13th SafePork 2019

ONE HEALTH – Tear down interdisciplinary walls

BERLIN | GERMANY

26–29 AUGUST 2019

Proceedings

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PRE-HARVEST FOOD SAFETY CONCEPTS

P1

Water pipe deposits in swine nursery units as a possible reservoir of Salmonella?

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Introduction

The quality of drinking water is crucial for the health, welfare and performance of swine. As a consequence of poor water quality, undesirable substances as well as microorganisms can be introduced into the food chain. The farmer himself is responsible for ensuring that water is suitable for animal nutrition in accordance with legislation and that technical installations are sufficient, so that the risk of water contamination is minimized. So far, there is neither a guidance for risk assessment according to inorganic and organic deposits nor biofilms in drinking water installations on farms. It is known, that components in water originating from deposits/ biofilms can cause a bad taste of drinking water. Hence, this might lead to a decreased uptake of water by the pigs. It is also discussed that biofilms might be a reservoir for pathogens.

Materials and Methods

Deposits in drinking water installations in 15 piglet rearing farms were sampled and analyzed for their physical, chemical and microbiological characteristics. Based onto results from analysis of deposits from the first five farms, a practical approach for a risk assessment on farms was elaborated and tested on ten farms. Deposits were classified with respect to their inorganic proportion and by microbiological culture methods. Different cleaning concepts were tested under laboratory conditions on the respective pipes containing farm-specific deposits.

Results

In four farms *Escherichia coli* and *Salmonella enterica* (predominantly *S.* Typhimurium var. Copenhagen) were isolated in a number of biofilms from water pipes. The antibiotic resistance patterns of respeceive isolates were compared with those from isolates originating from routine samples or from those reported in literature. Cleaning concepts based onto alternating applications of basic and acid cleaning substances combined with mechanical flow impulses were successful to remove most of the deposits.

Discussion and Conclusion

Inorganic deposits and biofilms are farm-specific with a high variation between farms depending on water origin, pipe installation, dosage of substances by water, technical devices and operation. The results of the study suggest, that water pipes might be a reservoir for zoonotic *Salmonella* strains and that pigs consuming faecally contaminated drinking water are at risk to be infected. Furthermore Salmonella detection may be of importance for the prevalence of seroreagents in the context of salmonella monitoring. The fact, that pathogens were most frequently detected in the periphery of the pipeline system near to the drinkers, suggested that predominantly a retrograde bacterial contamination from drinkers takes place on farm. In addition the resistancey patterns and the minimal inhibitory concentrations of antimicrobial substances of the potentially pathogenic microorganisms did not differ from those reported in other studies or routinely tested. If a high load of *E. coli* or *Salmonella* is detectable in water pipes of nursery systems, water origin, pipe installation and drinker technique should be checked and a pipe cleaning procedure might be recommendable.

Acknowledgement

The authors thank the Federal Ministry for Economic Affairs and Energy (BMWI) for the financial support under the German Federation of Industrial Research Associations (AiF) for the research project “drinking water hygiene” IGF-Project Number 18952N/2.

P2

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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P3

Lipid-caused antagonism of the bactericidal activity of thymol and thymol-β-D-glucopyranoside is not overcome by emulsifiers

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Introduction

Strategies are sought to reduce the carriage and dissemination of zoonotic pathogens and antimicrobial resistant microbes within food-producing animals and their production environment. Thymol is an essential oil shown to be a potent bactericide *in vitro* but demonstration of its efficacy when fed to animals has been inconsistent, due largely to its lipophilicity which limits its passage and subsequent availability in the distal gastrointestinal tract. Conjugation of thymol to glucose to form thymol-β-D-glucopyranoside can decrease absorption of the conjugate, thereby promoting passage to more distal intestinal sites where pathogens primarily reside, yet *in vivo* efficacy of the conjugate remains suboptimal. It is possible that hydrolysis and absorption of thymol-β-D-glucopyranoside and free thymol may still have been rapid enough within the proximal small intestine to preclude their delivery to the cecum and large intestine. Considering that modern swine diets often contain 5% or more fat, we hypothesized that even at 60 to 80% apparent digestibility there may be passage of enough residual undigested lipid to the distal intestinal tract to sequester free or conjugated thymol within lipidic microenvironments, thereby limiting the availability and subsequent effectiveness of these biocides.

