© 2016 Universities Federation for Animal Welfare
The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK
www.ufaw.org.uk

Animal Welfare 2016, 25: 355-363
ISSN 0962-7286
doi: 10.7120/09627286.25.3.355

The associations between animal-based welfare measures and the presence of indicators of food safety in finishing pigs

I Alpigiani[*†], *C Bacci*[†], *LJ Keeling*[‡], *MD Salman*[§], *F Brindani*[†], *S Pongolini*[#], *PL Hitchens*[‡] *and S Bonardi*[†]

[†] Department of Veterinary Science, University of Parma, Parma, Italy
[‡] Swedish University of Agricultural Sciences, Department of Animal Environment and Health, Uppsala, Sweden
[§] Animal Population Health Institute, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, USA
[#] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Parma, Italy
* Contact for correspondence and requests for reprints: irene.alpigiani@nemo.unipr.it

Abstract

Stressful housing and management practices affect animals, potentially increasing their receptiveness to pathogens. Since some pathogens do not lead to clinical signs of sickness, subclinical pigs could enter the food-chain, contaminating carcases and offal at slaughter, representing a threat to human health. Here, we assess the feasibility of a new approach (using animal-based welfare outcomes) to investigate the association between the animal welfare status of finishing pigs on-farm and the occurrence of Yersinia enterocolitica *and* Salmonella enterica *in slaughtered pigs in Northern Italy. Thirty batches of finishing pigs were assessed for animal-, resource- and management-based measures according to the Welfare Quality® protocol for pigs on-farm and at slaughter. A sample of five individuals per batch was tested for* Y. enterocolitica *and* S. enterica *in tonsils and in mesenteric lymph nodes, respectively, and gross pathological changes were recorded. Environmental faecal samples per batch on-farm were tested for the same pathogens. Univariable logistic regression models were used to investigate the association between batches of pigs that were positive to* Y. enterocolitica *and* S. enterica *and indicators of poor welfare. The animal-based measures of welfare, greater on-farm mortality and poor human-animal relationship, were found to be associated with* Y. enterocolitica. *This study provides a good indication of the validity of this approach, but there is a need for larger-scale studies in the future to confirm the magnitude of the associations between these animal welfare and food safety indicators.*

Keywords: *animal-based measures, animal welfare, finishing pigs, food safety,* Salmonella enterica*,* Yersinia enterocolitica

Introduction

Animal production systems and practices differ worldwide and it is already established that some risk factors, mainly related to the provision of resource and management procedures, influence the prevalence of pathogens in food animals (Bahnson *et al* 2006; Garcıa-Feliz *et al* 2009). Risk factors related to the animals' behaviour are rarely investigated under routine commercial settings. However, even within the same system there can be variation between individuals in how they are affected by the resources available on the farm and by the management routines. Thus, it is becoming increasingly common in animal welfare assessment to make observations of animals and well-developed pig assessment protocols are now in place (eg Welfare Quality® 2009) using mainly animal-based measures. Animal-based welfare indicators are favoured for providing the most reliable insight into how well the animal is coping with the environment, irrespective of the animal production system (Whay *et al* 2003; EFSA 2012). It is thought that good welfare, as demonstrated by animal-based measurements, may reduce the risk of food-borne pathogens in farms, but this has not been explored in actual observational studies. Establishing associations between animal-based welfare outcome measures and the presence of specific infection could further motivate farmers to avoid practices associated with these poor welfare outcomes.

The mechanism by which stress can alter the outcome to infections in animals is well-known by scientists. Stress affects both pathogens and hosts and it is generally agreed that there is a dual mechanism linking reduced animal welfare (eg high levels of stress) and increased risk of infection. Firstly, stressed animals are more susceptible to new infections and may carry more pathogens in the intestine and lymphatic tissue (Rostagno 2009). This is because bacteria in the gastrointestinal tract respond to the presence of stress-induced catecholamines with increased microbial growth and pathogenic processes (Rostagno 2009; Verbrugghe *et al* 2011). In the host, catecholamines enhance gastric pH, thereby increasing gastrointestinal permeability to food-borne pathogens, allowing eventual pathogens to pass the gastric barrier and thus facilitating microbial invasion. Secondly, increased intestinal motility in subclinical carriers

Table 1 Number of batches of slaughter pigs from each farm, type of farm and number of farms supplying each of the three slaughter plants.

