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Original article

Serologic profile of a cohort of pigs and antibody response to an autogenous vaccine for *Actinobacillus suis*

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Abstract – *Actinobacillus suis* is a commensal opportunistic pathogen in swine. However, in recent years, an increasing prevalence of clinical signs associated with *A. suis* has been observed in high health status herds in North America. The objectives of the study were to assess the kinetics of antibodies to *A. suis* in pigs from a herd showing clinical signs of *A. suis* infection and, to evaluate the antibody response in gilts following vaccination with an autogenous vaccine. An enzyme-linked immunosorbent assay (ELISA) using a saline extract of boiled-formalinized whole cells of a field strain as the coating antigen was standardized. This ELISA was used as a tool for monitoring, in a comparative way, the variations in *A. suis* antibody levels. The herd selected for the serologic profile was negative for *Actinobacillus pleuropneumoniae* infection and showed clinical signs of *A. suis* infection in 16 to 19-week-old pigs. A cohort of 20 pigs was blood sampled at 5, 8, 12, and 16 weeks of age. The lowest level of serum antibodies was observed between weeks 8 and 12, this probably corresponding to a decrease in maternal immunity. A marked increase in the antibody response was seen at 16-week of age, at the approximate time of onset of *A. suis* clinical signs in the herd. The evaluation of serum antibody responses to an autogenous vaccine revealed that the humoral immunity of gilts further increased following vaccination although the level of antibodies was already high prior to vaccination. The magnitude of the response to vaccination was higher when the level of antibodies was low prior to the first injection. The ELISA test seems to detect antibodies against the O-chain LPS.

***Actinobacillus suis* / autogenous vaccine / ELISA / serologic profile / pig**

Résumé – Profil sérologique chez une cohorte de porcs et réponse humorale à un autovaccin pour *Actinobacillus suis*. *Actinobacillus suis* est généralement considéré comme un agent pathogène opportuniste chez le porc. Toutefois, en Amérique du Nord, une augmentation de la prévalence des signes cliniques associés à *A. suis* a été notée récemment dans des troupeaux de haut statut sanitaire. Les objectifs de cette étude étaient d'évaluer la cinétique des anticorps chez des porcs provenant

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d'un troupeau dans lequel on observait des signes cliniques associés à *A. suis* et d'évaluer la réponse humorale suite à l'administration d'un autovaccin chez des cochettes. Un ELISA utilisant un extrait salin à chaud et formalinisé d'une souche isolée de cas clinique comme antigène de surface a été standardisé. Cet ELISA a été utilisé pour évaluer de façon comparative les variations des taux d'anticorps pour *A. suis* dans différents groupes de porcs. Le troupeau sélectionné pour le profil sérologique était négatif pour l'infection à *Actinobacillus pleuropneumoniae* et démontrait des signes cliniques associés à *A. suis,* chez les porcs de 16 à 19 semaines d'âge. Des prises de sang ont été effectuées sur une cohorte de 20 porcs à 5, 8, 12, et 16 semaines d'âge. Le niveau d'anticorps le plus bas a été observé entre 8 et 12 semaines d'âge, reflétant vraisemblablement une baisse de l'immunité d'origine maternelle. Une augmentation importante du taux d'anticorps est survenue à 16 semaines d'âge, approximativement au moment où les signes cliniques étaient observés dans ce troupeau. L'administration d'un autovaccin aux cochettes a induit une augmentation du taux d'anticorps même si celui-ci était déjà élevé avant la vaccination. L'amplitude de la réponse à la vaccination était d'autant plus élevée que le taux d'anticorps était faible avant la première vaccination. Le test ELISA semble détecter la chaîne O des LPS.

***Actinobacillus suis* / autovaccin / ELISA / profil sérologique / porc**

1. INTRODUCTION

Actinobacillus suis is considered as a commensal opportunistic pathogen in pigs [9, 13, 14]. In recent years, the prevalence of problems associated with *A. suis* infection has increased in North America, especially in high-health-status herds [8, 11, 14]. In young animals, *A. suis* is responsible for acute and rapidly fatal septicemia or localized infection such as endocarditis, polyarthritis, respiratory distress or neurological disturbances [10, 11, 14]. In older animals, clinical signs are rare, but fever, anorexia, abortion, cough, pneumonia, erysipelas-like lesions, and acute death have been reported [10, 13, 14].

