REVISITING THE ROLE OF PIG SEROLOGY IN THE CONTEXT OF *SALMONELLA* CONTROL PROGRAMS IN COUNTRIES WITH HIGH PREVALENCE OF INFECTION - A PRELIMINARY STUDY

Raúl C. Mainar-Jaime[1*], A. Casanova-Higes[2], S. Andrés-Barranco[2], J.P. Vico[3]

[1]*Dpt. de Patología Animal, Instituto Agroalimentario de Aragón -IA2- (Universidad de Zaragoza-CITA), Avda. Miguel Servet, 177, 50013, Zaragoza, Spain. *E-mail: rcmainar@unizar.es*
[2]*Centro de Investigación y Tecnología Agroalimentaria de Aragón, IA2, Zaragoza, Spain*
[3]*IRNASUS-CONICET-Universidad Católica de Córdoba, Córdoba, Argentina*

Abstract

In this study, we assess whether on-farm serology may be useful for predicting *Salmonella* shedding at slaughter. Serology on serum samples collected 60 and 90 on fattening and three days before slaughter predicted somewhat shedding at slaughter with no significant differences among them. Pigs with higher OD% values at these point times would have higher risk of shedding when arriving to slaughter. On-farm serology may help to predict to some extent the risk of *Salmonella* shedding at slaughter in seropositive fattening units, which may allow for prompt on-farm and slaughter interventions to mitigate the risk of shedding when pigs arrive to slaughter.

Introduction

Most *Salmonella* national control programs in Europe have been based on the categorization of pig herds according to risk levels based on serological results, but most of them have not reported of any overall significant success on *Salmonella* infection reduction in fattening pigs or on the number of human cases attributable to pigs or pork. The United Kingdom suspended its meat juice testing for *Salmonella* antibodies in 2012 and moved towards an “on-farm risk assessment” approach based on a scoring system[1]. Belgium discontinued its serological program in 2015 but kept advisory veterinarians on the field (Brossé, 2015), and Germany, although still keeps its initial program, has not detected a significant reduction of category III farms (Blaha, 2017). The limited diagnostic accuracy of the tests used, the small number of animals sampled, and the likely lack of herd representativeness of the samples used, could have played a part in the wrong *Salmonella* characterization of a pig herd and the misperception of its true *Salmonella* risk. Spain, a country of high *Salmonella* prevalence and without any control program yet, may require a different strategy to tackle this problem.

Salmonella shedders are an important source of slaughter and carcass contamination (Argüello *et al*., 2013), thus from a pig farm´s perspective, a major goal to address should be the reduction of the number of pigs shedding *Salmonella* that arrive to the slaughterhouse. Focusing on the prevention of *Salmonella* shedding at slaughter may be far more important than focusing on detection of seropositivity at this stage. Pigs shedding *Salmonella* at slaughter seroconverted earlier during the fattening period

[1] http://www.pigprogress.net/Health-Diseases/Health/2012/6/UK-New-direction-for-Zoonoses-National-Control-Programme-ZNCP-PP008961W/

than non-shedder pigs (Casanova-Higes *et al*., 2016), therefore serology may help to predict the risk of shedding at slaughter.

Material and methods

The pigs considered for this preliminary study belonged to 6 control groups (batches A to F), of approximately 50 animals, included in previous studies carried out between 2010 and 2016 in a small fattening unit (N≈110) for other purposes. The farm was in NE of Spain and was known to be *Salmonella* positive. Pigs had been individually identified and only those that had been blood sampled at 30 (30d), 60 (60d), 90 (90d) days in the fattening unit and within 3 days before slaughter (BS), and for which a minimum of 25g of fecal (FEC) samples were collected at slaughter, were considered for this study. In addition, 25g of mesenteric lymph nodes (MLN) were also collected at slaughter from these pigs to assess their infection status. For serological analysis the HerdCheck Swine *Salmonella* ELISA (IDEXX Laboratories, ME, USA) was used. Bacteriology on FEC and MLN samples was performed according to the EN ISO 6579:2002/A1:2007.

