

Protection of pigs against experimental *Salmonella* Typhimurium infection by use of a single dose subunit slow delivery vaccine.

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Abstract

Infections caused by septicemic strains of *Salmonella* are significant animal health as well as food safety concerns for the North American swine industry. Among the various strategies to control these infections at the herd level, development of vaccines are attractive alternatives. In this study, based on previous studies of immune response to various proteins following natural and experimental infections of pigs by *Salmonella*, we designed a subunit slow delivery vaccine and tested it in an experimental model of infection. The selected immunogenic protein was cloned and purified by chromatography. The purified protein was then incorporated in PLGA (a polymer that is slowly degraded within the animal's gastro intestinal system) microspheres and given orally once to groups of pigs (n=8) while control animals (n=8) received only PBS. Animals were challenged orally 4 weeks after the vaccination with 10^8 cells of a virulent strains of *Salmonella* Typhimurium. Animals were examined twice a day and clinical signs evaluated using a predetermined scoring grid. Pigs were sacrificed 12 days later and bacterial cultures of various organs, electron microscopy and evaluation of IgA response by ELISA were performed. No significant difference was found at bacteriology and ELISA but marked differences in clinical signs were observed between vaccinated and non vaccinated animals. None of vaccinated animals showed fever exceeding 40°C while it was observed in 5 out of 8 non vaccinated. Only one of vaccinated pigs showed mild diarrhea while severe diarrhea was observed in all control animals. Different sizes of microspheres were observed in intestinal crypts of vaccinated animals at electron microscopy. We concluded that this vaccine can protect pigs against clinical signs associated with experimental infection by *Salmonella* Typhimurium.

Introduction

Although *Salmonella* is, in pigs, most often associated with sub-clinical infections, the Typhimurium serovar can cause severe clinical signs such as septicaemia and may as well result in mortalities that can have significant economical impacts in affected herds. This serovar is often among the most important serovar recovered from humans and it is therefore critical to reduce the number of affected animals. When herds are affected by clinical outbreaks of *S. Typhimurium*, it was shown that the bacteria is present in most animals/pens and environmental samples (Letellier and al 1999), increasing the likelihood of meat contamination. It is therefore important to control these infections at the herd level both for productivity and food safety point of views. Since the host-adapted serovar *Choleraesuis* was in the past, and is still, associated with similar clinical signs, most vaccines, live or autogenous, were developed to protect pigs against this serovar. Nevertheless, a few live vaccines were proposed to reduce *S. Typhimurium* clinical signs (Roesler et al, 2004). In this study, we report the development of a sub-unit slow delivery vaccine against *S. Typhimurium* in pigs and results of a pre-clinical protection trial using an experimental model of infection.

Material and Methods

Selection of immunogenic proteins. The protein to be included in vaccine was selected by western blots using various strains of *S. Typhimurium* as antigens and antisera. Bacteria were

own in different conditions (low and high osmolarity, low iron, low pH, ...) to ensure that the targeted protein would be expressed in various phases of infection. The antisera used to detect immunogenic proteins were recovered from over a hundred of animals that survived episodes of clinical salmonellosis in various herds. The selected protein (p 37) was recognized by all antisera and was expressed in all types of growth conditions.

Purification of protein. The selected protein was sequenced and cloned into *E. coli* M15 using pRSET2.1::gapAREZ as vector. Using the histidine tail the protein was purified by affinity chromatography (FPLC).

Microspheres production. The selected matrix was a co-polymer of (poly (DL-lactic-co-glycolic) acid) (PLGA), a non toxic, non teratogenic, FDA approved molecule. The delivery rates are controlled by the relative proportion of copolymers. The end products of this polymer are CO₂ and H₂O. Moreover, it is known that 11 µm and less microspheres are well absorbed by intestinal mucus at Peyer's patches level (Tabata et al., 1996). This compound also possesses adjuvant properties (Igartua et al., 1998). The P37 protein was incorporated into PLGA in a pre-determined ratio with co-incorporation of albumin to improve P37 incorporation rates. The resulting microspheres were lyophilized until the protection trials.

