Epidemiology and control of Salmonella in pork - some of the questions

Peter R. Davies and Julie A. Funk

College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606, USA

"Attempting to unravel the epidemiology of Salmonella infections in both humans and animals continues to provide employment for many health professionals. There is little to suggest that a 'magic bullet' is on the horizon."

R. Ashley Robinson, August 1996 (1)

It is an intimidating task to provide an overview of the epidemiology and control of Salmonella in pork, and attempt to 'set the stage' for the presentation of the impressive body of new information promised at this symposium. From its modest inception in Ames, Iowa, in 1996, where 31 papers were presented, the gathering has blossomed, with 73 papers presented the second symposium in Copenhagen in 1997 and about 130 submitted for the current meeting. The common stimuli for our efforts are familiar: increased concern about foodborne disease as a public health issue in developed countries, and the rising importance of product safety in international trade in food. Last year, an international symposium on Salmonella control in poultry was also convened in the USA. Despite the greater historic importance, relative to swine, of food safety concerns associated with Salmonella infection of poultry, and correspondingly greater research efforts, it is sobering to cite the authors of the introductory paper at that meeting: 'In poultry, the risk factors for spread of Salmonella throughout a flock are poorly known'.(2)

Epidemiology and Control of Salmonella - the Scope

At least in the USA, the politically correct perspective on food safety embraces the 'farm to table' concept - acknowledging that all participants in the chain of food production and consumption bear some responsibility for reducing the risk of foodborne disease. But at a mundane level, the partitioning of the overall responsibility and accountability, and the implementation of appropriate control measures across the production-to-consumption continuum remains somewhat fuzzy notion. For historic and practical reasons, formal recognition of specific food safety responsibilities (read 'regulation') continues to be concentrated in the slaughter/processing and, to a lesser extent, retail/distribution segments of the continuum. Despite the logical (and politically favorable) appeal of controlling foodborne disease by reducing the prevalence of pathogens in food animal populations (preharvest), the HACCP/Pathogen Reduction Act of 1996 stopped short of involvement at the preharvest level. The likely wisdom of that decision resides in the fact that current epidemiologic knowledge of most (at least bacterial) foodborne pathogens in animal populations is inadequate to enable reliable and cost-effective control measures to be mandated.

The technical feasibility of preharvest control of Salmonella has been amply demonstrated by the Swedish poultry and swine industries (3), an example that is frequently held up by U.S. consumer groups and diverse advocates for increased regulation of agriculture. However, perhaps the most eloquent statement of the difficulty and cost of implementing the 'Swedish model' for Salmonella control is that, despite its apparent success, after some 40 years it has not yet been adopted by any major swine or poultry producing nation. Without attempting to delve into the sociologic, economic and other factors that may explain this, it is apparent that there is interest, in many pork producing countries, in achieving Salmonella control by means other than the Swedish model. Again, paucity of epidemiologic knowledge appears to be a major constraint on the development of alternatives.

Epidemiology and Control of Salmonella – Breaking Down the Monolith

The intricacy of the ecology and epidemiology of Salmonella is difficult to overstate. To establish a framework for this discussion, we have distilled the 'monster' into three components, illustrated in Figure 1. Regardless of the segment(s) of the farm-to-table continuum one wishes to address, we feel this simple model may be applied to tease apart, or weave together, the concepts involved in Salmonella control. For brevity, and the bias and limitations of our experience, this paper will elaborate this model in the context of swine production (preharvest), with occasional reference to 'post-harvest' segments.

A recent review of Salmonella control in poultry (2), presented 2 models for control at the production level:

1) Exclusion – preventing the introduction of any Salmonella serotypes into the population (introduction-biosecurity in our model).

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2) Non-exclusion – accepting the introduction of Salmonella into the population, and introducing measures to reduce infection or contamination during production (transmission-process control in our model).

Clearly, neither the exclusion/non-exclusion options presented for poultry, nor the three components of our model, should be viewed as mutually exclusive. Our challenge, across the entire production-consumption continuum and within each of its segments, is to identify the optimal mix of control measures to deliver the most cost-effective reduction in risk of human illness.