Pore-forming protein toxins belonging to the RTX (repeats in the structural toxin) group of toxins, very similar to APXI and APXII of *Actinobacillus pleuropneumoniae* [1, 3, 6], have been suggested as potential virulence factors. *A. suis* isolates from healthy and diseased pigs are similar [18]. However, a recent study has demonstrated differences among strains in two of the major surface antigens, the capsule and the LPS, with more than one type of LPS O chain with common core being found [15].

Despite the fact that *A. suis* is sensitive to a wide range of antibiotics [10, 14], the rapid onset of clinical signs makes effective treatment difficult [18]. Currently, no serodiagnostic tests or vaccines are commercially available to help controlling *A. suis* infections. Autogenous vaccines are sometimes used in herds with clinical problems, but their efficacy has not been critically evaluated so far [16].

The purpose of this study was to develop and standardize an experimental strain-specific ELISA for the detection of serum antibodies against *A. suis*, and to evaluate the serologic profile in pigs from a herd showing clinical signs, as well as the serum antibody response to an autogenous vaccine against *A. suis.*

2. MATERIALS AND METHODS

2.1. Animals

A cohort of 20 pigs was selected from a herd having clinical problems associated with *A. suis* infection. In the selected herd, respiratory problems and sudden deaths were occurring in 16 to 19-week-old pigs. The herd was known to be serologically negative for all serotypes of *A. pleuropneumoniae* as routinely tested by a recognized ELISA test [4].

The antibody response to an autogenous vaccine was evaluated in gilts newly introduced into a breeding herd. Gilts were randomly divided into two groups: a vaccinated group ($n = 36$) and a control group ($n = 29$).

2.2. Autogenous vaccine

An autogenous vaccine (bacterin) was prepared by a commercial laboratory (Gallant Custom Laboratories Inc., Cambridge, Ontario, Canada) with two field isolates of *A. suis* (AS1 and AS2) recovered from the affected herd. Since this herd presented some clinical problems due to *Haemophilus parasuis*, a field isolate belonging to this bacterial species was also included in the vaccine. A placebo solution was prepared with only the *H. parasuis* strain. Superfos alhydrogel with [Al_2O_3 – 2% and Al [OH_3] – 3%] was used as the adjuvant and added to a final concentration of 25% for both solutions. The treatment group was injected intramuscularly in the neck with 2 mL of the autogenous vaccine, whereas the controls were inoculated with 2 mL of the placebo solution. Each animal received a second injection two weeks later.

2.3. Blood sampling

For the serologic profile, blood samples were collected from each pig at 5, 8, 12 and 16 weeks of age. For the evaluation of the antibody response to the autogenous vaccine, blood samples were collected from each animal before the first injection and 2 and 5 weeks after the first injection.

