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Estimation of the *Salmonella enterica* prevalence in finishing swine

H. S. HURD[1]*, J. D. McKEAN[2], R. D. GRIFFITH[3] AND M. H. ROSTAGNO[1]

[1] *Pre-Harvest Food Safety and Enteric Diseases Research Unit, USDA, Agricultural Research Unit, Ames, IA 50010, USA*
[2] *Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011, USA*
[3] *Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA*

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SUMMARY

The study objective was to evaluate three methods of *Salmonella enterica* prevalence estimation in swine herds (faecal culture, culture of abattoir-collected samples, and serum ELISA). From each of six swine herds, we necropsied approximately 100 finishing pigs (>70 kg); one-half on farm and the other half at the abattoir, after transport and approximately 2·5 h holding. We collected the same samples for *S. enterica* culture at both locations (1 g faecal, 10 g caecal contents, ileocaecal lymph nodes, superficial inguinal lymph nodes, 25 g of gluteal muscle for serum ELISA). On farm, the 1 g faecal sample only detected 13·3% (2/15) of all positive pigs necropsied on farm. However, with abattoir and on-farm results combined, the faecal sample detected 57·4% (74/129) of positive pigs. Abattoir-collected samples provided prevalence estimates much higher than on-farm collected samples (39·9 *vs.* 5·3%; $P<0{\cdot}001$). This study shows that faecal samples have a low sensitivity for detecting infected pigs and that abattoir-collected samples overestimate the on-farm *S. enterica* prevalence. For most herds, serology overestimated the on-farm culture prevalence.

INTRODUCTION

In food animals, *Salmonella enterica* prevalence estimation can serve multiple purposes: to estimate the on-farm prevalence for risk factor analysis, intervention assessment and producer feedback, and to predict the food safety risk of products entering the food chain. In pork production, the more *S. enterica* that is carried into the plant, via the pigs, the greater the risk of equipment contamination and final product contamination [1, 2]. Therefore, pre-harvest (pre-slaughter) prevalence data can be useful in improving pork safety.

Prevalence estimation is affected by the sampling strategy (number tested, stratified or cluster sampling, etc.) and the diagnostic test in use. Diagnostic test considerations include cost, logistics, specificity, and sensitivity. Overall, sensitivity is affected by analytical sensitivity and diagnostic sensitivity. The analytical sensitivity describes a test's ability to detect some quantitative amounts of a target in a positive sample. The ability to correctly identify an infected animal (diagnostic sensitivity), is a function of the pathophysiology of the agent, the volume of sample tested, the concentration of the target, and the probability that the target is present in the given sample.

* Author for correspondence: H. S. Hurd, USDA, ARS, National Animal Disease Center, P.O. Box 70, Ames, IA 50010-0070, USA.

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Diagnostic sensitivity is particularly problematic with an intermittently shed organism, such as *S. enterica*.

The diagnostic tools available for pre-harvest *S. enterica* prevalence estimation are limited. Generally, three tools can be identified: organism detection in faeces, organism detection in post-mortem samples collected at the abattoir, and detection of serum antibodies. Organism detection methods may include PCR (polymerase chain reaction), which targets a part of the *S. enterica* genome, antigen capture ELISA tests, which detects cell proteins, and organism isolation by culture. Faecal culture of individual pigs is commonly used in the pork industry to estimate prevalence. However, the reported diagnostic sensitivity on known positive pigs is not high ($\sim$80–90%) [3, 4]. Rectal swabs are occasionally used, but have been shown to have an extremely low sensitivity [5]. In studies of infected humans, for example, over one-third of infected patients were not detected by 3 days of rectal swabbing. Reasons for low sensitivity of faecal samples include competition between *S. enterica* and other enteric bacteria, low numbers of *S. enterica* in the sample, and intermittent faecal shedding from the infected host [4, 6].

