Diagnostic notes

Serologic basis for assessment of subclinical *Salmonella* infection in swine: Part 2

Isabel Turney Harris, DVM, PhD

This is Part 2 of a two-part series. In Part 1, topics included control of subclinical salmonellosis in swine, ELISA tests to detect *Salmonella* serum and meat juice antibodies and the sensitivity and specificity of these tests, national *Salmonella* surveillance programs, serological tests used in the monitoring programs, and correlation of serological test results with culture results.

Detection of serotypes of importance

For optimum sensitivity, the *Salmonella* ELISA should incorporate antigens capable of detecting antibodies to the predominant serotypes in the geographical area where animals are to be tested.¹ The Danish mixed-ELISA (DME) detects O antigens from about 93% of isolates found in pigs in Denmark.² In the United States, a study in which pooled pen fecal samples from 37 farms were cultured resulted in 286 *Salmonella* isolates, 92% containing O antigens capable of producing antibody detectable by the DME.³ In the report of serotypes most frequently isolated from swine by the National Veterinary Services Laboratory (Ames, Iowa), 91% were in O antigen groups included in the DME.⁴ When *Salmonella* is isolated on a premise, multiple serotypes are usually identified.⁵

Herd test compared to individual test

At its present level of sensitivity and specificity, the *Salmonella* ELISA is applied in the field as a herd test, not an individual animal test. The response of the individual animals sampled are evaluated in order to make a decision on the status of the whole herd.⁶ Therefore, at any sampling time point, pigs in the sampled group may be at different stages in their serologic responses, depending upon when they were exposed to *Salmonella*, the infective dose, and the degree of immunologic response detectable by the test. The results of the individual test determinations are pooled to make an interpretation on the status of the herd. Evaluation of the herd as the unit is useful because infection levels may vary greatly between groups. Clinical trials on the effectiveness of intervention strategies designed to reduce subclinical infection are necessarily conducted on groups. To be valid, methods of intervention must be tested and applied to groups, not individuals, because of the dynamics of *Salmonella* infection in the herd.⁷

Several studies have reported the variability of serologic responses in different groups of animals and within groups of animals sampled at the same time and at different times. In one study, seroprevalence ranged from 0 to 80% in individual buildings at a single locus in a multi-site system.⁸ In another study, in which two groups of pigs from three different herds were sampled twice at a 2- to 3-week interval (I. T. Harris, unpublished data), the proportion of animals that were positive by culture, serology, or both varied between groups and within groups of pigs sampled at different times (Figure 1). Between collection of the first and second samples in the first group of pigs, the proportions of positive serologic and cultural results both increased. In the second group of pigs, the proportion of seropositive animals decreased between the first and second samplings, while culture results remained about the same.

Department of Microbiology, College of Agriculture, Iowa State University, Ames, Iowa

Address correspondence to: Dr Isabel Turney Harris, Department of Microbiology, 207 Science I, Ames, IA 50011; Tel: 515-294-7058; Fax: 515-294-6019; E-mail: iharris@iastate.edu.

This article is available online at http://www.aasv.org/shap.html.


Differences in experimental and field use of ELISA tests

*Salmonella* ELISA tests are useful as individual pig tests in research situations, where pigs are experimentally infected with pure cultures of known serotypes of *Salmonella*. The “cutoff” selected to distinguish positive and negative results may be different for the test under research conditions compared to the same test used in field studies because of potential variations in the time when the pigs are exposed to *Salmonella*, the infective dose, and the degree of immunologic response detectable by the test.⁹ Since the color change read as optical density by the ELISA reader is a continuous variable, test samples are compared with control samples of known antibody concentration, or known reactivity or lack thereof. Control samples are usually produced by experimentally infecting animals with pure cultures of known serotypes of *Salmonella* and collecting their sera. Likewise, sensitivity and specificity determinations are made by comparing serological results to cultural results of swabs collected from the same experimentally infected animals, if culture is used as the "gold standard." Sensitivity and specificity may be quite different in a field situation compared to research conditions. The animals might have antibodies to other Entero-bacteriaceae that cross react with components of the ELISA; the infectious dose of *Salmonella* may be low (ie, ≤10^3 colony forming units); there may be *Salmonella* serovars of varying antigenicity; there may be varying levels of immunocompetence in the animals exposed; there might be multiple serovars of *Salmonella* on the farm, or serovars containing antigens not included in the ELISA; or there might be an effect on the antibody response of concurrent infections in the herd or vaccine or antibiotic use in the herd. It has been reported that an avirulent vaccine used to prevent salmonellosis in pigs due to *Salmonella* serovar Choleraesuis does not induce antibodies detectable by the DME.⁹
Our laboratory began a project to determine the seroprevalence levels of salmonellae in pigs. Two groups of pigs (Groups 1 and 2) in the same herd were sampled individually by collecting serum samples and rectal swabs on two occasions 2 to 3 weeks apart (samplings a and b).

**Figure 1:** Proportion of pigs that were positive, negative, or both for Salmonella serovars when tested both serologically (Danish mix-ELISA; positive cutoff value of ≥30 OD%) and by culture of rectal swabs. Two groups of pigs (Groups 1 and 2) in the same herd were sampled individually by collecting serum samples and rectal swabs on two occasions 2 to 3 weeks apart (samplings a and b).