Introduction – Biosecurity:

This component of the model addresses sources or routes by which Salmonella are introduced into the animal-production environment, and the attendant role of biosecurity measures (defined here as steps taken to prevent the introduction of an infectious agent into a herd) required to reduce the risk of introduction. The practically ubiquitous nature of Salmonella is one of the main reasons we are gathering today. The host range of Salmonella spans the entire vertebrate phylogenetic tree, from amphibians and reptiles to marine mammals and primates. Salmonella have also been isolated from invertebrates such as snails and cockroaches.(4,5) Combined with its myriad animate lifestyle options, the ability of Salmonella to survive and even multiply in the external environment presents a monumental challenge if we aspire to rear animal populations free of Salmonella. The worldwide trend towards larger herd sizes of most agricultural species in no way aids the cause, as the risk of introduction of Salmonella inevitably increases as the volume of inputs (animals, feed, water, etc.) to herds increases. In discussing the (absolute) exclusion strategy in poultry production, McCapes and Riemann point out that such an approach would require complete redesign and reconstruction of production systems based on principles of sanitary engineering, and incorporating sanitation, security and surveillance measures.(2) Further, they claim that the ‘exclusion program’ itself, if employed throughout the poultry and other food animal industries would represent the largest and most expensive animal health program in the history of the world. However, acceptance of the idea that complete exclusion of Salmonella from animal populations may be an unrealistic goal in the long term, does not mean that biosecurity measures are not vital to risk minimization programs.

We have probably done enough research to know that potential sources of Salmonella introduction are many, and that the relative importance of these sources will vary among farms and over time. The overall importance of reducing the risk of introducing Salmonella to a herd is inversely proportional to its existing Salmonella level. That is, adequate biosecurity for swine populations free of Salmonella must be of the utmost priority, but is arguably

less crucial in herds that are already highly contaminated. A conclusion, based on review of a considerable body of swine research in Holland, was that ‘the role of on-farm contamination cycles is so important that the role of other factors is difficult to ascertain’.(6) However, it is obvious that attempts to control Salmonella in infected populations would be frustrated by regular reintroduction of infection. This is particularly the case when our understanding of transmission of Salmonella within swine populations, and therefore our ability to control horizontal spread following introduction, is far from perfect. While acknowledging the broad range of potential sources of Salmonella introduction into herds, we will limit our discussion to incoming pigs and feed.

Question 1: How important is Salmonella control in feed?

The animal-feed/animal-infection/human-infection axis is an historically important and well-documented facet of the epidemiology of Salmonella. While feeds of animal origin have received the most attention as sources of Salmonella, it is now well recognized that feeds of plant origin, such as soybean meal, are often contaminated with Salmonella. Arguably, all raw feed components should be regarded as potentially contaminated, and complete exclusion of contaminated inputs is impractical. Hence appropriate process control and decontamination steps in feed mills are essential to avoid dissemination of contaminated feed to herds. Without disputing the established role of feed as a potential source of Salmonella, there are some grounds on which to question the central role traditionally afforded to feed. Most notably, the serotypes of Salmonella isolated from feed are frequently not those most commonly in animal populations nor human cases.(6-9) In poultry, the serotypes of overriding human health significance (S. enteritidis and S. typhimurium) are relatively uncommon in feed compared with other serotypes.(10) Similarly in Denmark, where S. typhimurium is of greatest epidemiologic significance in pigs and people, this serotype is rarely found in swine feed.(11) These findings, together with the observation of a negative association between the presence of ‘non-typhimurium’ serotypes in feed and the presence of antibodies (ELISA using S. typhimurium/S. choleraesuis LPS antigens) in Danish herds (11) raise questions about how to consider contamination of feed, and particularly:

Question 2: Should all Salmonellae receive equal emphasis in control programs?

Although Salmonella are geographically ubiquitous, the predominating serotypes vary both temporally and geographically.(5) Currently, it is impossible to predict when and why a serotype will increase or decline in importance, and whether it will be a major or minor threat to human or animal health.(12) Perhaps buoyed by sporadic outbreaks of human disease associated with ‘rare’ serotypes, most medical bacteriologists and clinicians retain the conventional view that all Salmonella are pathogens, despite the fact that few serotypes cause most human and animal
disease. Also, Cherubin argued that reports of foodborne outbreaks are biased towards rare serotypes because clusters of cases are more readily recognized and publication bias towards reports of unusual outbreaks. While S. typhimurium, S. enteritidis, S. infantis and S. heidelberg are regularly ranked among the most frequent isolates from humans and animals in many countries, the vast majority of serotypes appear to be of minimal epidemiologic importance. Wierup suggested that Salmonella surveillance should encompass all Salmonella but control could be focussed on serotypes responsible for most disease. The response to this question has enormous implications for the logistics and cost of control programs.