Median OD% values and their 95%CI were estimated for each sampling time and for each batch of pigs. Overall estimates of prevalence of shedding (FEC+) and infection (MLN+) at slaughter were also calculated for each batch. The relationship between log-transformed OD% values at each sampling time (30d, 60d, 90d and BS) and shedding at slaughter was assessed by logistic regression analysis after adjusting by batch. When a significant association was found, Receiver Operating Characteristic (ROC) curves were constructed and the area under the curve (AUC) estimated for the ELISA. Estimates of probability of shedding *Salmonella* were calculated from logistic regression equations. Statistical analyses were performed with STATA software (STATA, StataCorp, L.P., USA).

Results

A total of 233 (76.1%) control pigs met the inclusion criteria and were included in the study. The number of sampled pigs varied among batches (Table 1). A total of 101 (43.3%; 95%CI: 36.9, 49.8) pigs shed *Salmonella* spp. at slaughter and in 97 (41.8%; 95%CI: 35.4, 48.2) the bacterium was isolated from MLN.

Table 1. Proportion of slaughter pigs shedding *Salmonella* and infected with *Salmonella* for each batch of pigs analyzed.

	Batch (no. of pigs)					
	A (25)	**B (28)**	**C (49)**	**D (48)**	**E (41)**	**F (42)**
% of shedders at slaughter (95%CI)	60 (39.4, 80.6)	21.4 (5.2, 37.6)	69.4 (66, 82.8)	75 (62.3, 87.7)	9.7 (0.3, 19.2)	14.3 (3.2, 25.3)
% of infected pigs at slaughter (95%CI)	76 (58, 94)	18.5 (2.8, 34.2)	40.8 (26.5, 55.1)	68.7 (55.1, 82.3)	7.3 (0, 15.3)	40.5 (25,55.9)

Serological results differed among batches (Figure 1). The OD% values for pigs from batches B and C remained quite low for all sampling times. In contrast, for batches A, D, and F, OD% values increased significantly after first sampling on day 30. For batch E, OD% values remained similar along the fattening period with some increase in the last sampling. No relationship was observed between ELISA results and shedding at slaughter when serum samples were collected on day 30 on fattening (*P*=0.79), but a positive significant relationship was found between OD% values and shedding at slaughter for samplings on days 60, 90 and BS (*P*-values <0.01).

Figure 2 depicts the ROC curves for the ELISA test with regard to shedding at slaughter when performed at 60 and 90 days on fattening and BS. No differences were observed regarding AUC among these three sampling times (AUC≈0.83). Since batches B and C presented very low OD% values along the entire fattening phase (median OD%≤10), a further ROC analysis was performed excluding them. Results remained similar, although the AUC increased somewhat (AUC≈0.87).

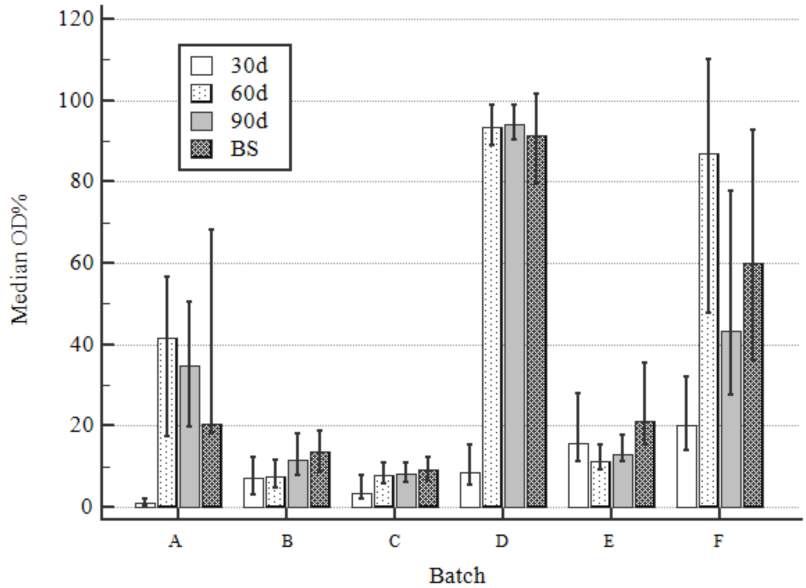

Figure 1. Median OD% values and their 95%CI for pig serum samples collected on day 30 (30d), 60 (60d), 90 (90d) on fattening and before slaughter (BS) for six batches of pigs (A to F).