Slaughter plant	Farm	Number of batches	Type of farm
I	A	2	Intensive
	B	2	Intensive
	C	2	Intensive
	D	2	Intensive
	E	2	Intensive
II	F	2	Intensive
	G	2	Intensive
	H	2	Intensive
	I	4	Intensive
III	L	2	Semi-free-range
	M	2	Organic
	N	2	Semi-free-range
	O	2	Organic
	P	2	Intensive
Total	**14**	**30**	

enhances faecal shedding of micro-organisms. This is the result of the neurohormonal outflow response to stress with the consequent contamination of environment and healthy animals (Cogan *et al* 2007; Verbrugghe *et al* 2011).

Modern intensive farming systems experienced by animals are sufficient to cause chronic physiological stress responses and may thus contribute to a higher incidence of animal disease (De Passillé & Rushen 2005). For example, Callaway *et al* (2006) indicated that social stress, such as mixing weaned pigs, can influence the intestinal population of *Salmonella* spp, with a subsequent increase in the faecal shedding of the pathogen. Dowd *et al* (2007) further demonstrated that even routine animal management practices, eg transfer to other pens or weighing, can cause stress-induced changes in the gastrointestinal tract that would affect the shedding of any pathogen acquired by the animals.

Salmonellosis and yersiniosis are swine infectious diseases which may be associated with clinical signs, but which may also cause a subclinical carrier state. Carrier pigs may shed *Salmonella* spp in the gastrointestinal contents and mesenteric lymph nodes up to four weeks post-infection (Rostagno *et al* 2011) and subclinical pigs entering the slaughter plant increase the risk of ending up as contaminated carcases, with implication for human health for both zoonotic pathogens (Bonardi *et al* 2013). In the present study, *Yersinia enterocolitica* and *Salmonella enterica* were selected as food safety indicators. Our choice was based on the following considerations: i) both pathogens are frequently found in pig populations in EU countries (EFSA & ECDC 2013, 2014); ii) pigs may carry human pathogenic strains of *Y. enterocolitica* and *S. enterica* (Nesbakken *et al* 2006; Fosse *et al* 2009), thus contaminated pork could represent a threat to human health; iii) the pig is the single most important reservoir of human pathogenic serotypes of *Y. enterocolitica*, such as bio-serotypes 4/O:3 and 2/O:9 (Fredriksson-Ahomaa *et al* 2007); iv) moreover we suppose that there could be a difference between the two selected microorganisms. Pigs contamination by *Y. enterocolitica* in low prevalence pig herds seem to be less influenced by the environment than *S. enterica*, as the role of external sources on transmission of the micro-organism (pen floors, tap water, equipment, pets and other animals) is minimal (Pilon *et al* 2000). For these reasons, *Y. enterocolitica* infection in pigs might be expected to be more influenced by the animal welfare status of the pig, as demonstrated by animal-based measures of welfare, compared to *S. enterica*.

In the last decade, some attempts have been made to establish an integrated approach, based on the interface between environmental conditions, animal health and welfare, and the quality of the animal products throughout the entire food-chain from breeding to slaughter (Petersen *et al* 2002), but progress in this multidisciplinary area is still slow. Other studies have aimed at developing a Hazard Analysis and Critical Control Points (HACCP)-based approach to animal welfare (Von-Borell *et al* 2001), or animal health (Noordhuizen & Frankena 1999). Nevertheless, more effort is needed to use the predictive value of animal health and welfare indicators and to identify associations with food safety hazards.

Here, we present a study aimed at assessing the association between the animal welfare status of finishing pigs on-farm and the status of *Y. enterocolitica* and *S. enterica* carriage on-farm and at slaughter. The presence of *Y. enterocolitica* and *S. enterica* was used as a proxy for food-borne pathogens in general. Our intention was to explore the potential of this approach to determine the association. There are limited examples of this type of study in the literature. Bull *et al* (2008) investigated the association between *Campylobacter* spp and flock health indicators of broilers in the UK and found a link between health (rejections due to infections) and welfare (dermatitis) which led them to conclude that improving health and welfare may also reduce *Campylobacter* levels. Moreover, Smith *et al* (2011) studied the association between gross pathology lesions and serum prevalence of *S. enterica* in finishing pigs. However, in the present study, food-borne pathogens are linked to a wide range of welfare outcome indicators with a view to defining an approach that can be carried out under commercial settings. Therefore, this study represents the first attempt to investigate the association between welfare outcome indicators (animal-based measure of welfare) and food-safety indicators (*Y. enterocolitica* and *S. enterica*) in finishing pigs.

