

## A prevalence study of *Salmonella* spp., *Yersinia* spp., *Toxoplasma gondii* and porcine reproductive and respiratory syndrome virus in UK pigs at slaughter

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### SUMMARY

An abattoir-based study was undertaken between January and May 2013 to estimate the prevalence of *Salmonella* spp. and *Yersinia* spp. carriage and seroprevalence of antibodies to *Toxoplasma gondii* and porcine reproductive and respiratory syndrome virus (PRRSv) in UK pigs at slaughter. In total, 626 pigs were sampled at 14 abattoirs that together process 80% of the annual UK pig slaughter throughput. Sampling was weighted by abattoir throughput and sampling dates and pig carcasses were randomly selected. Rectal swabs, blood samples, carcass swabs and the whole caecum, tonsils, heart and tongue were collected. *Salmonella* spp. was isolated from 30.5% [95% confidence interval (CI) 26.5–34.6] of caecal content samples but only 9.6% (95% CI 7.3–11.9) of carcass swabs, which was significantly lower than in a UK survey in 2006–2007. *S. Typhimurium* and *S. 4,[5],12:i:-* were the most commonly isolated serovars, followed by *S. Derby* and *S. Bovismorbificans*. The prevalence of *Yersinia enterocolitica* carriage in tonsils was 28.7% (95% CI 24.8–32.7) whereas carcass contamination was much lower at 1.8% (95% CI 0.7–2.8). The seroprevalence of antibodies to *Toxoplasma gondii* and PRRSv was 7.4% (95% CI 5.3–9.5) and 58.3% (95% CI 53.1–63.4), respectively. This study provides a comparison to previous abattoir-based prevalence surveys for *Salmonella* and *Yersinia*, and the first UK-wide seroprevalence estimates for antibodies to *Toxoplasma* and PRRSv in pigs at slaughter.

**Key words:** *Salmonella*, *Toxoplasma gondii*, veterinary pathogens, *Yersinia enterocolitica*, zoonotic foodborne diseases.

### INTRODUCTION

Foodborne diseases are estimated to cost the UK nearly £1.5 billion per year [1]. *Salmonella* spp. is the second most frequently reported cause of food

poisoning in the UK [2], with an estimated 33 000 cases per year [3]. There has been a reduction in reported human cases over the last 5 years, which is in part due to the successful implementation of National Control Programmes (NCPs) in the poultry sector [2, 4]. The declining risk from poultry meat and eggs means that the proportion of human cases attributable to pork and pork products may rise. The European Food Safety Authority (EFSA) recently estimated that over 50% of *Salmonella* infections in

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humans across the European Union (EU) may be attributable to pigs and pork [5] while the contribution within the UK has been estimated at 11.7% [6].

Between 2006 and 2008, EU-wide *Salmonella* prevalence surveys were conducted to obtain baseline and comparable data for all Member States for both breeding and slaughter pigs (Commission Decisions 2008/55/EC and 2006/668/EC). The results for slaughter pigs indicated that UK levels of *Salmonella* were above the EU average, with *Salmonella* isolated from 21.7% of ileo-caecal lymph node samples and 15.2% of carcass swabs (compared to an average of 10.3% and 8.3% across the EU, respectively) [7, 8].

An EU-wide and UK National Control plan for *Salmonella* in pigs was expected to begin by 2015 [9] although the focus has since moved to increased abattoir-based controls [Commission Regulation (EU) No. 217/2014] with no targets set for primary production. In order to ascertain an updated prevalence estimate prior to the implementation of these controls, a prevalence study was launched in 2013. This provided an opportunity to simultaneously investigate the prevalence of other organisms of public and/or animal health relevance in UK pigs, including *Toxoplasma gondii*, *Yersinia* spp. and porcine reproductive and respiratory syndrome virus (PRRSv).

*Yersinia* was identified in an EFSA Opinion on pig meat inspection published in 2011 as one of the four major public health hazards [10]. Pigs are considered to be the primary reservoir of human pathogenic *Y. enterocolitica* strains, mainly because of the high prevalence of such strains in pigs and the genetic similarity between human and porcine isolates [11]. Estimates of *Y. enterocolitica* intestinal carriage in British pigs have been reported previously at 10.2% [12] and 26.1% [13]. Human cases of yersiniosis in the UK are rare compared to other European countries, most likely due to less frequent consumption of raw pork [14, 15]. Nevertheless, EU Member States are recommended to gather prevalence data for pigs at slaughter at regular intervals [16] and the survey provided an opportunity to fulfil this recommendation.

An estimated 350 000 people become infected with *Toxoplasma* each year in the UK, of which 10–20% are symptomatic [17]. Tissue cysts are highly infectious for both people and animals and undercooked meat has been identified as an important source of human infection. However, there is little data on meat contamination in the UK and further studies have been recommended to establish the seroprevalence for antibodies to *Toxoplasma gondii* in UK

livestock [17]. A recent serological survey had been carried out in sheep [18] but there had never been a UK-wide serological survey in pigs.

