Université de Montréal

Evaluation of an autogenous vaccine used in sows to protect piglets against *Streptococcus suis* disease

Par
Alison Jeffery

Département de pathologie et microbiologie
Faculté de médecine vétérinaire

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Ce mémoire intitulé

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Présenté par
Alison Jeffery

A été évalué par un jury composé des personnes suivantes :

Marie-Lou Gaucher
Présidente-rapporteuse

Mariela Segura
Directrice de recherche

Marie-Ève Lambert
Membre du jury

Résumé

Streptococcus suis est une bactérie pathogène qui cause d'importantes pertes économiques dans l'industrie porcine à travers le monde. Comme il n'existe pas de vaccins commerciaux en Amérique du Nord, l'utilisation d'autovaccins administrés aux cochettes/truies pour induire des anticorps passifs chez les porcelets représente une alternative intéressante pour les producteurs. Cependant, il n'existe aucune production standardisée de ces vaccins et le produit final peut être très différent d'un laboratoire agréé à l'autre. Dans la présente étude, un vaccin autogène (« bacterin ») polyvalent contenant les sérotypes 1/2, 2, 5, 7 et 14 de *S. suis* a été préparé par un laboratoire agréé et utilisé dans un programme de trois doses administrées aux cochettes par voie intramusculaire. La réponse humorale (anticorps) chez les cochettes ainsi que le transfert passif d'anticorps aux porcelets ont été évalués. Contrairement à ce qui avait été publié précédemment avec un vaccin autogène produit par une autre compagnie, la réponse anticorps accrue observée chez les cochettes vaccinées était suffisante pour améliorer le transfert d'anticorps maternels aux porcelets âgés de 3 à 5 semaines. Cependant, les porcelets resteraient encore sensibles à la maladie à *S. suis* qui apparaît souvent pendant la deuxième partie de la période en pouponnière. Le niveau élevé d'anticorps n'a pas affecté l'excrétion de *S. suis* (ainsi que celle de sérotypes spécifiques de *S. suis* inclus dans le vaccin) chez les cochettes et les porcelets. Bien que tous les traitements antibiotiques aient été absents pendant l'essai, l'effet protecteur clinique du programme de vaccination avec le vaccin autogène n'a pas pu être évalué, car des cas limités d'infection à *S. suis* étaient présents pendant l'essai. D'autres essais pour évaluer l'utilité de la vaccination des cochettes/truies avec des vaccins autogènes pour protéger les porcelets de pouponnière devraient être réalisés. Il est nécessaire, pour les futurs essais sur le terrain, de toujours inclure un groupe témoin non vacciné, d'éliminer si possible tout traitement antimicrobien dans l'élevage et de confirmer l'étiologie des

cas cliniques par un diagnostic en laboratoire lors de l'évaluation de l'effet protecteur de tels vaccins autogènes.

Mots-clés : *Streptococcus suis*, vaccin, bactérines autogènes, porc, étude de terrain, réponse immunologique, vaccination.

Abstract

Streptococcus suis is a bacterial pathogen that causes important economic losses to the swine industry worldwide. Since there are no commercial vaccines available in North America, the use of autogenous vaccines applied to gilts/sows to induce maternal antibodies to protect piglets is an attractive alternative for producers. However, there is no universal standardization in the production of such vaccines and the final product may be highly different among licenced laboratories. In the present study, a polyvalent autogenous vaccine ("bacterin") with *S. suis* serotypes 1/2, 2, 5, 7 and 14 was prepared by a licenced laboratory and used in a three-dose program given to gilts intramuscularily. The humoral (antibody) response in gilts as well as the passive transfer of antibodies to piglets were evaluated. Different from what was previously published with an autogenous vaccine produced by a different company, the increased response seen in vaccinated gilts when compared to non-vaccinated animals was sufficient to improve maternal antibody transfer to piglets of 3 to 5 weeks of age. However, piglets would still remain susceptible to *S. suis* disease that often appears during the second part of the nursery period. The high level of antibodies did not affect *S. suis* (as well as that of specific serotypes of *S. suis* included in the vaccine) shedding by both, gilts and piglets. Although all antibiotic treatments were absent during the trial, the clinical protective effect of the vaccination program with the autogenous vaccine could not be evaluated, since limited *S. suis* clinical cases were present during the trial. Further trials to evaluate the usefulness of gilt/sow vaccination with autogenous vaccines to protect nursery piglets should be done. There is a need, for future field trials, to always include a control non-vaccinated group, to eliminate if possible any antimicrobial treatment in the farm and to confirm the etiology of clinical cases by a diagnostic laboratory when evaluating the protective effect of such autogenous vaccines.

Keywords: *Streptococcus suis*, vaccine, autogenous bacterins, swine, field study, immunological response, vaccination.

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List of abbreviations

APCs	Antigen presenting cells
BCR	B cell receptor
CC	Clonal Complexes
CNS	Central nervous system
CPS	Capsular polysaccharide
CSR	Class switching recombination
CTLs	Cytotoxic T lymphocytes
EF	Extracellular factor
FBPS	Fibronectin-binding protein
Fhb	Factor H-binding proteins
IFN	Interferon
Ig	Immunoglobin
IL	Interleukin
ISCOMS	Immune-stimulating complexes
MHC	Major histocompatibility complex
MLST	Multilocus sequence typing
MRP	Muramidase-released protein
NK	Natural killer cell
OPA	Opsonophagocytosis assay
PAMPs	Pathogen-associated molecular patterns
PRRs	Pattern recognition receptors

Sao	Surface antigen one
SHM	Somatic hypermutation
SLY	Suilysin
SspepO	Secreted *Streptococcus suis* putative endopeptidase O
SspepC	Secreted *Streptococcus suis* putative endopeptidase C
Ssna	Secreted *Streptococcus suis* nuclease A
ST	Sequence types
TD	T-dependent
TI	T-independent
Zmp	Zinc metalloprotease

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I. Introduction

Streptococcus suis (*S. suis*) causes great economic losses to the pork industry worldwide, affecting mostly post-weaned piglets [1, 2]. This pathogen is also an emerging zoonotic agent. In both pigs and humans, it can cause meningitis and septicaemia. Serotype 2 is the most common cause of disease in humans and pigs [3]. Infections in humans had been usually considered as sporadic infections in people working with pigs or pork-derived products, such as pig farmers, veterinarians, abattoir workers, pork transporters, meat inspectors and butchers [3]. However, important outbreaks that occurred in Asia have changed the perspective of the threat posed by this pathogen to human health [4]. In swine, control of *S. suis* has mainly been through prophylactic or metaphylactic use of antibiotics. However, there is rising concerns of antibiotic resistance. As a result of this, new regulations within Canada have started to strictly regulate the use of antibiotics in livestock. As complexity of *S. suis* epidemiology increases (multiple serotypes, multiple strains within those serotypes that have a high phenotypic diversity), it has been difficult to develop a universal vaccine. Thus, there is currently no commercial vaccine in North America to control *S. suis*. Several vaccines such as subunit, live-attenuated and bacterin-based vaccines have been developed for *S. suis* control, but results are still experimental. The use of bacterial autogenous vaccines has increased in popularity as they are a relatively low-cost preventive strategy for swine producers and can include several serotypes in one vaccine. However, effectiveness of these vaccines remains controversial. These vaccines are composed by the strain(s) isolated from diseased pigs within a farm and produced by an accredited laboratory, and then applied to the original farm. Field studies evaluating the protective capacity of autogenous vaccines produced by licenced laboratories are limited and presented contradictory results [5-8]. Absence of protective responses from these vaccines have been attributed to the failure of whole-bacterial antigens to elicit an

immune response due the inactivation processing, production of antibodies to antigens not associated with protection, and/or the use of inappropriate adjuvants [9, 10]. However, it is difficult to compare studies with different autogenous vaccine manufacturing procedures [5, 6], as they may use different adjuvants, bacterial concentrations as well as conditions in which the pathogen is grown and killed, among other variables. There are limited field studies evaluating immunological response and the protective capacity of autogenous vaccines coming from different manufacturing companies. In addition, no field studies have evaluated the usefulness of an autogenous vaccine in the complete absence of antimicrobials on the farm.

The hypothesis of the thesis is that vaccination of sows/gilts with available autogenous vaccines is not highly protective for piglets at the end of the nursery period, independently of the company producing the vaccine, mainly due to a relative short duration of maternal antibodies and/or absence of effect on bacterial shedding. The objective of this thesis was to evaluate the immune response and protective capacity induced by a *S. suis* autogenous vaccine, manufactured by a vaccine company (that has never been previously tested), applied to gilts and to evaluate transfer of maternal immunity to their piglets. The specific objectives were:

1. To study the immune response of a three-dose autogenous vaccine program in gilts:

- To evaluate and characterize the magnitude and profile of the antibody response in pregnant gilts,
- To measure the level of maternal immunity transfer to their litters, and
- To characterize *in vitro* the protective potential of antibodies in gilts and piglets.

2. To evaluate the clinical protective capacity in piglets induced by the gilt autogenous vaccine program:

- To measure and characterize *S. suis* bacterial levels in saliva in both gilts and piglets and the effect of the vaccine to decrease bacterial shedding, and
- To characterize the protective effect in the field through clinical and bacteriology follow up.

II. Literature review

1. *Streptococcus suis*

1.1 General characteristics

Streptococcus suis (*S. suis*) is an encapsulated gram-positive microorganism and one of the major bacterial pathogens that causes important economic losses in the swine industry [11, 12]. Almost 100% of pigs worldwide are carriers of *S. suis* [4]. This bacterium is known as one of the main causes of bacterial-induced death in 5 to 10 week-old pigs causing mainly septicemia with sudden death, meningitis and arthritis [3, 11]. It is also known to cause endocarditis, myocarditis and polyserositis in swine [3, 13-16]. With proficient and efficient treatment, prevention of this infection is important for pig welfare and husbandry [17]. *S. suis* is also an emerging zoonotic pathogen worldwide, known to cause meningitis, septicemia, endocarditis and other diseases in humans [17, 18]. From the public health perspective, this pathogen presents a growing interest not only because of its zoonotic potential, but also by its antimicrobial resistance [19], which is a rising concern worldwide. It is thus important to establish prevention strategies for *S. suis* other than the use of antimicrobials. There are a number of serotypes (described below), but serotype 2 is one of the most virulent and is commonly isolated in pigs and humans worldwide [15, 18, 20].

1.1.1 Serotypes

Strains taken from diseased animals are serotyped to complete the diagnosis [4]. *S. suis* strains found in neurological or systemic tissues (brain, meninges, joints and the heart) are considered primary pathogens while strains recovered from lungs are considered as opportunistic and those present in the upper respiratory tract are mostly commensal [21]. Originally, thirty five (35) serotypes of *S. suis* have been identified based on the antigenicity of their capsular polysaccharide (CPS) [4, 20, 22]. Reference strains have originated from diseased pigs, clinically

healthy pigs, diseased humans, calves and a lamb [3]. However, certain serotypes such as 20, 22, 26, and 33 have been recently more carefully analyzed, due to new diagnostic techniques, which has led to their re-classification as a different *Streptococcus* species [3, 16, 22]. Therefore, 29 true serotypes of *S. suis* are currently recognized [16, 22, 23]. Additionally, individual pigs are known to be colonized by more than one serotype of *S. suis* at a time [3].

There are different serotyping techniques that can be used to confirm *S. suis* infection such as the co-agglutination test and the capillary precipitation test or the Neufeld's capsular reaction, both using reference antisera [4]. These tests have been used in many laboratories across North America; however, some serotypes may cross-react due to common antigenic determinants. In particular, the following serotypes have been described to cross-react: 1/2 and 2, 6 and 16, 2 and 22, and 1 and 14 [4]. The use of molecular serotyping by PCR amplification of serotype specific *cps* genes is now commonly used. Not using antisera, this method does not require animals for serum production. However, this method cannot differentiate between serotypes 2 and 1/2, as well as serotypes 14 and 1, as these pairs do not possess distinctive *cps* genes [4]. For these serotypes, new techniques have been developed since traditional PCR is not able to differentiate between the serotypes [24-26]. Lacouture *et al* (2020) developed mismatch amplification mutation assay (MAMA)-PCR that was able to correctly serotype 148 isolates that were previously known to be serotypes 1, 2, 1/2 and 14 [24]. In addition, Thu *et al* (2021) also validated a multiplex PCR to differentiate between serotypes 1 and 1/2, and serotypes 2 and 14 [26]. Rapid high resolution melting assay is also another new development to differentiate between these serotypes, as it is based on a single-nucleotide polymorphism with capsular polysaccharide synthesis gene cluster K [25]. These new techniques allow for the identification and the acquisition of further information

on serotypes 2 and 14, which possess high zoonotic potential as well as serotype 1/2, which is frequently isolated in North America [24].

Distribution and prevalence of *S. suis* serotypes (in diseased pigs) differ country to country (**Figure 1**). In South America, serotype 2, 1/2, 14, 7 and 9 are the most prevalent while in Asia the most prevalent serotypes are 2, 3, 4, 7 and 8 [4]. In Europe, there is lack of reports on serotype distribution. Reports from Spain and the Netherlands have shown a relatively similar serotype distribution, with serotype 9 being the most prevalent followed by serotypes 2, 7, 8 and 3 [4]. In North America, the most prevalent serotype in the United States is 1/2, followed by 7 and 2 [21]. Similarly, in Canada the most predominant serotype is 1/2, as well as serotype 2, followed by serotype 7 [27]. Worldwide, serotype 2 is the most common serotype isolated from diseased human and pig cases [3, 20, 22].

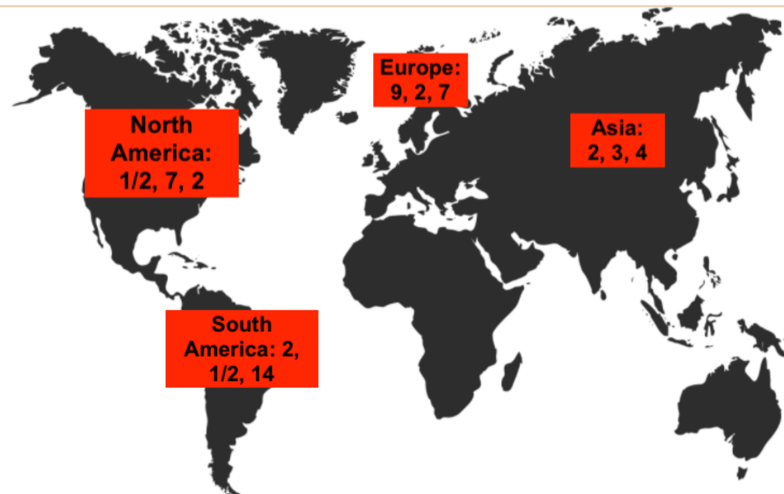


Figure 1*:* Geographical distribution of the three most prevalent *S. suis* serotypes frequently isolated from diseased pigs from January 2002 to September 2019 (Image created by Alison Jeffery).

In 2014, most *S. suis* zoonotic infections have been caused by serotype 2 (74.7% of total global cases) and serotype 14 (2.0% of total global cases) [4]. Unconfirmed or unknown *S. suis* serotypes (based on biochemical identification) account for 23% [4]. In addition, rare cases associated with serotypes 4, 5, 7, 9, 16, 21, 24 and 31 have been identified [4]. The majority of clinical human cases have been reported in Asia, particularly in Vietnam, Thailand, and China. Important *S. suis* human outbreaks emerged in China (1998 and 2005) and Thailand, making this bacterium a primary health concern in this part of the globe [4]. Recently, there has been new reports of *S. suis* outbreaks in Australia and New Zealand. However, there is lack of serotyping data from these countries [4, 28, 29]. In Western countries, humans who are in contact with infected pigs or work with contaminated pork-derived products are most at risk for *S. suis* infections [11].

1.1.2 Sequence types

Genetic and phenotypic diversity exists within serotypes of *S. suis*, possibly attributing to virulence of a strain. Therefore, the classification based on Multilocus Sequence Typing (MLST) is used to compare seven housekeeping genes between strains [4]. These housekeeping genes control cellular function and genetic similarity within strains, thus allowing classification as "sequence type" (ST). As of November 2019, a total of 1245 sequence types have been recorded in the MLST database for *S. suis* [22]. Laboratories around the world use the MLST database to determine sequence types of strains isolated from pigs and humans [4, 22]. This database is a trustable tool to efficiently differentiate and compare strains between laboratories. Unlike cross-reactivity observed with serotyping, household genes are very specific to each ST, avoiding potential errors in classification [30].

Regarding *S. suis* serotype 2, ST1, ST25 and ST28 dominate the world population [30, 31]. ST1 is considered the most virulent, as majority of isolates derived from clinical cases associated with septicemia, meningitis and arthritis in swine and humans, especially in Europe and Asia (Figure 2) [30]. ST7 is endemic to China, where it has been responsible for the 1998 and 2005 epidemics, while North America varies with most strains classified as either ST25 or ST28 [4].

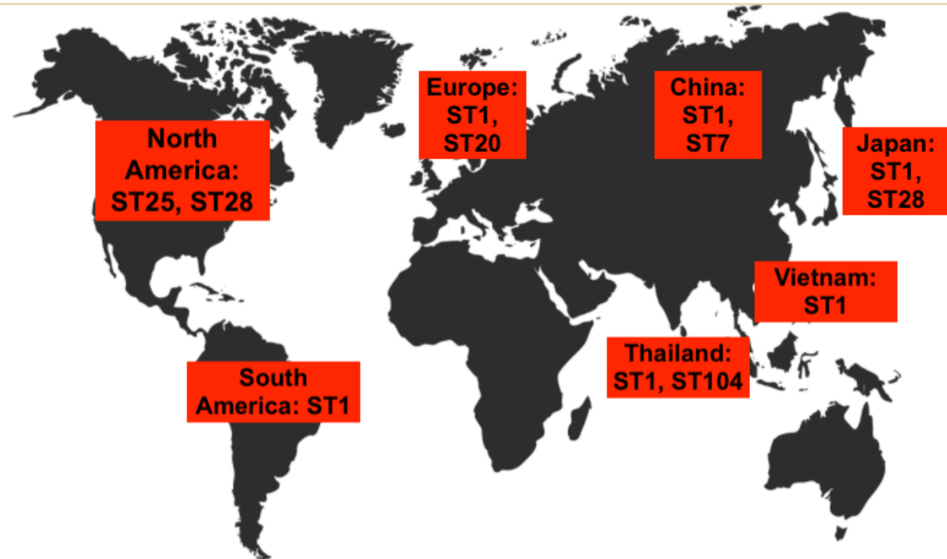


Figure 2: Worldwide distribution of predominant *S. suis* serotype 2 sequence types (ST) of isolates from both clinical pig and human cases of infection (Image created by Alison Jeffery).

