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A biomolecular DIVA-strategy for Salmonella spp. - diagnostics in Swine

Lindhaus H.[1], Högemann M.[1], Bischoff H.[1], Haneke J.[1], Behr K.-P.[1], Menke T.[1], Helmer C.[1]

[1]AniCon Labor GmbH, Höltinghausen, Germany

Introduction

Salmonellosis remains of utmost significance regarding all levels of food production, in particular for public farmers, practitioners, food industry and public health authorities.

Decades ago, Germany together with many other current member states of the European Union agreed on measures to reduce *Salmonella* spp. infections as the cause of foodborne diarrhoea.

Hence, for Swine Production in Germany a monitoring system based on antibody detection was implemented and used to categorize farms in three different categories (low, medium, high number of positive samples). Once a farm enters category 3 (high positive) a plan of action against *Salmonella* spp. infection has to be worked out including adjustments of biosecurity, disinfection, feeding and the vaccination program. In order to accompany these measures antigen-detection is mandatory to verify the success. An evaluation of Salmonella serovars in food samples divided into animal species revealed that in 2015 *Salmonella Typhimurium* (ST) was the dominant serovar related to pig meat stated by Dr. Istvan Szabo at the Symposium "Zoonosen and Lebensmittelsicherheit" in 2016. Next to traditional diagnostic tools for antigen detection like cultivation and typing of isolates, biomolecular techniques have broadened the diagnostic spectrum. This study reviews the traditional diagnostic tools and points towards a new approach regarding the Differentiation of Infected and Vaccinated Animals (DIVA) of *Salmonella Typhimurium* - field strains and *Salmonella Typhimurium* - vaccine strains.

Material and Methods

In total 511 samples were examined over a period of 6 months at the AniCon Labor GmbH. The sample material was divided in faecal and environmental swabs. Furthermore, pigs were sampled 1 week and 2 weeks post vaccination. All submitted samples were derived from pigs which were vaccinated with a commercial modified live vaccine (Salmoporc) produced by IDT Biologika in Dessau. The vaccine strain is a *Salmonella* Typhimurium - Mutant.

All samples were enriched with buffered peptone water (BPW) at 37 degrees for at least 16 hours according to DIN EN ISO 6579-1:2017. After that 1 ml of the BPW were pipetted into a sterile tube. The majority of the 1 ml were implemented in a modified Rappaport - Vassiliadis - Soy Broth and incubated at 42°C for another 24hours. Material of all samples, which indicated bacterial growth in form of a swarming area, was then transferred to a Xylose-Lysin-Desoxycholat-Agar. The next step was the cultivation of a *Salmonella* spp.-isolate on Columbia agar for 24hours at 37°C in order to perform serum-agglutination and further differentiation between field- and vaccine strain using a commercial test kit developed by IDT Biologika GmbH, Dessau; Germany. The commercial test kit is based on the evaluation of bacterial growth characteristic in two selective media and can be performed on cultivated *Salmonella Typhimurium* - strains only.

A smaller aliquot of the 1 ml peptone solution was used to perform biomolecular detection of *Salmonella* spp. with the Kylt® *Salmonella* spp. DNA Extraction and Real-Time PCR Detection Kit according to the manufacturers´ instructions.

Subsequently, all positive samples were further examined with the new Kylt® ST DIVA Real-Time PCR Detection Kit according to the manufacturers´ instructions.

Results

Out of 511 samples 375 samples were negative for *Salmonella* spp. in all detection methods. In 136 out of 511 samples Salmonella spp. was detected by *Salmonella* spp. - Screening - PCR.

I. A successful cultivation of *Salmonella* spp. was achieved in 62 out of 136 *Salmonella spp.* - Screening - PCR positive samples. The isolates were typed by serological agglutination in the following descending order: *Salmonella Typhimurium* (n=44), *Salmonella Ohio* (n=8), *Salmonella Infantis* (n=6), *Salmonella Derby* (n=1) and others (n=2).

Differentiation of the 44 *Salmonella Typhimurium* isolates was performed by using a DIVA-method developed by IDT Biologika, Dessau, Germany. 27 isolates were identified as vaccine strains, 11 isolates were identified as field strains and 8 isolates were not tested.

The biomolecular DIVA-method revealed the following results using the same buffered peptone enrichment as used for the cultivation of the isolates: ST vaccine strain positive (n=24), ST field strain positive (n=9), ST vacc & ST field strain (n=9) and ST not detectable (n=18).

II. Furthermore, a number of 64 positive samples, from which no successful cultivation was possible, remained. The biomolecular DIVA-method showed the following results: ST vaccine strain positive (n=46), ST field strain positive (n=3), ST vaccine strain & ST field strain positive (n=7) and not detectable (n=8).

Discussion and Conclusion

This study reveals the successful attempt to establish a biomolecular DIVA method which is able to differentiate between *Salmonella Typhimurium* - field strains and *Salmonella Typhimurium* - vaccine strains. It can be carried out from faecal and environmental swabs which were enriched in buffered peptone water at 37 degrees for at least 16 hours. Hence, the differentiation could be performed within one day after sample receipt. Furthermore, non-viable as well as viable genome sequences of *Salmonella Typhimurium* could be differentiated. This ST DIVA PCR-method is most successfully used in combination with a *Salmonella* spp. Screening - PCR. Utilising this diagnostic approach would not only decrease the examination costs and speed up the process of result reporting but also increase the sensitivity for the detection of *Salmonella* spp. in pig herds. In summary, the new biomolecular ST DIVA-strategy for *Salmonella* spp. has the potential to be used as a monitoring tool in *Salmonella Typhimurium* vaccinated pig herds.