PRRSv has a significant economic impact on pig farming with direct and indirect costs associated with production losses, increased mortality, treatment, and disruption to breeding programmes. The cost to a 500-sow herd in the first year of infection has been estimated at £52 000 and £94 000 in a growing herd and breeding herd, respectively [19]. Better understanding of the prevalence and epidemiology of PRRSv in the UK is needed in order to target control measures appropriately and assess the effectiveness of interventions. The survey provided an opportunity to carry out the first UK-wide study of PRRSv in pigs at slaughter.

## MATERIALS AND METHODS

### Study design

The study design was consistent, where possible, with the technical specifications for the previous EU baseline survey for *Salmonella* in slaughter pigs (Commission Decision 2006/668/EC). The target sample size for that survey was 600 pigs, based on sample size calculations using an estimated prevalence of 50% with an accuracy of 4% and 95% confidence [7]. Additional sample size calculations showed that by sampling about 600 pigs in this survey, it would be possible to determine (with 80% power and 95% confidence) a 20% overall change (reduction or increase) in *Salmonella* prevalence. In anticipation of non-responses or inadequate samples, a further 10% of additional pigs were scheduled for sampling. The target population was all slaughtered pigs (finishers plus cull sows and boars) in the UK.

### Abattoir recruitment and sampling schedule

Sampling was scheduled to take place between 14 January 2013 and 12 April 2013 at 14 high-throughput abattoirs that together processed 80% of all pigs slaughtered in the UK. Abattoirs were recruited by the British Pig Executive (BPEX) and the Food Standards Agency (FSA) organizations in Great Britain and Northern Ireland.

Sampling was weighted so that the number of carcasses to sample in each abattoir was proportional to their annual throughput; the total number of pigs scheduled for sampling by each abattoir thus ranged between 12 and 98. The total number of carcasses to

be sampled by each abattoir was then stratified by calendar month. The dates of sampling within each month and the carcass to be sampled on each of those days were then both randomly selected using Microsoft Excel (Microsoft Corp., USA). For most of the abattoirs, the maximum number of pigs sampled per day was one pig (three abattoirs) or two pigs (seven abattoirs). In the four remaining abattoirs, the maximum number sampled per day was three pigs (two abattoirs), five pigs (one abattoir) and six pigs (one abattoir); however, these were rare exceptions and these abattoirs most commonly sampled fewer pigs per day.

### Sample and data collection

Samples were collected by trained staff of the FSA in Great Britain and the Veterinary Public Health Unit of the Department of Agriculture and Rural Development (DARD) in Northern Ireland. Eight samples were collected along the processing line from each selected carcass: one rectal swab immediately post-stun; one blood sample post-bleed; the whole caecum, tonsils, heart and tongue at the evisceration point; and two pre-chill carcass swabs (Polywipe™ pre-moistened blue sponge swabs, MWE, UK). The carcass swabs were taken from opposite sides of the carcass, using one single sponge for all four sites described in Annex A of Standard ISO 17604 (i.e. the hind limb, abdomen, mid-dorsal region and jowl); two sites were swabbed with one side of the sponge and two sites with the other side. Each sample was separately packaged and labelled with a unique identifier. Samples were chilled and transported by courier to the Animal and Plant Health Agency (APHA) at Bury St Edmunds with minimum delay in an insulated shipping box that held samples between +2 °C and +8 °C for up to 72 h. Samples were refrigerated until bacteriological examination.

A standardized data collection form was also completed by trained personnel after sampling. The requested information related to the abattoir processes and sampled carcass, including the farm of origin.

### Eligibility criteria

All samples were taken from carcasses deemed fit for consumption by the competent authority. The following were excluded: carcasses that were totally condemned or for which the intestinal tract was condemned; pigs with a live weight of <50 kg; pigs that

had undergone emergency slaughter; and pigs kept in the UK for <3 months prior to slaughter. Bacteriological examination was to be carried out within 24 h of the samples arriving at the laboratory; any samples examined more than 96 h after sample collection were excluded.

### Microbiological methods

#### *Salmonella* spp.

*Salmonella* isolation was performed at APHA Bury St Edmunds following Annex D of ISO 6579:2002. The caecum was opened aseptically and the contents were thoroughly mixed. The whole carcass swab and 10 g caecal contents were suspended in 90 ml buffered peptone water (BPW) (Merck 1-07228-500) for pre-enrichment while the rectal swab was suspended in 20 ml BPW (sample to BPW ratio 1:10). The pre-enrichment culture was incubated at 37 °C for 16–20 h then subcultured into selective modified semi-solid Rappaport–Vassiliadis (MSRV) (Mast DM440D) medium (with novobiocin at 0.001%) and incubated for up to 48 h at 41.5 °C. MSRV plates were examined at 24 h for growth typical of *Salmonella*, suspect growths were subcultured onto Brilliant Green agar (BGA) (Oxoid CM329) and xylose lysine desoxycholate media (XLD) (Becton Dickinson 278820). MSRV plates without spreading growth were re-incubated for a further 24 h and the process repeated. BGA plates and XLD media were incubated for 18–24 h at 37 °C and examined for the presence of *Salmonella*-like colonies. Presumptive *Salmonella* spp. colonies were confirmed using standard biochemical and serological procedures. There are no sensitivity and specificity estimates for this test. All strains isolated and confirmed as *Salmonella* spp. were sent to APHA Weybridge for serotyping according to the White–Kauffmann–Le Minor scheme [20]. Isolates of *S. Typhimurium* and *S. 4,[5],12:i:-* were phage-typed using the Public Health England, Colindale scheme [21].