Through MLST, clonal complexes (CC) have been identified within the *S. suis* population [22]. Clonal complexes consist of STs sharing 6 out of 7 alleles with at least one ST [31]. Clonal complexes are named based on the ST that contains the greatest number of variants at a single locus [31]. CC that have been linked to causes of infections in humans and pigs have been CC1, CC16, CC20, CC25, CC28, CC94, CC104, CC233/379 and CC221/234 [22]. In Europe, Asia, Australia and South America (Argentina), CC1 was found while CC20 (ST20) was more important in the Netherlands [22]. CC16 and CC94 were mainly described in Europe, with some human cases reported in Thailand [22].

1.2 Epidemiological aspects

1.2.1 Transmission of infection

S. suis is considered a normal inhabitant of swine, as it resides in the upper respiratory tract of pigs, specifically in the tonsils and nasal cavities, as well as the genital tract and possibly digestive tract of pigs [3, 4, 15]. Healthy pigs are also known carriers of the bacterium, and contribute to bacterial shedding and further dissemination throughout the herd [3, 13, 17]. Carrier pigs can harbour the organism in their tonsils and may never develop the disease [32]. Introduction of potentially virulent *S. suis* strains by carrier pigs into herds could result in the onset of clinical disease [3, 11]. Vertical transmission of the pathogen occurs during birth through the vaginal canal through vaginal colonization [3, 11, 17]. Horizontal transmission occurs through oro-nasal route amongst pigs [3, 13, 15, 17, 20]. Recently, aerosolization has been identified as a possible route of transmission within a pig barn [3, 20]. Therefore, it is hypothesized that *S. suis* could possibly infect pigs and humans through the respiratory route [11, 20].

It is uncertain if environmental contamination, fomites, and insect vectors contribute to the transmission of *S. suis* [33, 34]. Regarding *S. suis* serotype 2, survival of the bacterium in dust can be up to 54 days at 0˚C and up to 25 days at 9˚C. However, it could not be isolated at room temperature in dust [35]. In addition, this serotype can survive in the feces for 104 days and 10 days at 0˚C and 9˚C, respectively, as well as up to 8 days at 22-25˚C [35]. In water, survival is seen for up to 10 minutes at 60˚C, revealing the possibility of contamination during the scalding process in abattoirs [35]. Though isolation of *S. suis* has been reported from feed troughs of piglets and sows [34], the oral route has not yet been proven nor the survival of *S. suis* in feed (fine pellet or crumb feed, with or without formic acid) [3, 36]. *S. suis* survival in the stomach contents is also unknown [36]. In swine production, it is also suggested that stress may facilitate the bacterial

systemic invasion in pigs, as it lowers immunity or resistance to *S. suis* and results in clinical disease [1, 3, 17].

In humans, *S. suis* can cause meningitis, septicemia, arthritis and sometimes streptococcal toxic shock-like syndrome resulting in rapid death [15, 23]. Close contact with pigs, wild boars or pork are important risk factors for humans [4, 14, 17]. Individuals who work directly with pigs or pork have a 1500 times higher risk of being infected with *S. suis* than those who do not [13]. Human transmission occurs through skin wounds when working with infected animals or contaminated meat [1, 13, 18, 20]. Undercooked contaminated meat is also an oral route for humans [20]. Cultural practices in countries, such as Vietnam and Thailand, that include the eating of raw meat may also contribute to the important prevalence of human transmission of *S. suis* [20]. In addition, wild boars are known to carry *S. suis* in some countries. In Spain, wild boars and domestic pigs were both shown to have the presence of serotype 9, thus this is a risk factor for outdoor commercial farms in countries with a large wild boar population [3].

1.2.2 Pathogenesis

The early mechanisms used by *S. suis* strains to colonize and invade the host are not well known [3, 32]. Strains may reside in the tonsils for long periods of time after colonizing the respiratory mucosal surfaces and without producing the disease [3, 11]. Before hematogenous and/or lymphogenous dissemination and systemic disease, colonization of the mucosal surfaces is the first step but the exact mechanism(s) is/are unknown [11]. To overcome this first innate immune barrier, it is suggested that *S. suis* causes damage to epithelial cells or decreases mucus production [3, 11]. Since the tonsillar lymphoid tissue has deep epithelial invaginations, it is possible that *S. suis* remains hidden from the immune system after adhesion and invasion of the

epithelial cells [32]. It is also proposed that *S. suis* can overcome the complement immune system at the mucosal surface [11]. Immunoglobulin A (IgA) is crucial part of the host's immune defence at mucosal levels [20]. A study reported that *S. suis* has IgA1- protease which is thought to increase invasive capacity of the mucous membrane to reach the blood stream [20]. However, this conclusion may be questionable based on three main considerations: Firstly, porcine specific or cross-reactive IgAs against *S. suis* have never been documented; secondly, no IgA protease activity against human IgAs was detected in any of the *S. suis* strains evaluated in a subsequent study, and thirdly, it was demonstrated that the zinc metalloprotease (Zmp) encoded by the *iga* gene does not have IgA protease activity [37]. Segura *et al.* (2016) provides further details on potential mechanisms in regards to mucosal barrier breakdown [11].

The gastrointestinal tract is thought to be a possible secondary site of infection in piglets [3, 11]. Studies have shown that the bacterium is able to translocate from the intestine into the blood stream to cause disease in different tissues and organs [1, 3, 11]. However, these studies included *S. suis* in gastric acid-resistant capsules. There is suggestion that *S. suis* is unable to survive gastric pH of 4.7 [36]. Passage of *S. suis* through the stomach is not well understood and may differ in different age groups of pigs, thus more studies are needed [3, 32].

After cell invasion of the mucosal barrier, the pathogen must survive the attack of the innate immune system [11, 20, 32]. The bacterium has multiple virulence factors and it is able to spread to the bloodstream by overcoming the host immune system [18, 20]. There are over 20 virulence factors that have been identified within different serotypes and strains of *S. suis*, including Muramidase-released protein (MRP), Suilysin (SLY), the extracellular factor (EF), capsular polysaccharide (CPS) and different pili [1, 3, 15]. Not all of these factors are present in all strains of *S. suis*, and the presence or absence of any or all of these proteins are not necessarily associated

with lack of virulence [3], suggesting that *S. suis* virulence is multifactorial. *S. suis* also has multiple adhesins that work together to adhere and invade the host, such as Fibronectin-binding protein (FBPS), enolase, and a Streptococcal histidine triad protein gene *htpsC* [18, 20, 32]. These proteins adhere to host cells or components of the extracellular matrix such as fibronectin, fibrinogen, plasminogen and collagen [20, 32]. *S. suis* capacity to form biofilm at the surface of the endothelium could contribute to the onset of symptoms such as endocarditis [20]. The CPS of *S. suis* plays an important role in the regulation of biofilm formation. Non-encapsulated strains have a higher likelihood of forming biofilm, which suggests that receptors enabling the interaction between bacteria and host cells may be hidden by the capsule [20].

Suilysin (SLY), which is produced by some strains, has been reported to contribute to the pathogenesis of *S. suis* as it is a hemolysin and contains cytotoxic properties [20, 38]. SLY causes necrosis, apoptosis, and cell lysis [39]. The role of SLY in bacterial adherence and host-cell invasion is not fully understood; however it is suggested that SLY facilitates bacterial invasion into the epithelium and promotes bacterial survival in the bloodstream [39]. Survival in the bloodstream may also be enabled by CPS and cell wall components that efficiently avoid phagocytosis [3, 11, 16, 20]. CPS is thought to be a key virulence factor that provides protection against the immune system, but other virulence factors must be present for full virulence in the host [3, 11, 18]. Factor H is part of the complement system that prevents major pro-inflammatory responses that could be harmful for the host [20]. However, many bacterial pathogens have the ability to bind factor H to their cell surface to avoid complement attack and opsonophagocytosis. In *S. suis*, recruitment of factor H to the bacterial surface is multifactorial and it seems to modestly occur through two factor H-binding proteins (Fhb and Fhbp) and the CPS [40].

After the bacterium has spread to different organs, inflammation will play an important role supporting the pathogenesis of *S. suis*-induced septicemia and meningitis [3, 18]. Hyperactivation of the immune system occurs when *S. suis* exposes its bacterial cell wall components and triggers a pro-inflammatory response that can lead to septic shock [3, 20]. Indeed, an increase in the release of proinflammatory cytokines is associated to rapid disease progression with a high rate of mortality [32]. This rapid disease progression happens over a short incubation period and can lead to sepsis and toxic shock-like syndrome [32]. If the host survives through the initial and rapid proinflammatory response caused by *S. suis*, the pathogen will invade the central nervous system (CNS) and may cause meningitis [32]. The two barriers that represent the largest interface between blood and brain extracellular fluids are the blood-brain barrier and the blood-cerebrospinal fluid-barrier, which are formed by the brain microvascular endothelial cells and the choroid plexus epithelial cells, respectively [3]. The mechanism(s) by which the bacterium can cross these barriers are not fully understood. Invasion into the blood-brain barrier's microvascular endothelial cells by *S. suis* serotype 2 depends on adhesins, cell wall components and interactions with host cell extracellular matrix proteins [16, 32]. In addition, different proteins or surface components of *S. suis* interact with porcine microvascular endothelial cells and promote the release of cytokines, which help the bacteria entering the blood-brain barrier [16, 18]. It is also possible that SLY contributes to bacterial crossing of the blood-brain barrier [20] and the blood-cerebrospinal fluid barrier, by inducing increased permeability and allowing bacterial adherence to the extracellular matrix [16, 20]. By using SLY, *S. suis* is capable of upregulating important cell adhesion molecules during inflammation that promotes leukocyte migration [39]. After *S. suis* has entered the CNS, the release of proinflammatory cytokines induced by bacterial cell wall and surface components induces inflammation leading to CNS clinical signs [3].

1.2.3 Clinical signs

As mentioned above, *S. suis* carrier rate can be very high, and these healthy carrier pigs can disperse *S. suis* throughout the herd [3, 4, 17]. The incidence of the disease can vary but is usually less than 5%, mainly due to antimicrobial prophylaxis (described below). However, in the absence of prophylactic measures, disease rates can reach 20% [41]. The majority of *S. suis* clinical cases are observed at 5-10 weeks of age (mainly due to the decrease of maternal antibodies at weaning); exceptionally cases have been reported in pigs from a couple of hours old to 32 weeks in age [1, 3]. This pathogen is known to cause meningitis, septicemia, endocarditis, and arthritis [13-16]. Early signs of infection in pigs begin with a high rectal temperature, following with a fluctuating fever, poor appetite, depression and shifting lameness [3]. During this time, a detectable bacteremia or pronounced septicemia can be diagnosed and may persist up to 3 weeks if untreated [3]. Sudden death may occur in some animals without previous clinical signs. Neurological signs are seen when *S. suis* causes meningitis; this includes early signs of incoordination or unusual stances which then progress to inability to stand, eyes staring with little response to stimulus, paddling, convulsions, opisthotonos and nystagmus. Less common clinical signs are vegetative valvular endocarditis, rhinitis, abortion and vaginitis [3].

Mixed infections of *S. suis* with other swine viruses are commonly found in swine herds [42]. Infection with other viruses is suggested to make pigs more susceptible to *S. suis* diseases causing an increase of clinical signs and increased mortality [3, 42]. There is a clear synergistic co-infection between porcine reproductive and respiratory syndrome virus (PRRSV) and *S. suis*, but other important viral pathogens associated with *S. suis* coinfection are swine influenza virus and porcine circovirus 2 [42]. In North America, acute infections with virulent PRRSV significantly increased susceptibility to *S. suis* disease on farm [3]. In a study by Feng *et al* (2001),

piglets infected with PRRSV in utero are more likely to have *S. suis* infection and clinical disease when challenged by *S. suis* serotype 2 [43].

In humans, meningitis is the most frequent clinical manifestation, but septic shock along with organ failure, endocarditis, pneumonia, arthritis and peritonitis have also been reported [13, 22]. Patients who had acute meningitis also experienced symptoms such as headache, high fever, chills, nausea, and vomiting [13]. Chills, headache, vomiting, vertigo, abdominal pain, high fever were observed in cases of acute streptococcal toxic shock-like syndrome as well as hypotension, tachycardia, liver dysfunction and haemorrhage, acute renal failure and acute respiratory distress syndrome that can be followed by death [13]. Acute hearing loss is the most common side effect of meningitis [13, 15].

1.2.4 Measures to control infections caused by *Streptococcus suis*

Strains of *S. suis* differ genetically and phenotypically among serotypes and countries, thus vaccine development is a difficult task [1]. There is currently no commercial vaccine available for use in North America. Although the bacterium is sensitive to some antibiotics [13], prophylactic and metaphylactic antimicrobial treatments help prevent and control the disease spread in the herd [1]. However, there is increasing concern of antimicrobial resistance in human and animal health worldwide. Due to new Canadian regulations that came into effect December 2018, a prescription is now necessary to acquire antimicrobials for on-farm use. These new regulations will ensure that antimicrobials are used when necessary and justifiable, preventing over- and unnecessary use and potential increase in resistance [1, 44]. In addition to antimicrobial treatment plans, there are also preventative measures that can occur to help decrease the risk of disease in a herd, as well as prevent and decrease the need of antimicrobials.

1.2.4.1 Prevention of clinical expression of *S. suis* via correction of risk factors

Different factors are suggested to potentiate the risk of *S. suis* clinical disease in swine herds, such as concurrent viral infections, mixing of infected and naïve pigs, quality of the environment (cleanliness), overcrowding in pens and other management factors [3, 13, 17][33]. *S. suis* is known to be an opportunistic pathogen, coinfecting with other viral or bacterial pathogens [21]. As previously mentioned, viruses such as PRRSV and/or influenza virus are known to increase susceptibility to *S. suis* [3, 45].

Management practices that can be done to help reduce incidence of the disease include all-in/all-out practices, smaller rooms to assist with temperature fluctuations, grouping similar-aged pigs together, and cleaning between batches or groups of pigs [3]. Practices such as early medicated weaning and/or segregated weaning do not help in eliminating *S. suis* infection, as the bacterium is an early colonizer of piglets [1].

1.2.4.2 Treatments against *S. suis*

Choice of treatment to control *S. suis* clinical disease should be based on criteria such as the susceptibility of the organism, type of infection and the mode of administration [3]. Antimicrobial treatment is suggested based on knowledge of the local pattern of resistance [3]. *S. suis* is sensitive to some antibiotics. However, there is low resistance to certain antibacterial agents such as ampicillin, ceftiofur, enrofloxacin, florfenicol, penicillin and trimethoprim/sulfamethoxazole but high resistance to tetracycline [46, 47]. Even though penicillin G is used to treat and control this bacterial infection, penicillin-resistant strains have been isolated as well as other strains that are highly resistant to other antibiotics used to treat *S. suis* [13].

Amoxicillin rapidly achieves high plasma levels and diffuses into the extracellular space, so it is frequently used for treatment against *S. suis* [3]. Strains isolated from healthy animal's tonsils or animals at slaughterhouses are usually multi-resistant [3, 48, 49].

Treatment in drinking water or medicated feed is an effective route for administration as it is of low cost. However, it may be difficult for infected animals to obtain sufficient concentrations when they do less eating and drinking [3]. Route of administration (feed or water), animal competition affecting feed or water availability, and/or antimicrobial serum concentrations to kill the bacterium should all be considered when strategizing for treatment [3].

1.3 Immune responses to infection

1.3.1 Innate immune response

The innate immune system works as the first defence barrier against invading pathogens, helping the host to prevent and fight microbial infections using highly integrated and networked cells [11, 16]. The innate immune response does not require any previous exposure to an antigen and does not generate a "memory" of past exposures [3]. The first lines of defence include physical and chemical barriers, such as the epithelial and mucosal barriers, incorporating their secretions and antibacterial products which they produce [50, 51]. These barriers create a biochemical fence made up of mucus, antimicrobial peptides and molecules, and cytokines [11]. If a foreign body or pathogen is able to overcome this biochemical fence, the secondary line of defence includes polyreactive antibodies, the complement system and specialized resident cells such as phagocytes and innate lymphocytic cells. Among several functions, this secondary line of defence is

responsible for pathogen neutralization, opsonisation, phagocytosis and destruction as well as inflammation [30].

To recognize pathogens, the innate immune system relies on the interaction of pattern-recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs). Many innate immune cells in the body express these PRRs, such as monocytes/macrophages, dendritic cells, and natural killer (NK) cells, while PAMPs are present in all microorganisms (pathogenic or not) [50]. Besides resident cells, circulating sentinel cells are recruited to the inflammation site and they will further differentiate and become activated to fight the infection. These sentinel cells will eliminate the foreign bodies through endocytosis/phagocytosis. Monocytes in the blood, tissue macrophages, neutrophils and dendritic cells are the main phagocytic cells. Neutrophils, which are part of the granulocyte family of cells, are short lived but possess potent bactericidal functions. These cells release granules when activated, which directly and indirectly impair pathogen activity. Eosinophils and basophils cells are also part of the granulocyte family, but these cells are not considered professional phagocytes; they instead release chemokines and cytokines that will influence an inflammatory response. The release of cytokines also influences the adaptive immune response. Dendritic cells and monocytes, which mature to macrophages, are capable of antigen-presenting following endocytosis/phagocytosis. These antigen-presenting cells (APCs), further activate the adaptive immune response [51].

