DIFFERENTIAL TRANSLOCATION OF \textit{Salmonella} SEROVARS TO MESENTERIC LYMPH NODES OF PIGS

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Abstract In observational studies of growing pigs in North Carolina, we cultured paired samples of mesenteric lymph nodes (MLN) and cecal contents for \textit{Salmonella} using standard methods for selective enrichment and plating. Apparent \textit{Salmonella} prevalence was higher among cecal (39.2\%) than MLN (20.5\%) samples from the same animals. \textit{Salmonella} Typhimurium var. Copenhagen (STC) and \textit{S}. Derby comprised 84\% of all isolates at slaughter. For these two serovars we found an association between serovar and sample type. The odds of isolating STC from MLN rather than cecum were 5.7 (95\%CI 3.0 to 10.7) times higher than the odds for \textit{S}. Derby. The odds for isolating STC from MLN versus cecum were 58 times higher for groups where STC had been isolated from the pigs on farm than for groups where it was had not been detected on farm. These findings suggest biological differences among common group B \textit{Salmonella} serovars in the pig.

Introduction The conventional view of medical microbiologists, that all \textit{Salmonella} serovars are pathogenic, implies that all merit equal consideration in control programs. This view is supported in part by the frequent emergence of previously unfamiliar serovars in outbreaks of human salmonellosis (Cherubin, 1981). Beyond the few host-adapted serovars that show little or no propensity for cross-species infection (\textit{S}. Typhi; \textit{S}. Gallinarum/Pullorum; \textit{S}. Choleraesuis; etc.), there is minimal information available with respect to epidemiological heterogeneity among \textit{Salmonella} serovars. However, over many decades it has been clear that relatively few \textit{Salmonella} serovars are implicated in the vast majority of cases of human and animal salmonellosis. The underlying reasons for this have not been critically considered, but biological and epidemiological differences among serovars are one obvious possibility.

In field studies \textit{Salmonella} in North Carolina, we examined the distributions of serovars isolated in paired samples of mesenteric lymph node (MLN) and cecal contents of individual slaughtered pigs from 4 farms. Of primary interest was the question of whether the choice of sampling site would influence conclusions regarding the profile of serovars present in a swine population. However, at a mechanistic level, discordant results for these tissues might also provide some insight into the interactions of various serovars with the host.

Materials and Methods The experimental design and methods employed for this study have been described in detail elsewhere (Gebreyes \textit{et al.}, 2004). Longitudinal sampling of individually identified growing pigs was conducted on 2 cohorts of 60 pigs from each of 4 farms. The final fecal samples on farms were collected within 24 hours of slaughter. Following slaughter, paired samples of mesenteric lymph nodes (10g) and cecal contents (10 g) were cultured using standard methods of selective enrichment and plating. The total number of paired samples collected at slaughter was 405. Colonies with morphology typical of \textit{Salmonella} were screened biochemically then sent for serotyping to the National Veterinary Services Laboratory in Ames, Iowa. Comparison of farm and slaughter results was reported in detail by Gebreyes \textit{et al} (2004). This report compares results obtained from paired samples of cecal contents and MLN from individual pigs that were not detailed in that study. Serovars isolated from the slaughtered animals were described as concordant if the same serovar had been isolated from that group of pigs at slaughter, or discordant if the serovar had not been isolated at the farm.

Results The prevalence of positive cultures was significantly higher for samples collected after slaughter (29.8\%) than for fecal samples on farms (8.9\%). Prevalence was higher among 405 cecal samples (39.2\%) than 405 MLN samples from the same animals (20.5\%). Although multiple serovars were isolated both on farms and at slaughter, \textit{S}. Typhimurium var. Copenhagen (STC) and \textit{S}. Derby comprised 64\% of all isolates. For all groups, the number of serovars isolated from cecal samples (1 to 7; median 5) was greater (P = 0.03; Wilcoxon rank sum test) than for MLN samples.
(range 0 to 4; median 2.5). Overall, 84% of MLN isolates (n = 130) were concordant with serovars on the farm, while only 41% of cecal isolates were concordant (OR 7.4; 95% CI 4.2 to 12.8).

For the 2 predominant serovars we found a statistically significant association between serovar and sample type (MLN node vs. cecum). Of 84 isolates of S. Derby obtained at slaughter from 6 cohorts, 66 (78.6%) were from cecal samples. In contrast, of 119 isolates of STC among 6 cohorts, 45 (37.8%) were from cecal samples, and a similar distribution was seen among 18 isolates on S. Typhimurium in one cohort (44% from cecal samples). Across all other serovars (n = 93), the proportion of isolates obtained from cecal samples (69.9%) was similar to that for S. Derby. The odds of isolating STC from MLN rather than cecum was 5.7 times higher (95% CI 3.0 to 10.7) than the odds for S. Derby. Considering only those cohorts in which S. Derby or STC had been previously isolated from pigs on farm, there were 87 instances in which the corresponding serovar was isolated from MLN compared with 60 isolations from cecal samples. In contrast, when these serovars had not been isolated from a group on the farm, only 6 MLN isolates were obtained compared to 51 cecal isolates. However, these ratios differed greatly when stratified by serovar.

