. The average weight of the piglets on the day of the challenge was 14 kg. Blood samples were collected from the jugular vein before each immunization and before challenge for the determination of antibody responses (see below). Following the challenge, pigs were monitored three times per day over a period of nine days for the presence of clinical signs and mortality. A daily clinical score was calculated based on a clinical observation sheet [10]. Assessed were general behavior, locomotion (musculoskeletal alterations) and functional alteration of the central nervous system (CNS). Pigs having a clinical score = 3 in either category and a body temperature above 40 °C for two consecutive days were humanely euthanized. Blood was collected from each piglet before euthanasia for bacteriological analyses. A post-mortem examination procedure was conducted for all pigs. Swabs were collected from meninges and synovial fluid from affected joint cavities and seeded on

blood agar for bacterial recovery. Samples of liver and spleen were collected and cultured for bacterial recovery. Samples for bacterial isolation and serotyping were taken from all euthanized animals as well as survival animals at the end of the trial.

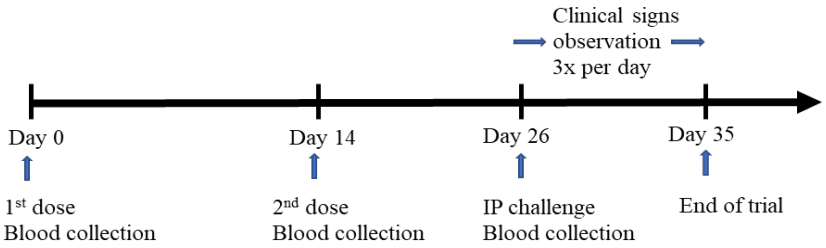

Figure A. Experimental design of the study for evaluation of immunogenicity and protection of bacterin vaccines formulated with different adjuvants. The experimental design was consistent for all tested vaccine formulations. IP; intraperitoneal injection.

Measurement of antibodies against *S. suis* serotype 2: Sera from vaccinated and control piglets were analyzed by an indirect ELISA for antibodies against whole *S. suis* bacteria, standardized using the challenge strain, as reported [11].

Results and Discussion: see Figures in Annexe

I. Survival rates and clinical signs:

Animals immunized with vaccines formulated with Alhydrogel® (survival rate of 40%), with Emulsigen®-D (survival rate of 33%) or with Quil-A® (survival rate of 40%) presented survival rates and clinical scores of locomotion, CNS, and behavior similar to those observed in corresponding placebo (control) animals (Figures 1A-C and 2A-C). Indeed, morbidity was high in both vaccinated and placebo animals for these three adjuvant groups. Clinical signs of locomotion in a form of limping, swollen joints, and difficulties in moving, were observed in 90 to 100 % of Alhydrogel®, Emulsigen®-D, and Quil-A® vaccinated piglets. CNS clinical signs were observed in 60 to 80% of vaccinated piglets in these three adjuvant groups with no statistical differences between vaccinated and control animals. Besides, there was no statistical difference amongst these three vaccine formulations in any of the clinical sign categories (Figure 4A). Furthermore, *S. suis* was isolated from the blood, synovial fluid, and organs of vaccinated piglets at a similar rate to that of placebo animals.

The survival rate of piglets vaccinated with Montanide™ ISA 201 or 206 VG was 60%, and although the survival rates of the corresponding placebo groups were 40% and 30%, respectively, the difference was not statistically significant (Figures 1D and F). An improvement in clinical scores was observed for both vaccinated groups compared to corresponding placebos but, as in the case of mortality, there was no statistical difference during nine days (Figures 3A and C). *S. suis* was isolated in 6 out of 10 vaccinated piglets in the Montanide™ ISA 206 VG group, which is an improvement compared to 9 out of 10 animals in Montanide™ ISA 201 VG group.

The survival rate of piglets vaccinated with Montanide™ ISA 61 VG was 100 %, which was significantly different compared to the corresponding placebo with a survival rate of 30 % (Figure 1E). Furthermore, clinical scores in all categories were significantly lower in the vaccinated group compared to the placebo group (Figure 3B). The vaccinated group did not have

a single case of meningitis while the placebo group had 7 out of 10 animals with aggravated CNS clinical signs. Although 7 out of 10 vaccinated animals showed clinical signs of lameness, swollen joints, and limping, the overall clinical score was low, the clinical signs were mild, and all animals were able to recover completely until the end of the trial. Only 3 out of 10 vaccinated animals had *S. suis* isolated from the joints or organs, with no isolation from the brain or blood. Opposite, *S. suis* was isolated from the joints, tissue samples, and/or blood of the majority of placebo piglets. Finally, clinical sign scores were significantly decreased in Montanide™ ISA 61 VG vaccine group compared to the other two Montanide™ formulations (Figure 4B).

II. Immunogenicity against *S. suis* serotype 2:

ELISA analyses of antibody titers against *S. suis* serotype 2 after immunization revealed different levels of immunogenicity induced by the vaccine formulations. Upon arrival, all piglets had high basal levels of antibody titers as detected using whole *S. suis* serotype 2 antigen, which suggests the presence of maternal antibodies acquired during the suckling period (Figure 5). These antibody levels decreased over time in all placebo groups, reaching the lowest level at 7 weeks of age (Figure 5). Furthermore, at five weeks of age (after the 1st vaccine dose), none of the vaccine formulations was able to increase the antibody titers compared to the corresponding placebos (Figure 5). Indeed, a significant increase in anti-*S. suis* antibody levels were only observed after the 2nd vaccine dose for all vaccine formulations (Figures 5B-F), except for that adjuvanted with Alhydrogel® (Figure 5A). In addition, the latter vaccine formulation did not induce antibody isotype switching, since there was no difference in IgM, IgG1, and IgG2 antibody titers between Alhydrogel® placebo and vaccine group at seven weeks of age (Figure 6A).

Emulsigen®-D and Quil-A® vaccine formulations induced a significant increase in antibody titers at seven weeks of age, after the 2nd vaccine dose (Figures 5B and C). When analyzing the antibody profile (Figure 6B), it was observed that Emulsigen®-D vaccine formulation induces an increase in IgG1 and, to a lesser extent of IgG2 against *S. suis* serotype 2. On the other hand, Quil-A® vaccine formulation induced isotype switching towards IgG1 only (Figure 6C).

All three Montanide™ vaccine formulations were able to induce a significant increase in antibody titers against *S. suis* serotype 2 at seven weeks of age, after the 2nd vaccine dose (Figures 5D, E and F). The highest increase in antibody titers was observed in pigs vaccinated with the vaccine formulated Montanide™ ISA 61 VG (Figure 5E). In terms of the isotype profile of induced antibodies, a significant and marked increase in both, IgG1 and IgG2 subclasses was observed in animals immunized with the three Montanide™ vaccine formulations (Figure 7).

Conclusions:

Adjuvants are key components of vaccine formulations and possess multiple properties able to increase the level (magnitude) of the vaccine-induced immunological response, reduce the number of doses, control the release of the antigen (depot effect) at the site of the injection and, importantly, to modulate the type of induced immunity. The latter effect may have a major impact on the vaccine-induced protection against clinical disease [12]. Facing both the lack of effective commercial vaccines and the forthcoming restrictions in the prophylactic and metaphylactic use of antimicrobials, swine producers have increased the use of autogenous vaccines (bacterins) to prevent and control *S. suis*

outbreaks. Nevertheless, as recently reviewed by Rieckmann *et al*. [5], there is a lack of scientific data on the actual efficacy of this type of vaccine and the immune response induced in vaccinated animals. Furthermore, only few studies have comparatively addressed the role of different adjuvants on the antibody response and/or protection induced by a *S. suis* bacterin vaccination [7, 8]. In the present work, we compared six commercial and widely used veterinary adjuvants under the same experimental conditions. Our systemic challenge model, using an intraperitoneal route of infection, showed high reproducibility and consistency of clinical results which is of the utmost importance when comparing the effect of adjuvants on a given vaccine formulation [13, 14]. **Using this model, our results showed that the type of adjuvant used in the vaccine formulation has a paramount effect on the protection of piglets against *S. suis* challenge.**

The results of this study were able to confirm previous findings on the limited or lack of immunogenicity and/or protection of bacterin vaccines adjuvanted with aluminum hydroxide [7, 15]. This adjuvant, commonly known as alum, has been one of the most extensively used aluminum salts as an adjuvant for swine vaccines so far. It can form a short-term depot and is inexpensive, safe and simple to formulate [12]. Although it has been used for over 90 years in human and animal vaccines, our data indicate that there is no scientific rationale to use aluminum hydroxide in *S. suis* bacterin vaccine formulations due to the low immunogenicity and lack of protection in weaned piglets against *S. suis* serotype 2 experimental infection. Since the antigen can also influence the adjuvant effect and appropriate formulation, more research on the use of aluminum adjuvants for *S. suis* vaccines in swine would be required [14, 16].