---

Possible intervention strategies of merit

Field studies have identified several procedures that reduce Salmonella seroprevalence. Basically, these encompass all in-all out practices, cleaning and disinfection between batches, and strict control over the introduction of external sources of Salmonella (in pigs or feedstuffs). Salmonellae may survive up to 3 months in wet feces and 13 months in desiccated material. The use of home-ground barley feed or fermented liquid feeds, the addition of organic acids to the feed, or all three, may reduce Salmonella seroprevalence in groups of pigs. Vaccination for Salmonella Choleraesuis in the United States, not widely practiced in other countries (for example, in Denmark, where Salmonella Choleraesuis does not occur), may reduce the occurrence of subclinical salmonellosis. The feeding of home-ground corn-soy rations, which is common in the United States but not in other countries, may account for differences in the prevalence of subclinical infection between US herds and herds in other countries. There is some evidence that subclinical Salmonella infection may affect the growth performance of swine. A study which compared growth in groups of pigs with different Salmonella seroprevalence levels showed that low seroprevalence groups gained more than pigs in high seroprevalence.
groups. High Salmonella seroprevalence may be an overall indicator of cleanliness, effectiveness of rodent control, stocking density, and other management procedures on the farm.

**Use of ELISA serology for subclinical Salmonella infection surveillance**

An increase in the number of laboratories using in-house Salmonella ELISA tests and the approval of commercial kits will make Salmonella serology more available to practitioners and producers. There has not been a large outbreak of human foodborne salmonellosis attributable to pork consumption in the United States. It remains to be seen if or when serologic surveillance is widely adopted in the United States. When reports regarding use and interpretation of Salmonella serological testing are examined, three facts are clear. First, serological testing is most useful on a herd level, to identify herds with elevated seroprevalence. Second, repeated testing of successive groups of pigs in a herd is important to gain an accurate assessment of the Salmonella status of the herd. Finally, serological testing should not be used to identify a pig or group of pigs as Salmonella-infected or Salmonella-free. In a herd with high seroprevalence, culture of pooled pen fecal samples may identify the serotypes present. Considerations in selecting a Salmonella ELISA include the ability of the test to detect indigenous serotypes, availability of the test, and cost per sample. Information on the “cutoff” value and an approximation of how the test generally compares with other tests used in the same geographic area are also important.

Experience with serological testing in other countries suggests that it be used for seroprevalence determination and ongoing monitoring to provide a means of establishing a baseline for the herd and identifying a change in seroprevalence. That is, the test should be able to determine whether intervention strategies are warranted and, if implemented, are effective. Serological testing for Salmonella serovars represents another tool in the management of on-farm food-safety procedures for swine producers and veterinarians.

**Appendix**

**Danish monitoring for slaughter pig producing herds**

To approximate Danish-type surveillance for a herd, first establish a baseline index. For 3 consecutive months, either collect serum samples from 10 to 20 animals close to slaughter, or obtain 10 to 20 meat juice samples from carcasses after slaughter. Assay the samples for Salmonella antibody with the DME or an equivalent ELISA. In the Danish sampling system, 60, 75, or 100 samples per year are collected from each herd, depending upon herd size. Calculate the seroprevalence of the group, i.e., the number of positive samples (as determined by the “cutoff value” of the specific test you are using) divided by the total number tested. Calculate a weighted average of the seroprevalence (weighted 0.2, 0.2, and 0.6, least to most recent test), which provides a figure roughly equivalent to the Danish Salmonella Index. If the weighted average is <40, the herd would be considered Level 1 (low seroprevalence). If it is 40 to 70, the herd is Level 2, and if it is >70, the herd is Level 3 (high seroprevalence). This procedure is described and an example of this calculation is provided by Alban et al. Danish control programs for Salmonella have recently been reviewed by Wegener et al and elimination and eradication procedures by Harris and Harris. Salmonella ELISAs should not be used to classify a particular group of animals as “infected” with Salmonella. An antibody response is a historical measure and does not mean the animal is still infected; lack of an antibody response does not mean the animal is free from infection.

![Figure 2: Seroprevalence of Salmonella serovars was monitored during a 4-year period in approximately 86 swine herds producing breeding stock. Herds submitted 10 to 20 samples monthly from pigs in the finishers, >5 months of age, for testing using the Danish mix-ELISA. Each herd was placed in one of three seroprevalence categories according to the weighted mean seroprevalence determined from three consecutive herd submissions. The categories corresponded to seroprevalence <1%, 1% to 39%, 40% to 70%, and >70%.](image-url)
Acknowledgements
The author would like to extend her sincere appreciation to the following individuals and granting agencies: Drs Dave Baum, Thomas Blaha, Eric Bush, Jan Dahl, Mike Daniels, Bill Christianson, Jeff Gray, D.L. Harris, N. Lee, Ann Letellier, Al Lownachan, Denis Matousek, Bent Nielsen, D. Nilubol, Bo Norby, L.L. Sorensen, Montserrat Torremorell, and Rick Tubbs; Chris Baum, Brad Chriswell, Matt Erdman, Kathy Ferris, Stephen Gaul, Ellen Martens, Jeanne Nugent, Stephanie Wedel, Yuhua Zhang, and Huiyuan Zhao; and the Food Safety Consortium (US Department of Agriculture [USDA]- Cooperative State Research, Education, and Extension Service #20013421110276); the National Pork Board (National Pork Producers’ Council #00–100); PIC, US; and the USDA (#00511109757).

References – non refereed

References – refereed