Materials and methods

Data collection and sampling were carried out from mid-April to mid-November 2012 in the north of Italy. The entire research study was carried out in accordance with the routine commercial activity of each slaughter plant without interfering with their operations either at the slaughter plant facility or on the farm.

Study design

Three slaughter plants and 14 farms were involved in the study (Table 1). The two semi-free-range farms were extensive 'Nero di Parma' breed pig rearing farms. The intensive farms supplied heavy breed pigs (Landrace, Large White and their hybrids) for typical Italian meat products.

Welfare assessment on-farm

All the animals in the pens belonging to each slaughter batch were assessed on-farm according to the sampling and practical information of the Welfare Quality® protocol for growing pigs (2009) which, whenever possible, uses animal-based measures of welfare. This protocol was also used by Temple *et al* (2011). Each slaughter batch was visited from one to five days before the day of slaughter. Observations were assessed on a binary scale, with the exception of mortality and space allowance ranges, which were reported on a three-point scale. For mortality, we compared the frequency with which the 'warning' and 'alarm' threshold levels of the Welfare Quality® protocol for pigs were exceeded (2009). Space allowance was described as m^2 per pig and the inverse transformation of the variable was used in the analysis ($1/[m^2/pig]$) in order to satisfy normality and aid interpretation.

Environmental faecal sampling

During farm visits, 30 environmental faecal samples belonging to each slaughter batch were collected. Aliquots of 30 g of faeces were placed into sterile bags using sterile equipment according to the Commission Decision (EC) 55/2008 (Annex 1 Part B; 2008). The sampling was at the batch level, because individual pigs could not be traced from the farm through the commercial slaughter. They were taken on-farm in order to remove the influence of stress due to the transport and lairage, which is known to increase faecal excretion and the number of carrier pigs. This was to evaluate specifically the role of on-farm stress on the presence of *Y. enterocolitica* and *S. enterica*.

Sample collection at the slaughter plant

For each slaughter batch of pigs, information concerning the origin of the animals, date of arrival, day of slaughter and live bodyweights were collected from the ante mortem record-sheets of the slaughter plant. On the day of slaughter, five individual carcases per batch were randomly selected for viscera inspection and sample collection, performed at the evisceration stage of the slaughter line.

Sampling of tonsils and mesenteric lymph nodes

From each of the five pigs belonging to each of the 30 batches, tonsils and mesenteric lymph nodes were collected, totalling 150 tonsil samples and 150 mesenteric lymph node samples. The tonsils were aseptically removed immediately after evisceration at the slaughter line and placed into sterile sampling bags. At the same time as the viscera inspection was carried out, mesenteric lymph nodes belonging to the same carcases were aseptically removed and collected into a sterile sampling bag. Tonsil and lymph node samples were transported to the laboratory under chilled conditions and tested on the day of collection.

Table 2 Scores description of the stomach assessment (adapted from Kopinski & McKenzie 2007).

Welfare measures	Score	Description
Stomach assessment	0	Shiny white squamous epithelium
	1	Gastritis
	2	Parakeratosis of *pars oesophagea* and thickened epithelium with little or no sloughing
	3	Erosion of squamous/glandular junction and start of ulcers (erosions and/or mild ulcers with extensive sloughing of the epithelium)
	4	Developed ulcers, haemorrhage and stenosis present

Table 3 Score description of viscera assessment (Welfare Quality® protocol for pigs at slaughter 2009).

Welfare measures	Score	Description
Pleura assessment	0	No evidence of pleurisy
	2	Evidence of pleurisy
Lungs assessment	0	No evidence of pneumonia
	2	Evidence of pneumonia
Heart assessment	0	No evidence of pericarditis
	2	Evidence of pericarditis
Liver assessment	0	No evidence of white spot liver
	2	Evidence of white spot liver

Gross pathology

From the same five randomly selected animals per batch, stomachs were assessed according to a modified version of the Kopinski and McKenzie (2007) scale; extending it by one grade for the gastritis gross lesion (Table 2).