1.3.2 Adaptive immune response

Adaptive immune response requires more time of antigen exposure than the innate immune response; indeed it occurs days after the initial barrier breach and development of the innate response [51]. Besides this feature, the unique characteristic of the adaptive immune response is

the generation of a memory response ready to respond to a secondary antigen exposure. The adaptive immune response relies on initiation through antigen-mediated stimulation of T lymphocytes and B lymphocytes via their antigen specific receptors [52, 53]. T lymphocytes are responsible for cellular immunity while B lymphocytes for humoral immunity. APCs will present antigens to T cells, inducing their differentiation into effector cells. CD4+ T lymphocytes, also called helper T (Th) lymphocytes, will contribute to activate B lymphocytes, or will secrete cytokines to amplify the activation of additional cells of the innate and adaptive immune systems. In contrast, CD8+ lymphocytes, also called cytotoxic T lymphocytes (CTLs), directly kill infected cells and produce cytokines for an amplified immune response [51, 53, 54]. On the other hand, the activation of B lymphocytes leads to the production of antigen specific antibodies that contribute to pathogen clearance [51].

Major histocompatibility complex (MHC) is an essential player for adaptive immunity. MHC class I and II allow antigen presentation to activate T lymphocytes [51]. Two classes of MHC exist:

- MHC class I: it is expressed on nearly all nucleated cells. MCH class I will be recognized by CD8+ T lymphocytes that monitor and eliminate infected cells.
- MCH class II: it is primarily expressed by APCs (dendritic cells, macrophages and B cells), and it will be recognized by CD4+ T lymphocytes. Activated CD4+ T lymphocytes will differentiate in subsets of Th cells, which produce different sets of cytokines and contribute to activate B lymphocytes.

T lymphocytes:

Activation through MCH class I and class II enables differentiation of mature naïve lymphocytes to mature activated lymphocytes. There are two types of T lymphocytes: CD4+ T lymphocytes and cytotoxic CD8+ T lymphocytes.

CD4+ T lymphocytes:

- Naïve CD4+ T lymphocytes will be activated by MCH-II (as described above). Depending on the produced cytokines, a different Th response (Th1 or Th2) will be developped. If APC produces IL-12, IFN-γ and/or IL-18, differentiation will be directed to a Th1 cell. If the APC is producing more IL-4, differentiation will be directed to a Th2 cell. Afterwards, Th1 cells will secrete type 1 effector cytokines such as IFN-γ, while Th2 cells will secrete type 2 effector cytokines such as IL-4, IL-5 and IL-13. These type 1 or type 2 effector cytokines induce differential B-lymphocyte class switching of antibodies [51].
- Besides their role in B lymphocyte activation, Th1 cells secrete IFN-γ which activates phagocyte microbicidal activity, up-regulates the level of MHC-II and cytokine expression, contributing to eliminate the infection [46, 51]. IFN-γ secretion also induces antibody class switching of B lymphocytes to IgG sub-classes (such as IgG2), boosting opsonophagocytosis [51]. Indeed, the effects of the Th1 response are well suited to respond to infections with both intracellular and extracellular pathogens. In the type 2 immune response, Th2 mechanisms are not well understood. Effector cytokines released during the type 2 immune response influence the activation of B lymphocytes, as well as isotype switching to type 2 IgG subclasses (such as IgG1). These antibodies play a role in allergies as well as in the immune response in mucosal membranes.

CD8+ T lymphocytes:

- Naïve CD8+ T lymphocytes recognition of MHC class-I peptide complex, usually on dendritic cells, drives their activation into effector CTLs [51]. Once CTLs are activated, clonal expansion will follow with migration to infection sites. Following this, they can eliminate infected cells by releasing the contents of cytotoxic granules.

B lymphocytes

B lymphocytes possess a B cell receptor (BCR) and are capable of recognizing specific antigens. The BCR is composed of membrane bound immunoglobins (IgM/IgD). When BCRs first recognize a new antigen, this leads to development of activated B cells which then differentiate into either plasma cells or memory B cells [51]. Plasma cells are responsible for secreting antibodies. IgM is the first immunoglobin secreted, in large amounts; however, IgM tends to be low-affinity as they have not gone through affinity maturation. These low affinity antibodies can limit the infection while the maturation of higher-affinity antibodies can take place to establish the longer and stronger humoral immune response. Higher affinity antibodies are a major source of protective humoral immunity. Generation of high-affinity plasma and memory B cells (switched from IgM to predominantly other classes and subclasses of antibodies) will occur in the germinal centre. This complex process is called somatic hypermutation (SHM) and class switch recombination (CSR). In the germinal centre, genetic selection of B cells will take place to have a greater affinity with the antigen. Plasma cells also achieve high rates of Ig secretions, providing high concentrations of specific (high affinity) antibodies that neutralize or opsonize antigens. There are two pathways for B lymphocyte activation: T-dependent (TD) response and T-

independent (TI) response. In the TD response, B lymphocytes rely on activation via T lymphocytes to initiate the humoral immune response, while the TI response does not require direct contact with T lymphocytes [51].

1.3.3 Antibody production

B lymphocytes express IgM on the surface (as part of the BCR), which will be secreted by plasma cells when antigen-recognition occurs. IgM is present in monomeric form in the membrane of B cells but secreted in pentameric form into the circulation. IgM is the first antibody class produced. They are less specific to the antigen but are strong activators of the complement. They have a short half-life of around 5 days. After class switching, B lymphocytes will undergo mutations to produce Igs such as IgA, IgG, IgE and IgD in swine. IgA are present in the gut, respiratory and reproductive tracts and in tears, saliva, and milk. IgA neutralizes toxins and pathogens that colonize the mucosal surfaces in order to prevent bloodstream entry. IgE antibodies are involved in allergic reactions and defense against parasites [51]. In pigs, 6 subclasses if IgG exist (IgG1-IgG6) [55]; however, there is little data on their roles in the immune system. It has been reported, however; that IgG2 could be more opsonizing than IgG1, and therefore possibly more effective in eliminating encapsulated bacteria [56]. Regarding protection against *S. suis,* studies have suggested that vaccine-induced IgG2 antibodies may be more likely to induce opsonophagocytosis than other isotypes, and thus confer protection against *S. suis* invasive systemic disease [57, 58]. In regards to isotype switching, adjuvant component in the vaccine formulation is a very important factor as it drives isotype switching.

1.3.4 Role of maternal immunity

Through colostrum, maternal antibodies are transferred from mother to spawn for protection as their adaptive immune system is maturing. Colostrum is mainly composed of IgG, but also includes IgM and IgA. Absorption of IgG from colostrum at birth is important for survival [59]. Absorption of colostrum and uptake via in the intestine is very important for neonates in the first 24 to 36 hours after birth. After this period, a process called “closure” occurs where intestinal mucosal cells are unable to absorb macromolecules. IgG antibodies absorbed from colostrum uptake are present in the bloodstream; however, these antibodies are known to supress vaccine-induced immune responses. These antibodies might reach titre levels similar to those of the mother. However, these serum antibodies are estimated to last around 6-10 days [60, 61]. Endogenous hormones, such as prolactin and cortisol, are essential for colostrum uptake as they assist in intestinal cell growth and the uptake of colostrum through these cells [62-64]. After closure of the intestinal barrier, lactogenic immunity takes place. When piglets are still suckling on the mother, local gut protection is guaranteed through the ingestion of milk. Once the mucosal cells close and absorption of colostrum halts, the passage of antibodies to the blood stops. IgA, which is a major immunoglobin in colostrum and milk, is produced by plasma cells in the mammary tissues. These cells are associated with mucosa-associated lymphoid tissue (MALT). Thus, the colostrum and milk will contain specific antibodies for pathogens that are directly linked with the mother’s immunity generated in the MALT [64].

2. *Streptococcus suis* vaccines

Despite intensive research leading to different vaccine-candidate antigens, no universally efficacious *S. suis* vaccine has been commercialised so far. There is lack of knowledge on molecular components, genetics and mechanisms involved in the pathogenesis of *S. suis*, all of which contribute to the challenges of researching and producing an safe and efficient vaccine [1].

Generally, *S. suis* vaccine studies provide contradictory results. This can be attributed to different vaccine compositions (adjuvant choice, antigen used), administration techniques (number of doses, antigen concentration, administration route), the study model (animal model being used, on farm study or experimental trial in a research facility, the use of a control group in field studies), the use of different laboratory protocols to prepare the vaccine, amongst multiple variables [1, 3]. The goal is to find an efficacious and universal vaccine that will protect against multiple strains/serotypes of *S. suis* and is inexpensive and safe for swine producers. Different vaccines such as live-attenuated, subunit and some bacterins (killed bacteria) are all experimental. There has been some focus on autogenous bacterins, which can be more beneficial to the producer and have showed some encouraging results.

2.1 Live-attenuated vaccines (experimental)

Live-attenuated vaccines contain non-virulent mutant strains or naturally non-pathogenic strains. With this type of vaccine, the challenge is to obtain a completely non-virulent strain which is safe and induces a strong immune response [1, 3] A few *S. suis* live-attenuated vaccines have been tested with varied results [65-76]. It is proven that the capsule (CPS) plays an important role in the pathogenesis of *S. suis* infection. Thus, different mutants that have been produced for live-attenuated vaccines include non-encapsulated mutants, which have shown to be avirulent [73, 77]. However, these studies had mixed results regarding antibody production and a reduction in clinical signs [66]. Therefore, some studies have tested vaccines with mutants that express CPS but lack the expression of some virulence factors which allows for a response against CPS while simultaneously reducing the pathogenicity of *S. suis*; however, results observed were variable [71, 78]. Another study using *S. suis* serotype 2 mutants deficient in expression of the serum opacity

factor (*ofs*) was applied to piglets. Though strains that do not express *ofs* are severely attenuated in virulence and a high humoral immune response was observed, the vaccine did not elicit a significant protection against a challenge with serotype 2 or serotype 9 [76]. Other studies have used naturally avirulent strains such as the #1330 strain of serotype 2, originally isolated from lungs of a pig with pneumonia at the Faculty of Veterinary medicine of the University of Montreal, or #05HAS68 strain of serotype 2, isolated from the tonsils of a healthy pig [65, 68, 72]. Two or even three doses of these avirulent strains were needed to induce protection against challenge in pigs or mice [58, 79, 80]. Nevertheless, safety would certainly be a concern using 'naturally' avirulent *S. suis* strains. Hyperthermia, lameness fever and convulsions, have been reported in experiments with pigs [3, 66, 69]. As well, the risk of zoonosis is an important factor with these vaccines, as it cannot be excluded that an attenuated strain for pigs is not completely attenuated in humans. Further research needs to be done to identify and eliminate risks of introducing a live vaccine strain into commercial herds [1, 3].

Some vaccine studies have also shown that there is potential of cross-protection between serotypes. Vaccine formulations with *S. suis* 1/2 provided protection against challenge by strains of *S. suis* 1/2, 1, and 2. These findings are in agreement with the reported cross-reactions between these serotypes. Accordingly, vaccines composed of either serotype 1 or 2 protected against serotype 1/2 as well, confirming epitope sharing between these three serotypes [70, 74]. Other studies have reported cross-protection induced by a live vaccine containing serotype 5 against challenge with *S. suis* serotypes 2 and 9 [67]. Finally, one study focused on a live vaccine containing a serotype 2 double-deletion mutant (SsPep/SsPsPC$^{-/-}$) that induced cross-protection against challenge with serotype 7 [75].

2.2 Subunit vaccines (experimental)

Subunit vaccines are composed of bacterial components, such as protein(s), the CPS, a fragment of the cell wall or different elements conserved between strains and/or serotypes of *S. suis* in order to provide protection against strains of heterologous serotypes [1]. Although information regarding subunit vaccine candidates have increased over recent years, data is still experimental and there is no commercial subunit vaccine available for *S. suis* control [3].

Albeit the rise of subunit vaccine studies over recent years, most studies focused on serotype 2 and protection against it [1]. Different proteins have been used in vaccination trials such as MRP, extracellular Factor (EF) [81], suilysin (SLY) [76, 82-86], surface antigen one (Sao) [87, 88], surface antigen two (SAT) [89], and galactosyl-(α 1-4) adhesin [90, 91]. Most of these studies have observed high levels of antibodies against these proteins but failure to induce a sufficient opsonizing (protective) response. However, the majority of proteins that showed protection against *S. suis* serotype 2 have not been tested for cross-protection with other serotypes or using a pig model. Among potential candidates, the Sao protein has been shown to induce cross-reactions among serotypes [92]. Sao-specific antibodies reacted with 28 of the 33 *S. suis* serotypes and 25 of 26 serotype 2 isolates which suggests high conservation among *S. suis* species [87]. Although there is evidence that Sao could be efficacious in a universal vaccine, more research is required [1]. Finally, a fibronectin-binding protein, known as enolase, has recently been studied. Enolase is expressed by all *S. suis* serotypes making it a good candidate for a universal vaccine. However, there are contradictory results concerning its protective capacity [93-95].

Studies also showed that antibodies against the CPS of *S. suis* serotype 2 have a potential to protect against *S. suis* [96, 97]. However, due to its carbohydrate nature, the CPS is a poorly immunogenic and generates mainly IgM antibodies with limited or absent levels of IgG antibodies

[98]. Non-encapsulated and encapsulated strains have been compared and results showed that the capsule is essential for a strong protective response [69]. Nonetheless, the CPS is used at the reference antigen for serotyping, thus it will induce protection against strains within the same serotype only. A potential solution to the poor immunogenicity of the CPS is the use of glycoconjugates that allows carbohydrate-based vaccines to produce an optimal immune response. A glycoconjugate vaccine based on the CPS of serotype 2 coupled with an immunogenic carrier protein (tetanus toxoid) demonstrated the capacity of this strategy to induce antibody production and protection in mice and pigs [98]. In conclusion, more research is required on subunit vaccines as it is difficult to develop a universal subunit vaccine encompassing the different proteins conserved within the the highly phenotypic and genotypic serotypes and strains of *S. suis* [99].

2.3 Bacterins

Bacterin vaccines contain killed bacteria from strains isolated from diseased animals. There are some vaccines (autogenous vaccines) that are applied in the field, but majority of bacterins are currently experimental. The effectiveness of these vaccines is controversial and comprehensive studies involving safety, immunogencity and protective efficacy are limited [1, 10, 100, 101]. As well, the choice of the adjuvant involved in bacterin vaccine formulation is critical for efficacy [10]. Immunogenicity and protective efficacy of these vaccines can also be effected by the condition in which they were inactivated, as well as bacterial concentration [10].

2.3.1 Commercial

Very few commercial bacterins are available and possess limited geographical distribution. Within these few commercial bacterins, incomplete information is available. Results obtained in experimental studies are paralleled by a field study that reported failure of a commercial serotype

2, oil-in-water adjuvant formulated bacterin to protect against nursery mortality among vaccinated pigs [102]. Another study reported a minor effect on *S. suis* serotype 2 colonization when using a commercial bacterin [103]. This vaccine is no longer marketed in North America.

2.3.2 Experimental

In general, the limited protective response obtained with bacterins has been attributed to failure of the whole-bacterial antigens to elicit an immune response due to loss of antigenicity caused by heat or formalin processing, production of antibodies to antigens not associated with protection, and/or lack of cross-reactivity. Conclusions from these studies are unconvincing [104-107].

As stated in section 1.1, distribution of *S. suis* serotypes geographically differs; however, most bacterin studies have been performed with serotype 2 with a few studies examining bacterins against other serotypes or evaluating cross-protection [1]. Experimental pig vaccination trials reported protection with a bacterin, but this protection was subjected to the adjuvant used in the formulation [69, 81]. The bacterin contained 10^9 formalin-killed cells, and a strong potentiating adjuvant seemed to be necessary to obtain protection when reducing the number of microorganisms contained in the bacterin. As mentioned, 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. Therefore, another study tested the hypothesis that protective antigens would be better preserved when using ceftiofur-inactivated *S. suis* as a whole cell bacterin formulation. Similarly to that observed with the formalin-killed bacterin, the efficacy of the ceftiofur-inactivated bacterin depended on the

adjuvant used [108]. The adjuvant choice can be another critical component when it comes to bacterins. The importance of adjuvants in the design of *S. suis* effective vaccines is discussed below. Another described strategy is the use of bacterins made with non-encapsulated mutants. Removal of the CPS by mutagenesis uncovers antigenic cell wall proteins, normally masked by the thick capsular shell. A stronger immune response elicited by the non-encapsulated mutant would be expected. Yet, and similarly to conventional bacterins, contradictory results were reported [48, 69].

