Association of *Salmonella* spp. in slaughter pigs with farm management factors

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Abstract

The objective of the study was to investigate the association of farm management factors with the detection of *Salmonella* in lymph nodes of pigs at slaughter. Samples were collected in German abattoirs in the course of the EU baseline study on *Salmonella* in slaughter pigs from October 2006 to September 2007. A minimum of 5 lymph nodes (≥15 g sample weight) were collected from pigs at slaughter and examined for *Salmonella* according to ISO6579:2003, Annex D. Data were collected using a standardized data capture form to be filled in by the veterinary officials. Additional information was available in a subsample (851/2569) of the pigs included in that study. The association of factors was tested using univariate and multivariate logistic regression. Separate analyses were run for a: *Salmonella* spp., b: specifically for *S.* Typhimurium, the most prevalent *Salmonella* serovar in the study population and c: for all other serovars grouped together.

Overall, 13.5 % of the 851 pigs included in this subset tested positive for *Salmonella* spp.. *S.* Typhimurium was detected in 8.2 % of the samples. Pigs were more likely to be positive for *Salmonella* in summer than in winter (OR 1.54, 1.03-2.30). However, this was only observed for *S.* Typhimurium, not for the other serovars. They were less likely to be positive for *Salmonella* in the south and in the east of Germany than in the Northwest. Use of home mill feed was negatively associated with the presence of *Salmonella* (OR 0.56, 0.37-0.85). Use of antimicrobials during the fattening period was not associated with prevalence of *Salmonella* spp. However, a significant positive association was detected between the use of certain antimicrobials and the prevalence of *S.* Typhimurium. This was not observed for the group of other serovars.

Results suggest that risk factors differ between *S.* Typhimurium and other *Salmonella* spp. and that control strategies should account for these differences. Furthermore, a critical review of antimicrobial use with respect to the interaction with the prevalence of *S.* Typhimurium in pigs is required.

Introduction

Salmonella spp. are among the main causative agents of foodborne infections in Europe (EFSA 2009). In the European Union, *S.* Typhimurium is the predominant serovar among *Salmonella* isolates from pigs and pork (EFSA 2009). This serovar is assumed to be endemic in the pig population and is able to cause persistent infections in pigs (Boyen et al. 2008). It can be introduced into fattening herds by a number of pathways e.g. purchase of carrier pigs, carry over from previous batches due to insufficient cleaning and disinfection or via rodents. Other routes like contaminated feed are also possible. Besides *S.* Typhimurium, a number of other serovars have been identified in pigs and pork.

The purpose of this study was to investigate farm management factors that were associated with the detection of *Salmonella* spp in lymph nodes of pigs at slaughter. More specifically, the study aimed to identify risk factors for *S.* Typhimurium on the one hand and for other serovars on the other hand, to test the hypothesis that risk factors differ between the groups of serovars due to differences in the epidemiology of the serovars.

Material and Methods

In the course of an EU-wide cross sectional study based on Dec. 2006/668/EC, lymph nodes of slaughter pigs were investigated between October 2006 and September 2007. Pigs were selected randomly per

abattoir and per month. The number of pigs to be sampled per abattoir was based on the capacity of the slaughterhouse, i.e. the number of pigs slaughtered at the abattoir during the year 2005. The study included those slaughterhouses that slaughtered 80 % of all slaughtered pigs in each region of Germany. Slaughterhouses were distributed all over Germany. Only one pig per slaughter batch was examined per day. A minimum of five lymphnodes (Lnn. iliocaecales) with a minimum weight of 15 g were collected during slaughter by official veterinarians or people authorised and instructed by the official veterinarians. Samples were transported to the laboratory with 48 hrs and examined for *Salmonella*. Primary isolation was performed at the regional state laboratories according to EN ISO 6579:2003, Annex D. Isolates were confirmed as *Salmonella* spp. and serotyped by the National Reference Laboratory for *Salmonella* at the Federal Institute for Risk Assessment in Berlin, Germany.