Protection trial. Groups of 8 cross-bred *Salmonella* negative pigs were administered orally 2 cc of microspheres or PBS (control pigs) at 3 weeks of age. At 7 weeks of age, animals were given orally 10⁸ cells of a virulent *Salmonella* Typhimurium DT 104 strains. Pigs were examined twice a day for 12 days using a pre-determined evaluation grid (the sum of clinical signs scores and diarrhea scores (on 4 levels of severity each) and then sacrificed. At necropsy, bacterial cultures and *Salmonella* counts in feces and internal organs were performed. Washes of intestinal mucus were also performed and IgA production was assessed using an ELISA, adapted from a previously described procedures (Côté et al, 2004). Finally, electron microscopy was done on small intestines of necropsied animals to check for the presence of microspheres in intestinal crypts.

Results

Animals that received the microsphere vaccine had clearly less clinical signs compared to the control group (figure 1). Only one of vaccinated animals showed a mild diarrhea while diarrhea, when severe, was observed in all control animals. None of vaccinated animals had temperature over 40° C while it was observed in 5 out of 8 control pigs. Although a trend to higher IgA levels was observed in vaccinated pigs, no statistical difference was observed between to groups of animals. Electron microscopy revealed the presence of microspheres in intestinal crypts and Peyer's tonsils 2 days after immunization (data not shown) and 12 days after the experimental challenge (figure 2), more than 5 weeks after vaccination. It was not possible to observe a statistically significant reduction of bacterial counts in tissues of both groups of animals.

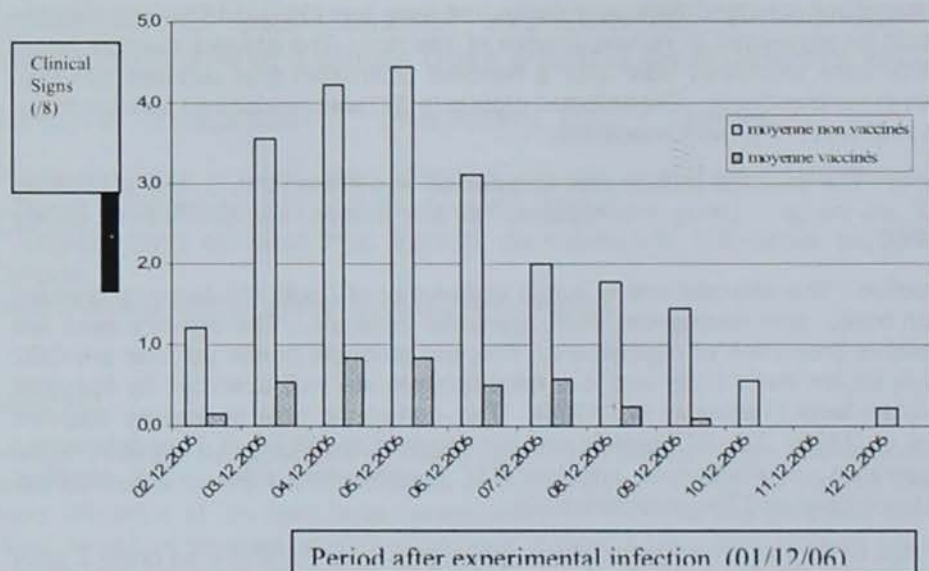


Figure 1. : Mean scores of clinical signs observed in pigs vaccinated (green) with P37 incorporated PLGA microspheres following a challenge with *Salmonella* Typhimurium DT104 strain compared to control group (in yellow).