As aforementioned, adjuvants have the potential to modulate the features of the antibody response induced by the vaccine. Indeed, the protection ability of the diverse immunoglobulin (Ig) classes and subclasses depends on the specificity and affinity with the targeted antigen and their biological functions. In pigs, the functionality of the different IgG subclasses has not been well characterized, in part due to the lack of appropriate reagents to differentiate the complexity of swine Ig variants [17, 18]. Nevertheless, based on available reagents, swine IgG2 has been suggested to correlate with better protection against *S. suis* infection [19]; yet contradictory results do exist on the swine IgG1 vs. IgG2 specific contribution to protection [20]. Despite the induction of humoral immunity in vaccinated piglets, bacterin formulations with Emulsigen®-D (O/W) and Quil-A® (saponin) failed to provide clinical protection against an *S. suis* serotype 2 experimental challenge and a biased IgG1 antibody response was observed. Another option that could be further explored is the combination of Emusligen®-D with aluminum hydroxide or Quil-A®. The combination of these adjuvants could potentially increase immune response as reported in previous vaccination studies against foot-and-mouth disease in pigs [21]. The obstacle to creating a vaccine with a combination of adjuvants against diseases in swine is mainly economic; the cost-benefit of such a combination should be evaluated.

Montanide™ adjuvants are commercial W/O or W/O/W emulsions used to formulate animal vaccines against different livestock diseases [22, 23]. In our work, *S. suis* vaccines formulated with one of these three MontanideTM adjuvants induced a mixed IgG1/IgG2 immune response. Bacterin vaccines formulated with Montanide™ ISA 201 VG or Montanide™ ISA 206 VG showed similar results characterized by partial, albeit not significant, clinical protection compared to the corresponding placebos. These results could be expected since both adjuvants are W/O/W emulsions and thus have a

similar mechanism of immune stimulation. On the other hand, the bacterin vaccine formulated with Montanide™ ISA 61 VG was the only formulation able to provide clear significant protection against homologous challenge and reduced morbidity. It should be noted that almost all piglets are early colonized by *S. suis* and it is not known how this early colonization may affect the response against a vaccine. Therefore, more studies confirming the data obtained in the current study are necessary.

→ The planned work for the project was 100% completed. An article was published and a second paper will be submitted in May 2022.

Complementary work:

With the contribution of additional funding by NSERC grants to M. Segura and M. Gottschalk, we also performed a field study on the immunological evaluation of an autogenous vaccine used in sows to protect piglets against *Streptococcus suis* infections. The MSc student Alison Jeffery (funded by the MITACS fellowship) was involved in this part of the work.

As stated above, autogenous vaccines are largely used as a preventive strategy; however, field studies on the immunological response induced by these vaccines are scarce. Previous studies performed in North America showed that autogenous vaccines increase antibody levels in vaccinated sows but the transfer of maternal immunity to piglets was either not improved or did not last longer than 18 days of age [11, 24]. Since manufacturing procedures are different amongst autogenous vaccine companies, in this study we assessed a sow vaccination program with an autogenous vaccine containing five *S. suis* serotypes (2, 5, 7, 14 and 1/2) from a manufacturer never studied before. The response induced by the vaccine and transfer of maternal immunity from sows to their litters were analyzed on a commercial farm.

Methods: Blood was obtained from gilts pre-vaccination and after three doses of the autogenous vaccine in both vaccinated (n=28) and placebo (n=25) groups. After farrowing, piglets (2/litter, n=106) were followed for serology up to 7 weeks of age. ELISA test was done to measure and characterize the vaccine-induced antibody response in sows and the passive immunity in piglets. Antibody functionality (i.e. the capacity of antibodies to kill *S. suis*), was measured via an *in vitro* opsonophagocytosis assay (OPA); this test is considered a correlation of protection in vaccine studies.
Results: Results targeting the response against serotypes 1/2, 2, 5, 7, and 14 showed that vaccinated sows present higher levels of antibodies than the placebo control group. Maternal antibody transfer to their litters was higher in piglets born from vaccinated sows compared to controls, which lasted until 3 to 5 weeks of age. A mixed IgG1/IgG2 response was observed. Antibody functionality (as measured by OPA) was high in one-week-old piglets but showed a decline at 5 weeks of age.
Discussion & Conclusion: This study provides evidence for the first time that a gilt vaccination program with an autogenous vaccine increases antibody levels in piglets after one week of age and up to 3 and/or 5 weeks of age depending on the *S. suis* serotype. Nevertheless, more studies are required to fully characterize the clinical protective effect of this vaccine during the complete nursery period.

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Knowledge Transfer:

- Published peer-reviewed articles

- Obradovic MR, Corsaut L, Dolbec D, Gottschalk M, Segura M. Experimental evaluation of protection and immunogenicity of *Streptococcus suis* bacterin-based vaccines formulated with different commercial adjuvants in weaned piglets. Vet Res. 2021 Oct 19;52(1):133. doi: 10.1186/s13567-021-01004-x. PMID: 34666827; PMCID: PMC8527783.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8527783/

- Presentation, poster or abstract from a scientific or industry meeting (please provide a copy or the link):

I. Invited conferences (by principal researchers)

1. **Gottschalk, M.** Straggling to control *Streptococcus suis* disease in the context of antibiotic reduction. 26th International Pig Veterinary Society (IPVS) Congress, Rio de Janeiro, Brazil, 21-24 June **2022**. **Keynote lecture**.
https://ipvs2022.com/en/congresso/full-programme

2. **Segura, M., and M. Gottschalk.** Vaccins autogènes contre *Streptococcus suis* : Mythe ou réalité ? Assemblée génerale des Éleveurs de porcs du Québec, Québec, Canada, July **2020**.

II. Poster or oral presentation (by students or staff involved in the project)

1. Jeffery, A., M. Gilbert, C. Lorelei, S. Cloutier, M.C. Frenette, C. Surprenant, M. Gottschalk, and M. Segura. Antibody response induced by a multiserotype *Streptococcus suis* autogenous vaccine used in sows to measure the immunoglobulin passive transfer to piglets. **26th International Pig Veterinary Society (IPVS) Congress**, Rio de Janeiro, Brazil, 21-24 June, **2022**. **Poster presentation**
https://ipvs2022.com/en/congresso/full-programme

2. Jeffery, A., M. Gilbert, C. Lorelei, S. Cloutier, M.C. Frenette, C. Surprenant, M. Gottschalk, and **M. Segura**. Immunological evaluation of an autogenous vaccine used in sows to protect piglets against *Streptococcus suis* infections. **13th European Symposium of Porcine Health Management**, Budapest, Hungary, 11-13 May **2022**. **Oral presentation**
https://www.esphm2022.org/index.php/programme/