To investigate potential factors that might be associated with the presence of *Salmonella* spp. in the lymph nodes, standardized data capture forms were provided to the official veterinarians at the abattoirs. These data capture forms were designed to collect the mandatory information required by Decision 2008/668/EC and to collect further information. A second data capture form was designed to collect data at the farm of origin. Filling of this second data capture form was not mandatory but strictly encouraged by the national and regional authorities. The requested information included production type, farm size, origin of pigs, origin of feed, type of feed, use of an "all in - all out" policy, use of antimicrobials and use of feed additives in feed or water. Information on pig density in the region of origin was collected from official German statistics.

All data were stored in an MSAccess database. Statistical analyses were carried out using SPSS, (Version 12, SPSS, Munich, Germany). To cover the variation in the structure of pig farms in Germany, Germany was divided into three regions for the analysis, namely the North-West, South-West and East. Regions were based on the location of the slaughterhouses. Season was included into the model as a binary variable with winter covering the months October to March and summer covering April to September.

Data were analysed in two steps. In a first step each variable was tested for its association with the three outcomes (positive for *Salmonella* spp., for *S.* Typhimurium or for *Salmonella* other than *S.* Typhimurium resp.). All pigs that were not positive for *S.* Typhimurium were regarded as negative in the latter analysis, even if they were positive for another serovar.

In a second step, all variables that showed an association with $p \leq 0.25$ were included in three logistic regression models with positive for *Salmonella* spp. (no=0 vs. yes=1), for *S.* Typhimurium and for *Salmonella* spp other than *S.*Typhimurium as binary outcomes. In a third step, those variables that did not show a significant association at $p<0.05$ with any of the three outcomes were removed from the final models.

Results

Of the 2569 data sets collected in Germany in the course of the EU-wide study, 851 (33.1 %) could be included in the risk factor analysis. The proportion of positive pigs (13.5 %) in this subset differed only slighty from the results obtained in the full data set (12.7 %)(EFSA 2008). Likewise, prevalence of *S.* Typhimurium in the subsample was similar to the complete sample (8.2 vs. 7.0 %). The regions South-West and East contributed slightly more to the subsample than to the complete sample 22.6 und 21.3 % vs. 17.6 und 15.0 %.

The predominant serovar was *S.* Typhimurium with 70 isolates (61 % of all isolates). Monophasic *Salmonellae* of group B were the second most frequent group of isolates (18 isolates, 15.7 %). *S.* Derby was identified in 8 of the included samples (7.0 %). All other serovars were infrequent, including *S.* Enteritidis (3 isolates).

Only few variables were associated with the outcomes in the univariate analysis. These included three feeding parameters (feeding home mill feed, use of probiotics and use of wet feed), season, region of abattoir, pig densitiy in district of origin, and use of one of the three antimicrobials tetracycline, amoxicillin and colistin.

Pig density in region of origin, wet feed and use of probiotics were dropped out of the multivariate model, because they did not show significant association with any of the three outcomes.

Using home mill mixed feed remained in the model. It was significantly negatively associated with *Salmonella* spp., with an odds ratio of 0.56 (0.37-0.85). The odds ratio was similar in the other two

models, indicating no substantial difference between *S.* Typhimurium and the other *Salmonella* serovars in this respect.
Salmonella spp. were more often isolated in summer (April to September) than in winter, with an odds ratio of 1.54 (1.03-2.3). The association was also observed for *S.* Typhimurium (OR=1.75, 1.05-2.91). However, it was not observed for the other serovars (OR=1.14, 0.62-2.10).
Region of the abattoir was also significantly associated with *Salmonella* spp. with the South-West and the East having lower odds of positive lymph nodes in pigs than the North-West. The difference between the North-West and the South-West was similar for *S.* Typhimurium and the group of other serovars. The difference was not observed between the North-West and the East for *Salmonella* spp. other than *S.* Typhimurium.
The use of the three antimicrobials tetracycline, amoxicillin and colistin was not significantly associated (p=0.113) with the overall prevalence of *Salmonella* spp. and the prevalence of *Salmonella* spp. other than *S.* Typhimurium in the slaughter pigs. This was different with *S.* Typhimurium. Prevalence of *S.* Typhimurium was associated with the use of the three antimicrobials. The odds ratio compared to the reference category (use of non of the three antimicrobials) was highest for amoxicillin, the combination of amoxicillin with either tetracycline or both other antimicrobials and the combination of tetracycline and colistin.