2.3.3 Autogenous

Autogenous vaccines are formulated (by a licensed company) from the isolate causing clinical problems on a specific farm and administered back to the original farm. To prepare the autogenous vaccines, samples are taken from affected animals and isolated bacteria are identified via bacteriology and serotyping before being inactivated and used in the vaccine. These vaccines are probably specific to the strains included in the vaccine and causing the clinical problems in the farm and cannot prevent an outbreak (as an initial outbreak is needed to isolate the strain(s) causing the disease in the herd). However, autogenous bacterins might positively resolve an existing infection overtime in a herd [1]. The efficacy of these vaccines has been poorly studied and results from field studies (when available) are controversial [10]. One of the disadvantages of this vaccine is that diagnostic error may result when a limited number of pigs are sampled. Thus, failure occurs to identify the *S. suis* strain or serotype associated with a recent outbreak in the farm [101]. Concerning autogenous vaccine scientific field studies, only 6 have been conducted to measure effectiveness of these vaccines [5-8, 102, 106]. Among these, one is written in German with no translation available, thus analysis is difficult [106]. A field study done in a nursery evaluated the

efficacy of an experimental autogenous serotype 2 bacterin prepared by an accredited company [102]. The experimental autogenous vaccine was made with the aluminum hydroxide adjuvant and administered intramuscularly. Piglets were vaccinated at weaning and 10 days later. Morbidity and mortality were recorded; however, serological responses were not studied. Mortality and morbidity rates between vaccinated and non-vaccinated groups were similar. Overall, the autogenous vaccine used in this study provided inconsistent results. In this study, an "experimental" autogenous vaccine was also tested using an oil-in-water adjuvant (Imugen®, Bayer Animal Health). Pigs that received the experimental autogenous vaccine "tended" to have lower morbidity/mortality. The authors suggested that it is always difficult to assess observations made in field trials, because there are many uncontrolled factors in commercial herds that can influence the results. Therefore, the effect of vaccination in reducing mortality of nursery pigs attributable to *S. suis* in this trial could not be definitively assessed. In the study by Lapointe *et al.* (2002), the researchers evaluated the antibody response to an "experimental" autogenous vaccine that was composed of a sonicated *S. suis* serotype 1/2 and formulated with the combination of two different adjuvants, Rehydragel® and Emulsigen® [8]. A control group of 200 piglets was included in the study with clinical observations on all 400 piglets and serological follow up on 36 piglets from each group. The vaccination trial was performed in a farrow-to-finish herd and vaccination of piglets occurred at 2 and 4 weeks following weaning. For serological monitoring, blood samples were collected from these pigs at weaning and subsequently at 2-week intervals until the pigs were 13 weeks old. A significant increase in the antibody response was observed in the vaccinated group, but the magnitude of this response was inversely correlated to the levels of maternal antibodies. Although serotype 1/2-associated clinical signs were present in the preliminary study, no outbreaks were reported during the field trial. Thus, protection conferred by the vaccine could not be properly

assessed. In another autogenous vaccine study by Hopkins *et al*. [7], the *S. suis* problem strain on farm was difficult to control with penicillin as the strain was discovered to be resistant to this antibiotic. Majority of clinical cases occurred between 6 and 9 weeks of age. A total of 540 pigs were included in the trial, separated into 5 cohorts. Of all the piglets included in the trial, 75% were vaccinated and 25% unvaccinated with exception of one cohort used a control group. With a vaccine manufactured by an accredited laboratory, vaccinations were given at weaning and 3 weeks later. The study measured direct, indirect, total, and overall vaccine effectiveness. In conclusion, direct effect of the vaccine was non-significant. In a recent study, Corsaut *et al*. (2020) [6] performed the first comparative field study on the immunological and protective response induced by autogenous vaccines applied to either piglets or sows in a herd with recurrent *S. suis* problems. Piglets from non-vaccinated sows received an autogenous vaccine during the first week and 3 weeks of age. On the other hand, sows received the vaccine at 5 and 3 weeks pre-farrowing and piglets were non-vaccinated. The vaccine was composed by *S. suis* serotype 7 strain for the piglet vaccination, and an additional *S. suis* serotype 9 strain was added for the sow vaccination. Both vaccines were formulated with an oil-in-water emulsion adjuvant (confidential formulation). Levels, isotype profile and opsonophagocytosis capacity of the serum antibodies induced by vaccination were evaluated. Vaccination of piglets failed to induce an active immune response. Vaccination of sows induced a significant increase in anti-*S. suis* antibodies, mainly composed of IgG1. Despite this antibody increase in vaccinated sows, transfer of maternal immunity to piglets was not different from the control group (i.e. piglets from non-vaccinated sows). Notably, levels of maternal antibodies in piglets were already very high with marked opsonophagocytosis capacity at 1 week of age, independently of the vaccination program. Yet, their levels decreased by 3 weeks of age, indicating possible absence of antibodies in the post-weaning high-risk period. These

observations correlated with lack of clinical protection in the farm. Overall, the piglet or the sow vaccination program performed in this study mostly failed to induce lasting protection in nursery piglets. Another recent study by Corsaut *et al*. (2021) [5], performed a field study on the immunological response induced by an autogenous vaccine applied in pre-parturient sows. Using a farm with recurrent *S. suis* serotype 7 problems, the study was divided in three experiments: (I) Sows received the vaccine at 7 and 3 weeks pre-farrowing. (II) Replacement gilts introduced to the herd received the vaccine at 4 and 7 weeks after their entry in quarantine and a boost 3 weeks pre-farrowing. (III) Gilts from experiment II received another boost 3 weeks pre-farrowing at their $3^{rd}/4^{th}$ parity. The vaccine was formulated with the adjuvant Alhydrogel^{TM}. Levels, isotype profile and opsonophagocytosis capacity of the serum antibodies induced by vaccination were evaluated in sows and maternal immunity in piglets. In sows (I), the vaccine induced a slight, albeit significant, increase in anti-*S. suis* total antibodies after 2 doses when compare to basal levels already present in the animals. These antibodies showed a high opsonic capacity *in vitro*, highlighting their potential protective capacity. A gilt vaccination program of 3 doses (II) resulted in a significant increase in anti-*S. suis* total antibodies and ensued a higher transfer of maternal immunity in piglets compared to control animals at 7 days of age; nevertheless duration of immunity was not improved at 18-day-old piglets. The vaccine response in both gilts and sows was mainly composed of IgG1 subclass, which was also the main Ig transferred to piglets. IgG2 subclass was also found in piglets, but its level was not increased by vaccination. Finally, a recall IgG1 response was induced by another boost vaccination at subsequent parities (III), indicating that the vaccine induced the establishment of a lasting memory response in the herd.

Albeit hard to compare due to clear experimental differences, altogether these studies suggest the need for optimization of the vaccination program and/or the vaccine formulation in

order to induce lasting maternal immunity in piglets (when vaccinating sows) or an active response able to overcome maternal immunity interference (when vaccinated piglets). Indeed, this promising approach requires extensive and comparative scientifically sound studies to evaluate the most efficacious way to prepare the vaccine, the adjuvant to be included, the number of doses, the real benefit of vaccinating sows, or piglets or both. Finally, it is important to remember that the overall efficacy of autogenous vaccines cannot be determined based on results obtained with one particular batch of vaccine prepared by a single licensed laboratory. Methods used for vaccine production, bacterial concentration and the adjuvant used (among other variables) may highly influence the results obtained.

2.4 Vaccination strategies

At birth, piglets have a relatively immature adaptive immune system [1]. For protection, piglets rely on maternal immunity obtained via colostrum and milk intake. Although these young animals may be the most fragile and at risk, *S. suis* is responsible for majority of clinical infections between 5 and 9 weeks of age (post-weaning). Albeit pre-weaning piglets are protected by colostrum-derived maternal antibodies, the life-span of these antibodies is limited in the nursery period [1, 102]. Majority of vaccination programs of autogenous vaccines (as described in 2.3.3) are administered to gilts/sows or piglets.

2.4.1 Immunization of piglets

Vaccination of piglets is more costly to producers in terms of product and labour. Piglets are often vaccinated with two vaccine doses at two or three weeks apart. The second dose is recommended to be given 10 days before the onset of the risk period in order to achieve seroconversion [109]. This makes the vaccination protocol a challenge as the risk period begins at

3 weeks of age and majority of clinical cases occurs between 5 and 10 weeks of age [3]. Another problem with piglet vaccination is the possibility of interference with circulating colostrum-derived maternal antibodies. Maternal antibodies in all species have been reported to induce a reduction of active vaccination efficacy [110]. However, there are limited studies preformed on maternal immunity interference of *S. suis* vaccination. The studies described in section 2.3.3 on the immunological characterization of the antibody response at approximately 1 week of age (before piglet vaccination) revealed impressively high levels of antibodies in piglets, most probably of maternal origin, and this independently of sow vaccination. Based on these observations, it could be expected a high maternal interference when vaccinating piglets during the first two weeks of age. In an experimental study by Baums *et al.* [60], a missing or weak immune response was observed after suckling piglet vaccination (two doses at 2 and 4 weeks of age) and it was potentially related to either inhibition by maternal antibodies and other colostrum components or immature adaptive immunity in suckling piglets. Therefore, more research is needed to evaluate the perfect age window for piglet vaccination in order to avoid maternal interference but confer protection at time of *S. suis* clinical signs onset.

2.4.2 Immunization of sows (transfer of maternal immunity)

Sow or gilt vaccination represents a more economical option to swine producers. Periparturient vaccination of sows could result in protective passive maternal immunity transfer to their piglets [1, 3]. Sow vaccination is typically performed 2 to 4 weeks apart, with the last dose given 3 weeks prior to parturition, allowing better colostrum immunity [1, 109]. Antibodies are unable to cross the placental barrier, thus they are acquired via colostrum intake (see below). Quantity of antibodies in colostrum can vary as age, parity, nutrition, vaccination, stressors,

environment and pathogen exposure contribute to colostrum quality [111]. Indeed, gilts produce less colostrum with lower Ig concentrations than multiparous sows [62]. Effect of colostrum also relies on piglet intake as well as uptake via the small intestine absorption [3]. As aforementioned, levels of maternal antibodies against *S. suis* are very high at 7 days of age, but significantly decrease thereafter [5, 6]; therefore, this passive immunity seems to be short-lived. Indeed, studies reported that at the moment of appearance of *S. suis* clinical signs, antibody levels in piglets were already very low, independently of the vaccination program used [5, 6]. Similarly to the findings of these field studies, an experimental study showed that neither application of *S. suis* bacterin to preparturient sows nor that to suckling piglets or both elicited protection in 8-week-old piglets, which was explained by the lack of opsonizing antibodies. Serum half-life for IgG in suckling piglets was estimated around 6 to 10 days [60]. A previously published serological cross-sectional profile of unvaccinated piglets also showed a significant decrease in anti-*S. suis* antibody levels after 2 weeks of age, with the lowest values occurring between 6 and 8 weeks [8].

2.5 Adjuvants used in veterinary medicine

Adjuvants are composed of chemicals, microbial components or mammalian proteins and enhance antigen presentation and stability [112, 113]. They can have several mechanisms of action such as influencing the onset, strength and duration of immune responses [53]. Indeed, an adjuvant has the capacity to increase vaccine-induced immune responses and enhance both innate and adaptive immune responses [53]. Vaccine-induced antibody response can be influenced by the choice and role of adjuvants as the efficacy of the vaccine depends on which antigen is targeted, as well as the specificity and affinity of the antibody towards the targeted antigen [1]. Choice of adjuvant is also important as they can cause nonspecific adverse effects which include fever,

arthritis, uveitis, anorexia, soreness and lethargy [112]. They can also cause inflammation and even granulomas or sterile abscesses [112].

For *S. suis* control, adjuvant choice is very important as adjuvants has the capacity to modulate the type of Ig class/subclass induced after vaccination. *S. suis* is an encapsulated pathogen and its CPS protects the bacterium against immune system clearance by phagocytic cells, thus allowing *S. suis* systemic dissemination. This natural resistance of *S. suis* is overcome if highly opsonic antibodies recognizing surface-exposed bacterial components, or the CPS itself, are present. These antibodies will induce rapid bacterial uptake by phagocytic cells and consequent destruction [88].Therefore, the isotype profile of vaccine-induced antibodies has been reported to be important when evaluating protection against *S. suis*, as this would be linked to the capacity of certain isotypes to induce opsonophagocytosis while other isotypes are supposed to be poorly opsonic [88]. Indeed the adjuvant used in the vaccine formulation can markedly influence not only the quantity (titers) but also the quality (isotype) of the antibody response induced by the vaccine. Nevertheless, few studies have compared the effect of different adjuvants in the same vaccine experimental trial or under the same conditions. Pallares *et al*. [108] studied ceftiofur-washed bacterins with 3 adjuvants, the oil-in-water emulsions Montanide ISA 25 and Montanide ISA 50 as well as a saponin adjuvant. The Montanide™ ISA 50 adjuvanted *S. suis* bacterin appears to be more efficacious than the Montanide ISA 25 formulation in delaying the onset of mortality, and decreasing clinical signs and lesions associated with *S. suis* serotype 2 challenge infection in piglets. Another study using protein-based subunit vaccines formulated with two different adjuvants (water-in-oil emulsion and aluminum hydroxide-based adjuvant) showed a superior capacity of the water-in-oil emulsion (Specol) in stimulating a protective immune response in pigs [81]. Furthermore, in a recent study by Obradovic *et al*. [9] evaluated a *S. suis* serotype 2 bacterin-

based vaccine formulated with six different commercial adjuvants (Alhydrogel®, Emulsigen-D®, Quila-A®, Montanide™ ISA 206 VG, Montanide™ ISA 61 VG and Montanide™ ISA 201 VG). Montanide™ ISA 61 showed a significant increase in anti-*S. suis* antibodies, including both IgG1 and IgG2. In addition, the vaccine formulation with this adjuvant showed protection against mortality and significantly reduced morbidity and severity of clinical signs. Other vaccines formulated with Montanide™ ISA 206 VG or Montanide™ ISA 201 VG also showed significant increase in antibodies and partial protection with reduction of severity in *S. suis* clinical signs. Finally, Alhydrogel®, Emulsigen®-D and Quil-A® vaccines induced low antibody responses and did not protect piglets against the *S. suis* challenge. This study highlights the importance of adjuvant choice in a vaccine formulation, and how immune response can be significantly influenced as well as the efficacy of the vaccine [69, 81, 108]. Common adjuvants used in veterinary medicine and their mechanisms of action are described below.

2.5.1 Aluminum salts

Mineral salts such as aluminum phosphate and aluminum hydroxide are adjuvants that are commonly used in veterinary medicine [53]. Aluminum phosphate and aluminum hydroxide are named as "alum" even though they both have different physical and adjuvant properties [53]. These adjuvants are inexpensive, safe and simple to formulate. Although alum has been used for over 90 years in human and animal vaccines, there are still unknowns about the mechanisms of immune stimulation. It has been believed that aluminum salts form a depot at the site of injection and enhance recruitment of APCs [53, 114]. The formation of antigenic depot in the tissue delays antigen release and induces immunity [115]. Recently, it has been suggested that absorption of

antigens on the surface of aluminum salts helps targeting antigens to APCs, leading to enhancement of antigen presentation by MHC molecules [53, 114]. Aluminum adjuvants activate dendritic cells via direct and indirect mechanisms. Phagocytosis of aluminum adjuvants followed by disruption of the phagolysosome activates NLRP3-inflammasomes resulting in the release of active IL-1β and IL-18. Aluminum adjuvants also activate dendritic cells by binding to membrane lipid rafts [116]. The use of aluminum adjuvant is limited by weak stimulation of cell-mediated immunity. Besides, aluminum salts primarily enhance Th2-driven antibody responses and will have little effect on Th1-type responses which are essential for protection against many pathogens, including *S. suis* [114]. Indeed, the study of Obradovic et al. [96] confirmed previous findings on the limited or lack of immunogenicity and/or protection of *S. suis* bacterin vaccines adjuvanted with aluminum hydroxide.

2.5.2 Oil-based emulsions

Oil-based emulsion adjuvants contain a formulation of oil and aqueous phases and are stabilized by a surfactant [112]. Generally, oil-based emulsions are stronger inducers of the immune system than alum but there are increased injection site reactions and they might induce granulomas [112]. Oils that are metabolizable are a better choice as adjuvants as they have a improved safety record than adjuvants with mineral oil [112]. Furthermore, mineral oils could be contaminated by carcinogenic polycyclic aromatic hydrocarbons [112]. There are several types of oil-based emulsions: oil-in-water, water-in-oil, and multiple emulsions including water-in-oil-in-water and oil-in-water-in-oil. As recently reviewed by Burakova *et al*. [117], water-in-oil (W/O)

emulsion is a dispersion of water droplets within continuous oil phase. Antigen is entrapped in the water phase surrounded by a continuous oil phase and slowly released upon breakdown of oil after injection. The depot effect at the injection site preserves the antigen from fast clearance by phagocytosis and the liver and, therefore, extends the time available for immune cell recruitment and antigen processing. The most well-known example of W/O emulsion adjuvants are Freund's adjuvants. However, Freund's adjuvants produce strong adverse reactions, which have prevented their use in animal and human vaccines. Successfully commercialized W/O emulsions are available under the brand name Montanide™ (SEPPIC, France) and are utilized in veterinary vaccines, including for pigs [53, 112, 115, 117].

Another type of emulsion utilized in vaccines is oil-in-water (O/W) emulsions, formed by the dispersion of oil droplets in the aqueous phase. Unlike W/O emulsions, O/W emulsion-based adjuvant does not form an antigen depot at the injection site. Instead, the oil droplets facilitate the chemokine-driven immune cell recruitment and the differentiation of macrophages and dendritic cells. MF59 (a squalene O/W vaccine adjuvant) demonstrated better adjuvanticity in stimulating cell-mediated immune response against influenza virus than alum. For veterinary applications, several commercially available O/W adjuvants exist under the brands of Montanide™, Emulsigen® (MVP Technologies, USA), and MetaStim® (Fort Dodge Laboratories, USA). These adjuvants are used in livestock vaccines against various economically important bacterial and viral antigens [53, 112, 114, 115, 117].

In attempts to overcome the issues with local reactions and high viscosity associated with W/O emulsions, research efforts have been devoted to develop multiphasic water-in-oil-in-water (W/O/W) emulsions as vaccine adjuvants. However, multiple emulsions have a very fragile structure and their formulations present great challenges. Currently, only few W/O/W emulsion

adjuvants are available on the market under the Montanide™ brand. These adjuvants demonstrate effectiveness and provide protection for different livestock species against several economically important pathogens, including influenza viruses [117].

2.5.3 Saponins

Saponins are complex amphipathic compounds adjuvants made from crude extracts from plants [53, 112]. The most prominent saponin-based adjuvant is Quil-A® (Brenntag Biosector A/S, Denmark). Quil-A® is a heterogeneous mixture of water-soluble saponins extracted from *Quillaja saponaria*, a tree indigenous to South America. Due to its toxicity, Quil-A® is not suitable for human vaccines; but it is widely used for veterinary applications, including pigs [53, 112, 117]. The purified fraction of *Q. saponaria* (named as QS-21), is currently in many clinical trials for human vaccines. Moreover, QS-21 is used as an adjuvant in a commercially available vaccine for feline leukemia. Studies on the immunoregulatory activities of Quil-A® and QS-21 have demonstrated that they can elicit cell-mediated immune responses with the stimulation of both Th1 and Th2 lymphocytes as well as cytotoxic lymphocytes, therefore generating both, type 1 and type 2 antibodies responses [53, 112]. Burakova *et al*. [117] proposed that for livestock applications, the approach of searching in nature for *Q. saponaria* analogues with less toxic saponins would be more economically feasible.

The combination of cholesterol, phospholipids, and purified fractions of Quil-A® in immune-stimulating complexes (ISCOMs) helps to improve the stability and reduce the toxicity of saponins. ISCOMs show a cage-like structure that assists in preserving and delivering the antigen to APCs. Indeed, ISCOMs enhance antigen uptake and prolong retention by dendritic cells in draining lymph nodes, inducing activation of dendritic cells, CD4+ Th1 and Th2 immune

responses, and induce high concentrations of long-lasting antibodies [53, 113, 114]. ISCOMs can also effectively stimulate CD8+ T cell responses. Thus, ISCOMs have been employed in licensed vaccines for veterinary use [117].

In conclusion, numerous natural and synthetic substances can be used as adjuvants to improve the efficacy of animal vaccines. Some of them, such as aluminum compounds, emulsions, and saponins have already been used in licensed products; whereas others are still evaluated experimentally. Finding the appropriate adjuvant or combination of adjuvants is one of the major challenges in animal vaccine development.