3. Jeffery, A., M. Gilbert, C. Lorelei, S. Cloutier, M.C. Frenette, C. Surprenant, M. Gottschalk, and **M. Segura**. Immunological evaluation of an autogenous vaccine used in sows to protect piglets against *Streptococcus suis* infections. **Banff Pork Seminar 2022**, Banff, Alberta, Canada, 11-13 January **2022**. **Oral presentation: 1st Prize - R.O. Ball Young Scientist Award** (See Photo below)

4. Jeffery, A., M. Gilbert, C. Lorelei, S. Cloutier, M.C. Frenette, C. Surprenant, M. Gottschalk, and **M. Segura**. Immunological evaluation of an autogenous vaccine used in sows to protect piglets against *Streptococcus suis* infections. **Le Porc Show**, Québec, Canada, 23 & 30 November **2021**. **Poster presentation**
https://leporcshow.com/exposants/domaine-de-recherche-vaccines/?salon=8681

5. Jeffery, A., M. Gilbert, C. Lorelei, S. Cloutier, M.C. Frenette, C. Surprenant, M. Gottschalk, and **M. Segura**. Immunological evaluation of an autogenous vaccine used in sows to protect piglets against *Streptococcus suis* infections. **The Allen D. Leman Swine Conference**, Minnesota, USA, 18−21 September **2021**. **Poster presentation**
https://drive.google.com/file/d/1rw0I-jDReKzNv91WGrCKecfHSyhIyoMU/view (poster ##55)

6. Jeffery, A., M. Gilbert, C. Lorelei, S. Cloutier, M.C. Frenette, C. Surprenant, M. Gottschalk, and **M. Segura**. Immunological evaluation of an autogenous vaccine used in sows to protect piglets against *Streptococcus suis* infections. 13e Symposium du FRQNT-Centre de Recherche en Infectiologie Porcine et Avicole (CRIPA), St-Hyacinthe, Quebec, Canada, 4 and 11 June **2021**. **Oral presentation**
https://www.cripa.center/_files/ugd/c91739_96b24f9522eb4e4ab2001848152ad782.pdf

7. Obradovic, M., L. Corsaut, M. Gottschalk, and **M. Segura**. Evaluation of the immunogenicity and protective efficacy of an experimental *Streptococcus suis* autogenous vaccine formulated with six different adjuvants. Le Porc Show, Quebec, Canada, 9 December **2020**. **Poster presentation**

- Popular Press Articles and communications:

- Segura, M., and Gottschalk, M. *Streptococcus suis* autogenous vaccins: what do we know? **PIG333**, *In press*, **2022**.

- Gottschalk, M., and M. Segura. *Streptococcus suis* disease in pigs. **Factsheet, Pork Information Gateway** (web magazine), **2021**. https://porkgateway.org/resource/streptococcus-suis-disease-in-pigs/

- Jeffery, A., M. Gilbert, C. Lorelei, S. Cloutier, M.C. Frenette, C. Surprenant, M. Gottschalk, and M. Segura. Immunological evaluation of an autogenous vaccine used in sows to protect piglets against *Streptococcus suis* infections. **Networking Morning with the Rural Ontario Institute, Advanced Agricultural Leadership Program**, St-Hyacinthe, Quebec, Canada, 17 November **2021**. ***Short oral presentation*.** https://www.cripa.center/general-7-1

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"You have really talented employees right now. There may be some shortage of labour, but you have to really find ways to keep those employees. They have the potential to be champions for you. Let's work on those efforts."

Lesley Kelly offered the adult members of her own family as examples in a frank and energetic discussion on mental health and ways to help each other deal with stresses and trauma. She went on to describe the five defences her family has used to help weather the S.T.O.R.M.

*Stress: Some stress is normal and helps us grow. Stress becomes harmful when it is overwhelming or prolonged. Signs and symptoms of harmful stress can be both physical and emotional, including headaches, chest pain, teeth clenching, irritability, diet and sleep changes and lack of ability to concentrate.

*Take action: Stress is real and people in its grip need a lifeline.

*Open communications: Kelly's family meets regularly and they talk about the things they are going through. Those talks have brought the family closer and has spilled over into other relationships, she said. One of the exercises they use is to rate their stress on a scale of one to 10, and then letting each other know their rating at the start of each day.

*Relationships: Kelly said she and her family have learned to process conflict differently now as a win-win rather that a right-wrong. "For us, to have a win-win is to keep the dialogue open. So now, we process conflict with empathy, understanding and curiosity to help us get through conflict times."

*Management: These are the things you do to support yourself and those around you.

— continued on page 16

Allison Jeffery

Winners of R.O. Ball Young Scientist Awards. Ben Willing, awards chair, Carley Camire (L) Allison Jeffery (R)

Photo courtesy of Banff Pork Seminar

ANNEXE - FIGURES

SEGURA *et al*.

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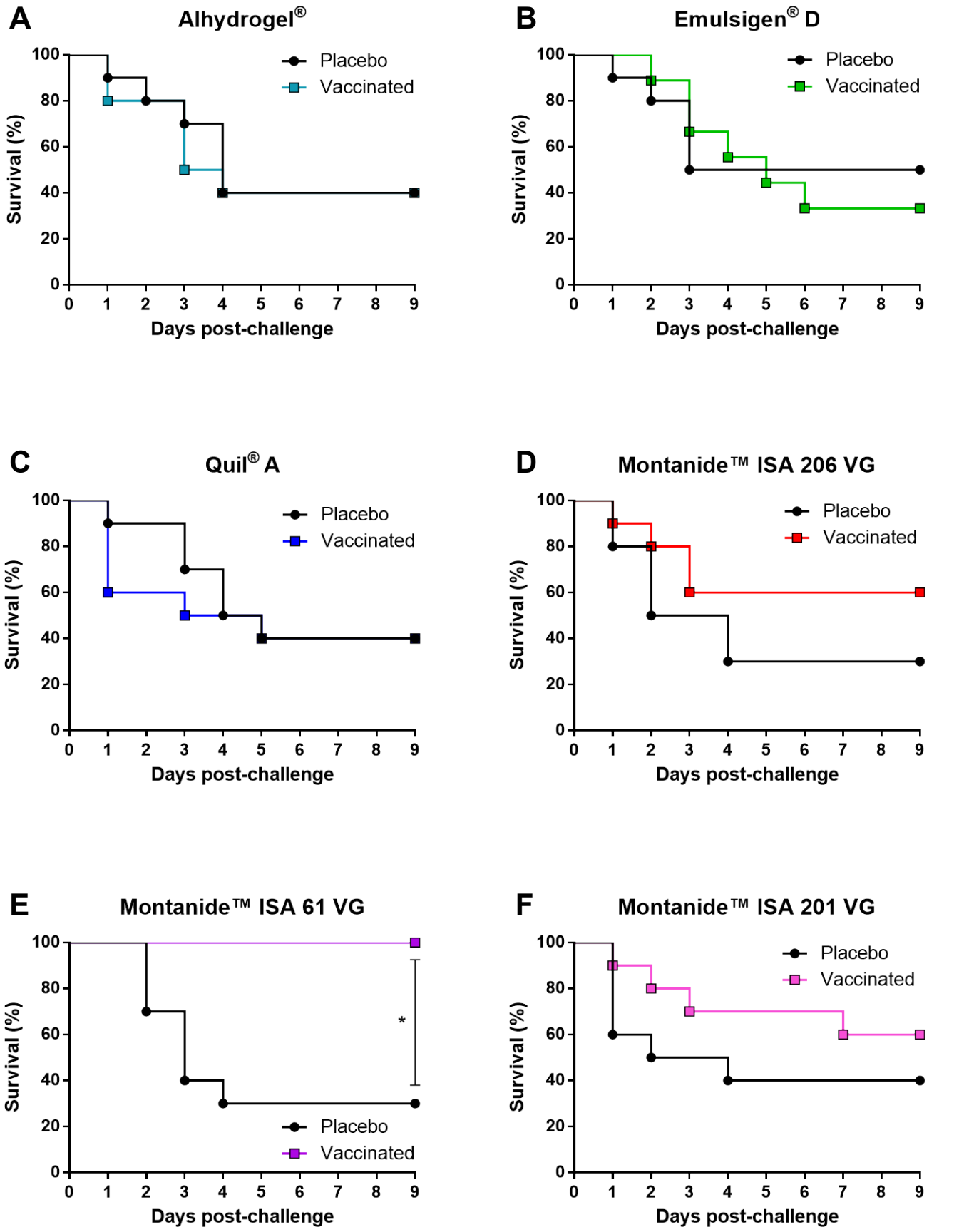
A
Alhydrogel®
Placebo
Vaccinated
Survival (%)
Days post-challenge
B
Emulsigen® D
Placebo
Vaccinated
Survival (%)
Days post-challenge
C
Quil® A
Placebo
Vaccinated
Survival (%)
Days post-challenge
D
Montanide™ ISA 206 VG
Placebo
Vaccinated
Survival (%)
Days post-challenge
E
Montanide™ ISA 61 VG
*
Placebo
Vaccinated
Survival (%)
Days post-challenge
F
Montanide™ ISA 201 VG
Placebo
Vaccinated
Survival (%)
Days post-challenge