#### *Toxoplasma gondii*

The blood sample (EDTA plasma), heart and tongue were sent by courier to the *Toxoplasma* Reference Unit, Public Health Wales for testing. The Sabin–Feldman Dye Test was used for serodiagnosis [22]. The heart and tongue tissue from seropositive pigs have been stored for possible future molecular investigations using nucleic acid amplification testing (NAAT). The Dye Test is the recognized international gold standard test for *Toxoplasma* serology but, in the

absence of any large cohort sensitivity/specificity evaluations, no absolute measurement of sensitivity and specificity was performed.

#### *Yersinia* spp.

*Yersinia* spp. was isolated by the cold enrichment method at APHA Bury St Edmunds. A tonsil scrape was added to one universal of phosphate buffer solution (PBS) and a carcass swab was rinsed in PBS to achieve a ~10% v/v suspension. Tonsils are the sample type recommended by EFSA for pathogenic strain recovery [16]. Samples were stored at 2–8 °C and subcultured weekly; 0.1 ml was subcultured onto *Yersinia* selective agar (Oxoid CIN MED PO0287A) for three successive weeks. Plates were incubated at 30 °C and examined at 24 h and 48 h. Identification of *Y. enterocolitica* was confirmed by colony morphology and API 20E biochemical strips. The sensitivity and specificity performance for detecting *Yersinia* have not been defined.

#### PPRSv

The blood (EDTA plasma) samples were tested for antibodies to PRRSv by ELISA at APHA Weybridge, using the IDEXX PRRS X3 enzyme-linked immunoassay (IDEXX Laboratories Inc., USA) according to the manufacturer's instructions. The sensitivity for the ELISA was estimated to be 98.8%, while the specificity was 99.9% (J.-P. Frossard, personal communication).

#### Analysis

Questionnaire data and laboratory test results were registered on a survey-specific Microsoft Access database then cleansed and analysed using Microsoft Excel and Stata v. 12 (StataCorp., USA). The prevalence was calculated using the survey command (svy) in Stata to account for clustering of pigs within farms. Variation by month and age group, and associations between the presence of organisms on the same carcass, were investigated using  $\chi^2$  tests, while taking into account within-farm clustering. Where multiple sample types were tested for the same organism, the agreement between sample types was examined using kappa tests.

## RESULTS

### Sample and data collection

A total of 654 pigs were scheduled for sampling in the allotted time period, although 5.2% were rearranged for sampling in late April/early May. Samples were

collected from 648 pigs and for most (>96%) pigs all eight samples were taken. Two pigs originated from the Republic of Ireland and one pig had not been kept in the UK for 3 months prior to slaughter, so these were excluded. A further 19 pigs were excluded as testing was carried out >96 h after sample collection. Consequently, 626 pigs were included in the analysis.

These pigs originated from 439 farms, with between 1 and 10 pigs from each. Most of the pigs were from farms in England (81.7%), followed by Northern Ireland (13.4%), Scotland (4.5%) and Wales (0.3%), which is comparable with the UK pig population [15]. More than half (53.5%) were from finishing-only farms, a fifth (20.3%) were from farrow-to-finish farms, and the production type for the remainder (26.2%) was reported as not known. The majority (89.5%) of pigs were aged <12 months with 9.0% reported as being aged >12 months. Most (77.6%) were kept in controlled housing or all-in/all-out systems. Only 3.5% were reported as born outdoors and kept in controlled housing since weaning and a further 0.5% were kept outdoors until slaughter, which is lower than expected although the housing information was not known for 18.4% of pigs.

#### *Salmonella* spp.

A total of 619 caeca, 625 rectal swabs and 624 carcass swabs were tested for *Salmonella* spp. The caecal contents were collected to measure *Salmonella* carriage and the carcass swab to measure carcass contamination; the rectal swab was taken to explore whether these could offer a simpler and more efficient method for monitoring carriage in the future. The prevalence of *Salmonella* in caecal samples was 30.5% [95% confidence interval (CI) 26.5–34.6], in rectal swabs 24.0% (95% CI 20.5–27.5) and in carcass swabs 9.6% (95% CI 7.3–11.9). Caecal carriage varied by abattoir from 11.3% to 46.8% and carcass contamination ranged from 0.0% to 21.0%. The prevalence ratio of carcass contamination:carriage by abattoir ranged from 0.0 (0.0:27.3%) to 1.17 (13.2:11.3%) with an average of 0.31 (9.4:30.5%). Two abattoirs had a higher prevalence on carcasses than in caeca.

