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Short communication

Epidemiological analysis of the dynamic and diversity of *Salmonella* spp. in five German pig production clusters using pheno- and genotyping methods: An exploratory study

J. Niemann [a,*], E. Tietze [b], I. Ruddat [a], A. Fruth [b], R. Prager [b], W. Rabsch [b], T. Blaha [c], C. Münchhausen [a], R. Merle [a], L. Kreienbrock [a]

[a] Institute of Biometry, Epidemiology and Information Processing, WHO Centre for Research and Training in Veterinary Public Health, University of Veterinary Medicine Hanover, Bünteweg 2, 30559 Hanover, Germany
[b] Robert Koch Institute, Wernigerode Branch, Division Enteropathogenic Bacteria and Legionella, National Reference Centre for Salmonellae and Other Bacterial Enteric Pathogens, Burgstraße 37, 38855 Wernigerode, Germany
[c] Field Station of Epidemiology, University of Veterinary Medicine Hanover, Büscheler Straße 9, 49456 Bakum, Germany

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ABSTRACT

An exploratory study in five conventional pig production clusters was carried out to investigate the dynamic and diversity of *Salmonella* spp. within different production stages and sample site categories (pooled feces, direct and non-direct environment). Observing two production cycles per production cluster, a total of 1276 samples were collected along the pig production chain. Following a microbiological examination via culture, 2246 subcultures were generated out of 285 *Salmonella* positive samples and analysed by pheno- and genotyping methods. Based on a combination of serotyping, MLVA (multiple-locus variable-number tandem repeat (VNTR) analysis), PFGE (pulse-field gel electrophoresis) and MLST (multilocus sequence typing), an amount of 22.3% *Salmonella* positive samples were characterized in clonal lineages and its variants.

Within each production cluster, one main clonal lineage could be identified and persisted over both production cycles with a large diversity of variants and a wide distribution in sample site categories and production stages. Results underline the importance of biosecurity with emphasis on the environment to prevent persistence and circulation of *Salmonella* within herds. Furthermore, the combined implementation of MLVA, PFGE and MLST with conventional culture techniques for isolate classification could be successfully applied as an effective and valuable tool for identifying similar pattern of *Salmonella* occurrence within pig production clusters.

1. Introduction

With salmonellosis as one of the major bacterial food-borne zoonotic diseases the dissemination of *Salmonella* along the food chain is an important ongoing challenge within the scope of consumer protection and public health. Although, various studies focusing on pigs and pork products have been carried out on pre- and postharvest level and many risk factors and preventive measures have been reported over the last years, effective intervention strategies often fail: This is might be due to the incomplete understanding of transmission dynamics in terms of the spatial and temporal distribution of *Salmonella* (Barber

* Corresponding author. Tel.: +49 511 953 7835; fax: +49 511 953 7974.
E-mail addresses: Jana-Kristin.Niemann@tiho-hannover.de, niemannja@aol.com (J. Niemann).

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et al., 2002; Fosse et al., 2009). Although PFGE is still considered as the gold standard for the subtyping of *Salmonella*, the method is limited with regard to being less effective in discriminating closely related strains (Lindstedt et al., 2004; Wattiau et al., 2011). Hence, various other alternative methods to characterize *Salmonella* strains with a higher discriminatory power may be used (Wattiau et al., 2011). However, only a small number of studies additionally utilizing MLVA in their investigations to analyse *Salmonella* strains have been performed so far (Kirchner et al., 2011; Nathues, 2011; Arguello et al., 2013; Prendergast et al., 2013).

Therefore, the aim of this exploratory investigation was to gain a better understanding at the genotype level of the spatial and temporal dynamic and diversity of *Salmonella* spp. throughout the production chain over multiple production cycles. Pooled fecal and environmental samples were collected within farrowing, weaning and fattening and were microbiologically examined for *Salmonella* via culture. By combining serotyping, MLVA, PFGE and MLST, *Salmonella* isolates were assigned to clonal lineages and variants and their dynamic and diversity within each pig production cluster were described.