Figure 1 Kaplan-Maier survival rate curves. Piglets were vaccinated with the equivalent to 10^9 CFU/mL of *S. suis* strain P1/7 bacterin formulated either with Alhydrogel® (**A**), Emulsigen®-D (**B**), Quil-A® (**C**), Montanide™ ISA 206 VG (**D**), Montanide™ ISA 61 VG (**E**), or Montanide™ ISA 201 VG (**F**). All piglets were challenged intraperitoneally with 5×10^9 CFU of *S. suis* strain P1/7. Each vaccine formulation group of vaccinated piglets ($n = 10$) had a corresponding placebo group of piglets ($n = 10$) challenged at the same time. The clinical signs observations were conducted three times per day, nine days post-infection. *, $P < 0.05$.

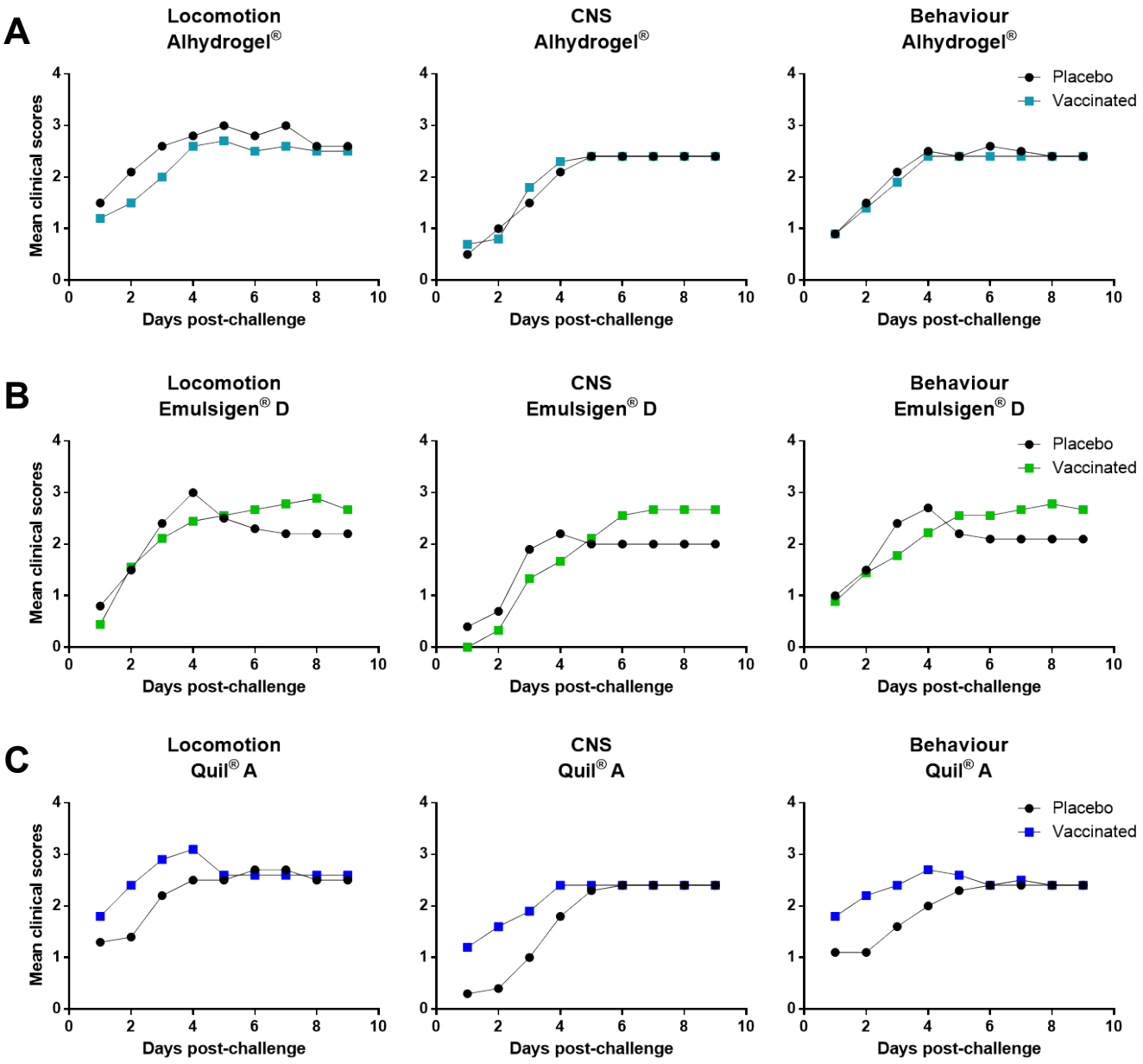

Figure 2 Kaplan-Maier mean clinical scores. Piglets were vaccinated with the equivalent to 10^9 CFU/mL of *S. suis* strain P1/7 bacterin formulated either with Alhydrogel® (**A**), Emulsigen®-D (**B**), or Quil-A® (**C**). All piglets were challenged intraperitoneally with 5×10^9 CFU of *S. suis* strain P1/7. Each vaccine formulation group of vaccinated piglets (n = 10) had a corresponding placebo group of piglets (n = 10) challenged at the same time. Clinical signs of locomotion/lameness, central nervous system (CNS), and behavior change were recorded three times per day, for nine days.