Evaluation of viscera was also carried out before any further manipulation of these organs was executed, to detect pneumonia, pleurisy, pericarditis and white spot liver, according to the Welfare Quality® protocol for growing pigs at the time of slaughter (2009) (Table 3).

Table 5 Association between presence of *Yersinia enterocolitica* and on-farm welfare indicators assessed at the pen level (n = 159) for finishing pigs.

Variable	Odds Ratio*	95% CI	*P*-value
Farm type			
Intensive	Ref		
Organic	0.13	0.01, 1.57	0.108
Semi-free-range	0.17	0.01, 2.03	0.161
Farm size			
Small	Ref		
Medium	3.47	0.26, 46.26	0.347
Large	7.32	0.53, 102.04	0.139
Number of pens on-farm	1.63	0.97, 2.74	0.067
Farm mortality	**1.87**	**1.18, 2.97**	**0.008**
Lower space allowance	1.70	0.18, 16.42	0.646
Absence of enrichment material	6.41	0.98, 41.69	0.052
Absence of outdoor access	2.94	0.51, 16.99	0.228
Floor type			
Concrete	Ref		
Slatted	2.72	0.39, 19.07	0.315
Ground	0.28	0.02, 3.46	0.318
Poor human-animal relationship	**16.17**	**2.28, 114.80**	**0.005**
Poor body condition	4.00	0.70, 22.75	0.118
Manure on the body	1.40	0.49, 4.00	0.530
Wounds on the body	0.32	0.07, 1.57	0.161
Lameness	0.79	0.79, 2.26	0.967
Ruptures and hernias	2.57	0.22, 29.96	0.451
Tail-biting	–		

* Adjusted for clustering of batches at the farm-level (n = 30).
Results displayed in bold are significant at $P < 0.05$.

Microbiology

Detection of Yersinia enterocolitica *and* Salmonella enterica

Aliquots of 10 g of both tonsil and faecal samples were analysed for the presence of pathogenic *Y. enterocolitica* according to the ISO 10273:2003 method and enumerated by direct plating following Bonardi *et al* (2014). *Y. enterocolitica* species identification was performed using the API® 20E system (bioMérieux, Marcy l'Etoile, France) incubated at 30°C for 48 h. *Y. enterocolitica* biotyping was carried out according to the modified Wauter's scheme (Bottone 2015). Serotyping was performed by slide agglutination with commercially available O-antisera for the serogroups O:1-2, O:3, O:5, O:8 and O:9 (*Yersinia enterocolitica* Antisera Set® - 293756, Denka Seiken, Japan).

Mesenteric lymph nodes and faecal samples were tested for *Salmonella* spp. Aliquots of 10 g of mesenteric lymph nodes were tested according to the ISO 6579:2002 method. Ten grams of pooled faecal samples belonging to each batch and collected on-farm were analysed following the ISO 6579:2002/Amd.1:2007 method. Enumeration of *S. enterica* was not carried out as the sample size was not deemed large enough to generate reliable enumeration data, and purely the presence or absence of *S. enterica* was of interest. Isolates showing typical *Salmonella* biochemical reactions were tested by slide agglutination with an O-omnivalent *Salmonella* serum (Denka Seiken, Tokio, Japan). Agglutinating cultures were tested for biochemical properties with the API® 20E micro-substrate system (bioMérieux), incubated at 37°C for 24 h. *Salmonella* serotyping was performed according to the White-Kauffmann-Le Minor scheme by slide agglutination with O and H antigen specific sera (Staten Serum Institute, Copenhagen, Denmark).

Data description and analysis

The conceptual hypothesis was that individuals, pens and batches positive for poor welfare would also be *Yersinia* and *Salmonella*-positive. Welfare indicators (animal-, resource- and management-based measures) were linked to the corresponding food safety indicators to investigate the relationship among these two characters of the study batches.

In the individual-level analysis, an individual was considered positive for *Y. enterocolitica* if it was detected in the individual's tonsils. An individual was considered positive for *S. enterica* if it was detected in the individual's lymph nodes. Binary terms (0 = negative; 1 = positive) were used to indicate food safety status. The individual was considered 'positive' for an animal welfare indicator (eg stomach lesion) if it had a poor welfare score for that indicator (not scored 0).