**Question 3: How important are incoming breeding stock?**

Both the Swedish and Danish Salmonella control programs emphasize monitoring of suppliers of replacement breeding stock. The logic for this is indisputable, and particularly for conventional farrow-to-finish systems where market hogs are raised on the same farm as the breeding herd. However, the relative contribution of incoming breeding stock to the risk of infected pigs being sent to slaughter is likely to vary among production systems, and most obviously in the case of multiple-site production where different phases of production are reared on separate farms.

Although results of earlier studies of farrow-to-finish farms indicate that sows and boars play an important role in maintaining Salmonella infection on farms, more recent publications indicate that breeding herds can be a minor source of infection of finished pigs. A review of numerous studies conducted in Holland concluded that 'nowadays it does not frequently occur that Salmonella spp. are carried from breeding or multiplying herds to finishing farms and the consumers thereafter'. The same authors estimated, by methods that were not clearly elucidated, that 'under current conditions, Salmonella spp. imported from the breeding farm probably play a role in about 1-10% of all infections that take place in the finishing period'. In Denmark, removal of 10 week old pigs from breeding farms infected with S. typhimurium to clean finishing facilities appeared to be effective in preventing infection at market age. Our own cross-sectional and cohort studies of multiple-site systems in North Carolina were also in line with those observations, with different serotypes predominating in the breeding and finishing phases, and the serotype profile of fecal isolates changing between the nursery and finishing phases and within the finishing phase. However, it is also clear that Salmonella infection can occur in very young pigs and absolute exclusion of preweaning infection is unlikely to be reliably achieved in commercial herds using contemporary 'early weaning' technologies alone.

Rather than convince us that Salmonella in breeding stock is unimportant, these observations raise questions about the likely benefits to be gained, at the market hog level, through intensive screening and control of breeding stock when transmission in 'downstream' segments is poorly controlled. However, there are other aspects to consider. Firstly, breeding stock themselves constitute an important sector of the supply of pork products to consumers. While most studies have concentrated on market hogs, some reports of Salmonella infection in sows have shown a high (20%-84%) prevalence of infection. These isolation rates suggest that products derived from culled breeding stock are potentially important sources of human infection.

Secondly, the market dominance of few, large commercial breeding-stock companies in the USA has created a situation in which human foodborne pathogens could quickly become widely distributed in the industry. This is probably not of great importance with respect to Salmonella in general (because of the multitude of collateral sources of herd infection), but could markedly impact the rate of dissemination of specific 'epidemiologically significant' strains throughout the industry. The potential public health and political effects are most obvious in the case of strains carrying multiple antimicrobial-resistance determinants, such as S. typhimurium DT 104. Again, there may be merit in differentiation among Salmonella in order to concentrate control efforts in breeding stock suppliers on those strains of greatest (current) epidemiologic significance.

**Transmission – ‘Process Control’**

Arguably, the resources warranted to prevent Salmonella from entering herds would be less if we possessed effective means for controlling transmission of the organisms after they were introduced. The term 'process control' has been borrowed from manufacturing and here is used to denote the steps taken during a process to ensure that a product meets its specifications -- or in our case, the steps taken to reduce the risk of transmission of Salmonella to and among pigs on infected farms. As an approach to quality control, process control is viewed as superior to 'end-of-line' inspection, a philosophy underpinning the HACCP legislation in the USA. In contrast to manufacturing (and feedmills or slaughterplants), where steps in process control are often very specific and quantifiable, the concept of process control in live animal production is more nebulous. The basis for developing measures that reduce risk of Salmonella transmission among pigs should reasonably be based on understanding of the epidemiology of transmission.

Traditionally, control measures for Salmonella on swine farms have been based on principles of improved hygiene and management that theoretically should reduce the introduction and transmission of organisms shed in feces. However, current concepts regarded as 'good management' in the USA (for example, segregated early weaning, multiple-site production) are aimed to control swine diseases and optimize production.
developed without specific regard to control of asymptomatic infections with human foodborne pathogens. Our investigations in North Carolina indicate that these modern systems are highly effective in excluding Trichinella and Toxoplasma, (30) but are not adequate to ensure control of Salmonella. (8) Even in Sweden, the failure of rigorous measures of hygienic control to eliminate Salmonella on some farms has necessitated herd depopulation. (3) At this time, we argue that we do not have a set of control procedures that can be applied to contaminated commercial farms to invariably control Salmonella. In part, this is probably because hygienic measures target control of Salmonella "outside the pig", while the epidemiology of transmission is dominated by Salmonella infection "inside the pig" and pig-to-pig transmission. Also, while we have been understandably fixated on fecal-oral transmission, there is evidence that we have been underestimating the significance of aerosol transmission.