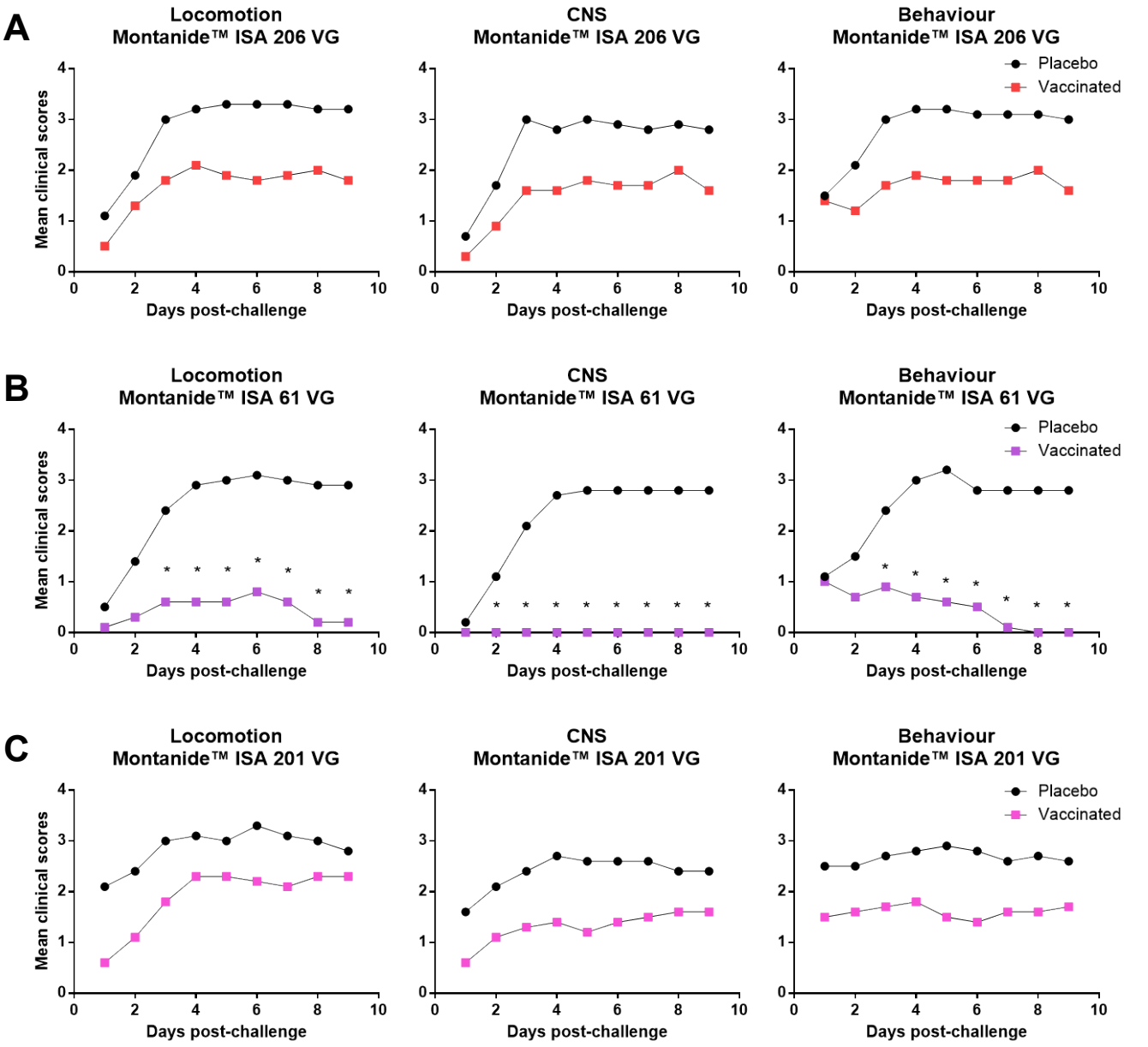

Figure 3 Kaplan-Maier mean clinical scores. Piglets were vaccinated with the equivalent to 10^9 CFU/mL of *S. suis* strain P1/7 bacterin formulated with either Montanide™ ISA 206 VG (**A**), Montanide™ ISA 61 VG (**B**), or Montanide™ ISA 201 VG (**C**). All piglets were challenged intraperitoneally with 5×10^9 CFU of *S. suis* strain P1/7. Each vaccine formulation group of vaccinated piglets (n = 10) had a corresponding placebo group of piglets (n = 10) challenged at the same time. Clinical signs of locomotion/lameness, central nervous system (CNS), and behavior change were recorded three times per day, for nine days. *, $P < 0.05$.

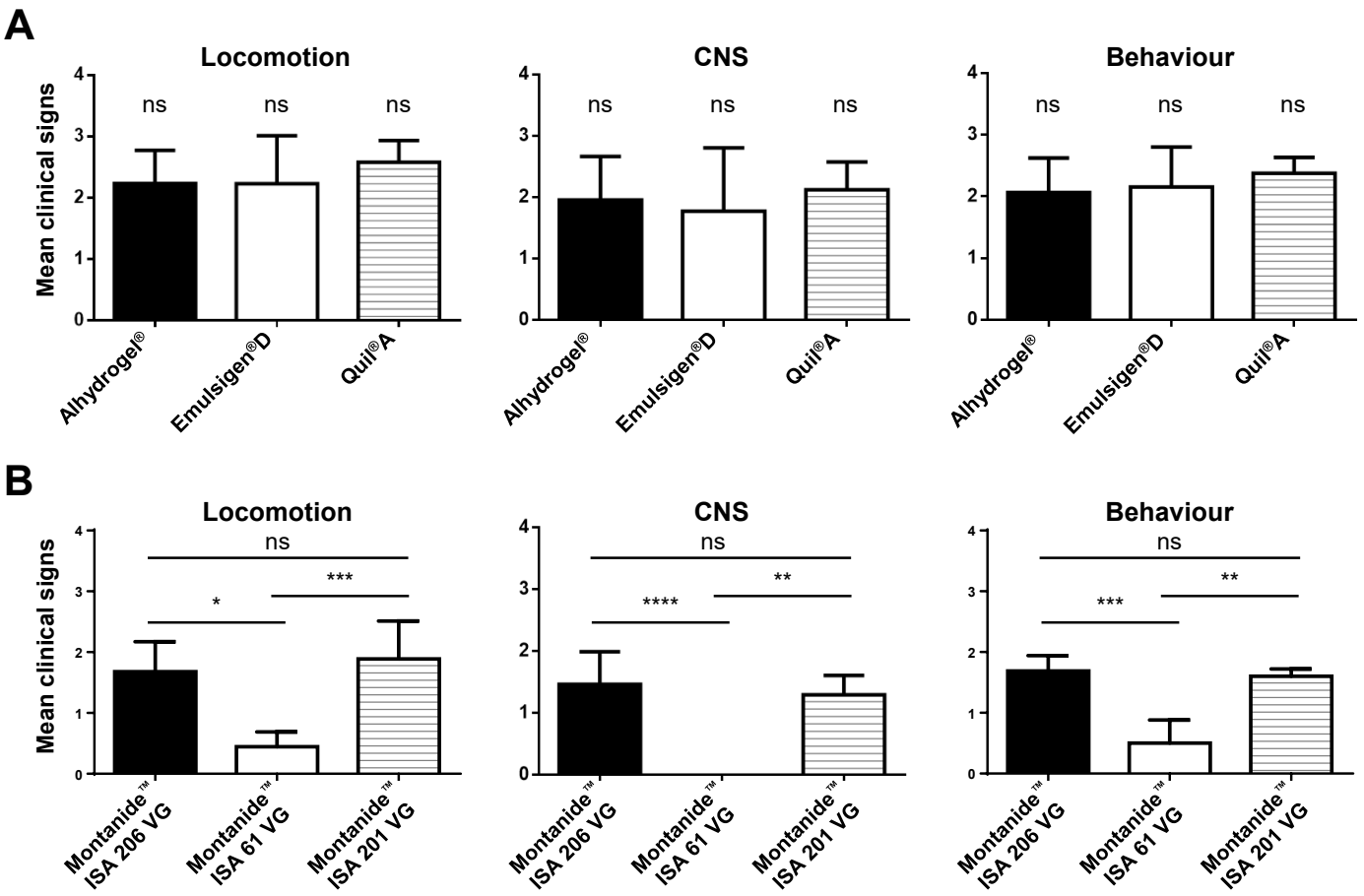

Figure 4 Comparison of the mean clinical scores [locomotion, central nervous system (CNS), and behavior]. Piglets were vaccinated with bacterin vaccines formulated with Alhydrogel®, Emulsigen®-D, and Quil-A® (**A**), and piglets vaccinated with bacterin vaccines formulated with Montanide™ ISA 206 VG, Montanide™ ISA 61 VG, and Montanide™ ISA 201 VG (**B**). The error bar shows the standard deviation of the mean value of clinical scores of 10 piglets. (ns) not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