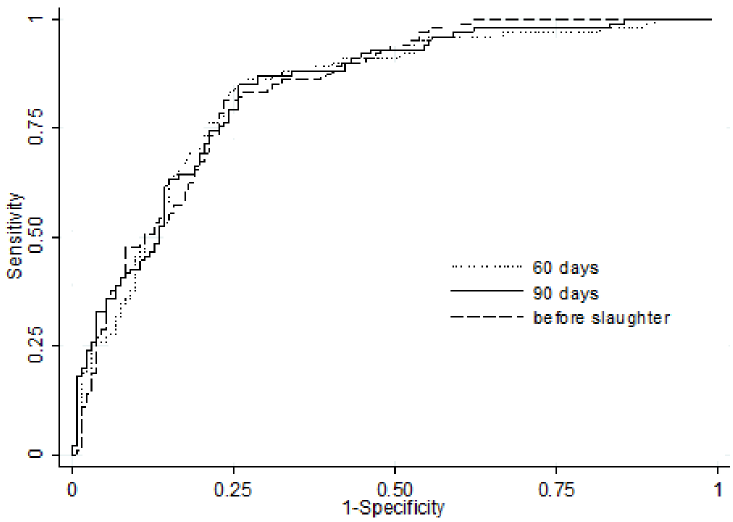

Figure 2. Receiver Operating Characteristic (ROC) curves estimated for prediction of shedding when an ELISA test was used on serum samples collected at 60 and 90 days on fattening, and before slaughter.

Estimates of the probability of shedding *Salmonella* spp. at slaughter with regard to OD% values for a pig sampled on day 90 on fattening from batch A are shown in Figure 3. When all batches were considered in the logistic regression analysis the probability of shedding *Salmonella* spp. for a pig showing an OD%=10 was as high as 43%. This risk increased significantly up to 65% for an OD%=40. When only batches A, D, E and F were considered these probabilities were 39.7% and 66% respectively.

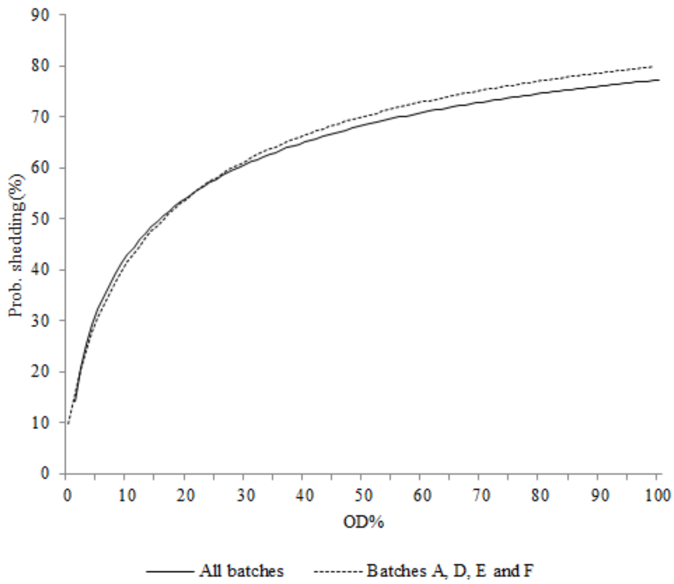

Figure 3. Estimated probability of shedding *Salmonella* at slaughter for a pig sampled on day 90 from batch A. Estimates are calculated for all the batches together (All batches) and also for those batches that showed increasing OD% values along the fattening period (A, D, E and F).