2.4. ELISA

An indirect ELISA was adapted and standardized for this study from a method described by Gottschalk et al. [4]. Since preliminary results showed no significant serological differences when either of the two isolates were used for the antigen preparation (results not shown), the field isolate AS1 was chosen as the source for the ELISA antigen throughout this study. This specific isolate was classified as a serotype 2 ([12], MacInnes, personal communication). Two different antigens were tested in preliminary studies: the whole bacteria as used in the bacterin and a saline boiled extract. Similar results were obtained with both antigens; however, a lower inter- and intra-plate variation was observed with the crude extract, which was subsequently used for the ELISA. For the preparation of the saline boiled antigen, a 6-hour culture was washed off the plates of trypticase soy agar (Difco Laboratories, Detroit, MI, USA) with 0.85% (w/v) saline containing 0.5% (v/v) formalin. After overnight storage at 4 °C, the bacterial suspension was spectrophotometrically adjusted to a concentration of approximately 1×10^9 CFU·mL^{-1} (confirmed by viable count), boiled for 1 h, centrifuged and the supernatant was collected and filtered on a 0.22 µm pore size filter (Millipore Corp., Bedford, MA, USA). Each well of U-bottomed styrene plates (Corning Costar Corporation, Cambridge, MA, USA) was coated with 50 µL of the saline extract diluted 1/100 in carbonate buffer (pH 9.6). Plates were incubated overnight at 4 °C, drained and washed with phosphate buffer saline (PBS) containing 0.05% of Tween 20 (Sigma Chemical, St. Louis, MO, USA) (PBS-T20). Sera were diluted 1/800 in PBS-T20 and 50 µL of this dilution were added to each well. Serum of a specific pathogen free (SPF) pig and serum from a pig hyperimmunized with the autogenous vaccine were used as the negative and positive controls, respectively, and added to each plate. Plates were incubated 30 min at room temperature. A volume of 50 µL of commercial horseradish peroxidase conjugated goat anti-swine IgG heavy and light chains (Jackson Immunoresearch Laboratories Inc., West Grove, PA, USA), diluted 1/3000 in PBS-T20, was added to each well for 1 h at room temperature. For the

visualisation, 100 µL per well of 2.2'azino-bis (3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) (Sigma Chemical) dissolved in 0.05 M citrate buffer (pH 4) with 0.5 M $H_2 0_2$ were added. The absorbance was measured at 414 nm on a kinetic microplate reader (Molecular Device, Menlo Park, CA, USA) after 15 min of incubation at room temperature. Results were reported as S/P ratios (the optical density obtained for each serum divided by the optical density of the positive control). All sera from an animal in the vaccination trial or serologic profile were tested on the same plate. Each serum was tested at least three different times and the average of ELISA S/P ratios was accepted only when the coefficient of variation within the plates was below 10%. The ELISA was used as a tool to allow comparison of antibody levels in different animal groups and was not used for diagnostic purposes.

2.5. Immunoblotting

Material (whole cell bacteria used in the bacterin and the crude extract used in the ELISA test) separated by SDS-PAGE [7] was transferred from the slab gel to nitrocellulose using the methanol-Tris-Glycine system of Towbin et al. [17]. Electroblotting was performed in a transblot apparatus (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada) at 30 V for 16 h. Unreacted sites on the nitrocellulose paper (NCP) were blocked with a 2% (w/v) solution of casein in Tris-saline (CTS, 10 mM Tris-HCl, 0.9% NaCl pH 7.4) for 1 h at room temperature. The NCP was then incubated with an appropriate dilution of the positive or negative control serum in the same buffer for 2 h at room temperature and washed with CTS. Rabbit and anti-porcine immunoglobulin (Jackson Immunoresearch Laboratories Inc.) conjugated to horseradish peroxidase in CTS buffer was then added and the mix incubated at room temperature for 90 min. After several washings in CTS, the reacting bands were visualized using the method of Hawkes [5] using 10 mL of 0.3% (w/v) H_2O_2 in CTS.

2.6. Statistical analyses

The effects of age on S/P ratios of the animals in the serologic profile were evaluated with a repeated measures ANOVA with age as a within-subject factor (SAS v. 6.12, Cary, NC, USA). Animals were then divided into two groups according to their S/P ratio at 5 weeks of age; the level of measured antibodies were compared using a repeated measures ANOVA with age as within-subject factor and group as a between-subject factor. A similar repeated measures ANOVA was used to examine the effect of vaccination. Tukey's post-hoc tests were used to examine differences between pairs of means. Finally, differences in the magnitude of the response to the vaccination were analyzed using a linear regression model. Level for statistical significance was set at 0.05.