Caecal carriage of *Salmonella* ranged from 25.9% in pigs aged <6 months to 40.7% in pigs aged >12 months ( $P=0.21$ ) while *Salmonella* presence in the carcass swabs ranged from 7.3% to 10.9% in the different age groups, respectively ( $P=0.79$ ). In contrast, *Salmonella* positivity in the rectal swabs was highest in

Table 1. Agreement of *Salmonella* results between sample types

Sample type	Rectal swab		Carcass swab	
	Positive	Negative	Positive	Negative
Caecal contents				
Positive	72	117	25	164
Negative	78	351	33	397
	Agreement: 68.5%	Kappa: 0.21	Agreement: 68.2%	Kappa: 0.07
Carcass swab				
Positive	25	35		
Negative	125	438		
	Agreement: 74.3%	Kappa: 0.12		

younger pigs (<6 months: 21.8%; 6–12 months: 26.0%; >12 months: 7.1%;  $P = 0.008$ ). Caecal carriage did not vary significantly between months ( $P = 0.43$ ).

Of the 618 pigs from which both caecum and rectal swab were taken, 11.7% tested positive in both. There was a fair agreement between the caecal content and rectal swab results (kappa = 0.21) (Table 1). However, the caecal sample identified significantly more positive pigs ( $P = 0.006$ ). The agreement between caecal contents and rectal swab results was also tested for each of *S. Typhimurium*, *S. 4,5,12:i:-*, *S. 4,12:i:-* and *S. Derby* in turn; the kappa values ranged between 0.16 (*S. 4,12:i:-*) and 0.37 (*S. Derby*). More than half of the positive carcass swabs were from pigs that were not carrying *Salmonella* (Table 1).

Twenty *Salmonella* serovars were identified, as well as two rough strains (Table 2). The most commonly isolated serovars were *S. 4,[5],12:i:-*, *S. Typhimurium*, *S. Derby* and *S. Bovismorbificans*. No pigs or carcasses tested positive for *S. Enteritidis*. *Salmonella* Choleraesuis was isolated from three rectal swabs and one carcass swab; two of the three pigs with this serovar originated from the same farm. One third (24/72) of the pigs that were positive for both caecal content and rectal swab tested positive for different serovars in the two samples (Table 3). Ten carcasses were contaminated with a serovar that was not found in the *Salmonella*-positive caecal sample from the same pig. Definitive phage-type DT193 was the most common phage type of *S. 4,[5],12:i:-* and *S. Typhimurium*, although roughly a third of the *S. Typhimurium*-positive rectal swabs were phage-type U288 (Table 4).

### *Toxoplasma gondii*

Plasma samples from 626 pigs were tested for antibodies to *Toxoplasma*. For six pigs, it was not possible

to test all dilutions of blood required by the Dye Test. While no antibodies were detected at the dilutions tested, we cannot unequivocally exclude the possibility that lower levels of antibody might have been detected at lower dilutions so these pigs were excluded. Of the remaining 620 pigs, 46 were seropositive giving a seroprevalence of antibodies to *Toxoplasma* of 7.4% (95% CI 5.3–9.5). Seropositivity ranged from 5.5% in pigs aged <6 months to 11.1% in pigs aged >12 months ( $P = 0.42$ ).

### *Yersinia* spp.

Overall, 624 carcass swabs and 620 tonsil samples, from 624 pigs, were tested for *Yersinia*. The prevalence of carriage, determined by tonsil examination, was 32.9% (95% CI 28.8–37.0), while carcass contamination was 1.9% (95% CI 0.8–3.0). Carriage did not vary significantly by month ( $P = 0.22$ ).

Most of the positive pigs (87.3%) and carcasses (91.7%) carried *Y. enterocolitica*. A further 21 (10.3%) pigs were infected with *Y. pseudotuberculosis*; four had *Y. kristensenii* and two had *Y. frederiksenii* *intermedia*. The prevalence of *Y. enterocolitica* carriage in tonsils was 28.7% (95% CI 24.8–32.7) while the prevalence on carcasses was 1.8% (95% CI 0.7–2.8). The prevalence of *Y. pseudotuberculosis* carriage in tonsils was 3.4% (95% CI 2.0–4.8).

Approximately one third of the pigs aged 6–12 months were carrying *Yersinia*, this was greater than the approximate quarter of pigs with *Yersinia* aged either <6 months or >12 months ( $P = 0.22$ ). All of the positive carcass swabs were from pigs aged 6–12 months.