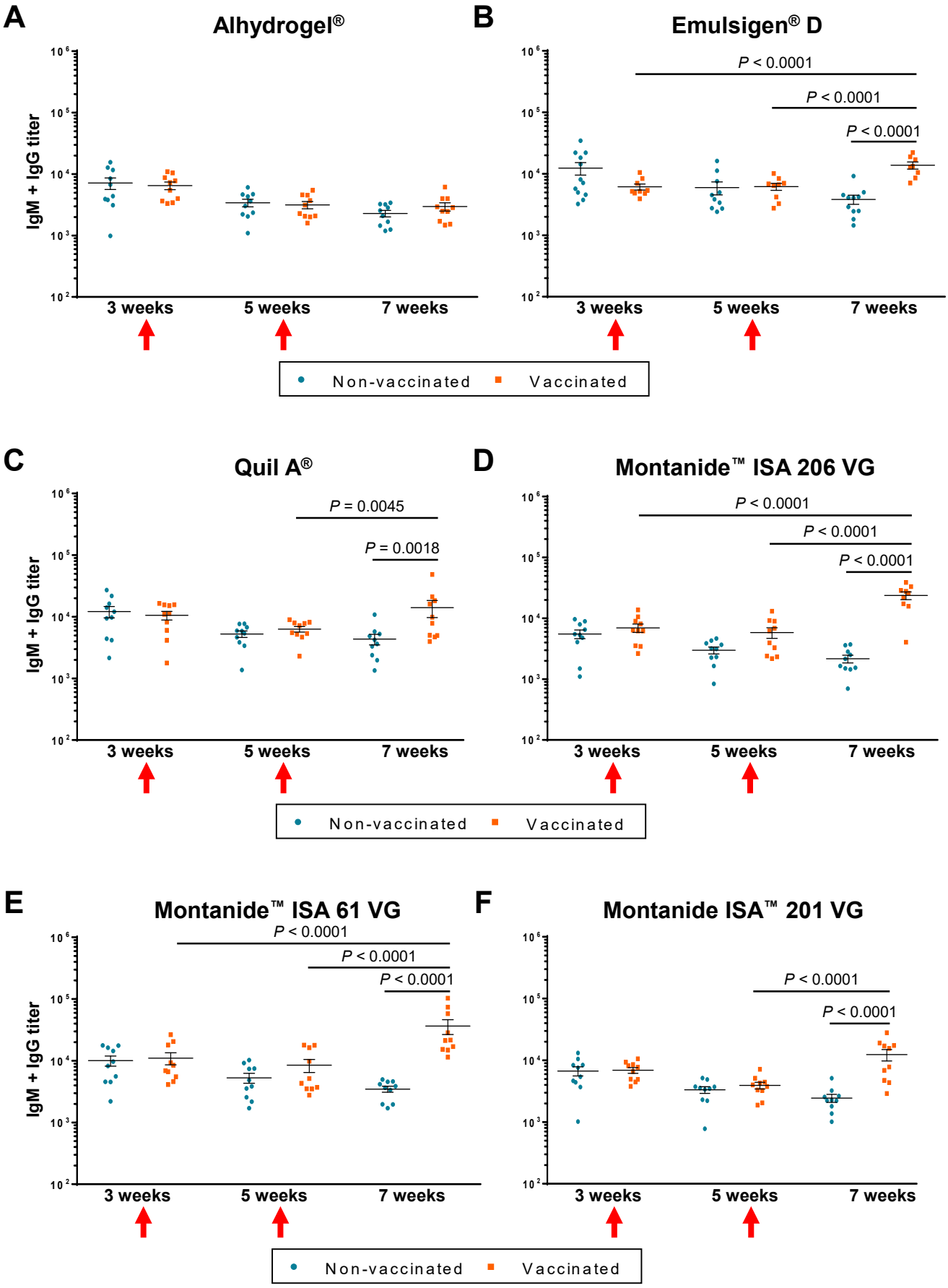
A
Alhydrogel®
B
Emulsigen® D
C
Quil A®
D
Montanide™ ISA 206 VG
E
Montanide™ ISA 61 VG
F
Montanide ISA™ 201 VG
IgM + IgG titer
3 weeks
5 weeks
7 weeks
P < 0.0001
P = 0.0045
P = 0.0018
Non-vaccinated
Vaccinated

Figure 5 Total Ig [IgM + IgG] levels against *S. suis* serotype 2 in piglets as determined by ELISA. Piglets were vaccinated with the equivalent to 10^9 CFU/mL of *S. suis* strain P1/7 bacterin formulated either with Alhydrogel® (**A**), Emulsigen®-D (**B**), Quil-A® (**C**), Montanide™ ISA 206 VG (**D**), Montanide™ ISA 61 VG (**E**), or Montanide™ ISA 201 VG (**F**). The 1st dose was given at 3 weeks of age and the 2nd dose at 5 weeks of age (red arrows). Each vaccine formulation group of vaccinated piglets (n = 10) had a corresponding placebo group of piglets (n = 10). Antibody titers for individual piglets are shown with horizontal bars representing mean ± SEM. Values significantly different are shown in the graph with the corresponding P-value.