Discussion

There were significant differences between the regions concerning the prevalence of *Salmonella* spp. in the sampled pigs. The population density for pigs and other livestock species is highest in the region with the highest prevalence. A higher population density favours spread of endemic pathogens, as there are more potential shedders and more potential recipients at the same time. This potential role of population density is underlined by the effect of population density in the univariate model. Furthermore, in a region with a high *Salmonella* prevalence, farms purchasing pigs are at greater risk of buying positive pigs that in turn may affect the other pigs in the premises. As *Salmonella* do not only occur in pigs but can be found in poultry also, an interaction between the different animal populations also cannot be excluded. However, further investigations into the association of region with the prevalence of *Salmonella* are required.

The detection of *S.* Typhimurium in the lymph nodes of slaughter pigs was positively associated with the use of certain antimicrobials in the herds. In line with these results an American study showed that the use of subtherapeutic dosages of chlortetracyclin increased shedding of *Salmonella* in fattening pigs (Funk et al. 2007). Likewise, use of tylosin as a growth promoter was associated with an increased proportion of seropositive pigs in a dutch study (van der Wolf et al. 2001). However, a potential causal relationship between the use of these antimicrobials and the prevalence of *S.* Typhimurium in the slaughter pigs cannot be deduced from cross sectional studies. More intervention studies on a significant number of animals are needed to proof this association.

Pigs are usually not treated with antimicrobials to combat *Salmonella*. The presence of other diseases requiring treatment in the pig herds may be an indicator of an insufficient management, e.g. hygiene, ventilation etc.. Such deficiencies may also support the spread of *Salmonella* within the herd. Treatment of pigs is usually not effective in eliminating *Salmonella* (Ebner und Mathew 2000; Roesler et al. 2005), although it may have been effective in controlling the other diseases.

Moreover, the presence of other disease may have interfered with infection resistance of the pigs and via this route have influenced the presence of *Salmonella* in the lymph nodes. Further research into the interaction of the use of antimicrobials in the herds and the presence of *Salmonella* in slaughter pigs is clearly required.

The negative association of the use of home mill feed with the prevalence of *Salmonella* is an interesting finding that raises questions concerning the role of feed as a source of *Salmonella* and as a potential factor increasing the viability of *Salmonella* in the gastrointestinal tract of pigs. Feeding has been identified as a risk factor for shedding of *Salmonella* before (Bahnson et al. 2006; Lo Fo Wong et al. 2004). Lo Fo Wong et al. (2004) recognized a protective effect of non pelleted feed over pelleted feed in a Scandinavian study

and ascribed this to structure and composition of the feed. Pelleting of feed was not recorded in our study. However, it is likely that home mill feed was not pelleted, while purchased feed may have been pelleted.

This finding calls for further research into the effect of feed on the prevalence of *Salmonella* in slaughter pigs, although there is already a substantial body of literature on this topic. The association was only marginally stronger for *Salmonella* spp other than *S.* Typhimurium indicating no substantial difference between these two groups of serovars in this respect.

The results of this study show that the risk factors associated with the prevalence of different serovars of *Salmonella* vary suggesting differences in the epidemiological characteristics such as reservoir, survival in the environment, infection dose and spread within and between animals between these serovars. Specific features of *S.* Typhimurium may need special attention when reduction of the *Salmonella* prevalence in pig herds is the target. The association of the use of antimicrobials with the prevalence of *S.* Typhimurium underlines the need for a prudent use of this group of drugs.

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