### PRRSv

A total of 621 plasma samples were tested for antibodies to PRRSv, of which 362 were seropositive

Table 2. *Salmonella serovars identified from each sample type*

Serovar	Caecal contents			Rectal swabs			Carcass swabs		
	No. of positive samples	% of all positive samples (n = 189)	Prevalence (n = 619)	No. of positive samples	% of all positive samples (n = 150)	Prevalence (n = 625)	No. of positive samples	% of all positive samples (n = 60)	Prevalence (n = 624)
4,12:i:-	33	17.5%	5.3%	18	12.0%	2.9%	16	26.7%	2.6%
4,5,12:i:-	32	16.9%	5.2%	29	19.3%	4.6%	12	20.0%	1.9%
Agona	2	1.1%	0.3%	—	—	—	—	—	—
Bovismorbificans	20	10.6%	3.2%	23	15.3%	3.7%	5	8.3%	0.8%
Choleraesuis var. Kunzendorf	—	—	—	3	2.0%	0.5%	1	1.7%	0.2%
Derby	27	14.3%	4.4%	28	18.7%	4.5%	8	13.3%	1.3%
Dublin	—	—	—	1	0.7%	0.2%	—	—	—
Goldcoast	3	1.6%	0.5%	4	2.7%	0.6%	1	1.7%	0.2%
Hindmarsh	1	0.5%	0.2%	—	—	—	—	—	—
Kedougou	6	3.2%	1.0%	5	3.3%	0.8%	—	—	—
Livingstone	—	—	—	1	0.7%	0.2%	—	—	—
London	5	2.6%	0.8%	3	2.0%	0.5%	—	—	—
Mbandaka	1	0.5%	0.2%	—	—	—	—	—	—
Newport	—	—	—	1	0.7%	0.2%	—	—	—
Ohio	1	0.5%	0.2%	—	—	—	—	—	—
Panama	6	3.2%	1.0%	4	2.7%	0.6%	1	1.7%	0.2%
Reading	8	4.2%	1.3%	3	2.0%	0.5%	1	1.7%	0.2%
Rissen	3	1.6%	0.5%	4	2.7%	0.6%	2	3.3%	0.3%
Stanley	5	2.6%	0.8%	3	2.0%	0.5%	2	3.3%	0.3%
Typhimurium	36	19.0%	5.8%	20	13.3%	3.2%	9	15.0%	1.4%
O_ROUGH:i:-	—	—	—	—	—	—	1	1.7%	0.2%
O_ROUGH:r:1,5	—	—	—	—	—	—	1	1.7%	0.2%

giving a seroprevalence of antibodies to PRRSv of 58.3% (95% CI 53.1–63.4). The lowest seroprevalence was found in pigs aged >12 months (32.1%, 95% CI 15.0–49.3) and the highest in pigs aged <6 months (68.5%, 95% CI 54.5–82.5) ( $P = 0.002$ ).

### Co-infections

Six hundred and six pigs were tested for all of the following: *Salmonella* caecal carriage, *Yersinia* tonsil carriage, *Toxoplasma* antibodies and PRRSv antibodies. A fifth (19.5%) of these pigs tested negative for all of these organisms while one pig had evidence of having been infected with all four. The combinations of infections are shown in Table 5. Based on these 606 pigs, there was no evidence that PRRSv-seropositive pigs were more likely to carry *Salmonella* ( $P = 0.15$ ) or *Yersinia* ( $P = 0.69$ ) or have antibodies to *Toxoplasma* ( $P = 0.51$ ) nor any evidence that they were more likely to have another infection of any type (*Salmonella* carriage and/or *Yersinia* carriage and/or *Toxoplasma*

antibodies) ( $P = 0.23$ ). There was no significant association between the pairwise combinations of the other pathogens: 10.4% of pigs were *Salmonella* and *Yersinia* positive ( $P = 0.77$ ); 2.5% positive for *Salmonella* and *Toxoplasma* ( $P = 0.70$ ); 2.1% positive for *Toxoplasma* and *Yersinia* ( $P = 0.55$ ). There was weak evidence that pigs carrying *Salmonella* were more likely to have at least one of the other three infections as well (80.7% vs. 71.9%,  $P = 0.03$ ).

### DISCUSSION

The abattoirs participating in this survey processed 80% of the UK pig slaughter throughput. This coverage combined with the randomized sampling approach provides robust and representative prevalence estimates. However, the sampled population included 9% of pigs aged >12 months which was greater than the proportion of culled sows against all pigs slaughtered in 2014 (243 000 culled sows among 10 227 000 total slaughtered; [23]). This difference in population

Table 3. Combinations of *Salmonella* serovars identified in pigs for which both intestinal samples tested positive (n = 72)

Caecal contents	Rectal swab											
	4,12:i:-	4,5,12:i:-	Bovismorbificans	Derby	Goldcoast	Kedougou	London	Panama	Reading	Rissen	Stanley	Typhimurium
4,12:i:-	5		2									1
4,5,12:i:-	1	8		2								
Agona				1								1
Bovismorbificans			6									
Derby				11							1	
Goldcoast	1				1							
Kedougou						1						
London							1					
Ohio	1											
Panama	1							1				
Reading				1					3			
Rissen										1		
Stanley											1	
Typhimurium		2	2	1	1	1	1					9

may have biased the results. Analysis of the association of each test with age detected only one significant association with younger pigs more associated with being seropositive to PRRSv. This association may reflect the fact that active and primary infection with PRRSv is more likely to occur during the rearing period up to slaughter age, at around 6 months age, than later and antibody levels may then fall with age in the absence of challenge.