2. Materials and methods

2.1. Study design

The study was performed from January 2012 to December 2012 in five conventional pig production clusters in Lower Saxony consisting of fattening pig farms including the farms of origin from which they regularly purchase piglets. Beside the willingness of farmers to participate voluntary, each herd was selected due to the following criteria: (1) the results of the German serological *Salmonella* monitoring (seroprevalence) in finishing pig herds (2) previous bacteriological detections on the pig fattening farm and (3) representing conventional pig farms with indoor housing and all-in-all-out production. Hence, the classification of the herds in three *Salmonella* categories is depending on the seroprevalence (category I: 0–20%, II: 20–40%, III: >40%) according to the German legislation (Anonymous, 2007). A number of potential herds with a classification in category II or III were selected according to our criteria and were included in a preliminary investigation to ensure a current occurrence of *Salmonella*. Finally, five pig production clusters with a bacteriological *Salmonella* detection in pooled fecal samples from the fattening unit were selected by chance from the pool of positive ones for the final study. The production clusters A–C represent farrow-to-finish farms with 170 (A and B) and 950 (C) sow units for producing their own pigs for slaughter (A: one location; B and C: multi-site). The production clusters D and E represent fattening pig farms which purchased their pigs in each case from one supplier only (D and E: multi-site). Production system D and E were stocked from suppliers with 300 and 420 sow units, respectively. In system D the weaning period was conducted on the fattening pig farm itself.

2.2. Sampling and sample site categories

The *Salmonella* prevalence on farm was ascertained by analysing environmental and pooled fecal samples. According to Nathues (2011), three sample site categories were defined: (1) *pooled fecal samples* from the pen floor, (2) *the direct environment* which involved defined locations of examined compartments within the range of direct animal contact (pen walls and panels, pen floor, nipple drinkers, feeders, toys) and (3) *the non-direct environment* which included defined locations without a direct animal contact of examined compartments (ventilation, pipes, passageway), farm premises (hygiene sluice, central passageway, animal scale) and mobile vectors (boots of the farmer, guide boards) as well.

For the environmental sampling, sterilized gauze swabs (about 35 cm cotton cloth (Tubular bandage tg®, Lohmann und Rauscher, Germany)) were soaked with buffered peptone water (BWP®, Oxoid Ltd., UK). Predefined locations of environmental samples included swabbed surface areas of approximately 850 cm^2 which were evenly distributed in a sampled compartment or farm accommodation. Surfaces of mobile equipment (guide boards, boots of the farmer) as well as nipple drinkers, feeders and toys were swabbed completely. Pooled fecal samples were taken with a gauze sock (45 cm elastic cotton tube (Tubular bandage tg®)) from each pen within an examined compartment by pacing over the whole pen floor area with one gauze sock pulled over one boot for the sampling process.

2.3. Sampling protocol

Inside each production cluster, two batches of pigs were followed and examined repeatedly within the production stages of farrowing, weaning and fattening. Within a production cycle one compartment on every production stage was included in the sampling process which was based upon eight time points for collecting samples as presented in Fig. 1. A possible residual *Salmonella* contamination of the direct and non-direct environment was checked by collecting samples directly after the cleaning and disinfection procedures in examined compartments previous to re-stocking (sampling times 1, 3, and 5). After re-stocking the compartment with pigs

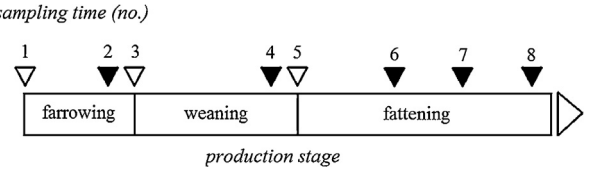

Fig. 1. Graphic presentation of the sampling protocol throughout an examined production cycle including one compartment at each production stage of farrowing, weaning and fattening in five German pig production clusters. The numbers (no.) from 1 to 8 above the white and black arrows are indicating the eight time points for sampling (▽: cleaning & disinfection control of an empty compartment before housing in the animals (nos.: 1, 3 and 5); ▼: sampling of pooled fecal, direct and non-direct environment samples including the examined compartment with animals, farm premises and mobile equipment (nos.: 2, 4, 6, 7 and 8)).