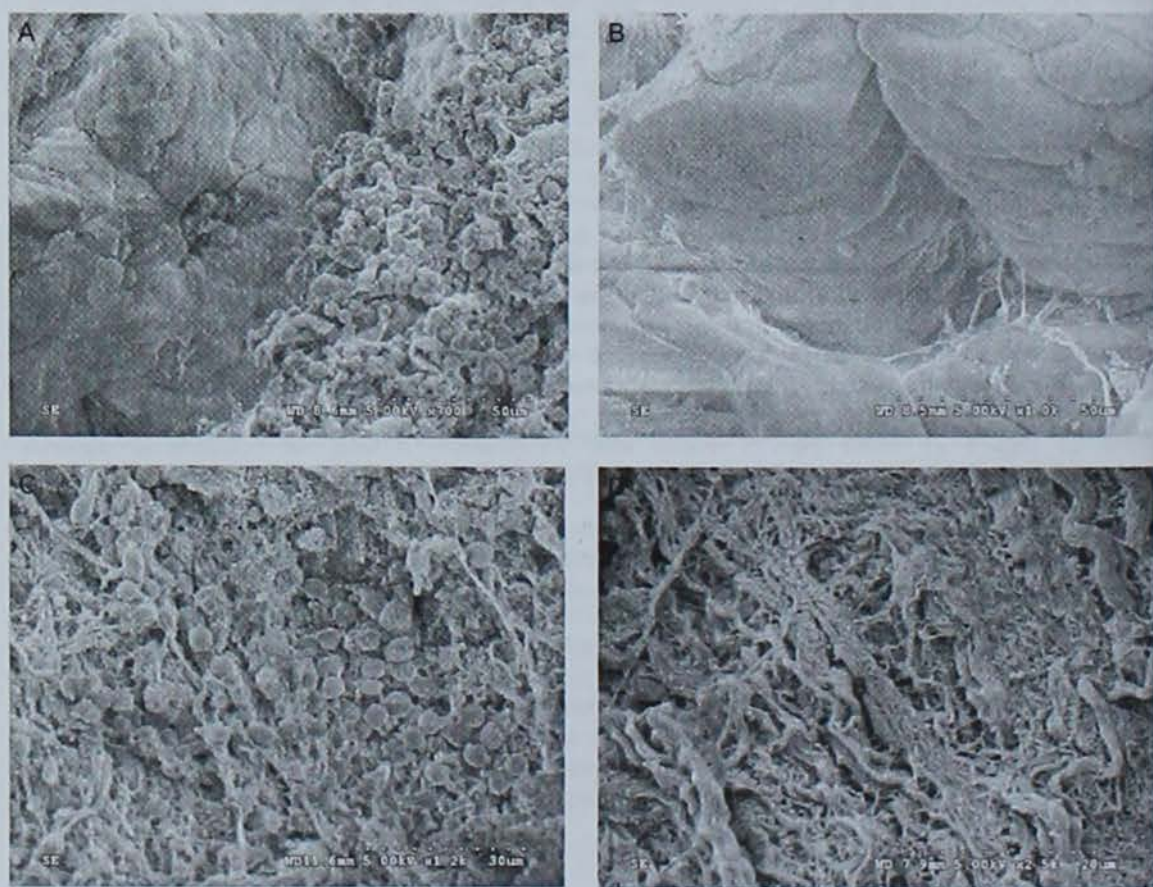


Figure 2. Electron microscopy of intestinal crypts (A) and palatine tonsils (C) of vaccinated and control pigs (B,D)

Discussion

Use of sub-unit vaccines can be beneficial to avoid undesirable effects seldom seen with live vaccine. In addition, when the selected antigen is embedded within an appropriate matrix, it may be delivered into the host gradually, avoiding repetitive vaccine administrations. Results obtained in this study suggest that co-polymer of PLGA can be used to protect immunogenic proteins in swine and to slowly deliver the antigens within the intestinal tract. Indeed, even 5 weeks after vaccine administration, it was possible to observe microspheres in the intestinal tract of pigs suggesting that the vaccine will be efficiently delivered for a prolonged period of time. Moreover, the fact that the end products of these compounds are non toxic would eliminate any concern related to food safety.

Since relatively high dosages of bacteria were used to ensure reproduction of clinical signs and given the low numbers of animals that were used, it was not unexpected to not observe significant difference in bacterial counts in various organs. Further studies with different experimental designs will have to be conducted to assess the ability of this vaccine to reduce shedding of *Salmonella* or to assess cross protection against other serovar.

Conclusion

Use of a subunit slow delivery vaccine composed of the P37 protein embedded within PLGA microspheres succeeded in protecting animals against an experimental infection by *Salmonella* Typhimurium. It is suggested to conduct further research to assess the efficacy of this vaccine to protect animals against the disease in field conditions or to reduce the carriage in sub-clinical infections.

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