Material and Methods

Freshly voided feces collected from 25 kg conventionally-reared pigs maintained on unmedicated feed were mixed (0.5% wt/vol) with ½-strength Mueller Hinton broth prepared under 100% N_2 gas. Fecal suspensions were then inoculated with novobiocin- and nalidixic-acid resistant (NN-resistant) challenge strains of *Salmonella enterica* serovar Typhimurium (NVSL 95-1776) or *Escherichia coli* K88 to achieve initial concentrations of approximately 10^6 colony forming units (CFU)/mL. The ½-strength broth was used to avoid excessive acid production within the fecal suspensions and the NN-resistant inocula, grown overnight at 37°C in tryptic soy broth supplemented with 25 µg of novobiocin/mL and 20 µg/nalidixic acid mL, were used to facilitate recovery and differentiation of the challenge strains from indigenous fecal microbes. The resultant suspensions were distributed (5 mL/tube) under a constant flow of 100% N_2 gas to 18 x 150 mm crimp top tubes that had been preloaded with or without 0.3 mL of vegetable oil and with or without small volumes (≤ 0.5 mL) of a 600 mM stock solution of thymol-β-D-glucopyranoside or thymol, prepared in ethanol, to achieve 6 mM upon addition of fecal suspensions. Control tubes were preloaded with 0.2 mL ethanol. In another experiment, fecal suspensions preloaded as above with oil and thymol-β-D-glucopyranoside were tested without or with additions of bile salts or taurine (0.6 or 8 mg/mL, respectively) added to assess the impact of bile acid-based micelles or their de-conjugation on pathogen survivability. The emulsifying agents Tween 20 or Tween 80 (each at 1% vol/vol) or polyoxyethylene (40) stearate (at 0.2% vol./vol) were also tested to assess their potential impact on the bactericidal activity of thymol-β-D-glucopyranoside. Tubes were closed with stoppers and incubated at 39°C for 24 h. The NN-resistant S. Typhimurium and *E. coli* K88 were enumerated via viable cell count on Brilliant Green or MacConkey agars supplemented with 25 µg novobiocin/mL and 20 µg naladixic acid/mL. Log_{10} CFU of NN-resistant S. Typhimurium and *E. coli* K88 were tested for treatment effects using a general analysis of variance and LSD separation of means. All incubations were conducted with n = 3 experimental units per treatment condition.

Results

The bactericidal effect of 6 mM free or conjugated thymol against S. Typhimurium and *E. coli* K88 are presented in Figure 1A and B. When expressed as log_{10}-fold reductions of CFU/mL, the addition of 3% added vegetable oil decreased (P < 0.05) the anti-*Salmonella* effects of thymol and thymol-β-D-glucopyranoside by 90 and 58%, respectively, compared to CFU reductions achieved during cultures without added oil (6.1 log_{10} CFU/mL). Addition of vegetable oil decreased (P < 0.05) the anti-*E. coli* activity of free and conjugated thymol by 86 and 84%, respectively, compared to reductions achieved in cultures incubated without added vegetable oil (5.7 log_{10} CFU/mL). Inclusion of taurine (8 mg/mL) or bile acids (0.6 mg/mL) had no effect on the antagonist-effect of vegetable oil on the bactericidal activity of thymol-β-D-glucopyranoside (not shown) and this antagonist effect was not overcome by further addition of the emulsifiers polyoxyethylene (40) stearate (0.2%), tween 20 or tween 80 (each at 1%) (Figures 2).