In the pen-level analysis, a pen was considered positive for *Y. enterocolitica* and/or for *S. enterica* if it was detected in the environmental faecal samples originating from the batch, or if it was detected in an individual from the respective pen. The pen was considered 'positive' for an animal welfare indicator (eg manure on the body) if above 50% of the animals in that pen had poor welfare (not scored 0).

Univariable logistic regression models were fitted for each of the animal welfare indicators: i) containing individual-level predictors and individual-level outcomes (presence of *Y. enterocolitica* in tonsils; presence of *S. enterica* in lymph nodes) with adjustment for clustering of individuals within batches at the farm-level; and ii) containing pen-level

https://doi.org/10.7120/09627286.25.3.355 Published online by Cambridge University Press

predictors and batch-level outcomes (presence of *Y. enterocolitica* in pooled faeces and/or presence of *Y. enterocolitica* in the tonsils of an individual from that pen; presence of *S. enterica* in pooled faeces and/or presence of *S. enterica* in the lymph nodes of an individual from that pen) with adjustment for clustering of pens within batches at the farm-level.

Initially, we fitted mixed-effects logistic regression models, adjusting for batches at the farm-level as a random effect; however, these models frequently did not converge or they provided very wide confidence intervals. The limiting factor here was likely the sample size at the batch level. For the individual-level models, especially those for *Y. enterocolitica*, the likelihood-ratio tests indicated that a standard logistic regression model was a better fit for the data when compared to the mixed-effects logistic models. Odds ratios (OR) with 95% confidence intervals (CI) are presented. Statistical analyses were conducted using Stata, version 13.1 (StataCorp, College Station, TX, USA).

The sample size was insufficient for reliable statistical results regarding the enumeration data of *Y. enterocolitica*, and therefore no analysis on enumeration data was performed.

Results

Data description

Thirty batches of finishing pigs were included in this study. The mean (± SD) size of the farms was 6,351.7 (± 5,281.4) pigs and ranged from 13 pigs (on a semi-free-range farm) to 16,000 pigs (on an intensive farm). The total number of pens was 159 and the average number of pens per batch was 5.3 (range 1 to 11 pens). The total number of animals scored according to Welfare Quality® protocol was 3,749 with a mean of 125 (± 51.2) animals per batch.

Food safety indicators

A total of 23 tonsils out of 150 (15.3%) were positive for *Yersinia enterocolitica*. The number of batches with *Y. enterocolitica* in tonsils was 15 out of 30 (50.0%). The number of batches with a *Y. enterocolitica*-positive faecal sample at the farm was one out of 30 (3.3%). Only one batch (3.3%) was positive to *Y. enterocolitica* in both tonsils and faeces. Seventeen samples (73.9%) were found to be positive by direct plating (mean concentration 1.9 × 104 CFU g^{-1}) with counts ranging from 1.0 × 102 to 5.8 × 104 CFU g^{-1}. Six additional tonsils were found to be positive to *Y. enterocolitica* by enrichment methods. Twenty-two *Y. enterocolitica* isolates (95.7%) belonged to the human pathogenic bio-serotype 4/O:3 and one (4.3%) belonged to the bio-serotype 1A/ONT. For more details on the *Y. enterocolitica* detection in these pigs, see Bonardi *et al* (2014).

A total number of 16 lymph nodes out of 150 (10.6%) were positive for *S. enterica*. The number of batches with *S. enterica* in lymph nodes was ten out of 30 (33.3%). The number of batches with *S. enterica* in faecal material at the farm was seven out of 30 (23.3%). There were four batches (13.3%) which were positive to *S. enterica* both in lymph nodes and in faeces. The number of batches considered positive to *S. enterica* either in lymph nodes or in the faeces was 13 out of 30 (43.3%). The serovars isolated from lymph nodes were the following: four *S.* Derby (25.0%), three *S.* Typhimurium monophasic variant 1, 4,[5],12:i:- (18.6%), two *S.* London (12.5%), two *S.* Give (12.5%), two *S.* Rissen (12.5%), two *S.* Typhimurium (12.5%), and one *S.* Braenderup (6.2%). The serovars isolated from faecal material were three *S.* Typhimurium monophasic variant 1, 4,[5],12:i:- (42.8%), two *S.* London (28.6%), one *S.* Derby and one *S.* Give (14.3% each).