3. RESULTS

3.1. Serologic profile

A significant effect of age was found on S/P ratios. Animals in the cohort of 20 pigs had an average ELISA S/P ratio of 0.4 at 5 weeks of age. The S/P ratios subsequently decreased at week 8 and 12 to attain the lowest S/P ratios and increased significantly at 16 weeks of age (Fig. 1A). When an arbitrary cut-off of 0.35 was applied to animals of 5 weeks of age, a significant effect of age and group on S/P ratios and a significant interaction between age and group were found, suggesting that the effect of age on S/P ratios was different in the two groups. A total of 7 animals presented a S/P ratio lower than 0.35 at 5 weeks of age. These pigs did not show a decrease in the level of antibodies at week 8 or 12 (Fig. 1B) and the S/P ratio stayed stable at approximately 0.2. For

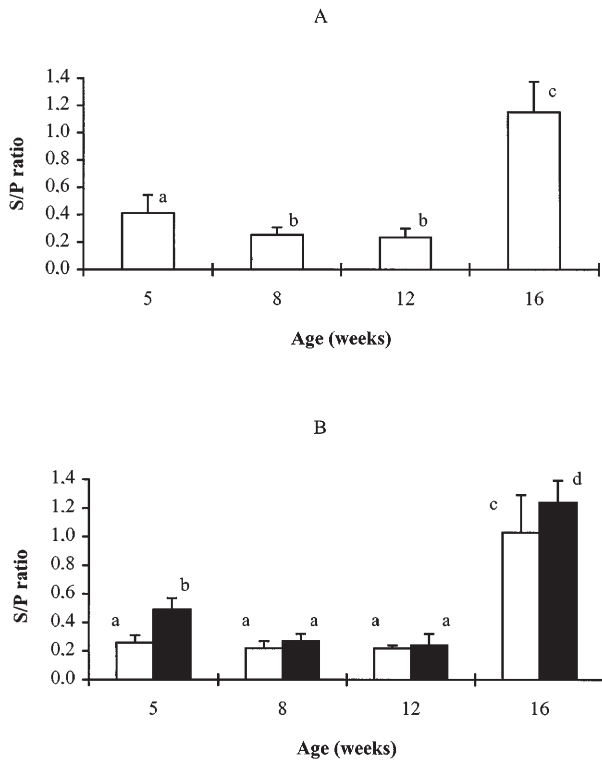

Figure 1. Average ELISA S/P ratios and standard deviation for *Actinobacillus suis* obtained at 5, 8, 12 and 16 weeks of age for a cohort of pigs (n = 20) (A). Average S/P ratios for pigs with a S/P ratio at 5 weeks of age ≤ 0.35 (n = 7, white band) and > 0.35 (n = 13, black band) (B). S/P ratio = optical density obtained for serum by ELISA divided by the optical density of the positive control. Different superscripts are statistically significant at $P < 0.05$.

the 13 other pigs, a significant decrease in the antibody level was observed at 8 or 12 weeks of age, reaching a level of 0.2 similar to the one of pigs having a S/P ratio lower than 0.35 at 5 weeks of age (Fig. 1B). Later, at 16 weeks of age, the two groups showed a marked and significant increase in the antibody response.

3.2. Serum antibody response to an autogenous vaccine

In both treatment and control groups of gilts, the antibody level was already high prior to the vaccination, with S/P ratios often greater than 1. During the experiment, the average S/P ratios in the control group remained stable (Fig. 2), whereas those of the vaccinated gilts increased significantly after the first vaccination and stayed high until the end of the experiment (Fig. 2).

In the vaccinated group, the magnitude of the response to the vaccination was smaller for the gilts showing a higher S/P ratio prior to the administration of the first dose of the autogenous vaccine. This phenomenon was not observed in the control group (Fig. 3).

Immunoblotting using an ELISA-positive serum from a vaccinated animal and both, the whole bacteria (as used in the bacterin) and the saline boiled extract used for the ELISA, revealed a ladderlike pattern in the high molecular weight region with both antigens (Fig. 4). This antigen most probably corresponds to the O-chain LPS, as previously shown by Gottschalk et al. [4] for *A. pleuropneumoniae*. Hence, antigens detected by ELISA were present in both, the bacterin