Serum antibody detection tests have been used since 1995 in the Danish *Salmonella* control programme, and more recently in other European countries [7–9]. Serology is recommended for use as a herd level test only, due to the historical nature of serum antibodies and the variability in results over time. Therefore, in Denmark, the results from three consecutive months of herd testing are used to classify herds into high, medium, or low categories of *Salmonella* prevalence [10].

Researchers have shown approximate but variable associations between serological results and other measures, such as faecal or caecal culture, pen faecal culture, and carcase swab culture. Serology seems to be able to distinguish between high and low prevalence herds [1, 8, 11]. However, precise and statistically quantifiable associations between serological and culture results was not always demonstrable [12, 13]. Additionally, the prevalence category (high, medium and low) of a herd may change over time, when measured with serology [14–16].

Another prevalence estimation tool available for food animals is collection, at the abattoir, of intestinal contents and other tissues [15, 17, 18]. Two shortcomings of this method include the possibility of in-plant tissue cross-contamination and rapid infection of pigs during transport and holding [19–21]. We have shown that finishing swine can be infected in the ileocaecal lymph nodes (ICLNs) and intestinal contents after only 2 h of exposure to a contaminated holding pen floor [22]. How this rapid infection affects on-farm prevalence estimation remains to be demonstrated.

There are two overlapping objectives of this report. The first is to estimate the sensitivity of faecal culture using known positive finishing swine necropsied on farm. The second is to compare *Salmonella* prevalence estimates provided by the three tools: faecal culture, culture of abattoir-collected samples and serum ELISA.

MATERIALS AND METHODS

Herd selection

Herds eligible for inclusion in this study were those enrolled in the Accelerated Pseudorabies Eradication Program (APEP). This was a voluntary effort of state and federal regulatory agencies (USDA, Animal and Plant Health Inspection Service) to depopulate swine herds that tested positive for the Pseudorabies virus in the last 12 months. The producer was reimbursed at fair market value for breeding stock and by weight for market animals. To avoid oversupply of pork, all APEP swine were to be euthanized at a commercial packing plant and sent for rendering. As producers within the state of Iowa were enrolling in the APEP from January 2000 to April 2000, they were invited to participate in this study. Herds were required to have a minimum of 100 animals weighing over 70 kg available for sampling. Final selection of herds included consideration of herd management, statewide location, timing of scheduled depopulation, and our data collection resources. Our goal was to test the first six qualifying and available herds. Herd selection was not intended to test hypotheses about the effect of management or herd size on *S. enterica* prevalence. Each herd provided its own control group.

Animal selection

From each herd, approximately 100 market or finishing swine were to be studied. One half was randomly assigned to on-farm necropsy, the other half to abattoir necropsy. The selection of 50 pigs for treatment (abattoir necropsy) and 50 for control (on-farm necropsy) would provide sufficient power to detect a *prevalence* difference of 25% with 95% confidence.

Except for breeding stock, all pigs over 70 kg were eligible for inclusion. We selected a predetermined number of pigs from each pen, based on total number of pens. Consequently, every eligible pig had an equal chance of being selected.

Sample collection

Three days before the scheduled depopulation, we collected a 1 g predepopulation faecal sample (PFEC) using a faecal loop (Jorgensen Inc., Loveland, CO, USA), and we attached alternately coloured and uniquely numbered ear tags to the study animals. The purpose of this faecal collection was to estimate the *S. enterica* status of the individual farm before any possible disruptions associated with pending depopulation. On the day of depopulation, we determined which ear tag colour group would be necropsied on farm and which at the abattoir. In all except herd 1, assignment was random. In herd 1, we selected pigs from the first three pens for farm necropsy and the next three for abattoir necropsy. For all herds, study pigs and herd mates were transported in disinfected commercial vehicles.