III. Scientific article

Immune response induced by a multiserotype *Streptococcus suis* autogenous vaccine used in sows to protect post-weaned piglets

Alison Jeffery[1], Mélina Gilbert[1], Lorelei Corsaut[1], Simon Cloutier[2], Marie-Christine Frenette[2], Charles Surprenant[3], Sonia Lacouture[1], Marcelo Gottschalk[1], and Mariela Segura[1]

[1]Swine and Poultry Infectious Diseases Research Centre, and Groupe de Recherche sur les maladies infectieuses en production animale, Faculty of Veterinary Medicine, University of Montreal, St-Hyacinthe, Québec, Canada. J2S 2M2

[2] S.V.A. Triple-V Inc., Acton Vale, Québec, Canada. J0H 1A0

[3]F. Ménard, Ange-Gardien, Québec, Canada. J0E 1E0

Corresponding author: Mariela Segura, Faculty of Veterinary Medicine, 3200 Sicotte, St-Hyacinthe, Qc, Canada. J2S2M2. mariela.segura@umontreal.ca

ABSTRACT

Streptococcus suis is a bacterial pathogen that causes important economic losses to the swine industry worldwide. Since there are no current commercial vaccines, the use of autogenous vaccines applied to gilts/sows to enhance transfer of passive immunity is an attractive alternative to protect weaned piglets. However, there is no universal standardization in the production of autogenous vaccines and the vaccine formulation may be highly different among licenced laboratories. In the present study, an autogenous vaccine made up of *S. suis* serotypes 2, 1/2, 5, 7 and 14 was prepared by a licenced laboratory and administrated to gilts using a three-dose program at 20 (pre-breeding), -16 and -3 weeks prior to farrowing. The antibody response in gilts as well as the passive transfer of antibodies to piglets was then evaluated. Different from what was previously published with an autogenous vaccine produced by a different company, the increased response seen in gilts was sufficient to improve maternal antibody transfer to piglets of 3 to 5 weeks of age. However, piglets would still remain susceptible to *S. suis* disease that often appears during the second part of the nursery period. The high level of antibodies did not affect shedding of *S. suis* (as well as that of specific serotypes of *S. suis* included in the vaccine) by both gilts and piglets. Although all antibiotic treatments were absent during the trial, the clinical protective effect of the vaccination program with the autogenous vaccine could not be evaluated, since limited *S. suis* cases were present during trial. Further studies to evaluate the usefulness of gilt/sow vaccination with autogenous vaccines to protect nursery piglets should be done.

INTRODUCTION

Streptococcus suis is a bacterial pathogen that causes important economic losses to the swine industry worldwide [3]. It affects mostly post-weaned piglets, causing mainly arthritis, meningitis, polyserositis, endocarditis and septicemia with sudden death [3]. A total of 35 serotypes had originally been described, although six of them have more recently been re-classified within other streptococcal species [4]. In Europe, few serotypes (mostly serotypes 2 and 9) are frequently recovered from diseased animals [4]. However, in North America, although most prevalent serotypes isolated from diseased animals are 1/2 and 2, a large number of serotypes are routinely isolated in both Canada and the United States [21, 27]. Isolates belonging to more than one serotype are also commonly recovered from diseased piglets within a single farm in North America [9]. Infections caused by the porcine reproductive and respiratory syndrome virus (PRRSV) is known to render pigs more susceptible to *S. suis* disease [3]. In addition, control of *S. suis* infections in swine productions is important as it has been reported to be an emerging zoonotic pathogen worldwide. There is a great risk attaining to those who have close contact with infected pigs or pork-derived products, such as, in Western countries, pig producers and employees, butchers, meat inspectors and swine veterinarians [31].

S. suis epidemiology is complex (multiple strains, multiple serotypes with a high phenotypic diversity) and difficulties in disease control and management are commonly reported in the field [1]. Different factors can contribute to development of the disease including immune status of the herd, mixing of naïve and infected animals, co-current infections, quality of the environment and other management factors leading to stress [3, 9]. Management practices, such as early medicated and segregated early weaning, do not eliminate *S. suis* infections, since piglets are infected very

early in life or even during farrowing [3]. Antimicrobials have been used (and still are where allowed) for metaphylactic and/or prophylactic treatment. However, there has been increasing concern worldwide around antimicrobial use and the susceptibility of *S. suis* on swine farms [17]. High rates of resistance to macrolides/lincosamides and tetracyclines are observed and attributed to the heavy use of antimicrobials in swine [17, 118]. Indeed, *S. suis* is an important antimicrobial resistance reservoir, with a high risk of transmission to other veterinary and human pathogens, due to the presence of mobile genetic elements carrying resistance genes transferable at high frequency within the species, as well, between bacterial species [119]. Until recently, *S. suis* has been considered as being susceptible to penicillin and amoxicillin. However, recent data showed increased resistance to these antibiotics [33, 120].

S. suis disease prevention should shift to focus on the management of the predisposing factors and, mainly, vaccines. A commercial efficacious vaccine has not been developed thus far, probably due to the high number of serotypes (with currently no known cross-protection between serotypes), and a high genetic variation amongst strains [1]. Bacterial autogenous vaccines (bacterins) have increased in popularity as these vaccines are relatively low expensive for swine producers and can include several serotypes in one vaccine formulation. These vaccines are composed by the strain(s) isolated from diseased pigs within a farm and produced by an accredited laboratory, and then applied to the original farm [1]. Field studies evaluating the protective capacity of autogenous vaccines produced by licenced laboratories are limited and presented contradictory results [5-8]. Absence of protective responses from these vaccines have been attributed to the failure of whole-bacterial antigens to elicit an immune response due the inactivation processing, production of antibodies to antigens not associated with protection, and/or the use of inappropriate adjuvants [9,

10]. There are limited field studies evaluating the immunological response and the protective capacity of autogenous vaccines from different manufacturing companies. Indeed, it is difficult to compare studies with different autogenous vaccines [5, 6], as they may use different adjuvants, bacterial concentrations as well as conditions in which the pathogen is grown and killed, among other variables. In addition, no field studies have evaluated the usefulness of an autogenous vaccine in the complete absence of antimicrobials on the farm.

Vaccination of gilts or sows (to elicit an enhanced passive maternal immunity) using an autogenous vaccine is more commonly used in the field as this method is less costly than piglet vaccination. However, published autogenous field studies showed so far limited and/or no increase of passive maternal antibodies in piglets during the nursery barn period [5-7, 102]. Two of these scientific field studies used vaccines manufactured within the same commercial vaccine company. Further research on length and duration of passive maternal immunity elicited by vaccines produced within different manufacturing companies is required.

In the present study, the immune response, the clinical protection in the absence of any antimicrobial treatment as well as the effect on bacterial shedding of a three-dose multiserotype *S. suis* autogenous vaccine produced by a company (not previously tested) was applied to gilts and evaluated from the farrowing period until 7 weeks in the nursery barn.

MATERIALS AND METHODS

Farm selection and herd health status

A 1000 farrow-to-wean sow operation in Canada with external gilt replacement and no commingling was selected. Piglets were weaned at 3 weeks of age and transferred to a separate off-site, all-in-all-out, three-room nursery facility for 10 weeks. The farm experienced recurrent *S. suis* problems at the nursery site. Post-weaned mortality cumulated to 1.65%, with 30% being related to *S. suis*-associated diseases in the presence of prophylactic, metaphylactic and curative antimicrobial treatments. This farm was selected with the objective to reduce not only mortality but also the use of antimicrobials. The operation had external gilt replacement from one source. External gilts quarantined for 30 days upon arrival to the sow farm. Pre-trial health status was established as PRRSV positive (stable) and *Mycoplasma hyponeumoniae* negative. The sow farm herd was declared negative of PRRSV in September 2020 during the trial. All external gilts were vaccinated against swine influenza, parvovirus, leptospirosis and erysipelas (Flusure XP x Farrowsure Gold®) and porcine circovirus type 2 and *Mycoplasma hyopneumoniae* (Circumvent PCV-MG2®) at arrival into barn quarantine. Piglets received no vaccination at entry of the nursery barn.

Samples (meningeal swabs, joint swabs, lung, heart, and brain) from the nursery were repeatedly submitted for complete diagnosis during at least 6 months prior to start the study. Serotyping of *S. suis* isolates was carried out at the diagnostic laboratory of the Faculty of Veterinary Medicine of the University of Montreal [1, 24, 121]. Final diagnosis of *S. suis* serotypes 2, 1/2, 5, 7 and 14-related diseases was established in this specific herd.

Vaccine preparation and administration

Autogenous vaccine was prepared by a licenced company. It was composed of *S. suis* serotype 1/2 (strain 506), serotype 2 (strain 526), serotype 5 (strain 507), serotype 7 (strain 503), and serotype 14 (strain 541). The adjuvant used is own by the company and no public information is available. The vaccine was administered concurrently (but not within the same injection) with another autogenous vaccine containing field strains of *Staphylococcus hyicus, Streptococcus dysglactiae* and *Staphylococcus aureus*. No ethical statement was required for the vaccine administration study as the protocol used was part of normal interventions in the farm and performed by the veterinarian in charge, as stated by the Animal Welfare Committee of the University of Montreal. For the blood collection for immunological studies, the protocols and procedures were approved by the Animal Welfare Committee of the University of Montreal (protocol number Rech-2014).

Immunization protocol

Out of the 70 gilts batch entered into the barn, vaccinated (n=28) and non-vaccinated (n=26) gilts were randomly selected. Gilts received 3 doses of the autogenous vaccine intramuscularly at 20 (pre-breeding), 16 and 3 weeks before farrowing. (**Figure 1)**. All piglets from both vaccinated (n=310) and non-vaccinated gilts (n=318) were tagged and enrolled in the trial (n=628). Of them, a total of 54 and 52 piglets from vaccinated and non-vaccinated gilts, respectively, were randomly tagged and numbered for serological follow up. All piglets were weaned into two nursery rooms in the same barn, with animals sorted according to their vaccination status (**Figure 1**). Other animals (not included in trial) were housed in the same facility.

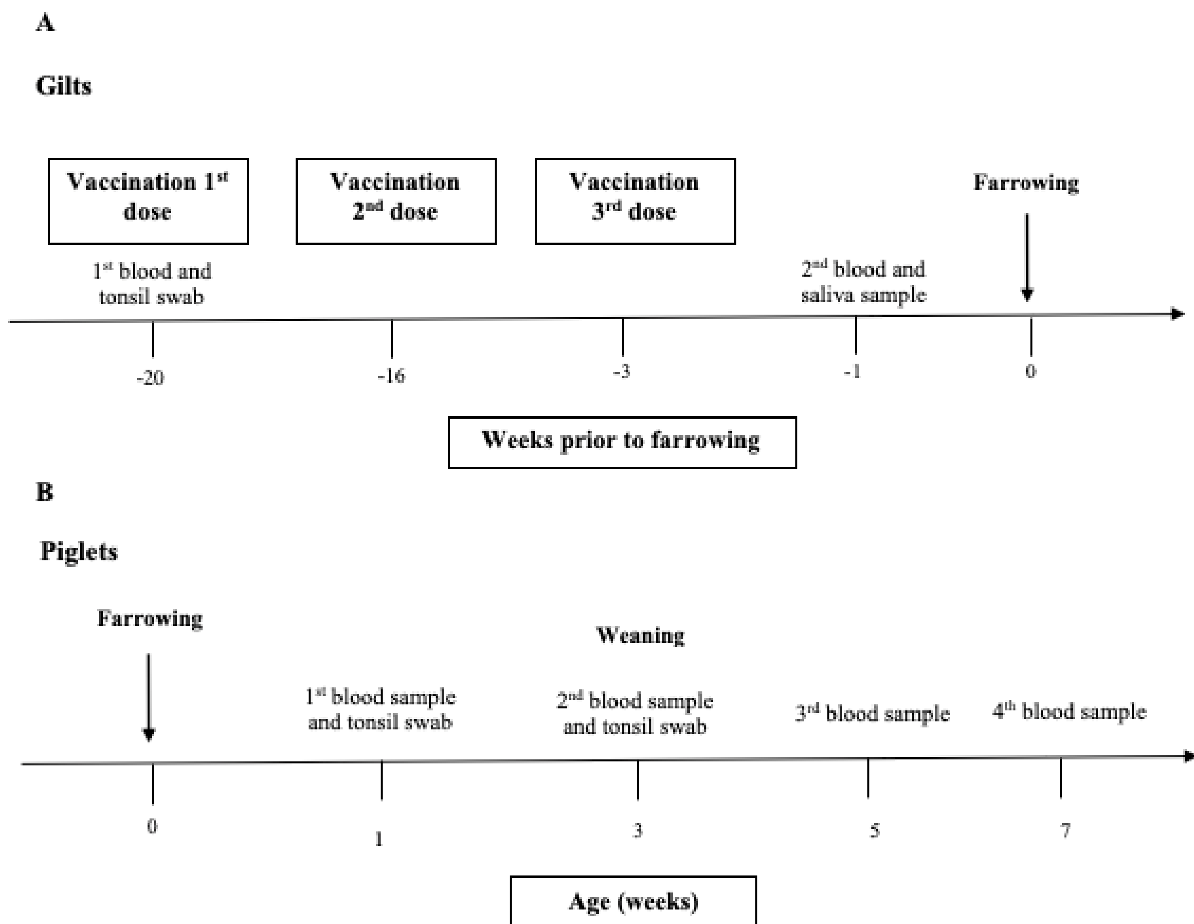


Figure 1: Experimental design of field study (A) Gilts received three doses of an autogenous vaccine via intramuscular injection at -20 (pre-breeding), -16 and -3 weeks prior to farrowing. Blood samples and tonsil swabs were taken in all gilts prior to the first vaccination. Final blood samples and saliva samples were taken -1 week before farrowing. (B) Two piglets were randomly selected per litter from vaccinated and from non-vaccinated gilt groups. Blood was taken from piglets at 1, 3, 5 and 7 weeks of age and tonsil swabs were taken from piglets at 1 and 3 weeks of age.

Blood and saliva/tonsil sampling

Blood samples were collected from gilts at -20 weeks (pre-breeding, before the first dose of the vaccine) and -1 week pre-farrowing (two weeks after receiving 3rd dose of the vaccine) **(Figure 1)**. At farrowing, two piglets from vaccinated and non-vaccinated gilts, respectively, were randomly selected, tagged, and numbered for the serological portion of the study. The remainder of piglets were tagged for clinical evaluation (n=628). Piglets included in the serological study

were sampled at 1 and 3 weeks in the farrowing room and at 5 and 7 weeks of age in the nursery barn. After blood collection, serum was recovered and stored at -80°C until analyses.

Tonsil swabs were collected from gilts at -20 weeks (before the 1st vaccine dose) and individual saliva samples (oral fluid collection via individual ropes) were collected -1 week pre-farrowing (two weeks after the 3rd vaccine dose) **(Figure 1)**. Saliva samples (oral fluid collection) were recovered for the second collection due to difficulties in obtaining tonsillar swabs encountered during the first collection. Piglet tonsillar swabs were collected at 1 and 3 weeks of age. Collected sera and saliva samples were stored at -80˚C until analyzed by ELISA and by opsonophagocytosis assay (serum samples for antibodies) and qPCR (tonsillar swabs and saliva samples for *S. suis* shedding) as described below.

Enzyme-linked immunosorbent assay (ELISA)

Strains of *S. suis* used in the autogenous vaccine were also used as the coating antigen for ELISA Polysorb plates (Nunc-Immuno; Thermo Scientific, Mississauga, ON, Canada). The ELISA protocol was adapted from Corsaut *et al* [6]. Briefly, bacteria were grown overnight onto 5% sheep blood agar plates at 37˚C, and isolated colonies were cultured in 5 ml of Todd-Hewitt broth (THB) (Becton Dickinson, Mississauga, ON, Canada) for 8 h at 37˚C with agitation at 120 rpm. Then, 10 µl of 1/1000 dilution of 8-h cultures were transferred into 30 ml of THB and incubated for 16 h at 37˚C with agitation at 120 rpm. Stationary-phase bacteria were washed in phosphate-buffered saline (PBS) at pH 7.3. Bacterial pellet was then suspended in ddH_2O and adjusted to a concentration equivalent at 10^8 CFU/ml. Plates were coated with 100 µl/well of the whole bacterial suspension, air-dried during two days at room-temperature (RT), and finally fixed with 50 µl/well

of 100% methanol. After evaporation of methanol, plates were stored at RT until use. For titration of antibodies, plates were washed with PBS-tween (PBS-T), then 100 µl of different 2-fold based dilutions of pig sera (in PBS-T) were added to each well and incubated for 1 h at RT. For titration of porcine total Ig [IgG + IgM] or IgM, plates were incubated with peroxidase-conjugated goat anti-pig total Ig [IgG + IgM] (Jackson ImmunoResearch, West Grove, PA) or IgM (BioRad, Mississauga, ON, Canada) antibodies, respectively, for 1 h at RT. For porcine IgG1 or IgG2 detection, mouse anti-porcine IgG1 or IgG2 (BioRad) was added for 1 h at RT. After washing, peroxidase-conjugated goat anti-mouse IgG (Jackson ImmunoResearch) was added for 1 h at RT. Plates were developed with 3,3',5,5'-tetramethylbenzidine (TMB; InvitroGen, Burlington, ON, Canada) substrate and the enzyme reaction was stopped by addition of 0.5 M H_2SO_4. Absorbance was read at 450 nm with an ELISA plate reader (Biotek, Santa Clara, CA). The reciprocal of the last serum dilution that resulted in an optical density at 450 nm (OD_{450}) of ≤ 0.2 (cutoff) was considered the titer of that serum. To control inter-plate variations, an internal reference positive control was added to each plate. This positive control was composed by a pool of serum of ten sows randomly selected on farm that showed high ELISA values against all 5 vaccinal strains (serotypes 1/2, 2, 5, 7 and 14) because of their natural exposition to these serotypes on farm. Reaction in TMB was stopped when an OD_{450} of 1.0 was obtained for the positive internal control. Optimal dilutions of the positive internal control sera and anti-porcine antibodies or conjugates were determined during preliminary standardization assays.

