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Evaluation of the efficiency of novel orally administered subunit vaccine to reduce the prevalence of Salmonella in swine under field conditions

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Introduction

Control of *Salmonella* sp. in swine production undergoes a systemic vision of the problem, and an integrated program focused on the main stages of production. Control measures at the stage of primary animal production are required for a reduction in the number of carrier and shedders animals that reach slaughter. Among the various tools available, vaccination is a traditional and consolidated concept in preventive veterinary medicine.

Salmonella sp. has on its surface large antigenic molecules (immunodominant molecules), membrane LPS, which are easily recognized by the immune system, and are the target of most line vaccines. These molecules tend to be specific to a particular serovar and / or serogroup (Arguello et al., 2012), and vaccines offering limited protection against heterologous serovars (Bearson et al., 2016).

To contribute to the solution of this problem, the aim of this research was to evaluate a subunit vaccine, based on secondary antigens, where a common genetic sequence for all *Salmonella* sp. was cloned into an expression plasmid, and inserted into *Bacillus subtilis*, which produced subunits (peptides) that were incorporated by microparticles, composing the mucosal vaccine. In order to be effective in controlling any serovar of *Salmonella* enterica (broad spectrum).

Material and Methods

The field trial was carried out on 20 swine fattening unit (pens held 10-20 pigs), belonging to the same agroindustrial integration system. The experimental unit was the swine batch, of which 10 were vaccinated (vaccinated group-VG) and 10 controls (control group-CG).

Two mL of the vaccine were orally administered at four ages. After the second dose of the vaccine, blood was collected with anticoagulant (n=32/group). Blood samples were collected during the first week of fattening (n=30/batch) and slaughter (n=30/batch). Mesenteric lymph nodes-MLN (n=30/batch) and faeces (n=20/batch) were collected at slaughter. Serological analysis was performed using a commercial-ELISA (Herd Check Swine *Salmonella*®IDEXX Laboratories, ME, USA), tested in three cut-off points (S/P relation, 10%, 20%, and 40% of optical density-OD).

The MLN and faeces were submitted to *Salmonella* isolation (ISO 6579: 2002), and the quantification, by most probable number technique- mNMP, following the ISO/TS6579-2:2012. The vaccine ability to induce phagocytic cells was evaluated. All statistical analyses were performed using commercial software SAS® 9.3: 2012.

Results

The group effect was not significant (p> 0.05) in any collection period for the two variables, the seroconversion at different cut-off points and the mean optical density. At slaughter, the isolation of *Salmonella* sp. from MLN in VG (115/300; 38.33%; IC 95%) presented a higher percentage than CG (90/300; 30%; IC 95%). The excretion of the agent in the faeces also had a significant group effect on the isolation of *Salmonella* sp. lower in CG (47/199; 23,62%; IC 95%) than in VG (66/200; 33%; IC 95%).

The quantitative method, mNMP was used to estimate the amount of *Salmonella* sp. positive isolates of faeces. There was statistical difference between the groups, VG presented a greater percentage of isolation. The CG was 0.07 (± 0.04) log NMP/g, while the VG ranged from> 0.16 to 0.06 log NMP/g. The F test of the analysis of variance detected a significant effect (p < 0.05) for the group in the faeces NMP.

Through the flow cytometry results it was possible to demonstrate that the activity of the phagocytic monocytes was altered by vaccination (p=0,067).

Discussion and Conclusions

The VG showed higher frequency of detection of *Salmonella* sp. than the CG, with a difference of 8.33% of carriers of *Salmonella* sp. in the MLN, 9.38% of shedders swine and 0.09 log in the faeces colony forming unit NMP at slaughter.

In addition to the effect of vaccination under carriers and shedders of *Salmonella* sp. was performed the immunological evaluation of the swine front of vaccine. It is known that the destruction of microorganisms phagocytosed by macrophages is due to the production of nitric oxide (NO) and other intermediates, which are produced due to the classic (Th1) activation of the macrophages through IFN-γ or LPS (Classen, Lloberas, and Celada, 2009). However, for intracellular bacteria, such as *Salmonella* sp., the ingestion of these by macrophages can provide a safe haven, protecting the bacteria from complement-mediated extracellular death. Eze et al. (2000) demonstrated that the virulent strain 16M of *Brucella melitensis* was efficiently phagocytosed by mouse peritoneal macrophages in the presence of

hyperimmune anti-LPS serum of *B. melintenis*. Once internalized, the bacterium multiplied efficiently in non-activated macrophages, and its elimination occurred only when the activation of macrophages by IFN-γ was induced. In this study, when evaluating all farms together, an increase in the phagocytic activity of peripheral monocytes was found in VG. Despite this, the data do not allow to infer if this increase of the phagocytic activity resulted in the effective direction of field strains by macrophages, or whether these cells have potentiated the multiplication of the pathogen serving as a replication site. The results of isolation in the faeces, MLN and mNMP point to the second hypothesis, once percentage of detection of *Salmonella* sp. was higher in the vaccinated group than in the control group.The vaccine tested had no effect on the seroprevalence of batches at the time of slaughter. It was concluded that the vaccination program with the oral subunit vaccine did not confer a reduction in the spread and amplification of infection on the farms that had an impact on the prevalence of swine carriers and shedders of *Salmonella* sp. at slaughter. These results allow us to state that the form of presentation of the antigen in the vaccine has not yet been sufficient to stimulate immunity that could withstand the field challenge.

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