At the abattoir, study pigs ($n=50$) were held together in a standard holding pen. After holding, they were stunned and exsanguinated. The carcases were then immediately diverted, before entry into the slaughter area, to necropsy in an open-top trailer. The ICLN and superficial inguinal lymph node (SILN) were collected using forceps and scissors scrubbed in 70% ethanol between each pig. The caecal contents ~30 ml were collected through a puncture in the caecum. The necropsy faecal sample (NFEC) was taken, through the rectum, using a sterilized faecal loop (1 g) for each pig. A 25 g portion of the gluteal muscle was collected for detection of *S. enterica* antibodies.

On the day following abattoir necropsy, pigs ($n=50$) selected to remain on farm were euthanized with a captive bolt gun. They were immediately moved to a central, on-site location where samples were collected in the same manner as at the abattoir.

Sample processing

All samples were placed on ice and transported to the National Animal Disease Center (NADC, Ames, IA, USA), where they were refrigerated (4 °C) until processed the following morning. For processing, the SILN (10 g) and the ILCN (5 g) were separately macerated in a sterile bag with a rubber mallet. Peptone water (Becton Dickinson, Sparks, MD, USA) (10 ml) was added and each sample was homogenized using a stomacher (Stomacher 400 Circulator, Seward Ltd, London, UK) at 260 r.p.m. for 1 minute. Then 1 ml of supernatant was added directly to each pre-enrichment medium (9 ml). Caecal and faecal samples (10 g) were added directly to each pre-enrichment broth (90 ml). For *Salmonella* cultures, pre-enrichment included separate tubes of GN–Hajna broth (Becton Dickinson) (24 h at 37 °C) and tetrathionate broth (Becton Dickinson) (48 h at 37 °C) followed by enrichment in Rappaport–Vassiliadis media (Becton Dickinson) (10 ml, 24 h at 37 °C). A portion of the Rappaport–Vassiliadis media was then streaked to Brilliant Green Sulfa (BGS) agar (Becton Dickinson) (24 h at 37 °C) and XLT4 agar (Becton Dickinson) (24 h at 37 °C), after which a single suspect colony was picked and transferred to triple sugar iron (Becton Dickinson) and lysine iron agar slants (Becton Dickinson) (24 h at 37 °C). Biochemically suspect isolates were further classified by agglutination using Bacto Salmonella O antiserum Groups poly A-I & Vi, B, C1 and E (Becton Dickinson). These isolates were then placed on TSA slants and shipped to National Veterinary Services Laboratories (Ames, IA, USA) for serotyping.

Gluteal muscle sections were frozen (−20 °C), upon arrival at the laboratory. Later, these samples were sent, to the laboratory of Dr D. L. Harris, Iowa State University, where the Danish mixed-ELISA for *S. enterica* antibodies was performed using the serum exudate (meat juice) [15]. The level of antibodies was measured in the ELISA by a colorimetric (wavelength 490) response expressed as optical density percent (OD%). Prevalence estimates at different levels of OD_{490}% were evaluated.

Analysis

A pig was defined as positive if any of the samples collected at necropsy were positive. The true farm prevalence (TFP) was defined as the number of pigs positive on farm divided the number tested. This TFP estimate, and its associated 95% confidence interval (CI), was compared to estimates provided by other sample types collected on farm or at the abattoir. The TFP was also compared to estimates provided by meat juice ELISA. For the ELISA, various cut-offs of the OD_{490}% were used to determine if a sample was positive [23]. The 95% CI was calculated with the

Table 1. Salmonella enterica *prevalence estimates provided by different sample types collected from finishing swine* (>*70 kg*) *4 days before depopulation, at on-farm necropsy, and at abattoir necropsy** (*6 herds*)