The study design and main laboratory methods were as similar as possible to the previous UK *Salmonella* study, although there were two main differences: the last study was over a 12-month period and there were separate surveys for breeding and finishing pigs. The level of *Salmonella* caecal carriage in this study was 30.5% (95% CI 26.5–34.6), which compares to carriage rates of 23.0% (95% CI 21.4–24.7), 23.4% (95% CI 19.9–27.3) and 22.0% (95% CI 18.7–25.6) in the 1999–2000 and 2003 GB abattoirs surveys, and the 2006–2007 UK study, respectively [8, 12, 24]. None of these surveys found a significant seasonal variation in carriage, so it is unlikely that the shorter period of sampling in this study had a significant effect. The UK breeding pig survey in 2008 found a *Salmonella* prevalence of 52.2% (95% CI 44.6–61.5) for breeding holdings and 44.0% (95% CI 37.8–50.9) for production holdings [25]. This was estimated from freshly voided pooled faecal samples collected on farm so results are not directly comparable. There was some indication that *Salmonella* caecal carriage was higher among the older, presumably breeding, pigs in the present study. However, the sample size in the youngest and oldest age groups was extremely small, so this result must be treated with caution. The inclusion of a relatively small number of adult breeding pigs in this survey is unlikely to have been responsible for the apparent increase in *Salmonella* carriage observed.

Previous studies have shown a significant association between the *Salmonella* status of caecal contents and carcass swabs [8, 26–28] with 70% of carcass contamination at slaughter thought to originate from infection in the same pig and 30% from cross-contamination [26, 29]. In this study, more than half of the positive carcass swabs were from pigs which were not carrying *Salmonella*, indicating that cross-contamination in the abattoir may have been responsible for more positive carcasses than in previous studies. However, carcass contamination was at a much lower level than intestinal carriage at 9.6% (95% CI 7.3–11.9). This was a significant decrease from the 2006–2007 survey of finishing

Table 4. *Phage types of Salmonella Typhimurium and Salmonella 4,[5],12:i:- identified from each sample type*

Serovar	Phage type	Caecal contents			Rectal swab			Carcass swab		
		No. of positive samples	% of all positive samples ( <i>n</i> = 189)	Prevalence ( <i>n</i> = 619)	No. of positive samples	% of all positive samples ( <i>n</i> = 150)	Prevalence ( <i>n</i> = 625)	No. of positive samples	% of all positive samples ( <i>n</i> = 60)	Prevalence ( <i>n</i> = 624)
4,12:i:-	120	6	3.2%	1.0%	1	0.7%	0.2%	1	1.7%	0.2%
	193	24	12.7%	3.9%	13	8.7%	2.1%	9	15.0%	1.4%
	208	—	—	—	1	0.7%	0.2%	—	—	—
4,5,12:i:-	UNTY	3	1.6%	0.5%	3	2.0%	0.5%	6	10.0%	1.0%
	104b	1	0.5%	0.2%	2	1.3%	0.3%	1	1.7%	0.2%
	120	4	2.1%	0.6%	2	1.3%	0.3%	—	—	—
	193	21	11.1%	3.4%	21	14.0%	3.4%	6	10.0%	1.0%
	U302	1	0.5%	0.2%	—	—	—	—	—	—
	U311	—	—	—	—	—	—	1	1.7%	0.2%
	U323	3	1.6%	0.5%	3	2.0%	0.5%	4	6.7%	0.6%
	NOPT	1	0.5%	0.2%	—	—	—	—	—	—
	UNTY	1	0.5%	0.2%	1	0.7%	0.2%	—	—	—
Typhimurium	32	2	1.1%	0.3%	—	—	—	—	—	—
	104	2	1.1%	0.3%	—	—	—	—	—	—
	104b	2	1.1%	0.3%	—	—	—	—	—	—
	120	4	2.1%	0.6%	—	—	—	—	—	—
	193	11	5.8%	1.8%	7	4.7%	1.1%	2	3.3%	0.3%
	208	2	1.1%	0.3%	2	1.3%	0.3%	1	1.7%	0.2%
	U288	6	3.2%	1.0%	6	4.0%	1.0%	2	3.3%	0.3%
	U302	3	1.6%	0.5%	3	2.0%	0.5%	2	3.3%	0.3%
	UNTY	4	2.1%	0.6%	2	1.3%	0.3%	2	3.3%	0.3%

NOPT, Not phage-typed; UNTY, cultures which did not react with any of the phages in the typing scheme.