Table 6 Association between presence of *Yersinia enterocolitica* and gross pathology at slaughter assessed at the individual level (n = 150) for finishing pigs.

Variable	Odds Ratio*	95% CI	*P*-value
Pleuritis	1.00	0.17, 5.86	0.996
Pneumonia	**3.69**	**1.67, 8.16**	**0.001**
Pericarditis	3.94	0.64, 24.28	0.140
White spot liver	0.75	0.16, 3.45	0.712
Stomach lesions	5.94	0.97, 36.48	0.054

* Adjusted for clustering of batches at the farm-level (n = 30). Results displayed in bold are significant at $P < 0.05$.

Animal welfare indicators

The proportion of animals in each batch with poor animal-based welfare measures (score 1 or 2) is presented in Table 4 (see supplementary material to papers published in *Animal Welfare* on the UFAW website: http://www.ufaw.org.uk/the-ufaw-journal/supplementary-material). Severe manure on the body, panic response to humans and mortality were the animal-based measures with the highest proportion of animals having the worst welfare scores.

Associations between animal welfare indicators and food safety indicators

Animal-based measures included in the analysis were: body condition, manure on the body, lameness, wounds on the body, tail-biting lesions, ruptures and hernias, panting, pumping (laboured breathing), mortality, human-animal relationship and presence of gross lesions in the viscera. The resource-based measures included in the analysis were absence of outdoor access, type of floor (soil and slatted vs concrete) and space allowance below 1 m^2 per pig. The absence of enrichment material was considered a management-based measure.

The animal-based measures: rectal prolapse, twisted snout, scouring, huddling and shivering were not included in the statistical analysis since the average score was zero for all batches. Bursitis, scouring and skin condition could not be recorded reliably for a variety of technical reasons, but mainly low light intensity in the building or dirtiness of the animals. Pumping and panting were initially included, but later excluded because they were observed on too few

doi: 10.7120/09627286.25.3.355

Table 7 Association between presence of *Salmonella enterica* and on-farm welfare indicators assessed at the pen level (n = 159) for finishing pigs.

Variable	Odds Ratio*	95% CI	*P*-value
Farm type			
Intensive	Ref		
Organic	–		
Semi-free-range	2.27	0.24, 21.20	0.473
Farm size			
Small	Ref		
Medium	0.33	0.03, 3.47	0.353
Large	0.76	0.07, 7.73	0.813
Number of pens on-farm	0.84	0.64, 1.10	0.194
Farm mortality	0.72	0.46, 1.12	0.145
Lower space allowance	**0.12**	**0.02, 0.87**	**0.036**
Absence of enrichment material	**0.14**	**0.02, 0.92**	**0.041**
Absence of outdoor access	0.75	0.14, 4.05	0.734
Floor type			
Concrete	Ref		
Slatted	1.01	0.16, 6.30	0.990
Ground	1.88	0.19, 18.24	0.587
Poor human-animal relationship	1.35	0.23, 7.82	0.741
Poor body condition	5.62	0.64, 49.56	0.120
Manure on the body	0.76	0.28, 2.08	0.589
Wounds on the body	2.16	0.49, 9.50	0.308
Lameness	0.36	0.09, 1.53	0.168
Ruptures and hernias	0.69	0.07, 6.96	0.754
Tail-biting	1.10	0.25, 4.78	0.896

* Adjusted for clustering of batches at the farm-level (n = 30). Results displayed in bold are significant at $P < 0.05$.

batches and required an Exact logistic regression due to zero cell counts, and thus could not account for clustering in batches at the farm-level.

Animal-based measures found to be associated with *Y. enterocolitica* included greater farm mortality and poor human-animal relationships (Table 5), and presence of pneumonia in the viscera of individual pigs (Table 6). Resource- and management-based measures were not found to be associated with *Y. enterocolitica*.

No animal-based measure was associated with *S. enterica* (Table 7). Resource- and management-based measures found to be associated with *S. enterica* included a greater space allowance per pig (ie farms with less m^2 per animal had lower odds of *S. enterica* on-farm) and the presence of enrichment materials (Table 7). None of the gross pathology lesions were associated with *S. enterica* (Table 8).