Sample location and type	Number positive	Number tested	Prevalence estimates (%)	95% CI
Pre-depopulation, 1 g faecal (PFEC)†	3	280	1·1	0–2·3
On-farm necropsy, 1 g faecal (NFEC)	2	281	0·7	0–1·7
On-farm caecal contents	5	281	1·8	0·2–3·3
On-farm lymph nodes‡	10	281	3·6	1·4–5·7
True farm prevalence (TFP)§	15	281	5·3	2·7–8·0
Abattoir prevalence§	114	286	39·9	34·2–45·5
Abattoir necropsy, 1 g faecal (NFEC)	72	286	25·2	20·1–30·2
Abattoir caecal contents	39	286	13·6	9·7–17·6
Abattoir lymph nodes‡	26	286	9·1	5·8–12·4

* Pigs were randomly selected, on farm, for abattoir or on-farm necropsy. Abattoir samples were collected after 2–4 h of transport in disinfected trailers and after ~2·5 h holding in an ante-mortem holding pen, before carcases entered the scald tank.

† Pre-depopulation results shown are only for pigs necropsied on farm for comparison to other on-farm collected samples.

‡ On-farm, lymph-node positives represent ICLNs only, as no SILNs were positive. At the abattoir, lymph nodes are combined results of ICLNs and SILNs.

§ Prevalence defined by the number of pigs positive by any sample type (1 g faecal, 10 g caecal contents, lymph nodes). Lower bound of confidence interval truncated at 0·0.

normal approximation to the binomial [24]. In the case of low point estimates, the lower bound of the CI might calculate to less than zero, but 0·0% is reported in our results.

The relative sensitivity of the 1 g faecal sample (NFEC), was estimated by comparing the proportion of pigs positive by that sample type to the proportion positive by: (1) caecal contents, (2) lymph nodes, (3) caecal contents or lymph nodes, and (4) all samples, including the faecal [25]. This analysis was conducted for samples collected on farm and for the combined on-farm and abattoir results. The same comparisons were used for determination of the kappa statistic for agreement between tests [24].

RESULTS

Table 1 shows the prevalence estimates provided by each of the sample types collected during on-farm and abattoir necropsy. The pre-depopulation faecal sample (PFEC) provided a *S. enterica* prevalence estimate of 1·1% (CI 0–2·3%). This estimate was similar to that from the NFEC collected 4 days later (0·7%, CI 0–1·7%). It is notable that these estimates were similar but came from different positive pigs. Only one pig positive on PFEC was also positive on NFEC. The point estimate from on-farm lymph-node culture (3·6%) was five times higher than the on-farm NFEC (0·7%). The on-farm lymph-node positives shown in Table 1 represent ICLNs only, as no SILNs were detected positive on farm. The TFP, combining results of all sample types, was estimated as 5·3% (CI 2·7–8·0%). This prevalence was higher than provided by any single sample type. The 95% CI for TFP did not overlap the PFEC (1·1%) and NFEC (0·7%), but did overlap the CI provided by the lymph nodes (3·6%).

As shown in Table 1, the prevalence estimates provided by abattoir-collected samples were much higher than the estimates from any on-farm collected samples. The overall abattoir prevalence was 39·9% (CI 34·2–45·5%) compared to 5·3% for TFP. Similarly, the estimate provided by each abattoir-collected sample was higher than the on-farm estimate from the same sample type. The prevalence estimate from abattoir NFEC was 25·2% compared to 0·7% for

Table 2. Salmonella enterica *isolation rates and seroprevalence for pigs necropsied on farm or at the abattoir. Numbers of samples tested are shown in parentheses. For each herd, percentages with the same superscript letter are significantly different between farm and abattoir for that sample type* ($P<0{\cdot}05$)