Discussion

In this study, serological results showed different on-farm pig *Salmonella* exposure experiences among batches, representing the expected variability for an infection like this one. In batch B, and particularly in C, pigs would have been infected at the end of the fattening period, i.e. within the last 10-15 days BS, with no time to develop detectable IgGs (Nielsen *et al*., 1995). The use of on-farm serology at any point time during the fattening period would have been useless in these two batches. In contrast, pigs from batches A, D, E and F experienced a significant increase of OD% values after day 30, which was compatible with pigs being exposed to the bacterium. Pigs from batch E showed OD% values relatively low and fairly constant along the fattening period. Thus, to explore the potential that serology may have on predicting *Salmonella* shedding at slaughter considering this variability of scenarios, this study initially took all the batches into account.

On-farm serology at the beginning of the fattening period, i.e. on day 30, was useless for the objective of predicting shedding at slaughter. This was an expected result as many pigs on day 30 may not have getting in contact with *Salmonella* yet, and some seropositive pigs at this time may have become seronegative BS (van der Wolf *et al*., 2001). However, a significant correlation was observed between serology on days 60 and 90 on fattening or BS and shedding at slaughter.

Analyzing pig serum in any of the last three sampling times yielded similar results as indicated by their corresponding AUC. Pigs with higher OD% values at these point times would have higher risk of shedding when arriving to slaughter (Figure 3). Estimates of the risk of shedding when batches B and C were not considered seemed to be somewhat more realistic since pigs from these batches were likely infected close to the date of slaughter, thus serology would yield biased results towards increasing the risk of shedding for pig showing low OD% values. Preventing infection during transport and lairage would surely improve the ability of on-farm serology to predict shedding at slaughter.

These results suggest that, for infections occurring time ahead of the slaughter date (between 15 and 45 days before), on-farm serology may help to determine to some extent the risk of *Salmonella* shedding at slaughter. For this purpose, a representative sample of pigs should be selected from the batch of interest. On-farm serology could be performed at any time after 60 days on fattening and until 2-3 days before slaughter, but as sooner is the serum analyzed, longer is the time available to respond to the risk. Thus, when shedding is likely, on-farm interventions could be promptly scheduled to try to mitigate the probability of shedding when pigs arrive to slaughter, and additional interventions could be implemented during transport or at slaughter. This approach would also allow for a more precise serological characterization of the pig farms through weighted averages of seroprevalence, as a representative sample of the pigs in the farm would be collected. Farms presenting low OD% values on day 90 would be expected to remain so for the rest of the fattening period if nothing wrong happens during the time remaining until slaughter. Special care on truck and lairage cleaning and disinfection procedures should be considered for these herds to prevent late infections and further shedding. A large-scale study to confirm the potential of this approach to reduce *Salmonella* shedding at slaughter is warranted.

Acknowledgements

Work funded by the National Institute for Agricultural and Food Research and Technology (RTA2012-24 and INIA-FPI 2014) and the Government of Aragón (DRU no. 2012-02-22-541-00-IFO-00770020052).

References

Argüello H., *et al*. 2013. J. Food. Protec.76(5): 899-911.

Blaha T. 2017. The German *Salmonella* serological monitoring programme. https://www.3tres3.com/ salmonela/el-programa-aleman-de-monitorizacion-serologica-de-salmonella_37930/(accessed 31 May 2017).

Brossé C. 2015. 2nd Int. *Salmonella* Symp. IDT Biologika - 12 Years of Directive 2003/99/EC: How far have we really come? Wörlitz, 23-25 September.

Casanova-Higes A., *et al*. 2016. Zoon. Pub. Health. doi: 10.1111/zph.12301.

Nielsen B., *et al*. 1995. Vet. Microbiol. 47, 205–218.

van der Wolf P., *et al*. 2001. Vet. Quart. 23(3):116-121