**Question 4: Is aerosol transmission important?**

The phenomenon of aerosol infection with Salmonella has been unequivocally demonstrated in several species, including the pig. (31) However, the relative importance of aerosol transmission, compared with ingestion, in transmission on-farms and during transport and lairage is unknown. The demonstration of Salmonella typhimurium in the intestinal tract and lymph nodes of pigs within hours of high-dose aerosol exposure of esophagotomized animals is remarkable, challenging our most entrenched presumptions about the course of infection. (31) However, perhaps the most compelling evidence for the likely importance of aerosol transmission comes from recent observations in poultry. About 3% of eggs were Salmonella positive following oral challenge of hens with $10^7$ to $10^8$ S. typhimurium DT 104, compared with 14% following aerosol challenge with 200 organisms. (32) This is consistent with earlier studies showing lower infective doses in mice and calves for the respiratory route compared with the oral route. (33) The fact that relative humidity markedly affected the survival of S. typhimurium in aerosols is also noteworthy and the importance of low-dose aerosol infection of swine warrants further investigation. Also, low dose infection of poultry has been achieved by the conjunctival route, and dust is considered an important vehicle of Salmonella transmission. (34, 35) Investigation of the effects of air quality and ventilation on Salmonella transmission in swine may be warranted.

The fact that our traditional approaches to Salmonella control have been founded more on first principles of disease transmission than field studies underpins the common call for more epidemiologic studies of foodborne pathogens in animal populations. If one accepts the need for such studies, many questions remain about how best to go about them.

**Question 5: What is the most appropriate outcome variable for epidemiologic studies**

Clearly the choice of an outcome variable can be influenced by many factors, not least of all the objective of the study. We will assume that the overriding objective for most epidemiologic studies is to identify "on-farm" risk factors for infection of pigs that will ultimately influence the probability of contaminated product being passed on to consumers. An ideal outcome variable would provide both a valid quantification of the Salmonella "status" of the unit of analysis (pig, pen, farm, company, etc.) in a study, and its attendant public health risk. However, few would argue that culturing Salmonella on finished product (although perhaps a reasonable indicator of public health risk) would be a reliable or efficient approach for studying on-farm risk factors, because of the multiple opportunities for contamination and decontamination during slaughter and processing.

We will discuss some attributes of 4 common outcome variables - fecal culture on farms, culture of intestinal contents or lymph nodes collected at slaughter, and serum antibody.

1. **Fecal samples collected on farms**: Two major disadvantages of collecting samples on farms are the cost and logistic difficulties of conducting farm visits, and the insensitivity of fecal culture. The sensitivity of one-time sampling of feces to detect Salmonella in individual pigs is poor, (36, 37) but is influenced greatly by methodologic factors. For example, we have shown that the probability of detecting Salmonella in feces is markedly affected by fecal sample weight, and that results of studies using rectal swabs are likely to be highly unreliable. (38) Better knowledge of the relative sensitivity of methods employed in field studies is required to enable meaningful comparison of data and also to allow appropriate adjustment of prevalence estimates at both the sample and herd levels. (39, 40) Moreover, consideration of individual and herd-level sensitivity allows researchers to compensate to some extent by increasing target sample sizes in study populations. Thus, imperfect sensitivity (individual test) may be of less concern in risk factor studies where the group rather than the individual is the unit of analysis. The fact that test qualities such as sensitivity and specificity can vary among herds may be of greater concern. (41)

Explicit details of on-farm sampling are often lacking from published studies, and it appears that little attention has been given to the question of sampling methods, particularly in large swine production systems. Using published statistical tables (42), for a cross-sectional study of a large multiple-site system we selected a sample size of approximately 90 pigs per herd to enable estimation of prevalence within 10% with 95% confidence. (19) We considered this to be acceptable accuracy for our goal of establishing baseline data of prevalence and serotypes. However, such tables assume diagnostic tests of perfect sensitivity, and do not address issues of clustering within herds, (19) multiple serotypes on farms, the occurrence of more than 1 serotype.
in individual animals,(36,43) or possible bias in enrichment methods.(4) All these are likely sources of measurement error or bias that together will reduce the accuracy of estimates of *Salmonella* prevalence, and serotype profile, in swine populations.