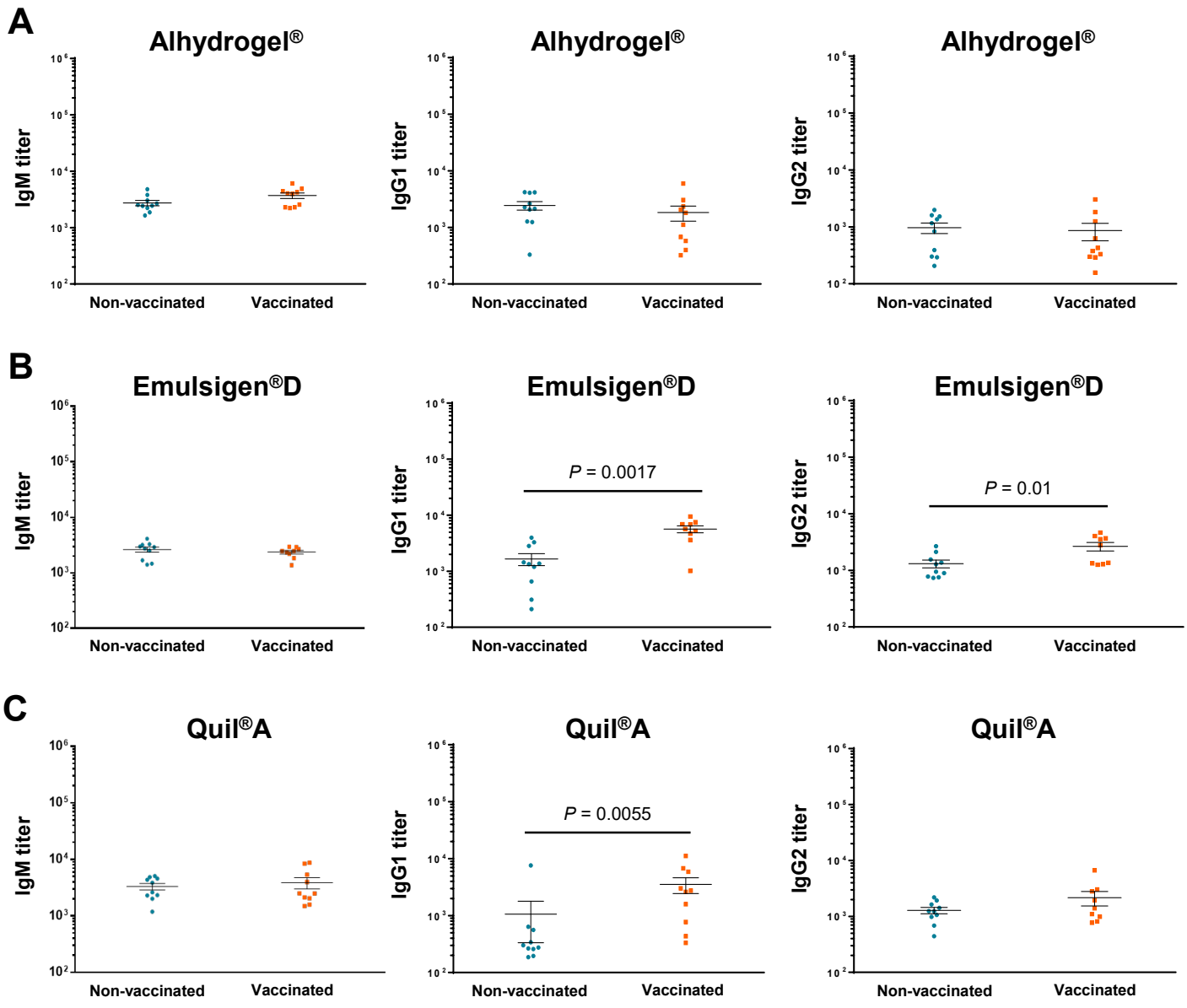

Figure 6 Isotype profile of antibodies against *S. suis* serotype 2 after 2 doses of vaccine in 7-week-old piglets as determined by ELISA. Piglets were vaccinated with the equivalent to 10^9 CFU/mL of *S. suis* strain P1/7 bacterin formulated either with Alhydrogel® (**A**), Emulsigen®-D (**B**), or Quil-A® (**C**). The 1st dose was given at 3 weeks of age and the 2nd dose at 5 weeks of age. Each vaccine formulation group of vaccinated piglets (n = 10) had a corresponding placebo group of piglets (n = 10). IgM, IgG1 and IgG2 titers for individual piglets are shown with horizontal bars representing mean ± SEM. *P*-values significantly different are shown.

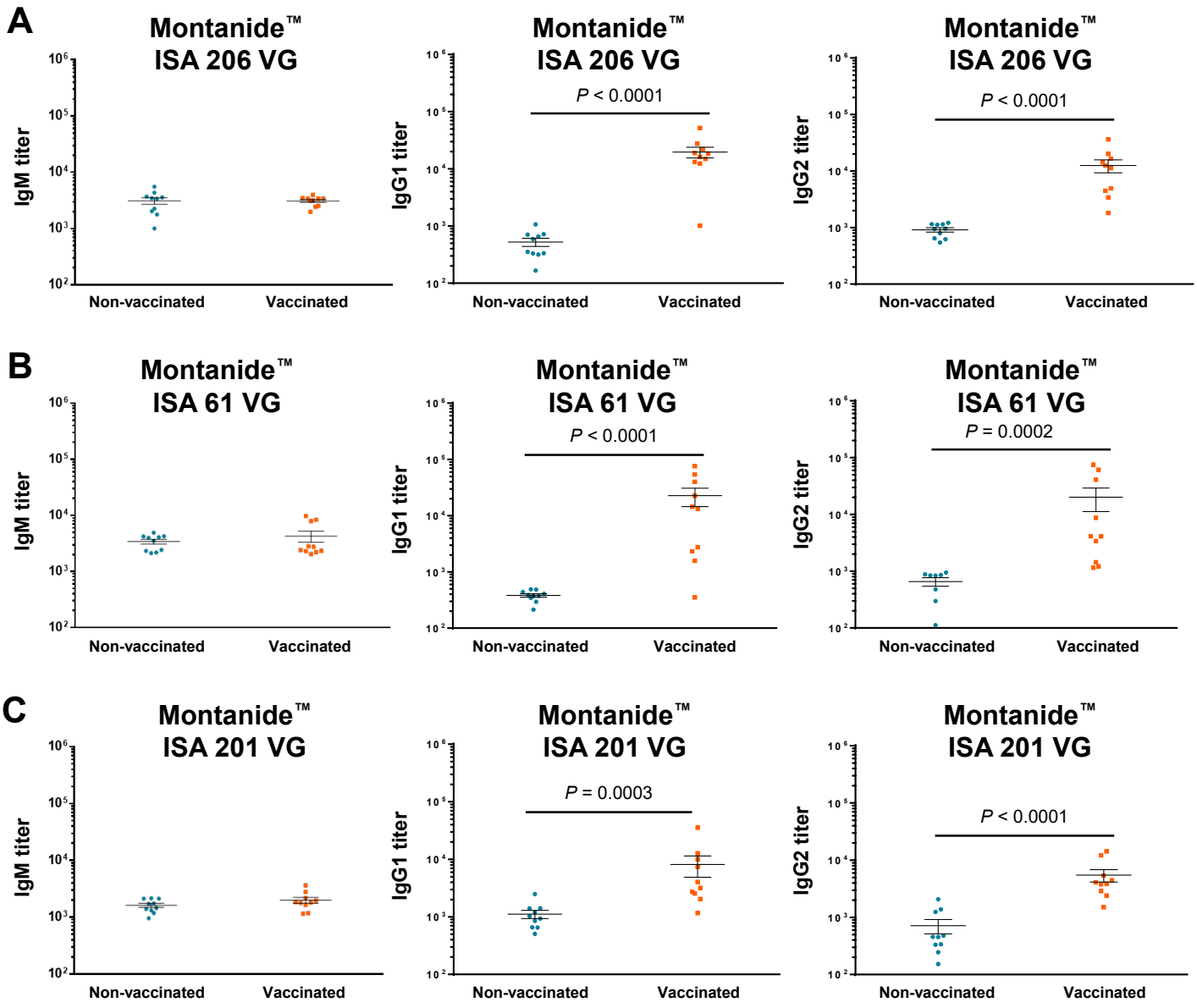

Figure 7 Isotype profile of antibodies against *S. suis* serotype 2 after 2 doses of vaccine in 7-week-old piglets as determined by ELISA. Piglets were vaccinated with the equivalent to 10^9 CFU/mL of *S. suis* strain P1/7 bacterin formulated either Montanide™ ISA 206 VG (**A**), Montanide™ ISA 61 VG (**B**), or Montanide™ ISA 201 VG (**C**). The 1st dose was given at 3 weeks and the 2nd dose at 5 weeks of age. Each vaccine formulation group of vaccinated piglets (n = 10) had a corresponding placebo group of piglets (n = 10). IgM, IgG1 and IgG2 titers for individual piglets are shown with horizontal bars representing mean ± SEM. *P*-values significantly different are shown.

ANNEXE II – Opsonophagocytosis assay (OPA test)

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In the original planned work, two approaches were proposed to measure the protective capacity of the different vaccine formulations:

1. **Challenge infection**: including the monitoring of clinical outcome (morbidity and mortality)

2. **Opsonophagocytosis assay or OPA test**: The OPA test evaluates the capacity of vaccine-induced antibodies to kill bacteria in the presence of phagocytic cells (derived from the blood of naïve pigs). The OPA test is considered an *in vitro* correlate of protection after vaccination (if a challenge infection is not performed or not successful) (Figure 1).

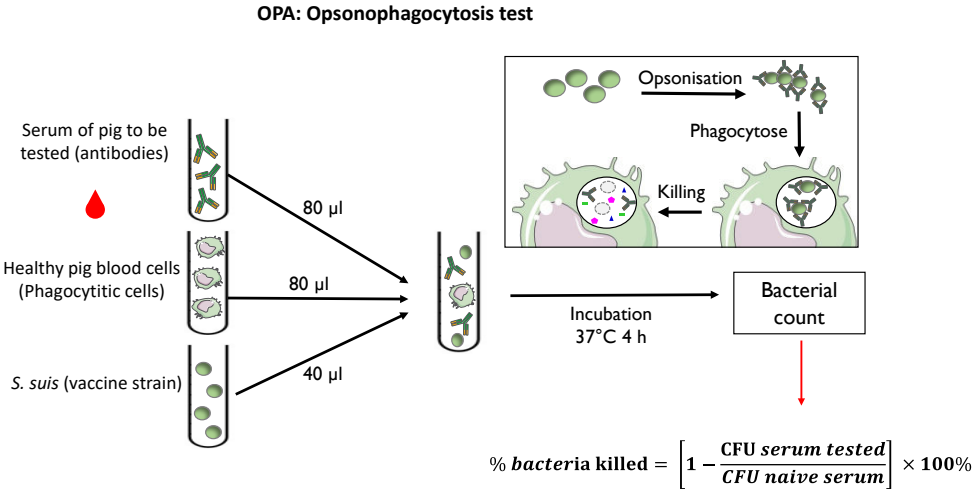

Figure 1. Schematic representation of the opsonophagocytosis assay (OPA test) methodology.