Sample type	Collection site	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5	Herd 6
Pre-depopulation faecal* (PFEC)	Farm	1·0 % (97)	1·1 % (94)	0 % (89)	4·4 % (90)	2·0 % (98)	0·0 % (98)
Necropsy faecal (NFEC)	Farm	0 % (48)	0 % (44)[a]	0 % (45)[b]	4·4 % (45)[c]	0 % (49)[d]	0 % (50)
	Abattoir	2 % (50)	30 % (50)[a]	36·4 % (44)[b]	31·1 % (45)[c]	51·0 % (49)[d]	2·1 % (48)
Caecal contents	Farm	0 % (48)	0 % (44)	0 % (45)[e]	4·4 % (45)[f]	6·1 % (49)	0 % (50)
	Abattoir	2 % (50)	4 % (50)	13·6 % (44)[e]	53·3 % (45)[f]	10·2 % (49)	2·1 % (48)
Lymph nodes†	Farm	6·3 % (48)	4·5 % (44)	2·2 % (45)[g]	4·4 % (45)	4·1 % (49)	0 % (50)
	Abattoir	6·0 % (50)	6 % (50)	27·3 % (44)[g]	0 % (45)	10·2 % (49)	6·3 % (48)
True farm prevalence (TFP)‡	Farm	6·3 % (48)	4·5 % (44)[h]	2·2 % (45)[l]	8·9 % (45)[j]	10·2 % (49)[k]	0 % (50)[l]
Any sample positive	Abattoir	10 % (50)[m]	38 % (50)[h,n]	54·5 % (44)[l,o]	68·9 % (45)[j,p]	61·2 % (49)[k,q]	10·4 % (48)[l]
Meat juice ELISA OD % >20	Farm	4·2 % (48)	95 % (44)	0 % (45)	26·7 % (45)	33·3 % (48)	12 % (50)
	Abattoir	4·4 % (45)	90 % (50)	0 % (42)	15·6 % (45)	25·5 % (47)	10·4 % (48)
	Combined farm and abattoir	4·3 % (93)	92·6 % (94)	0 % (87)	21·1 % (90)	29·5 % (95)	11·2 % (98)
Meat juice ELISA OD % >40	Farm	0 % (48)	77·3 % (44)	0 % (44)	13·3 % (45)	25·0 % (48)	8·0 % (50)
	Abattoir	0 % (45)	58 % (50)	0 % (42)	11·1 % (45)	21·3 % (47)	6·3 % (48)
	Combined farm and abattoir	0 % (93)[m]	67 % (94)[n]	0 % (87)[o]	12·2 % (90)[p]	23·5 % (95)[q]	7·1 % (98)

* A 1 g faecal sample taken 3 days before depopulation.
† Results from the ICLNs and SILNs are combined.
‡ TFP is defined as *S. enterica* isolation from any of the samples collected at necropsy (1 g faecal, caecal contents or lymph nodes). Cells with the same superscript letter are significantly different ($P<0{\cdot}05$).

on-farm NFEC, 13·6 *vs.* 1·8 % from caecal samples, and 9·1 *vs.* 3·6 % from lymph nodes, respectively. Similar differences between farm and abattoir were observed in each individual herd (Table 2). Only herd 1 did not have a significant difference ($P<0{\cdot}05$) in culture prevalence (TFP *vs.* any abattoir positive). Only herd 3 had much difference in the lymph-node-positive rates between farm and abattoir (2·2 *vs.* 27·3 %).

The relative sensitivities of the 1 g faecal sample collected on farm are shown in Table 3. On farm, the NFEC correctly detected 40 % (2/5) of pigs with *S. enterica* in the caecum. However, it missed all 10 of the pigs with positive ICLNs. On farm, the NFEC only detected 13·3 % (2/15) of all infected pigs. For all sample types, the kappa statistics were low (0·23–0·57).

Table 3 also shows the relative sensitivity of NFEC when results from on-farm and abattoir necropsies were combined. Many more samples collected at the abattoir were *S. enterica*-positive. With combined results, the relative sensitivity of NFEC was improved to 57·4 % (74/129), compared to on-farm only. The agreement as measured by the kappa statistic (0·68) could be considered as moderate [25]. However, NFEC still only detected 12·2 % (9/74) pigs with positive lymph nodes and 31·8 % of pigs with positive caecal contents.