However, perhaps the greatest concern for studies using fecal (or other) culture relates to temporal fluctuations and the reliability of point estimates of *Salmonella* prevalence. A longitudinal study of *Salmonella* in poultry buildings found little predictability or consistency in *Salmonella* isolation in individual barns over time, although considerable sampling error was likely.(44) In flocks of *S. enteritidis* infected birds from which approximately 1% of eggs were infected, considerable temporal clustering was seen, with up to 20% of eggs positive on a given day.(32) In longitudinal studies of cohorts of growing pigs in 2 multiple-site systems, we have observed marked variability in temporal patterns of *Salmonella* shedding.(24) In recent studies, prevalence of positive fecal samples declined from 21% to 5% in large samplings (50% of pigs) conducted 2 weeks apart in one finishing barn, and from 36% to 4% in the same individual pigs tested 1 week apart in another herd (unpublished data). In order to better evaluate the limitations of on-farm fecal sampling, we need more information on the dynamics of *Salmonella* transmission and shedding in naturally infected herds, and understanding of the mechanisms responsible for temporal fluctuations and clustering.

2. Culture of intestinal contents or lymph nodes at slaughter: Collecting samples at slaughter has been popular among researchers and is probably the most cost-efficient approach for conducting prevalence surveys. Obviously, the problems of imperfect sensitivity of culture also apply to sampling at slaughter and methodologic factors such as sample weight or number of lymph nodes cultured also have been shown to markedly influence results.(36,45) However, there are more vexing issues in using slaughter plant samples for farm-level studies. Firstly, the population of animals presented for slaughter represents a biased sample of the farm population.(46) However, given that a group is a normal marketing from a herd, it might be better considered a sample in time of the output of the herd, which is arguably more relevant in terms of potential public health risk than a random sample of the farm population. However, the validity of slaughter plant samples for use in studies of on-farm risk factors assumes that results are not affected by events occurring ‘beyond the farm gate’ – an assumption that is questioned by a considerable body of literature.

**Question 6: What does happen during transport and lairage?**

Some early investigators viewed contamination during transport and lairage to be the major source of *Salmonella* infection in slaughtered pigs.(36,47,48) In fact, in discussing the problem of defining the *Salmonella* status of farms, Newell and Williams (1971) stated ‘slaughterhouse studies cannot be used ... because of the risk of infection after leaving the farm or of contamination at or in the slaughterhouse’. (47) A study of cull sows in 1961 reported a rise in prevalence of culture positive rectal swabs from 9% to 80% from farm to slaughter.(49) Obviously, the possibility of changes of this magnitude during transport and lairage underlines the likely perils of using slaughter samples for ‘farm-level’ risk factor studies. Sampling of lymph nodes rather than intestines suffers the same concern, as lymph node infection was detected by 6 hours after experimental aerosol exposure.(31)

The phenomenon of changes in apparent *Salmonella* status at farm and slaughter is not restricted to swine, but is also documented in poultry and cattle.(50,51) Apart from methodologic issues (e.g., different samples at farm and slaughter in some studies), factors suggested to contribute to the ‘transport-lairage’ effect include 1) cross-infection from other animals or contaminated vehicles or facilities, and 2) increased populations of *Salmonella* resulting from stress of transport, including feed and water deprivation.(6) Perhaps the most convincing data illustrating the latter comes from studies of mice in which increases of 2-4 logs were observed in several organs (stomach and small and large intestines) after 1 to 2 days of feed and water withdrawal.(52) Several studies in poultry have also shown increased *Salmonella* shedding after feed withdrawal, as well as reduction in the infective dose required for feed deprived birds.(50) Preliminary studies in pigs suggested that both feed deprivation and transport may affect *Salmonella* shedding.(53) Given that multiple factors may impact the magnitude of the transport-lairage effect, not least being the *Salmonella* status of the group leaving the farm, is likely that it may vary considerably among plants and groups within plants.

Beyond the blurring of epidemiologic investigations, the transport-lairage effect has perhaps more serious implications for on-farm interventions and control programs. Studies of competitive exclusion that reduced *Salmonella* infection on farms gave variable results when slaughter samples were evaluated, with cross-contamination during transport being implicated.(54,55) Other interventions that reduce, but do not eliminate, *Salmonella* infection on farms may also be negated by pre-slaughter transmission within or among groups of pigs. Clearly there is a need for better understanding of the events in the transport and lairage phase and their underlying mechanisms.