Table 5. Co-infections identified in the 606 pigs that were tested for all of the microorganisms (*Salmonella* carriage in the caecum, *Yersinia* carriage in the tonsils, *Toxoplasma* antibody seropositivity and PRRSv antibody seropositivity)

Infection	No. of pigs	(%)
None	118	(19.5)
PRRSv	145	(23.9)
PRRSv, <i>Salmonella</i>	75	(12.4)
PRRSv, <i>Yersinia</i>	74	(12.2)
<i>Yersinia</i>	53	(8.7)
<i>Salmonella</i>	36	(5.9)
PRRSv, <i>Salmonella</i> , <i>Yersinia</i>	35	(5.8)
<i>Salmonella</i> , <i>Yersinia</i>	25	(4.1)
PRRSv, <i>Toxoplasma</i>	13	(2.1)
<i>Toxoplasma</i>	7	(1.2)
PRRSv, <i>Salmonella</i> , <i>Toxoplasma</i>	6	(1.0)
<i>Salmonella</i> , <i>Toxoplasma</i>	6	(1.0)
<i>Toxoplasma</i> , <i>Yersinia</i>	6	(1.0)
PRRSv, <i>Yersinia</i> , <i>Toxoplasma</i>	4	(0.7)
<i>Salmonella</i> , <i>Yersinia</i> , <i>Toxoplasma</i>	2	(0.3)
PRRSv, <i>Salmonella</i> , <i>Toxoplasma</i> , <i>Yersinia</i>	1	(0.2)

pigs when 15.2% (95% CI 12.4–18.3) of carcasses tested positive for *Salmonella* [8] potentially indicating improved control in abattoirs. The 2006–2007 survey found no significant seasonal variation in *Salmonella* prevalence although the highest levels occurred during January–March [8]. The reduction observed in carcass contamination is thus unlikely to be an artefact of study design and may reflect the introduction of new measures such as binging and increased scald/singe temperature or double singeing in many abattoirs in recent years.

Although there was a fair agreement between results from caecal and rectal sampling, the latter was less sensitive at estimating *Salmonella* carriage. This could be related to the smaller sample size (weight of material) collected on swabs or to the fact that infected pigs may not shed *Salmonella* continuously or have large variation in the quantity of *Salmonella* shed, leading to false-negative results [30]. The difference in sensitivity was particularly notable in the older age groups. This may be due to the nature of adult pig faeces being more dry and firm, and thus more difficult to collect via the rectal swab. The skin of adult pigs also tends to be less faecally contaminated than that of finishing pigs, as they are kept on straw and not transported or held in such large numbers.

The most common serovars found were *S. Typhimurium* and *S. 4,[5],12:i:-*, which together accounted for more than half of the positive caecal samples and carcass swabs, followed by *S. Derby* and *S. Bovismorbificans*. This differs to the previous survey when *S. Typhimurium* (40.3% of positive caeca and 49.5% of positive carcass swabs) and *S. Derby* (28.7% of positive caeca and 22.0% of positive carcass swabs) were most frequently recovered [8]. *Salmonella* 4,[5],12:i:- has been increasingly reported in both pig populations and human salmonellosis cases in many European countries in recent years [31]. The caecal prevalence of these monophasic variants of *Typhimurium* was 0.8% in 2007 whereas in this study it had risen to 10.5%. Their emergence may be, at least partly, responsible for the overall increase in *Salmonella* prevalence observed since 2007. Several cases of human illness from *Salmonella* 4,[5],12:i:- in the UK in the last few years have been traced back to hog roasts [32, 33] potentially reflecting the changing serovar distribution in pigs.

The prevalence of *S. Bovismorbificans* has also increased since 2007 in both caecal samples (3.2% vs. 0.7%) and carcass swabs (0.8% vs. 0.2%) [8]. This serovar has caused a number of foodborne outbreaks in Europe in recent years, in some cases associated with consumption of pork [34–36]. The isolation of *S. Choleraesuis* in this study is also of concern, particularly from a veterinary perspective since this serovar can cause substantial clinical disease in pigs [37], although it can also be invasive in the small number of human cases that occur. This serovar was not isolated in the 2006–2007 survey.

Control of *Salmonella* in pig herds and pig meat is complex and requires a multi-factorial approach to reduce contamination throughout the food chain. Results from this study indicate a rise in intestinal carriage but a potential reduction in carcass contamination during processing and, consequently, potential lower risk to public health from contaminated meat. Continued reliance on sound abattoir procedures to minimize or prevent carcass contamination is advisable while efforts continue to reduce the prevalence of *Salmonella* in pigs while on farms.