Opsonophagocytosis assay

The OPA test was performed as previously published [6]. One *S. suis* serotype (serotype 7) was used for this protocol as a representative serotype for the study. Whole blood of 4 to 8 week-old

piglets coming from a high health status herd was used as a source of phagocytic cells. These piglets originated from a farm without *S. suis* endemic infection and blood was intravenously collected in vacutainer sodium heparin tubes (Becton, Dickinson, Franklin Lakes, NJ, USA), and kept at RT. Using washed bacterial cultures grown as described above, final bacterial suspensions were prepared in complete cell culture medium (RPMI 1640 supplemented with 5% heat-inactivated fetal bovine serum, 10 mM HEPES, 2mM L-glutamine and 50 µM 2-mercaptoethanol; Invitrogen) to obtain a concentration of 2×10^6 CFU/mL. The number of CFU/mL in the final suspension was determined by plating samples onto Todd-Hewith agar (THA). Whole blood (containing approximately 1×10^8 leukocytes/mL) was mixed with the *S. suis* suspension to obtain a multiplicity of infection (MOI) of 0.01. Control and sample sera from immunized animals were added to a concentration of 40% v/v in microtubes to a final volume of 200 µl. Control sera came from naïve pigs (absorbed against different serotypes of *S. suis* and presenting negative ELISA values), and positive sera were obtained and pooled from sows (originated from the same farm and presenting high ELISA values). The tube tops were pierced using a sterile needle and incubated for 2 h at 37˚C with 5% CO_2, with gentle agitation. After incubation, viable bacterial counts were performed on THA using a spiral plater (Whitley Automated Spiral Plater, Whitley Wasp Touch, Frederick, MD). The percentage of bacterial killing was determined using the following formula:

% Bacteria killed= [1 − (bacteria recovered from sample tubes / bacteria recovered from negative control tube with control serum)] x 100

Quantification of total *S. suis* and *S. suis* serotypes 2 (and 1/2), 5, 7 and 14 (and 1) shedding

The technique qPCR was used to measure total total *S. suis* and *S. suis* serotypes 2 (and 1/2), 5, 7 and 14 (and 1) shedding in gilts as well as in 1 and 3 week-old piglets. Tonsil swab and saliva samples were centrifuged at 21 000 x *g* and the supernatant was removed. Pellets were then treated with lysozyme in 200 μM Tris HCl-EDTA-triton for 30 min at 37°C. QIAamp DNA kit (Qiagen, Toronto, Ontario, Canada) was used to extract DNA following the manufacturer instructions. qPCR was used to quantify the concentration of total *S. suis* as well as that of *S. suis* serotypes (2 (and 1/2), 5, 7 and 14 (and 1)) from tonsil swab/saliva samples. qPCR was performed using the EXOone *Streptococcus suis* oneMIX qPCR kit from Exopol (San Mateo de Gallego, Zaragoza, Spain) following the manufacturer's instructions.

Clinical evaluation of piglets

Clinical signs, mortality and euthanasia from all enrolled piglets were recorded by farm staff daily. Pigs were identified by ear tag colour and/or number and were followed until the end of the nursery period (10 weeks of age). Main clinical signs were listed as: arthritis, diarrhea, meningitis, pneumonia, prolapse, sudden death, and injury (from other animals). All antibiotic treatments were removed during the trial. Animals showing clinical signs were rapidly euthanized by farm staff. After euthanasia or mortality, meningeal swabs, joint swabs, and spleen tissue samples were collected and submitted for culture and, in case of positive isolation, *S. suis* serotyping. Culture was performed by the diagnostic laboratory at Faculty of Veterinary Medicine, University of Montreal). Isolation was performed by culture on blood agar, identification of the bacteria was done by MALDI-TOF [122] and confirmation by *rec-N* PCR [123]. Identified *S. suis* isolates were further serotyped using a multiplex-PCR [121].

Statistical analyses

ELISA data were log-10 transformed to normalize distributions. Unless otherwise specified, a linear mixed model was used with sampling time as the within-subject fixed effect, group (vaccinated or not vaccinated) as the between-subject fixed effect, and animal identification (id) as random effect. A priori contrasts were performed to compare pairs of means adjusting the alpha level downward for each comparison with the sequential Benjamini–Hochberg procedure. In the analysis of IgG1 and IgG2 subclasses, equal variance *t*-test was used to compare means according to the vaccinal status. For OPA analyses, data were arcsine square-root transformed to normalize distributions. Statistical analyses were performed using SAS 9.4 (SAS, Cary, NC, USA). The level of statistical significance was set at 0.05.

RESULTS

Total antibody levels induced by the autogenous vaccine increased in vaccinated gilts, but isotype profiles differ between serotypes

The autogenous vaccine contained five serotypes of *S. suis*: 2, 1/2, 5, 7 and 14. Before vaccination, levels of total Ig [IgG + IgM] in gilts against *S. suis* all serotypes tested were already high **(Figures 2A-2E**). After 3 doses of the vaccine, titers became significantly higher in vaccinated groups compared to the unvaccinated groups **(Figures 2A-2E).**

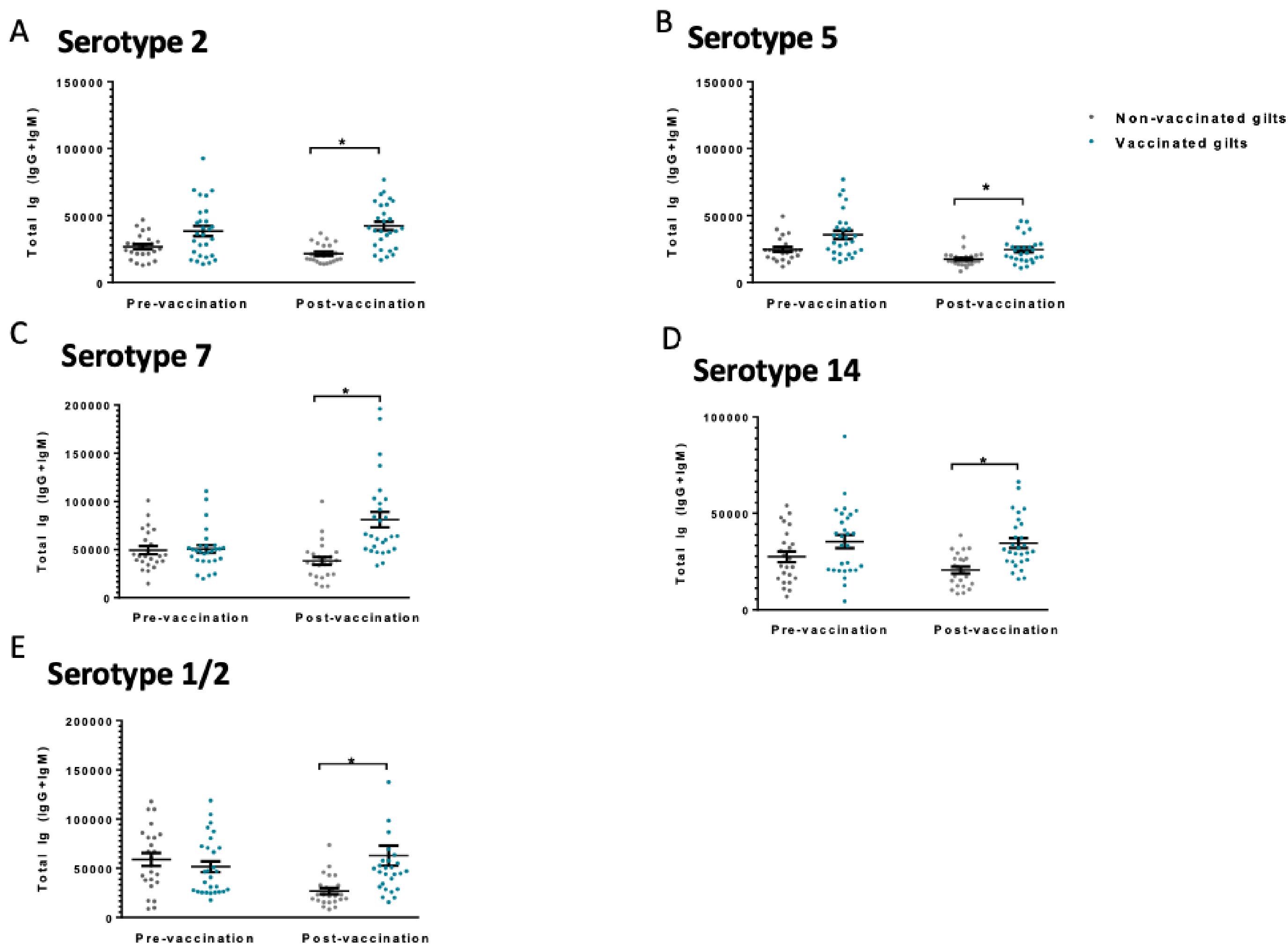
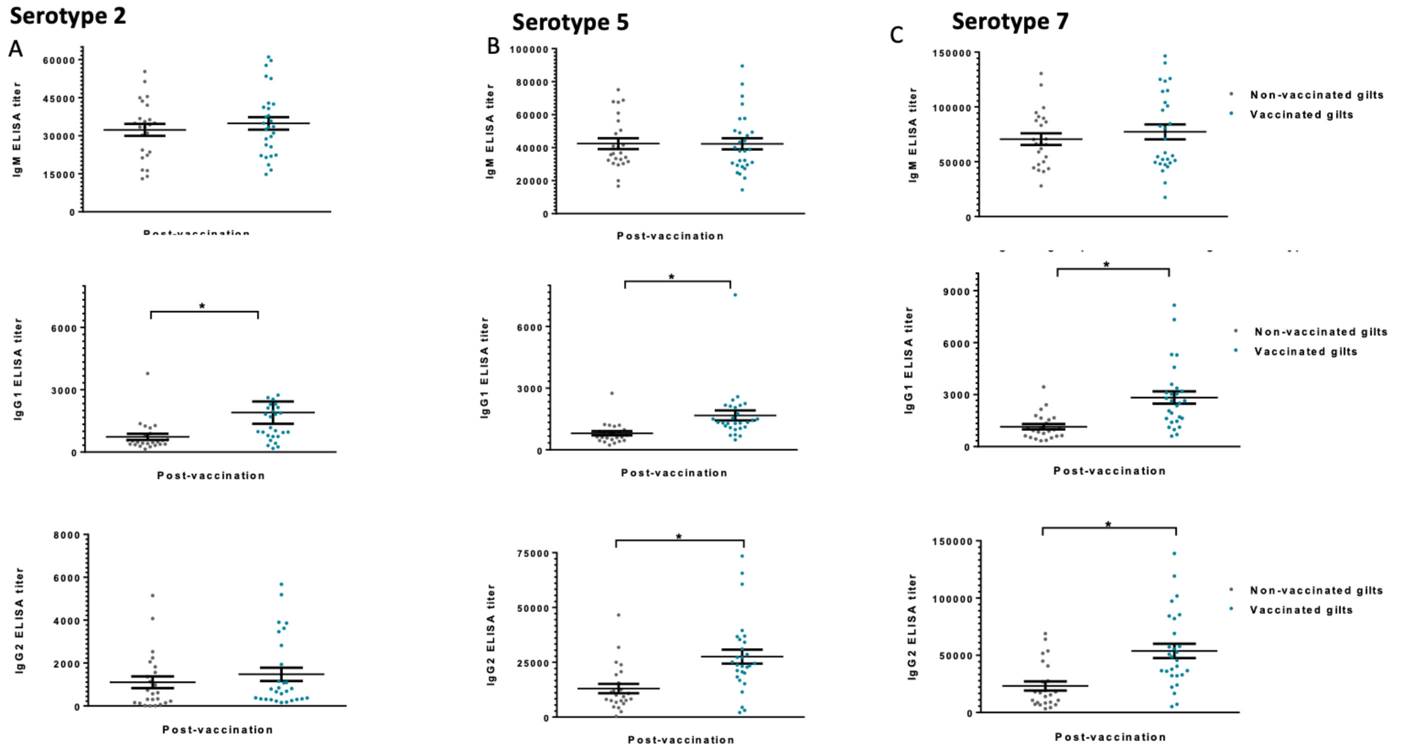


Figure 2: Kinetics of total Ig in gilts against S. suis serotypes (A) 2, (B) 5, (C) 7, (D) 14 and (E) 1/2. Total Ig [IgG + IgM] titers were determined by ELISA on serum samples collected pre- and post-vaccination (Figure 1), against all 5 vaccinal strains individually. Antibody titers for gilts are shown with horizontal bars representing mean ± standard error of mean (SEM). Significant values are shown with asterisks.

In addition to the increase of total Ig against *S. suis* tested serotypes, the goal of an efficient vaccine is also to obtain higher isotype switching from IgM to IgG. This was evidenced by a stable IgM level but an increased switching to IgG subclasses during a secondary immune response. This was observed for serotypes 2, 5 and 7 **(Figures 3A, 3B, 3C).** Indeed, the vaccine-induced immune response showed an increase in IgG1 antibodies for these serotypes ($p<0.05$) when comparing vaccinated to non-vaccinated gilts **(Figures 3A, 3B, 3C)**. For serotypes 5 and 7, IgG2 subclass was dominant with significantly higher titers in the vaccinated gilts when compared to the control

group **(Figures 3B, 3C)**. In addition, titers observed for the IgG2 subclass were also higher when compared to IgG1 ($p<0.05$). A clear increase of IgG1 and IgG2 was not observed for serotypes 1/2 and 14 (**Figures 3D, 3E)**.



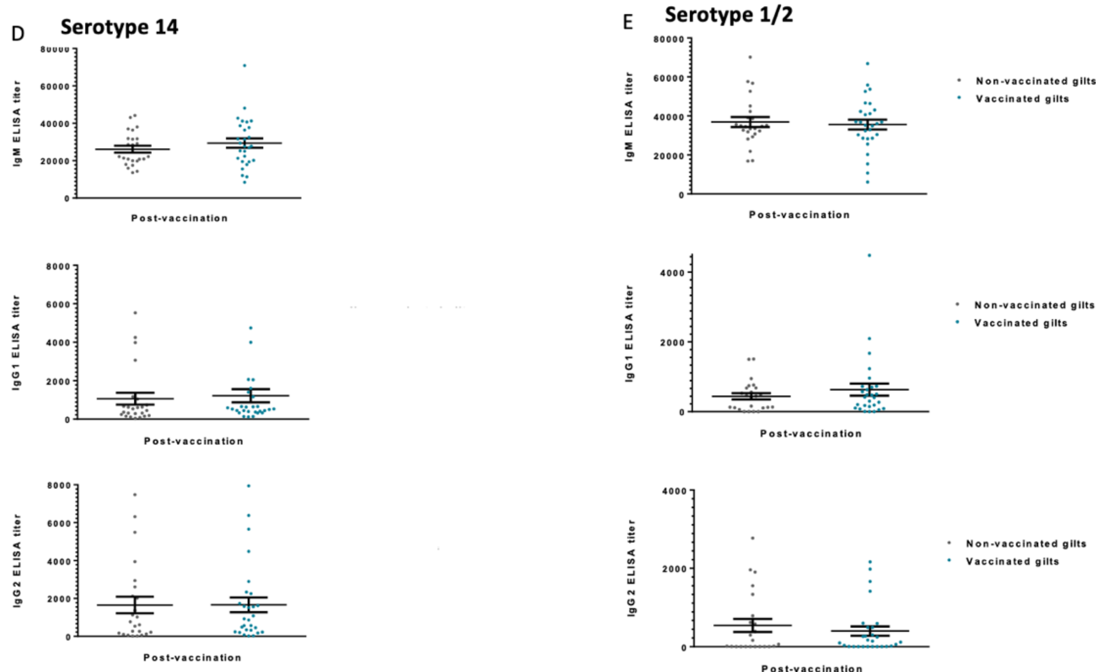


Figure 3: Isotype profile of antibodies in gilts against *S. suis* serotypes (A) 2, (B) 5, (C) 7, (D) 14 and (E) 1/2. Blood samples were collected pre- and post-vaccination to follow the immune response (Figure 1). IgM, IgG1 and IgG2 titers were determined by ELISA against all 5 vaccinal strains individually. Antibody titers for gilts are shown with horizontal bars representing mean ± standard error of mean (SEM). Significant values are shown with asterisks.

Maternal antibody transfer to piglets increased after gilt vaccination until 3 weeks for all serotypes and up to 5 weeks of age for select serotypes

The goal of sow vaccination programs is to increase maternal antibodies to their subsequent litters via colostrum intake. As higher levels of anti-*S. suis* Igs [IgG + IgM] were observed in gilts for all serotypes, significantly higher levels of Igs [IgG + IgM] maternal antibodies could be detected in piglets from vaccinated gilts until 3 weeks for serotypes 2, 7 and 14 and until 5 weeks for serotypes 1/2 and 5 (**Figures 4A-4E**). IgM titers for all serotypes were low and similar between the vaccinated and non-vaccinated groups (**Figure 5)**. For serotypes 2, 5 and 7, the predominant isotype profiles were both IgG1 and IgG2 in piglets born from vaccinated gilts (**Figures 5A-5C**).

However, for serotypes 1/2 and 14, isotype profiles of piglets from vaccinated or non-vaccinated gilts were similar (**Figures 5D-E**).