Another aspect of sampling at slaughter relates to the association of the location sampled with the contamination of carcasses or product. The popularity of sampling the cecum or lymph nodes of slaughtered pigs is explained by reports that these are the sites most likely to be culture positive.(36,56) Although the cecum have also been the traditional focus of *Salmonella* studies in poultry, recent data indicate the crop may be at least as important as a source of contamination at slaughter, despite generally lower counts of organisms in the crop than the cecum.(57) Interestingly, in one study of feed withdrawal, the prevalence of positive crops increased 5-fold, while cecal
prevalence was unchanged. Coprophagy by birds during feed withdrawal was considered an important factor.

In a recent study of gut lacerations at a slaughter plant in North Carolina, we found the highest prevalence of lacerations in the stomach (8.4%), and relatively few (0.9%) in the cecum. (58) In experimentally infected pigs, *Salmonella* were isolated from 29% of stomachs (compared with 67% of cecal samples and 42% of rectal samples) and up to 28 weeks after original exposure. (56) Regrettably, the same authors omitted to evaluate the stomach in a subsequent report of the populations of *Salmonella* in swine organs from the same study. (59) Significant increases in the numbers of *Salmonella* in the stomach, and other organs, occurred following feed deprivation of mice under conditions where coprophagy was improbable. (32) Few would question the likelihood of coprophagy by feed-deprived pigs, and thus its potential to contribute to cross-infection among pigs and herds during lairage. From a risk assessment perspective, there may be grounds for more extensive studies of the prevalence and distribution of gut lacerations in slaughtered pigs, and some consideration given to stomach contents as a potential source of carcass contamination.

3. Serum antibody: The inadequacies and cost of conventional culture methods and the convenience of modern serologic techniques have spurred the development of serologic testing for *Salmonella* infection in poultry, cattle and pigs. (60-62) Furthermore, serologic testing enables use of slaughter samples without the potential confounding effect of transport and lairage. Serologic testing has subsequently been used in epidemiologic studies in pigs, both at the farm and industry level. (63-65) The novel initiative by Danish swine industry to adopt serologic monitoring as the foundation for a nationwide control program (15) has elevated the level of debate about interpretation of serologic data, both in the context of epidemiologic studies and with respect to public health. A fundamental characteristic of serologic testing is that serum antibody will persist much longer after experimental infection than detectable fecal shedding, (60,62) which may be an advantage or disadvantage depending on the objectives involved. There is little question about the potential effectiveness of serologic testing for control of invasive, host-adapted serotypes such as *S. pullorum* in poultry. (66) However, as pointed out by Gast (67), the goals of serologic testing for foodborne *Salmonella* are more diverse and less clear than in the case of individual host-adapted serotypes. Implicit in the use of serologic detection with LPS antigen is the fact that not all serotypes will be given equal emphasis.

**Question 7:** How equivalent is the antibody response in LPS-based ELISAs following challenge with homologous and heterologous serotypes within the same serogroup?

The focus on the most epidemiologically significant serotypes (*typhimurium* and *infantis*) in pigs in Denmark is justifiable, but the assumption that the mixed ELISA used (including O-antigens 1,4,5,6,7, and 12 derived from group B and group C1 isolates) will 'theoretically detect antibodies after infection with most group *S. enterica* serovars (i.e., B or group C1 serotypes) that have been isolated from Danish pigs' (15) remains to be confirmed. The relatively modest antibody response, detected with a *S. typhimurium* LPS ELISA, to several other serotypes (including type B) was presented as evidence for the serotype specificity of the test in poultry. (60) Notably, the titer to *S. stanley* (group B - O:1,4,5,12,27:d:1,2) was 1:3200, compared with 1:102,400 to *S. typhimurium* (O:1,4,5,12:d:1,2) following equivalent challenge. At least in the USA, where group B serotypes other than *S. typhimurium* (e.g., *S. derby*, *S. agona*, *S. heidelberg*) are very prevalent in pigs (8,68,69), clarification of this question is essential for meaningful interpretation of data and application of serologic monitoring for herd screening. At least in North America the almost anomalous persistence of the swine-adapted *S. choleraesuis* as a prevalent swine pathogen (68) of apparently negligible significance as a foodborne pathogen (13), also complicates the interpretation (from a food safety perspective) of antibody titers to group C1 LPS antigens. Other factors seen to influence serologic responses include the infectivity and invasiveness of the organism, infective dose, host immune factors including age and immunocompetence, and the strain (within serotype) from which antigen is derived. (67)