The seroprevalence of antibodies to *Toxoplasma gondii* in pigs in this study was 7.4% (95% CI 5.3–9.5). Previous seroprevalence data for UK-reared pigs is sparse [17] although this figure is comparable with those published several decades ago [38, 39]. Comparisons with more recent serological surveys in other countries are problematical due to differences

in sampling procedures, serological testing methods and choices of threshold for positive cut-off values [17]. Nevertheless the estimate from this study falls broadly within the range of recent *Toxoplasma* antibody seroprevalence estimates from other European countries, including 4.7% in Ireland [40], 16.6% in Spain [41], 23.3% in Switzerland [42] and 26% in the Czech Republic [43]. It is difficult to gauge the precise public health implications of the findings, as the correlation between seropositivity and the number of viable *T. gondii* cysts in edible tissue has not yet been fully elucidated [17, 44]. In addition, the relative contribution of the foodborne route of transmission to the overall human disease burden, as well as the contribution of different food vehicles, is unclear [17, 44]. Thus, while the seroprevalence in this survey is considerably lower than that in a recent survey of sheep [18], the significance of this difference to UK consumers is uncertain. Nevertheless, the data provide a nationally representative baseline seroprevalence in pigs against which future survey results and the effectiveness of control measures can be monitored.

The prevalence of *Y. enterocolitica* carriage was significantly higher in this study than in the 2003 GB abattoir survey [28.7% (95% CI 24.8–32.7) vs. 10.2% (95% CI 8.9–11.5)] [12]. However, the sample and testing methods are not directly comparable as caecal samples were collected in the 2003 survey rather than tonsils. Higher rates of carriage were also found in the 2003 survey during December to May [12], and from January to March in a study by Bhaduri *et al.* [45], which includes the sampling time-frame for this study. If the methodology had been identical, lower isolation rates may have been observed.

Given that more than one quarter of the pigs were found to carry *Y. enterocolitica*, it is encouraging that so few carcasses ( $\leq 2\%$ ) were contaminated, indicating that the processes applied at the abattoir to reduce contamination are having a positive effect. This is similar to the prevalence of carcass contamination observed in pigs at slaughter in Italy [46]. Biotyping of the *Y. enterocolitica* isolates was not undertaken because of the low prevalence, and therefore low potential public health hazard, on the carcasses.

The seroprevalence of antibodies to PRRSv observed in pigs in this study was 58.3% (95% CI 53.1–63.4). The inclusion criterion of a live weight of  $\geq 50$  kg guarantees that the ELISA was detecting an active immune response rather than maternal antibody. Comparisons with previous surveys in Britain are complicated due to methodological differences.

Richardson [47] reported a similar seroprevalence of 56% but from non-randomized, non-structured testing of 356 pig herds between 2001 and 2003. Velasova *et al.* [48] reported a lower herd-level seroprevalence in 2008–2009, but in that case vaccinated herds were only classified as PRRSv-positive if there was evidence of virus from PCR testing of growing pigs [48]. Evans *et al.* [49] also reported a lower herd-level seroprevalence of 39.8% in 2003–2004, although an additional 26.2% of herds were classified as vaccinated. Pig veterinarians estimated at that time that growing pigs were vaccinated for PRRSv in between 10% and 55% of the herds they attended in East Anglia (S. Williamson, unpublished observations). Reports from 110 commercial pig units elsewhere in GB in 2013 also indicated that about 11% were vaccinating growing pigs (BPEX, Health and Welfare monitoring project). The lack of data on PRRSv vaccination status of the pigs in the current study is a limitation and the seroprevalence observed is likely to be an overestimate of the prevalence of field infection since vaccinal response is not differentiated. PRRSv is a pathogen of concern for the pig industry and controlling the disease is a target for the British pig industry's health and welfare strategy [50]. This study has provided an indication of the extent of PRRSv infection in the national herd and a baseline against which to evaluate changes over time.

There was no evidence for a significant association between the presence of multiple combinations of pathogens. Over 10% of the sampled pigs were positive for both *Salmonella* and *Yersinia* but the analysis result suggests that this was a function of the relatively high prevalence of each pathogen. However, previous studies have shown significant associations such as that between PRRSv presence and *Salmonella* shedding [51]. Associations between the presence of different pathogens can arise from synergistic effects, such as the dampening of the immune system by an initial infection, or from pathogens sharing similar risk factors. The lack of significant associations may have been influenced by mixing serology results, indicating historical infection, for PRRSv and *Toxoplasma* and bacterial detection for *Yersinia* and *Salmonella*.

A limitation of this study was the inability to provide true prevalence estimates, adjusted for test performance. The sensitivity and specificity for the use of the tests under these conditions were largely unavailable and would have been affected by sample type and the number of target organisms and competing flora in each individual sample. The apparent

prevalence was instead calculated, adjusted for clustering, to ensure comparability with previous UK studies.

This study will help government and industry policy-makers to clarify and prioritize work aimed at controlling these important infectious organisms along the pig meat supply chain. Through the extension and strengthening of links across government and industry, this study has also enabled a more collaborative and broad approach to addressing public and animal health concerns in relation to pigs in the UK.

#### APPENDIX. Consortium for Pig and Public Health

Steve Wyllie (APHA), Giles Paiba (APHA), John Tayleur (Defra), Mary Howell (FSA), Derek Armstrong (BPEX), Emma Bailey-Beech (BPEX), Richard Tedder (PHE), Edward Guy (PHW), Nicole Batey (VMD)

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#### DECLARATION OF INTEREST

None.

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