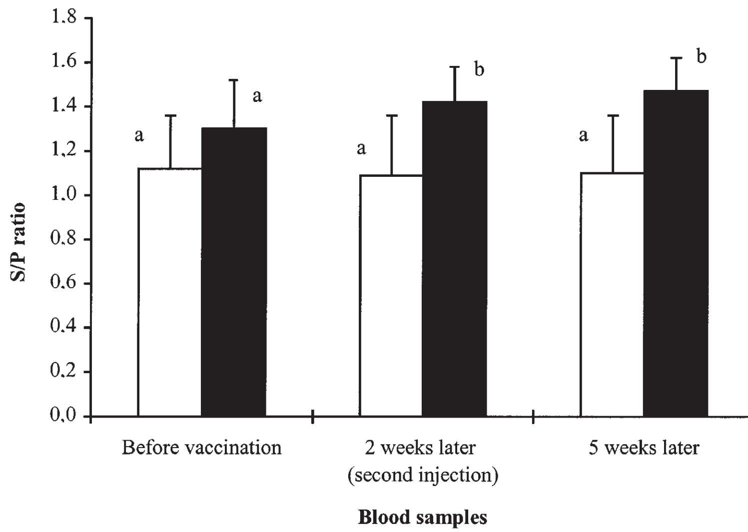

Figure 2. Average ELISA S/P ratios and standard deviation obtained before vaccination and 2 weeks (second injection) and 5 weeks later. Control group (white band) received a placebo ($n = 29$) and vaccinated group (black band) were injected with an *Actinobacillus suis* autogenous vaccine ($n = 36$). S/P ratio = optical density obtained for serum by ELISA divided by the optical density of the positive control. Different superscripts within a group are statistically significant at $P < 0.05$.

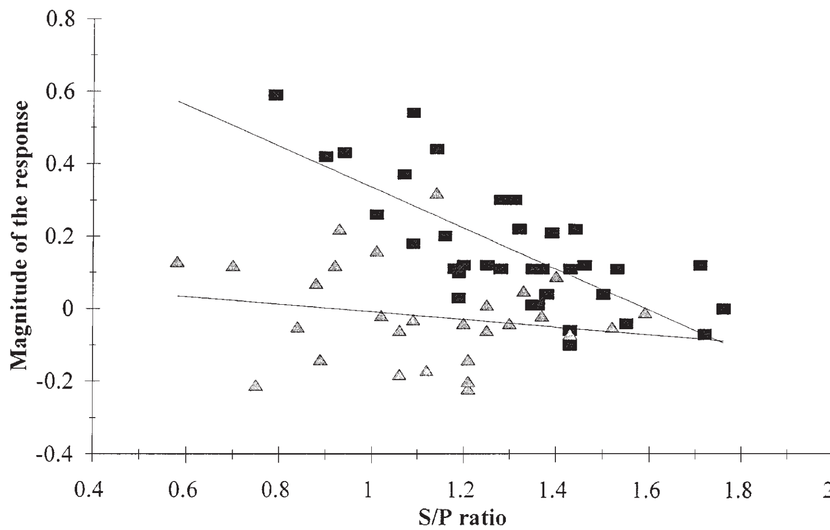

Figure 3. Relationship between the ELISA S/P ratio for *Actinobacillus suis* before the vaccination and the magnitude of the response 5 weeks after injection for the vaccinated group ($n = 36$, ■) ($y = 0.9 - 0.57x$) and control group ($n = 29$, ▲) ($y = 0.096 - 0.1x$). S/P ratio = optical density obtained for serum by ELISA divided by the optical density of the positive control.

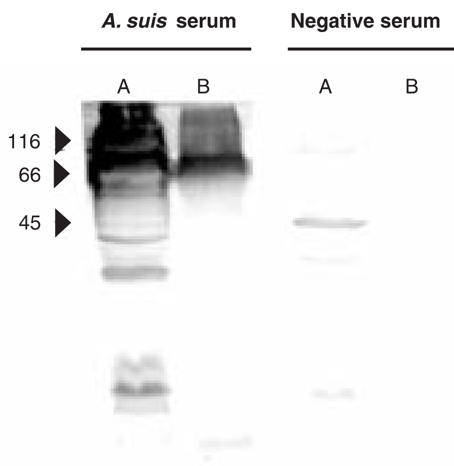

Figure 4. Immunoblot using a swine immunoserum raised against an *Actinobacillus suis* bacterin (field isolate AS1) or a swine negative control serum. Lanes A: whole cell bacteria (bacterin); lanes B: saline boiled extract (used in the ELISA test). Numbers on the left indicate the molecular weights (kDa) of the standards.