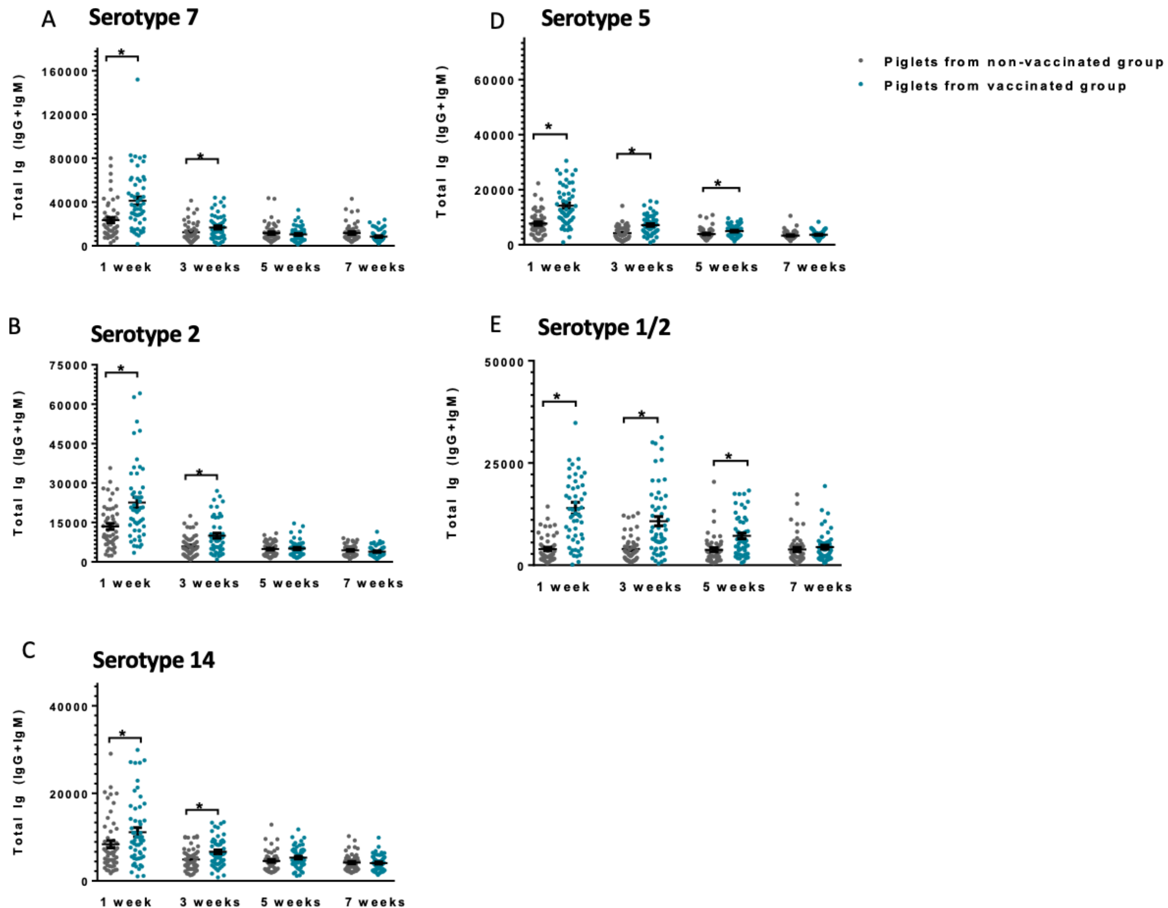


Figure 4: Kinetics of total Ig in piglets against *S. suis* serotypes (A) 2, (B) 5, (C) 7, (D) 14 and (E) 1/2 from either vaccinated or non-vaccinated gilts. Two piglets per litter were randomly selected and assigned to vaccinated or non-vaccinated groups. Piglets were sampled at 1, 3, 5, and 7 weeks of age. Total Ig [IgG + IgM] titers were determined by ELISA against all 5 vaccinal strains individually. Antibody titers for piglets are shown with horizontal bars representing mean ± standard error of mean (SEM). Significant values are shown with asterisks.

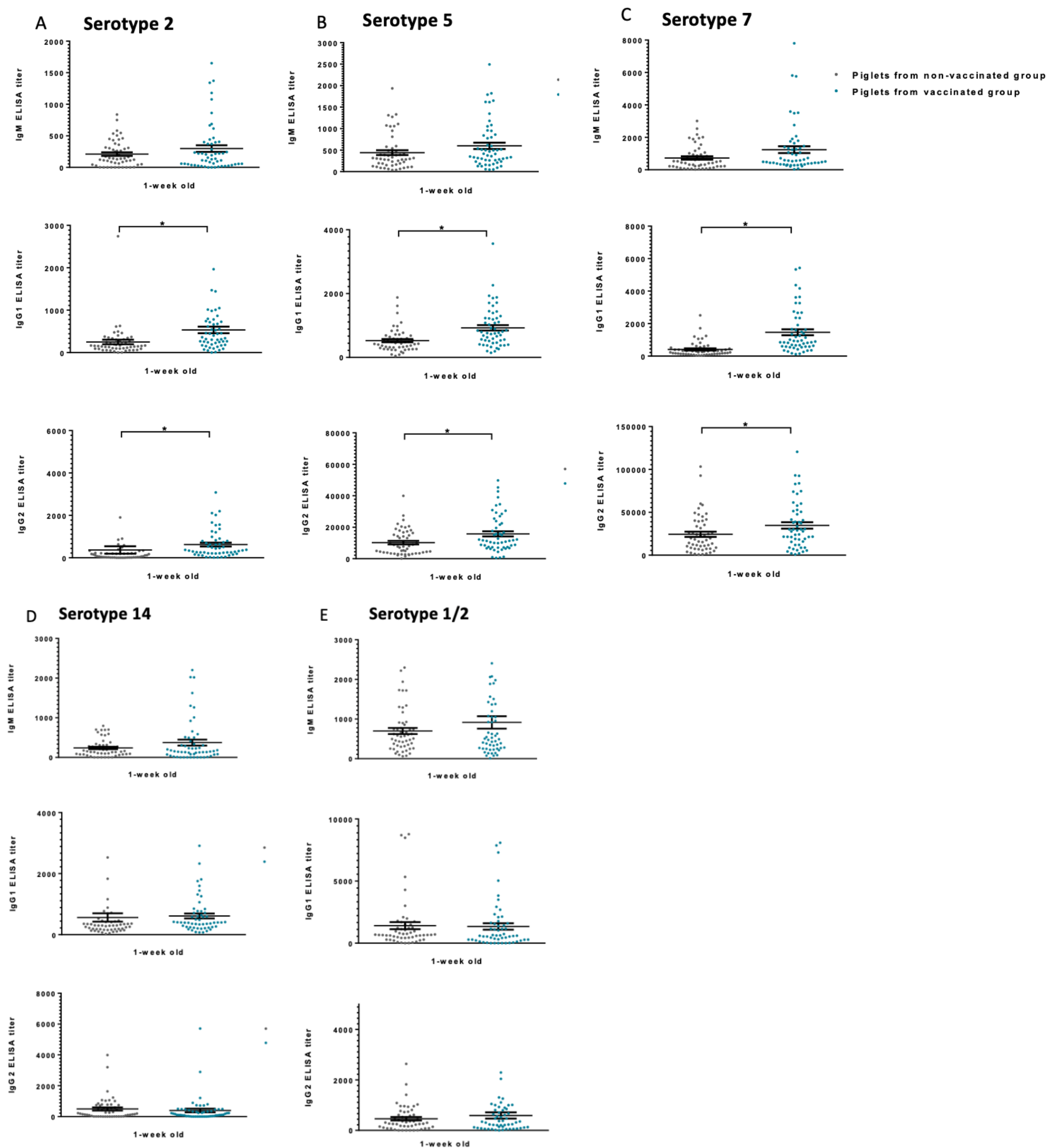


Figure 5: Isotype profiles of antibodies in piglets against *S. suis* serotypes (A) 2, (B) 5, (C) 7, (D) 14 and (E) 1/2 from either vaccinated or non-vaccinated gilts. Two piglets per litter were randomly selected from both vaccinated and non-vaccinated gilts. Total of 106 piglets were included in the serological study. Piglets were sampled at 1, 3, 5, and 7 weeks of age. Titers of IgM, IgG1 and IgG2 were determined by ELISA against all 5 vaccinal strains individually in samples of 1 week-old piglets. Antibody titers are shown with horizontal bars representing mean ± standard error of mean (SEM). Significant values are shown with asterisks.

Vaccination of gilts with the autogenous bacterin failed to improve the killing capacity of antibodies of piglets born in their subsequent litters

Functionality of the antibodies in both groups was evaluated using serotype 7 as a model. As showed in **Figure 6**, OPA activity of antibodies in gilts post-vaccination (1 week prior to farrowing) was very high (>80%) in both vaccinated and non-vaccinated groups. Regarding antibody functionality in piglets, OPA capacity was high for piglets at 1 week of age due to the maternal transfer of functional antibodies (**Figure 6**). However, there was no difference between piglets from vaccinated and non-vaccinated gilts. At 3 and 5 weeks of age, OPA capacity of the sera was significantly decreased when compared to 1 week of age.

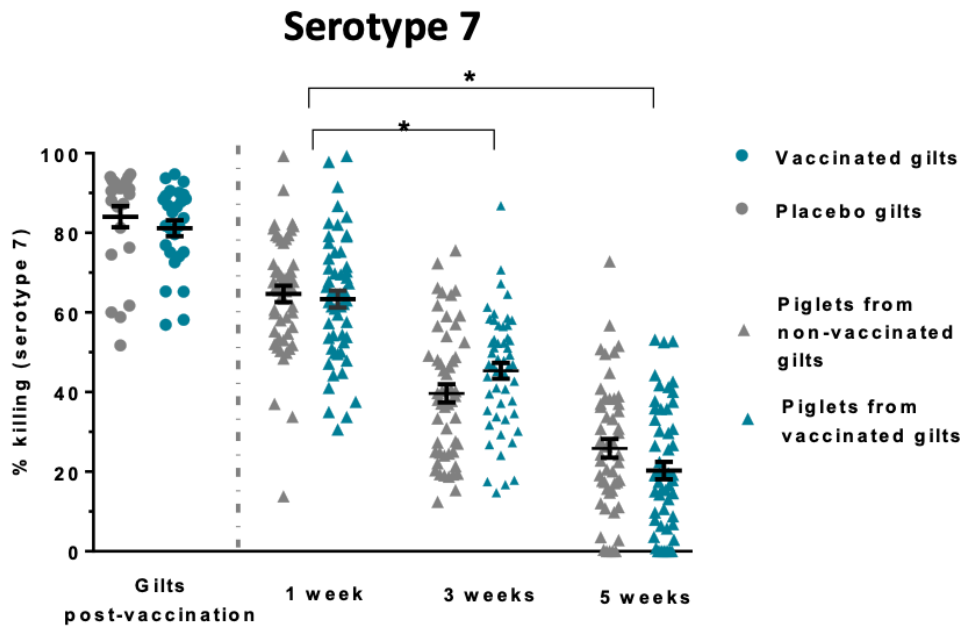


Figure 6: Opsonophagocytosis of *S. suis* serotype 7 induced by serum antibodies from gilts and from their piglets. Blood samples were collected at 1 week before farrowing from vaccinated and non-vaccinated gilts and from two piglets per litter at 1, 3, and 5 weeks of age (n=106) to evaluate their functionality in the opsonophagocytosis assay (OPA). Results are expressed at % of bacterial killing of individual sera, with horizontal bars representing mean ± SEM. Significant values are shown with asterisks.

Vaccination had no effect on *S. suis* shedding

Another goal of the study was to evaluate if the vaccination program was able to reduce *S. suis* (and/or specific serotypes) potential shedding either by gilts or piglets (or both). Using qPCR, **Figure 7** shows results on total *S. suis* species. As expected, all animals were highly colonized by *S. suis*. Vaccinated gilts did not show a significant decrease of bacterial load (copies/ml) when compared to the non-vaccinated gilts (**Figure 7**). In piglets, similar results to those of gilts were observed **(Figure 7).** When analyzing specific serotypes, bacterial loads were lower for gilts and piglets than those observed for *S. suis* species, with the exception of serotype 7. Although vaccination seemed to reduce bacterial shedding in gilts for serotypes 5, 7 and 14 (and 1) (**Figures 8A, 8C, 8D),** as opposed to serotypes 1/2 and 2 (**Figure 8B)**, differences were not significant. Gilt vaccination did not influence bacterial shedding in piglets for all serotypes tested (**Figure 8A-8D)**.

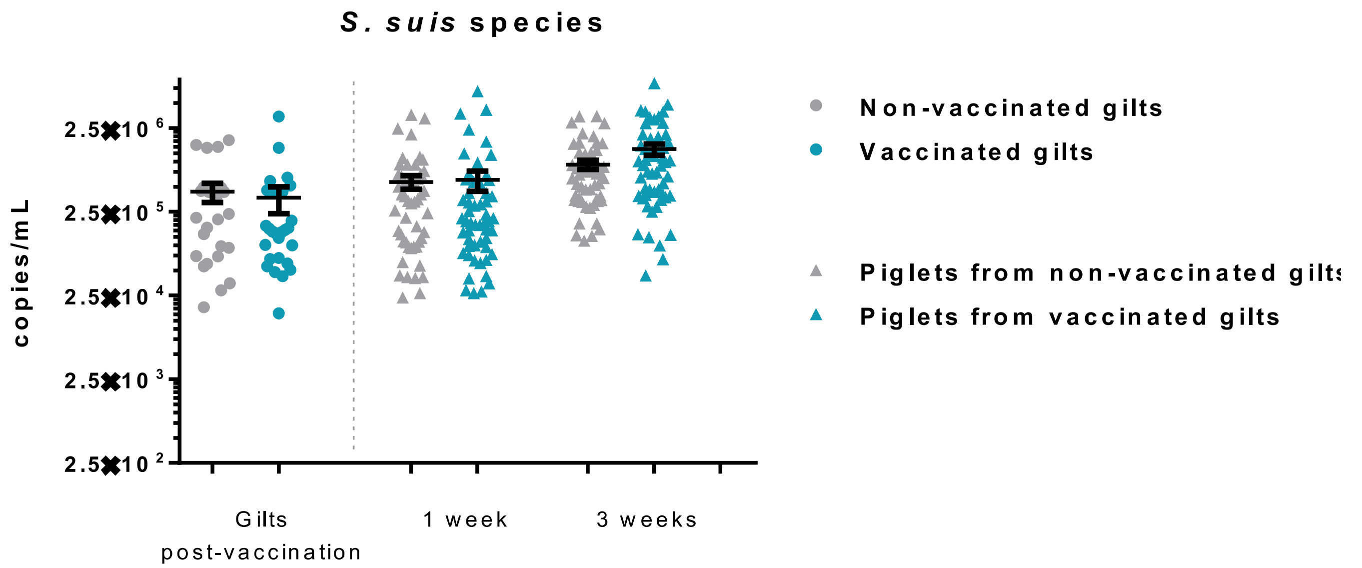


Figure 7: qPCR of total *S. suis* species. Tonsil swabs and saliva samples were collected at 1 week before farrowing from vaccinated and non-vaccinated gilts and from two piglets per litter at 1 and 3 weeks of age (n=106) to evaluate if the vaccine program influenced potential bacterial shedding. qPCR was used to quantify the concentration of total *S. suis* from tonsil swab and saliva samples. Results are expressed in copies/ml per sample.

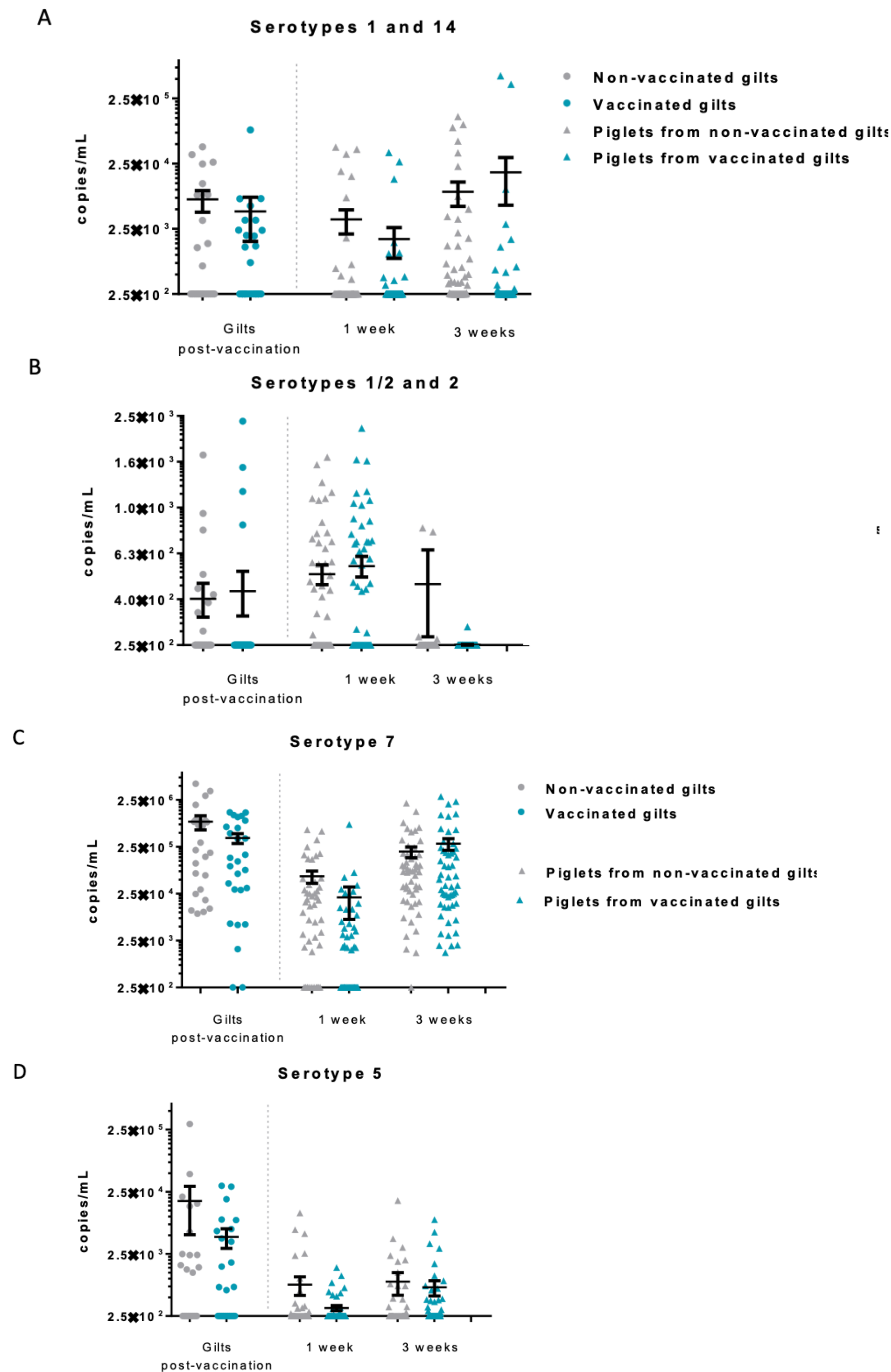


Figure 8: qPCR for each individual vaccinal strain; (A) serotypes 1 and 14, (B) serotypes 1/2 and 2, (C) serotype 7, and (D) serotype 5. Tonsil swabs and saliva samples were collected at 1 week before farrowing from vaccinated and non-vaccinated gilts and from two piglets per litter at 1 and 3 weeks of age (n=106) to evaluate if the vaccine program influenced potential bacterial shedding. Results are expressed in copies/ml per sample.