In Table 4, the TFP for all 6 herds, derived from on-farm necropsy is compared to the seroprevalence estimates provided by the Danish mixed-ELISA from meat-juice samples collected on farm, at the abattoir, and combined. The seroprevalence estimates using cut-off values of OD % >10, 20, 30 and 40 are shown. All seroprevalence estimates were higher than the TFP.

Table 2 shows the same data as Table 1 for each individual herd with ELISA results at the OD % 20 and 40 cut-offs. Herd seroprevalence estimates ranged greatly from 0 to 92·6 % (combined farm and abattoir). Herds 1 and 3 had no positive pigs at the OD % 40 cut-off. However, both of these herds had

Table 3. *Relative sensitivity and kappa statistic for agreement between 1 g faecal and other sample types for detection of* Salmonella enterica. *Samples were collected at necropsy of finishing swine* (>*70 kg*), *on farm or at the abattoir**

	Farm necropsy only ($n=281$)				Combined farm and abattoir necropsy ($n=567$)			
	Number positive by sample type	Number of same pigs faecal positive	Kappa	Relative sensitivity (%)	Number positive by sample type	Number of same pigs faecal positive	Kappa	Relative sensitivity (%)
Caecal contents	5	2	0·57	40·0	44	14	0·16	31·8
Lymph nodes†	10	0	0	0·0	74	9	0·09	12·2
Caecal or lymph nodes	15	2	0·23	13·3	74	22	0·18	29·7
Overall relative sensitivity of faecal sample‡	15	2	0·23	13·3	129	74	0·68	57·4

* Pigs were randomly selected, on farm, for abattoir or on-farm necropsy. Abattoir samples were collected after 2–4 h of transport in disinfected trailers and after ~2·5 h holding in an ante-mortem holding pen, before carcases entered the scald tank.

† On-farm, lymph-node positives represent ICLNs only, as no SILNs were positive. At the abattoir, lymph nodes are combined results in ICLNs and SILNs.

‡ Overall relative sensitivity of the faecal sample was based on combined culture results of 1 g faecal, caecal contents and lymph nodes.

Table 4. *Comparison of* Salmonella enterica *seroprevalence* estimates to estimates from culture of on-farm or abattoir-collected necropsy samples* (*1 g faecal, 10 g caecal contents and ICLNs*)

	Number positive	Number tested	Prevalence estimate (%)	95% CI
True farm prevalence (TFP)†	15	281	5·3	2·7–8·0
On-farm OD% >10	103	280	36·8	31–42
On-farm OD% >20	78	280	27·9	23–33
On-farm OD% >30	66	280	23·6	19–29
On-farm OD% >40	56	280	20·0	15–25
Abattoir OD% >10	87	278	31·3	26–36
Abattoir OD% >20	71	277	25·6	20–31
Abattoir OD% >30	59	278	21·2	16–26
Abattoir OD% >40	47	277	17·0	13–21
Combined‡, OD% >10	190	558	34·1	30–38
Combined‡, OD% >20	149	557	26·8	23–30
Combined‡, OD% >30	125	558	22·4	19–26
Combined‡, OD% >40	103	557	18·5	15–22

* Danish ELISA performed on serum exudates from gluteal muscle samples collected at necropsy.

† Prevalence defined by the number pigs positive by any sample type (1 g faecal, 10 g caecal contents and lymph nodes).

‡ Combined results include serum samples collected at on-farm and abattoir necropsy.

culture-positive pigs on farm. Herd 6 had no on-farm culture-positive pigs. However, the seroprevalence estimates were 11·2 and 7·1% for OD% 20 and 40, respectively. Attempts to associate TFP with categories of seroprevalence (high, medium and low), showed no correlation. For example, herd 2 with a

92·6% seroprevalence (OD% >20) had a TFP of 4·5%, whereas herd 1 with a 4·3% seroprevalence had a TFP of 6·3%.