As with culture, to some extent we should separate the scenarios of applying serologic testing for on-farm risk factor studies and for assessing potential public health risk. Most obvious in the case of antibody, is that it represents historical exposure which may not correlate closely with the microbiologic burden of animals at the point of slaughter. Because of this limitation, in assessing the usefulness of serology in poultry, it was concluded that serology was 'probably less useful as a primary tool to make decisions about the fate or disposition of flocks and their products.' (67) The same author considered that the most suitable application for serology is for verification of risk reduction programs, citing the example of *S. enteritidis*. Again, the application of serology for controlling specific serotypes is more straightforward that is the case with 'generic' *Salmonella*.

**Question 8:** What is hygiene and can we measure it?

If the respective shortcomings of these outcome variables are not sufficient grounds for pessimism about the efficiency of epidemiologic studies, they are supported by other considerations! In discussions of *Salmonella* control, 'hygiene' appears as ubiquitous as the bug itself. If we accept that hygiene is a central component of *Salmonella* control, it is implicit that it is also a major risk factor to be included in epidemiologic studies. Berends et al., reported that hygiene had the highest odds ratio among several putative risk factors evaluated, but again the methods were not described in detail. (6) These authors also commented that published studies have failed to provide insight into
differences in Salmonella status among farms and that it is currently not possible to identify specific elements of a 'hygiene' policy that should be considered crucial.

(6) A Danish study found disinfection between batches was associated with increased seroprevalence of Salmonella (70). It appears that detailed attempts to systematically define and measure hygiene are essentially absent among published studies. While it is hard to imagine that a study of risk factors for coronary heart disease that excluded assessment of smoking would clear the hurdle of peer review, we face a scenario in which a key risk factor (at least according to dogma), is typically ignored, presumably because of difficulty of measurement. In reporting the likely importance of feed related factors on seroprevalence of Salmonella, Stege et al., advised that information on feeding systems should be included in epidemiologic studies to avoid erroneous conclusions due to confounding with other risk factors. (70) In a pilot study on one farm, to evaluate the role of 'pen level' 'hygiene' in apparent clustering of Salmonella positive pigs that we have observed in field studies, we unexpectedly found a negative association between a subjective measure of fecal accumulation in pens and fecal shedding. (71) One might rightly contest that fecal accumulation does not equal 'hygiene', or that our subjective measure was not sufficiently reliable. However, the point remains that if we continue to emphasize the importance hygiene in control programs, how do we incorporate its measurement in epidemiologic studies?

The emphasis on 'external' control measures over 'internal' control of Salmonella may also have unintended negative consequences. It was hypothesized that preventive measures in the poultry industry may have contributed to the emergence and current dominance of invasive serotypes (S. enteritidis PT4 and S. typhimurium DT 104) in poultry in the United Kingdom. The invasiveness of strains of these serotypes appears to be correlated with attributes such as heat and acid resistance, ability to survive in aerosols or on surfaces, and enhanced resistance to disinfectants. (32) Similarly, the observation of declining diversity in Salmonella isolates from poultry was inferred to indicate a declining 'niche width' of the biotype available to the organisms, which in turn was speculated to be a possible outcome of declining diversity in host animals or increasing intensification of animal husbandry practices. (72)

Because the epidemiology of transmission is dominated by Salmonella infection 'inside' the pigs and pig-to-pig transmission, we should not be surprised that measures such as all-in/all-out management and improved hygiene do not always reduce the prevalence of Salmonella in herds. (3, 15). At the 2nd symposium in Copenhagen, a number of paper addressed various approaches targeting the 'host-agent equilibrium' (broadly intended to mean the probabilities of colonization, replication and shedding of Salmonella by pigs following exposure) of Salmonella in pigs. We will briefly address some of these options.

Question 9: What is the role of feed?