(autogenous vaccine) and the saline boiled extract. In addition, other antigens (mainly proteins) which can be observed by immunoblotting with the whole bacteria, were not detected by the ELISA used in this study. With the negative serum, a very few bands (which would also correspond to cross-reacting proteins) were only observed with the whole cell antigen.

4. DISCUSSION

Pijoan [12] reported that early weaning can help control certain infections, but the elimination of early colonizers such as *Streptococcus suis, H. parasuis* and *A. suis* remains questionable with this technique. Colonization by *A. suis* may occur in the first three weeks of age but vary between animals, so not all animals in a population of piglets will be colonized [12]. In such a situation, a vaccination program may be justified. Serologic profile could be used to determine the duration of passive immunity in a specific herd in order to help implementing the best vaccination strategy. The serologic profile of a cohort of 20 pigs revealed that the lowest S/P ratios were observed at weeks 8 to 12, corresponding to a decrease in maternal immunity. A marked increase in the antibody response was seen at week 16, at the approximate time of onset of *A. suis* clinical signs in this herd, which generally occurred between 16 to 19 weeks of age.

In 35% of the pigs of this cohort, the S/P ratio was low at 5 weeks of age and no further decrease in the level of serum antibodies was observed, suggesting that the level of passive immunity was already low for these pigs at that age. In contrast, 65% of the pigs had S/P ratio greater than 0.35 at 5-week-old and a decrease in passive immunity was subsequently observed, reaching at week 8 or 12 a level similar to the pigs having the lowest S/P ratio at 5 weeks of age. Possible explanations for lower or higher level of passive immunity in piglets are differences in level of immunity in sows or in absorption of antibodies by piglets. These results indicated that subpopulations of 5 week-old pigs with high and low maternal immunity were present in the infected herd.

The antibody response to an autogenous vaccine in gilts was assessed in a herd where the control strategy for *A. suis* was to vaccinate gilts and sows. The level of serum antibodies was already high in the control and treatment groups prior to the first injection, but varied considerably among animals, indicating that subpopulations were also present in that breeding herd. The use of the autogenous *A. suis* vaccine increased the specific humoral immunity of gilts, especially when the level of antibodies was low prior to vaccination. This suggests a possible interference between the natural active immunity present in herd and the vaccine. However, the use of an autogenous vaccine in a herd could help stabilize antibody levels in the whole population by increasing

humoral immunity of animals with lower levels.

Results of serologic profile and vaccination trial revealed that subpopulations were present in the two herds involved. The presence of subpopulations is a factor in the maintenance of bacterial transmission in herds with problems. Exposing all members of a population to the agent by an effective and strategically applied vaccination program may help produce more consistent immunity among animals and control transmission [2].

Different vaccination strategies could be adopted depending on the age of onset of clinical signs in herds involved. In this particular case, the vaccination strategy was, at the time, implemented to control clinical signs in young pigs. When problems occur in piglets, vaccination of sows may be a strategy because maternal immunity will then protect the pigs at the expected time of clinical signs. When clinical signs appear at a later stage, passive immunity may not protect and vaccination of piglets should then be considered.

The ELISA test used in this study should not be considered as being specific for *A. suis* and it can not be applied for diagnostic purposes. However, an ELISA test which is able to detect variations in the antibody titers of different groups of animals of a single herd against a bacterial pathogen, such as the one developed in this study, may represent a good alternative for the study of the kinetics of antibodies when an autogenous vaccine is to be used and thus can help when implementing a rational vaccination strategy. As used in this study, the ELISA test detected antibodies against the O-chain LPS which seems to be present in the bacterin.

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