No *S. suis* outbreak identified on farm, yet *S. suis*-associated clinical signs found

All antibiotic treatments were removed from the trial and a total of 318 piglets from vaccinated gilts and 310 piglets from non-vaccinated gilts were followed for clinical evaluation, morbidity, and mortality records. During the trial, animals presenting clinical signs were immediately euthanized, necropsied and submitted for bacterial examination. Any dead animals were also necropsied and submitted for bacterial examination. The overall mortality rate related to *S. suis*-associated disease (based on clinical signs) was 1.27%; with 0.31% in the vaccinated group and 0.96% in the non-vaccinated group (**Table 1**). However, mortality was mainly not due to *S. suis* as revealed by results from the diagnostic laboratory. Indeed, only 14 animals died: nine of sudden death, four of arthritis and two of meningitis. In the vaccinated group, 1 piglet died of *S. suis* which was confirmed as non-typable by the diagnostic laboratory. In the non-vaccinated group, 3 piglets died with diagnostic confirmation of *S. suis* (one serotype 2 and three untypable), while 10 piglets died from other diseases that were not related to *S. suis*. Untypable *S. suis* were all recovered with contaminating bacteria, so their role in disease may be questioned (possible post-mortem invasion). Indeed, *Erysipelothrix rhusiopathiae* was identified in 6 out of 14 of animals necropsied. Thus, no *S. suis* outbreak could be identified during the time of the trial on this farm, limiting the ability to properly measure vaccine efficacy (**Table 2**).

Table 1: Distribution of confirmed *S. suis* mortality among 318 vaccinated and 310 non-vaccinated piglets included in the trial

	Number of dead pigs		*S. suis*-related deaths		Total number of pigs
Vaccinated	1	0.31%	1	0.31%	318
Non-vaccinated	13	4.2%	3	0.96%	310
Total number of pigs	14		4		628

Piglets from vaccinated and non-vaccinated gilts (n=106) were followed clinically. *S. suis*-related mortality rate was calculated according to the etiology confirmation from the diagnostic laboratory bacteriology.

Table 2*:* Distribution of confirmed non-*S. suis* related mortality among 318 vaccinated and 310 non-vaccinated piglets included in the trial

	Number of dead pigs		*Erysipelothrix rhusiopathiae*-related death		Other causes of death		Total number of pigs
Vaccinated	1	0.31%	0		0		318
Non-vaccinated	13	4.2%	6	1.9%	4	1.3%	310
Total Pigs	14		6		4		628

Piglets from vaccinated and non-vaccinated gilts (n=106) were followed clinically. *Erysipelothrix rhusiopathiae*-related mortality rate was calculated according to the etiology confirmation from the diagnostic laboratory bacteriology.

DISCUSSION

Autogenous vaccines are very popular amongst swine producers, as they may represent an alternative to antibiotics. There is no current commercial vaccine available for *S. suis* control in North America. Most studies on bacterins used laboratory-made vaccines with a formulation that may be far from those used in the field [9, 60, 104, 107]. To the best of our knowledge, only four field studies (one of them performed 25 years ago) are available on the immunogenicity and/or clinical protection efficacy of autogenous bacterins manufactured by licensed companies [5-7, 102]. At least two of the recent trials used vaccines produced by the same company [5, 6]. In the first one, a two-dose vaccine program containing *S. suis* serotypes 7 and 9 was applied to gilts or piglets. Results showed that, despite a higher anti-*S. suis* levels in vaccinated gilts before farrowing, their piglets did not show higher maternal antibody levels against both serotypes when compared to those from non-vaccinated gilts [6]. Vaccination of piglets did not induce any seroconversion [6]. In the second study, using a three-dose autogenous vaccine program in gilts, containing *S. suis* serotype 7 strain, higher antibody levels were observed not only in vaccinated gilts but also in 7-day old piglets [5, 6]. However, at 18 days of age, levels of antibodies significantly dropped and were similar in piglets derived from vaccinated and non-vaccinated gilts, leaving animals unprotected at the riskiest period during in the nursery.

Manufacturing companies may use different laboratory protocols (growth conditions, media, etc.), adjuvants (types and final concentration), bacterial inactivation techniques as well as different bacterial concentrations. It is important to highlight the differences of laboratory protocols and manufacturing procedures used by different licensed vaccine companies [10]. Indeed, such procedures and compositions are, in general, part of confidential information. As some of the

previously mentioned studies contained autogenous vaccines that were manufactured by the same licensed vaccine company, it is unknown if similar results are obtained when the product is produced by a different company. In the current study, a multi-serotype autogenous vaccine manufactured from a different vaccine company was used. The immunological characterization of the antibody response confirmed that the basal antibody level before vaccination against *S. suis* in adult animals is very high, as previously shown [5, 6, 8, 60]. This can be explained by natural exposure of these animals to *S. suis* present in the farm. Indeed, tonsillar samples taken from gilts pre-vaccination revealed high bacterial loads of *S. suis* in most animals. After three doses of the autogenous vaccine, total Ig [IgG + IgM] antibody levels against *S. suis* in vaccinated gilts significantly increased for all five serotypes when compared to control group. However, isotype switching varied depending on the serotype, with low levels of IgG1 and IgG2 for serotypes 1/2, 2 and 14. A possible explanation for limited isotype switching in such serotypes could be due to antigenic similarity of those capsular polysaccharides, which are rich in sialic acid [4, 124].

Different from what was previously published with an autogenous vaccine produced by a different company [5, 6], the increased response seen in gilts was sufficient to improve maternal antibody transfer to piglets up to 3 weeks of age for all serotypes. Levels of antibodies against serotype 7 were surprisingly higher than those observed for other serotypes. These data along with a higher load of *S. suis* serotype 7 documented by qPCR (see below) may indicate a higher circulation of this serotype in the farm. In addition, and for the first time, maternal antibody transfer was significantly observed in piglets until to 5 weeks of age for serotypes 5 and 1/2. It is possible that the conditions used for the production of this autogenous vaccine (including the adjuvant used) made the product more immunogenic. As expected, autogenous vaccines produced by different

companies present different characteristics. Although maternal antibodies were still present for some serotypes at 5 weeks of age, it is not uncommon to observe clinical cases due to *S. suis* later in the nursery [6]. In such cases, a three-dose vaccination program with an autogenous vaccine evaluated in the current study would not induce a sufficient high level of antibodies to cover such period. Indeed, vaccination of gilts/sows may be useful when young piglets in the farrowing unit or early in the nursery are affected. Despite a clear increase in ELISA antibody titers for vaccinated gilts and for piglets from vaccinated gilts, no differences in the OPA test against serotype 7 were observed between vaccinated and non-vaccinated gilts as well as in piglets during the first 5 weeks of age. These results are similar to those previously reported for other autogenous vaccines [5, 6]. The lack of correlation between ELISA and the OPA tests may be due to differences in the sensitivity between both tests. Indeed, it is possible that the ELISA test detects both opsonic and non-opsonic antibodies. Since the clinical protection could not be evaluated (see below), the exact reasons for these differences remain unknown.

The potential effect of the autogenous vaccine to reduce total *S. suis* shedding (as well as that of the specific serotypes included in the vaccine) was evaluated. Samples consisted in tonsillar swabs (first sample set of gilts and those from piglets) as well as saliva (second set of gilt samples). It is accepted that *S. suis* is a normal inhabitant of tonsils [3]. However, saliva has also been shown to be a reservoir for this bacterial pathogen [125]. Results showed that although vaccination increase antibody titers, it did not reduce *S. suis* presence in tonsils/saliva of gilts, neither total *S. suis* or that of serotypes tested. Similar results were observed in piglets from vaccinated gilts. As expected, the total number of *S. suis* was in general higher than those of specific serotypes, an observation that was previously reported [125]. As mentioned before, serotype 7 was detected in higher copies

than other serotypes. It is unknown whether the levels of antibodies raised by the vaccine were not high enough to reduce shedding or, simply, pig colonization by *S. suis* does not depend on the presence of such antibodies. The last hypothesis seems to be plausible, since all adult animals are normally colonized by *S. suis* in the presence of high level of antibodies.

The evaluation of the impact of the application of an autogenous vaccine program on the development of *S. suis*-associated diseases in the field is not an easy task. Indeed, other pathogens may induce similar pathologies [9], the use of antimicrobials may prevent the development (and etiological diagnosis) of clinical signs and, finally, bacteriological follow up of clinical cases is rarely done in most studies. To our knowledge, there are no previous studies that completely eliminate the use of antimicrobials during evaluation of an autogenous vaccine program on farm, as it was done in the current study [5-7, 102]. In addition, *S. suis*-associated disease cases were sent for confirmatory necropsy, followed by bacteriology and *S. suis* serotyping (if present) for all clinical cases. Unfortunately, the clinical protective effect of the vaccination program with the autogenous vaccine could not be evaluated. Indeed, although some clinical diseases related to *S. suis* could be observed, limited confirmed *S. suis* cases were identified during trial. The spontaneous disappearance of *S. suis* cases at the moment of the autogenous vaccine application has previously been reported, and the cause(s) remain(s) unknown [8]. *Erysipelothrix rhusiopathiae* was mainly identified as causing the few cases of "*S. suis*-associated clinical signs", such as sudden death, meningitis and arthritis. Despite the fact that the farm used sow vaccination against that pathogen, there were no reports on its isolation from diseased piglets in the last years. It is possible that elimination of all antimicrobial treatments in the farm during the trial predisposed the appearance of such a pathogen. Results of the current study reinforce the need of having always

a non-vaccinated control group, removing antimicrobial treatments on the farm and etiological confirmation of clinical cases done by a diagnostic laboratory.

CONCLUSIONS

Presently, autogenous bacterins are important preventive tools intended to control *S. suis*-associated diseases. Results of the current study showed that autogenous vaccines produced by different licensed laboratories may induce different levels of antibodies. The hereby polyvalent autogenous vaccine tested in a three-dose program in gilts induced higher titers of passive antibodies for all serotypes, which last, for some of them, until 5 weeks of age in piglets, which was different from what has been reported so far. However, levels of antibodies in the late nursery period would still be very low to protect piglets. Although vaccination of gilts/sows is very popular in the field, there is still no study clearly showing that this approach induces high levels of passive antibodies to protect the whole period at risk for *S. suis* disease. In addition, it was shown that these antibodies did not have any influence on *S. suis* shedding and their protective capacity to reduce clinical signs could not be proved due to the absence of *S. suis*-associated diseases at the time of the trial evaluation. There is a need, for future field trials, to always include a non-vaccinated control group, to eliminate if possible any antimicrobial treatment in the farm and to use diagnostic laboratory when evaluating the protective effect of such autogenous vaccines.

IV. Discussion

As antibiotic use in swine farms decreases due to legislation and new meat markets (raised without antibiotics, organic, etc.), the need for alternative preventive measures increases. There is no current commercial vaccine available for *S. suis* control in North America, with limited studies available on the efficacy of autogenous vaccines manufactured by different licensed companies [5-8, 60, 103]. As *S. suis* is an important emerging zoonotic pathogen and is present in 100% of swine farms worldwide, control of this pathogen is imperative.

In this current study, a three-dose vaccine protocol was applied to gilts to measure the level of maternal immunity transferred to their piglets and its protective capacity against *S. suis* disease. Maternal immunity was for the first time observed in piglets up to 3 weeks of age for all serotypes, and 5 weeks of age for two out of the five serotypes tested. Indeed, the autogenous vaccine produced in this study was by far more immunogenic than two other vaccines previously described, and produced by another licensed laboratory [5, 6]. Differences between autogenous vaccines produced by these different laboratories are not known (for example, adjuvants used), as this is part of confidential information. Although high maternal immunity was measured, there was no *S. suis* outbreak in the herd at the time of the trial, precluding evaluation of the clinical protection conferred by the vaccine. This is an important limiting factor, as it was impossible to properly assess vaccine efficacy. A *S. suis* outbreak would have been more beneficial for the study to confirm if the autogenous vaccine induces proper protection. Due to this limitation, the goal and objective of the overall study was not fully reached. However, this study clearly confirmed the critical need for having a complete diagnostic confirmation of affected animals when the protective effect of an autogenous vaccine is evaluated. Indeed, previous reports also showed that the dynamics of *S. suis* infection in the herd might affect the conclusions of field studies [7, 102].

In the current study, sample size was large. Fifty-four (54) gilts were included in the study, 106 piglets were followed serologically and a total of 628 piglets were followed for clinical disease. Although more piglets could have been included in the serological study, the fact that two piglets per gilt were included, allowed clear interpretation of the passive transfer of maternal antibodies.

During the trial, antimicrobial treatment was, for the first time, completely removed from the herd. This aspect is highly important (and usually not possible to be accomplished in the field) as it allows researchers to clearly evaluate the protective activity of the vaccine, avoiding interference with the effect of any antimicrobial treatments. In the current study, there was important prophylactic antimicrobial use on the farm pre-trial, keeping mortality levels as low as 1.65%, with 30% pertaining to *S. suis*. In addition, when using antimicrobial treatments, clinical data analysis can be compromised by keeping mortality lower and affecting statistics [6]. In addition, the current study included necropsy and bacteriology in the analyses. This method was extremely valuable to the trial as it allowed for the detection of the presence of *Erysipelothrix rhusiopathiae* in affected animals from the studied herd. Indeed, 6 out of 14 affected animals were identified to have died from *E. rhusiopathiae*. The presence of this pathogen was probably not new and was previously not observed probably due to the routine use of antimicrobials in the herd.

A control group was included in the trial. This adds value to the project as it allows comparing vaccinated and control groups and the overall effect of the vaccine. However, during the current trial, the control and vaccinated animals were housed in the different rooms but were included with animals that were not involved the study, due to farm procedures. It would be more beneficial in the future to have a barn that could allow animals in the trial to be in their own rooms without non-included trial animals.

Another possible confounding factor in our study might be the timeline of the trial. The timeline of the project, from sampling the first animals in order to establish their *S. suis* status and their inclusion in the vaccine trial to the very end of the nursery period (piglets 10 weeks of age), spanned over 15 months. Although it is true that *S. suis* infection can be sporadic over a period of time [6, 7], data from clinical cases caused by *S. suis* in this farm were available during the last years and seroytpes involved were constantly the same. Indeed, if clinical cases had been present during the trial, they would have been most probably expected to be associated with the same serotypes as those previously identified in this herd. Unfortunately, the absence of *S. suis*-associated clinical disease during the trial did not allow confirmation of the serotypes involved. In terms of the sampling timeline, the study included piglets until 7 weeks of age. Since *S. suis* is typically seen in 5 to 10 week old animals [3], it would have been interesting to follow the animals until 10 weeks of age or until they were moved to the grower-finisher barn. Although this would have allowed for the evaluation of the clinical protective effect of a sow vaccination program during the complete post-weaning period, it has been proposed that protection obtained with passive maternal antibodies would be optimal at the beginning rather than at the end of the nursery period [3].

An opsonophagocytosis assay was used to evaluate the protective effect of the raised antibodies in the sera collected. Though this test has significance as it is considered in vaccinology as a correlate of protection, the current study only tested against serotype 7. It would be interesting to evaluate the protective effect of vaccine-induced antibodies against all 5 serotypes. However, this assay is tedious and requires the continuous availability of blood donors, thus there was difficulty in performing the assay for all the serotypes. Despite high levels of antibodies in vaccinated gilts and high levels of maternal immunity in piglets, opsonophagocytosis capacity of antibodies was

similar between groups. It can be concluded that the current assay may not be sensitive enough or that the produced antibodies are not opsonic.

In the future, repeating the study on a farm presenting with an outbreak of *S. suis* would be beneficial to evaluate the vaccine efficacy to protect the animals against clinical disease, although this is evaluated at the end of the study only. An additional piglet vaccination program after 3 weeks of age would be interesting to evaluate the potential increase of piglet antibodies and/or functionality of these antibodies. This would allow for a characterization of not only a passive vaccine program, but also evaluating the added bonus of an active immunization program.

This Master's thesis was an overall notable contribution to swine medicine as it characterized a *S. suis* autogenous vaccine program in gilts and, for the first time, measured passive maternal immunity in their subsequent litters up to 3 weeks of age for all serotypes and 5 weeks of age for two out of five serotypes. This study clearly showed that autogenous vaccines produced by different companies could induce different levels of antibodies. For the first time, antimicrobial treatments were completely eliminated and the etiological confirmation following clinical signs done by a diagnostic laboratory was performed. This study proves that quick and precise diagnostics in a herd, before and after the application of an autogenous vaccine, will allow for adequate diagnostics and herd health management. The use of gilt *Streptococcus suis* autogenous vaccines requires more characterization, specifically regarding passive antibody functionality and clinical protection in piglets, throughout the nursery barn period. 4

V. Conclusion and perspectives

Presently, autogenous bacterins are the only preventive tool intended to control *S. suis*-associated diseases. Results of this master's study showed that autogenous vaccines produced by different licensed laboratories may induce different levels of antibodies. The hereby polyvalent autogenous vaccine tested in a three-dose program in gilts induced higher titers of passive antibodies for all serotypes, which last (for some of them) until 5 weeks of age, which is different from what has been published so far. However, levels of antibodies would still be very low to protect piglets at the late phase of the nursery. Although it is a very popular vaccination program in the field, there is still no study that clearly showed that vaccination of gilts/sows induce high levels of passive antibodies to protect the whole period at risk for *S. suis* disease. In addition, it was shown that these antibodies did not have any influence on *S. suis* shedding and their protective capacity to reduce clinical signs could not be proved due to the absence of *S. suis*-associated diseases at the time of the trial conduct. There is a need for future field trials that will include a control non-vaccinated group, that will eliminate, if possible, any antimicrobial treatment in the farm and that will confirm the etiology of every single clinical case with the use of a laboratory when evaluating the protective effect of such autogenous vaccines. Overall, this study reinforces the need for complete follow-up after necropsy (bacteriology and serotyping) in on-field research trials. Quick and precise diagnostics in a herd will assist in accurate research results as well as herd health management. More research is needed regarding optimal vaccine formulation for piglets in the later stages of the nursery barn.

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