Overall, a minority of culture-positive pigs was seropositive. Of the 127 culture-positive pigs (farm and abattoir), only 23·6% were seropositive (OD% >40). For OD% cut-offs greater than or equal to 30, 20 and 10, respectively, 26·8, 31·5 and 35·4% of culture-positive pigs were seropositive.

DISCUSSION

This study provided a unique opportunity to compare faecal culture results with necropsy culture results on a large number of pigs ($n=567$). It also allowed comparison of abattoir-collected samples to on-farm collected samples, without in-plant cross-contamination. Herds in this study typified moderate-sized Midwestern United States producers. *S. enterica* serotypes recovered also represented a wide range of possibilities, as 22 different serovars were recovered [21].

The 1 g faecal sample only detected one-fifth (0·7% NFEC, 1·1% PFEC) of the truly infected pigs on farm (5·3%). The relative sensitivity (13·3%) of the 1 g faecal sample demonstrates that a faecal sample provides a very low probability of detecting an infected pig. It is particularly poor at detecting pigs infected in the lymph nodes (0% on farm, 12·2% farm plus abattoir). The relative sensitivity of the NFEC improved to 57·4% when abattoir-collected samples were included in the calculation. This result may be due to recent intestinal infection obtained at the abattoir [21, 22]. Recently ingested organisms will still be recoverable from gut contents and may be in higher quantities than in chronically infected animals.

Low sample volume (1 g) may partly explain the low sensitivity of NFEC. Funk et al. have shown that the relative sensitivity increases two-fold with a 10 g sample [26]. Therefore, our faecal prevalence estimate would have gone up to about 2% (Table 1). However, this prevalence is still only approximately half of the TFP measure by culture of multiple samples collected at necropsy.

On farm, the culture of ICLNs provided a prevalence estimate (3·6%) closest to the true prevalence (5·3%). Faecal samples collected on farm did not detect any of the 10 pigs with positive lymph nodes (Table 2). These observations are consistent with conventional wisdom that latent undetectable carriers are a common element of *S. enterica* epidemiology.

One reason that the TFP was higher than any individual sample type may be the collection of multiple samples from the same pig. Each necropsied pig provided four opportunities to recover *S. enterica*: faeces, caecal contents, ICLNs and SILNs. Therefore, multiple rounds of faecal collection, with replacement (resampling of animals permitted), should be considered for on-farm prevalence estimation. If some knowledge of the faecal test sensitivity is available, then the prevalence estimate could be adjusted upwards. However, a test with low sensitivity could detect no positive animals, making extrapolation to a herd prevalence difficult.

For all herds combined, serology consistently overestimated the TFP of 5·3% (Table 4). This finding is not surprising considering that serology reflects the exposure history of pigs, not the current infection status. However, Table 2 shows the correlation between TFP and serology is variable from herd to herd. Therefore, serology on a particular shipment of pigs may misrepresent infection status and food safety risk.

The prevalence estimate obtained by culturing pigs at the abattoir was seven times higher than the estimate provided by on-farm necropsy of penmates, 39·9 *vs.* 5·3%. This observation raises questions about the value of testing pigs at the abattoir for farm prevalence estimation. It also demonstrates that rapid infection and internal contamination of the gastrointestinal tract during transport and holding is an important complicating factor in on-farm prevalence estimation [18, 20–22]. It should be noted, however, that the confidence interval for abattoir-collected lymph nodes (9·1%) overlapped that of the TFP. Additionally, most herds had similar lymph-node prevalence estimates between farm and abattoir. This observation suggests that ICLNs may have some utility as an on-farm prevalence estimation tool.

In summary, this study suggests that (1) a single 1 g faecal sample will severely underestimate the TFP; (2) repeated faecal sampling will improve prevalence estimation; (3) a single cross-sectional serological sample provides an unreliable estimate of herd prevalence; and (4) abattoir-collected culture samples may not provide good on-farm prevalence estimates. We suspect these conclusions may also apply to other enteric pathogens in other food animals.

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