The reason of proposing both techniques was that experimental infections using virulent *S. suis* strains are not easy to reproduce and sometimes they lack reproducibility. In fact, presence of serious clinical signs in the field are frequently associated to co-factors, which are usually absent in research animal facilities. However, **our systemic challenge model**, using an intraperitoneal (IP) route of infection, **showed high reproducibility and consistency of clinical results** which is of the utmost importance when comparing the effect of adjuvants on a given vaccine formulation.

Indeed, the IP challenge model used in this study was able to reproduce typical clinical signs and lesions caused by *S. suis* infection in weaned piglets as those observed in the field (Figure 2). Challenged animals showed signs of depression, incoordination, and shifting lameness. In more severe cases, there were signs of septicemia and meningitis, characterized by convulsion, head inclination, ataxia, opisthotonos, paddling, and nystagmus. Necropsy revealed typical polyarthritis lesions with abundant fibrinopurulent exudate in joint cavities. Spleen was enlarged, with petechial hemorrhages indicating systemic infection (septicemia). The IP challenge model was able to produce consistent mortality and morbidity in all six experimental groups.

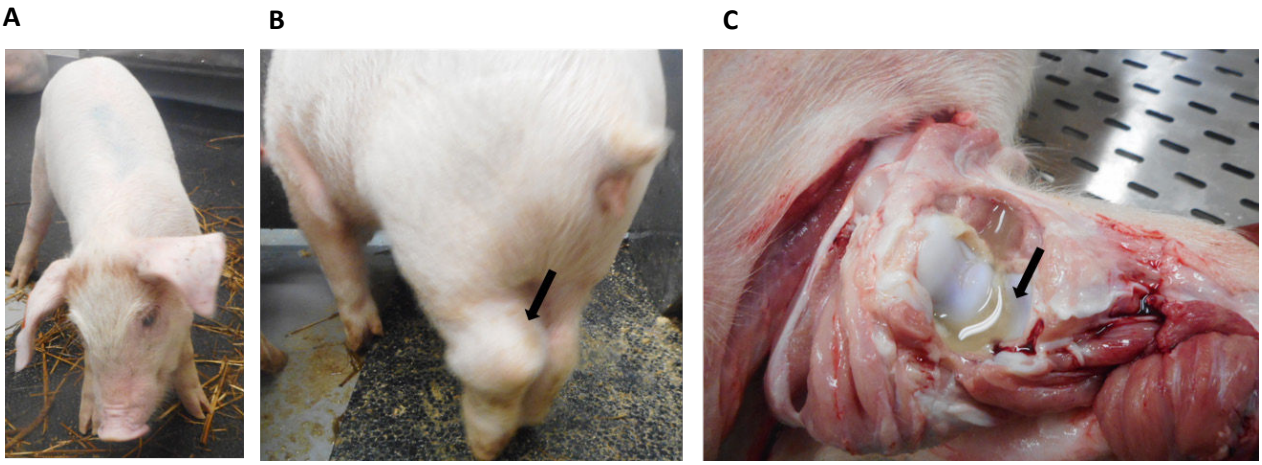

Figure 2. Typical clinical signs of *S. suis* disease observed in challenged piglets. The intraperitoneal challenge model used in this experimental study was able to reproduce typical *S. suis* clinical signs of meningitis (head inclination and incoordination) (**A**); lameness, swollen joints (black arrow), and polyarthritis (**B**); and characteristic lesions of fibrinopurulent exudate in swollen joints observed during necropsy (black arrow) (**C**). *S. suis* serotype 2 was isolated from the joint cavities, meninges, liver and spleen of diseased animals. Pigs having a clinical score = 3 were humanely euthanized.

Based on the success of our challenge model, we decided to not perform the OPA test for this experimental part of the study, as it will not provide any additional relevant information. Nevertheless, we allocated our resources to perform an OPA test with samples obtained in our complementary field study, as the obtained information would be of more practical interest (*considering that a challenge infection cannot be performed in the field*). Obtained data is presented in Figure 3.

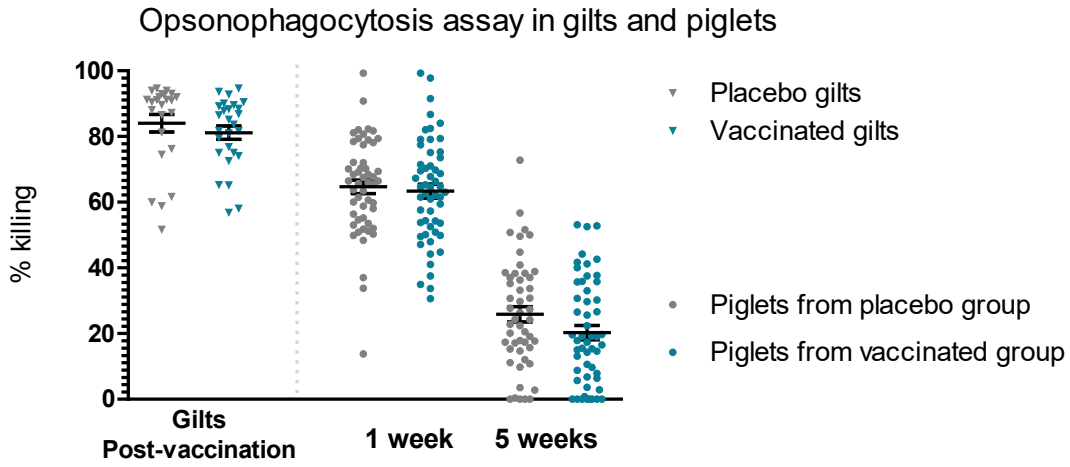

Figure 3. Blood was obtained from gilts after three doses of an autogenous vaccine program in the vaccinated group (n = 28) as well as from control placebo group (n = 25). After farrowing, piglets (2/litter, n = 106) were tagged and followed for serology up to 5 weeks of age. OPA test was done to measure antibody functionality (i.e. the capacity of antibodies to kill *S. suis* in vitro, as represented in Figure 1). Results are expressed as % of bacterial killing for individual sera, with horizontal bars representing mean ± SEM.

Results and Conclusion:

In vaccine studies, *in vitro* functional assays, such as the opsonophagocytosis assay (OPA test), complement ELISA titers for evaluating protection, and are largely used in human medicine as a correlate of immunity. The OPA test evaluates the capacity of vaccine-induced antibodies to kill bacteria in the presence of phagocytic cells. Such assays are normally performed using phagocytic cell lines or purified cell types, which underestimates the complexity of blood bactericidal activity. In this study, we standardized an OPA test using swine whole-blood as effector cells, in a format that requires small serum quantities. After incubation, viable bacterial counts are performed. The percent of bacterial killing is determined by comparing bacterial numbers in sample tubes with those in negative control tubes.

Results showed no statistically significant differences between vaccinated and non-vaccinated animals (gilts and their piglets) at any time point evaluated. Noticeably, the OPA activity of gilts antibodies was already very high before vaccination and thus no changes can be expected by a vaccination program of gilts. This observation may explain the usual lack of clinical disease in adult animals.

On the other hand, maternal-derived antibodies were already highly opsonizing in piglets of 1 week of age and thus able to induce bacterial elimination by phagocytic cells. Yet, the OPA activity of these antibodies was significantly reduced at 5 weeks of age, with no differences between vaccinated and control animals.

Two hypothesis might explain the lack of differences in OPA activity between piglets from vaccinated gilts and piglets from placebo control gilts: our OPA protocol either lacks sensitivity or vaccine-induced antibodies are not opsonic (i.e. lack functionality). More studies will be thus required to optimize the OPA test for swine field studies.