Perhaps the most exciting avenue for exploring Salmonella control in the pig relates to the effect of diet the intestinal microenvironment. A common feature of several field studies presented at the previous symposium in Copenhagen was the apparent influence of various 'feed-related' variables on Salmonella in pigs. (70) Field studies in Denmark found a lower prevalence of Salmonella on farms mixing their own feed and feeding liquid feed, suggesting that dietary factors might influence Salmonella prevalence. (70, 73) A study of 40 fattening farms in Holland found Salmonella in 19.4% of samples from farms using whey to compared with 64.4% for farms using water. (74) There is also some recent evidence, based on serological data, that fineness of grind of the feed can affect the prevalence of Salmonella, possibly via effects on intestinal flora or organic acid distribution. (75) There is growing evidence supporting the role of dietary factors on Salmonella, but minimal understanding of the mechanisms involved.

Question 10: Competitive exclusion (CE) – does the 'Nurmii concept' apply to swine?

The original CE concept was founded on the fact that chicks reared under intensive conditions had no contact with hens and little opportunity to develop normal intestinal flora. (76) These circumstances clearly do not apply in commercial swine production, where newborn pigs are commonly exposed to sow feces within the first moments of life. Another question related to the applicability of CE in swine is the radical dietary and intestinal changes occurring at weaning. Also, the fact that infection of pigs late in the finishing phase is probably of greatest epidemiological significance contrasts with the situation in poultry, where the susceptibility of very young birds to low dose infection is considered important. (77) However, pilot studies of CE in pigs to prevent colonization by S. choleraesuis indicated enough promise to warrant further investigation. (78)

Question 11: What is the potential for vaccination?

As with serologic monitoring, the historic triumphs of Salmonella vaccination have primarily been with host-adapted serotypes. Again the questions of serotype specificity and the breadth of the control effort are at the core of how vaccines might be incorporated into control of non-host-adapted serotypes important in foodborne disease. Generally, killed vaccines are thought unlikely to induce immunity that will inhibit colonization by non-host adapted serotypes. (79) With respect to food safety, the added challenge of vaccination is to prevent colonization of the intestinal tract rather than invasion of host tissues, which is the usual priority of the immune response. At least in chickens there appears to be no correlation between tissue invasion and gut colonization, and no consistently effective vaccine is available for non-host adapted serotypes in
poultry. Immunity generally appears to be serotype specific. Reports of cross-serotype protection often involve challenge soon after vaccination with the likely expression of non-specific immunity.

It is likely that recommendations for Salmonella control on farms will continue to be based on the ‘first principle’ standards (hygiene, rodent control, etc.). Most likely, effective control will depend on a combination of improved hygienic standards together with measures (and probably more than one) that modify the outcome of pig exposure.

**Decontamination/Test and Removal**

The third component of our model (dissemination-decontamination/test and remove) was not discussed in relation to on-farm control in poultry (2), probably because this approach is more familiar in the postharvest sectors. Because attention on preharvest control has been focussed almost exclusively on biosecurity and process control, there may be some opportunity to explore interventions that ‘decontaminate’ pigs in the immediate pre-slaughter period. Obvious post-harvest examples include the use of organic acid washes of carcasses, or irradiation of finished products - decontamination steps to reduce the risk of dissemination of contaminated product to ‘downstream’ sectors of the industry and consumers. Similarly, microbiologic monitor-

**Question 12:** Can effective process control at slaughter, including decontamination steps such as irradiation, render preharvest control unnecessary?

The complexity of Salmonella control on farms, and the resources likely to be required for achieving satisfactory control on farms continue to beg the question of the appropriate allocation of societal resources to address this problem. The difficulties may be even greater for control of Campylobacter, which are normal flora of food animals. McCapes and Riemann wrote, with a somewhat pugilistic tone, that ‘opposition to or hesitation to employ irradiation of poultry meat by so-called activist groups and industry is considered by the authors to be anti-social behavior of a most egregious nature as is the deafening silence of much of the regulatory community on the use of irradiation technology’. (2) Politicization of food safety in the USA has progressed to the point where new and uncertain scientific information may be rapidly taken on board by the media or other groups whose agenda it may serve. As scientists, it is important that we take pains to elucidate the limitations and caveats of our work.
References


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Footnotes

- The use of the term ‘level’ is generally to be discouraged. Here we use it loosely to indicate the overall *Salmonella* burden of a given farm—combining prevalence of infected pigs, numbers of organisms per pig, the sum of environmental contamination and reservoirs in other hosts.

- The Kaufmann-White system of serotyping is based on superficial antigens which may not correspond closely with genotype. Acknowledging the advances in bacterial genetics and typing methods, for this discussion we adhere to the conventional serotype classification.