In all 6 cohorts where S. Derby was isolated at slaughter, the numbers of cecal S. Derby isolates exceeded (total 66) the number of MLN isolates (18). In contrast, for STC there was no such consistent pattern. While the ratio of MLN to cecal isolates overall was of the order of 2:1 for STC, across 3 of the 6 cohorts the number of cecal isolates (total 20) exceeded lymph node isolates of STC (1 only). In these 3 cohorts, STC had not been isolated from pigs at the farm while for the remaining 3 cohorts in which MLN isolates exceeded cecal isolates, STC had been isolated from the pigs on farm. The odds for isolating STC from MLN versus cecum was 58 (95% CI: 7.4 to 457) times higher for the 3 groups where STC was found on farm than for groups were it was not detected on farm. The corresponding odds ratio (3.1) for S. Derby was not statistically significant (0.92 to 10.4).

Discussion These data were gathered in an observational study designed for other purposes (Gebreyes et al., 2004). Consequently interpretation of the data has to be cautious due to the possibilities of confounding or bias. The key outcome of the difference in prevalence and serovar profile between farm and slaughter and the likely role of infection during transport and lairage was discussed in detail by Gebreyes et al (2004). Regarding the results of the paired MLN and cecal samples presented here, the differences in prevalence are of less interest than the qualitative differences (distribution of serovars). The prevalence results are likely to be highly subject to methodological factors including sample weights (Funk et al., 2000). However, the observation that these two sources yielded markedly different serovar profiles implies that choice of samples may substantially influence the serovar profile observed in a study. Only 1 isolate per sample was serotyped, and the possibility that multiple serovars were present in samples must be acknowledged. However, this would be unlikely to explain the distribution of the results unless bias in colony selection was different for cecal and MLN samples. It is prudent to acknowledge that all studies of prevalence and serotypes of Salmonella are likely biased with respect to both sampling and bacteriologic methods (Rostagno et al., 2005). Although identical microbiological procedures were followed for both groups of samples, the sensitivity and selectivity of the enrichment procedures could vary between MLN and cecal samples which obviously present very different microbiological milieus.

At first glance, our data indicate that results from culture of MLN were more concordant with farm results than for were those from cecal samples. It could be inferred that MLN samples yield a better assessment of on-farm exposures than do cecal samples. However, it must be remembered that 65% of MLN samples were STC or S. Typhimurium and that potential differences in serovar biology may have confounding effects. The apparently higher concordance of MLN samples could arise from several scenarios. Assuming a high risk of exposure in lairage, the rate of colonization of the cecum may exceed that of the MLN. Recent experimental studies confirm that multiple serovars of Salmonella can colonize both alimentary and non-alimentary tissues (including MLN) within 3 hours of intranasal inoculation (Loynachan et al., 2004). However, the high challenge doses used and low numbers used in these experimental studies may not permit detection of variations among serovars that may occur with recent exposure. Another experimental study found differences among Salmonella serovars with respect to their rates of transmission among pigs (van Winsen et al., 2001). Our data strongly suggest that the propensity of STC to translocate to MLN was substantially greater than for S. Derby and other serovars isolated in this study.
Higher concordance of MLN with on-farm results would also be predicted if STC was also more likely to be detected on farm (i.e. higher rates of transmission or longer duration of shedding). Conversely, if isolates predominating in lairage had low propensity to translocate to MLN, this would also lead to the pattern we observed. In this study, we speculate that the relative affinity of STC for MLN compared to S. Derby may reflect differential risks of translocation to, or persistence in MLN, consistent with greater invasiveness and pathogenic potential in the pig.

While the list of Salmonella serovars isolated from pigs is extensive, S. Derby and S. Typhimurium have been consistently among the most prevalent serovars worldwide over many decades (Davies et al., 2004a, b; Rajic et al 2005). However, while S. Typhimurium is similarly common among other food animal and human isolates, Salmonella Derby is generally an uncommon serovar among isolates from species other than swine (Sarwari et al., 2003). Nonetheless, its potential to cause human disease is well established. S. Derby was responsible for one of the largest outbreaks of human salmonellosis in the USA (Cherubin, 1981), and over many years has remained a common serovar in human salmonellosis in Hong Kong (Ling et al., 2001).

Conclusions Sampling procedures as well and microbiological methods can substantially influence serovar patterns observed in field studies of Salmonella. Differences observed in the distribution of serovars in paired samples of MLN and cecal contents most likely reflect biological diversity among serovars in their propensity to translocate to the MLN of pigs. Assuming that such diversity may also be associated with infectivity and duration of infection in pigs, the predominant serovars on farms are likely confound results of studies comparing on-farm and post-slaughter prevalence of Salmonella.

References