Discussion

We explored a practical approach to estimating the magnitude of association between selected relevant animal welfare and food safety indicators. This study confirms the feasibility of this approach and justifies a larger study to obtain a better estimate of the magnitude of the relationships. A noteworthy finding, despite the small size of the study, was the strong association between the fear reaction of the pigs on the farm when the stockperson entered the pen, and presence of *Y. enterocolitica*.

In general, the resource and management factors that would be expected to be related to food safety indicators from other studies were also significant, or close to it, in this study for one or both pathogens investigated. For example, Fosse *et al* (2009) and Zheng *et al* (2007) have studied the associations of *Y. enterocolitica* and *S. enterica* with potential risk factors on-farm and it is well documented in the literature that the size of the farm, its type of production, and related management, influence the prevalence of the pathogens. Our study could not support the finding by Smith *et al* (2011) of positive associations between *Salmonella* and pneumonia, white spot on the liver, peritonitis and pericarditis, but this may reflect the smaller number of animals in this study compared to their larger abattoir-based study. Although, we did find an association between pneumonia and *Y. enterocolitica*.

The novelty of our approach is the emphasis on the link between a wide range of animal-based welfare indicators and food safety indicators. This approach had not been tested in a systematic way before in pigs, probably due to the lack of feasible and science-based indicators of animal welfare. We found strong associations on a pen level between high on-farm mortality and a poor human-animal relationship on the incidence of *Y. enterocolitica*. The importance of good stockmanship skills has long been promoted because of its links to production (Hemsworth 2003) and this study now supports its link to food safety.

The prevalence of a poor human-animal relationship was very high (70.0% of the batches) in this study. Fear of humans is greatly influenced by management, breed and age (Temple *et al* 2011) and stress is often related to animal management practice (Hemsworth 2003; Rostagno 2009). Considering the high prevalence of fear towards humans, we could suppose that these finishing pigs were stressed by the routine farm management practices. The most likely proposed mechanism for this association to infection with *Y. enterocolitica* is that stress impairs the immune system at

https://doi.org/10.7120/09627286.25.3.355 Published online by Cambridge University Press

the same time as it increases shedding of pathogens from already infected pigs on the farm. That is, both the presence of this pathogen on the farm increases and the likelihood of a previously uninfected pig becoming infected increases. Heavy-breed pigs, which represented the majority of the animals observed in the study, are slaughtered at older ages (at least nine months according to some typical Italian meat product specifications) compared to light-breed pigs and so would have had a longer fattening phase in which to be exposed to chronic stress.

This study also supports the logical link between indicators of reduced clinical health and infection, although with the exception of the association between *Y. enterocolitica* and pneumonia, and mortality these associations were weak. Associations that tended towards significance might nevertheless be worthwhile following up in a future larger study. Quantification by enumeration of the presence of *Y. enterocolitica* in a larger study may also provide useful information on the effect of different levels of contamination on animal-based welfare outcomes. Of particular interest is that the animal-based welfare indicator 'poor human-animal relationship' was only significantly associated with the food safety indicator '*Y. enterocolitica*'. This finding may suggest that *Y. enterocolitica* is especially sensitive to reduced animal welfare and health and less sensitive to the contaminated farm environment than *S. enterica*. Supporting this is the evidence that *Y. enterocolitica* is subject to an animal-to-animal transmission pattern whereas the external and environmental influences are minimal (Pilon *et al* 2000). The hypothesis that *Y. enterocolitica* may be useful as an 'iceberg' food safety indicator to establish possible associations between animal-based welfare outcome indicators and food safety, could be investigated in the future.

Table 8 Association between presence of *Salmonella enterica* and gross pathology at slaughter assessed at the individual level (n = 150) for finishing pigs.

Variable	Odds Ratio*	95% CI	*P*-value
Pleuritis	1.60	0.42, 6.10	0.493
Pneumonia	0.58	0.11, 3.15	0.531
Pericarditis	–		
White spot liver	1.88	0.44, 8.02	0.392
Stomach lesions	0.46	0.14, 1.50	0.196

* Adjusted for clustering of batches at the farm-level (n = 30).

Potential of this approach for future studies

This study was conducted to explore the possibility of linking specific animal-based welfare indicators to specific food safety indicators in the pork meat chain and to test the underlining hypotheses for their association. The study confirms the potential of the approach and some interesting findings to be explored further, but was not without limitations.

Welfare and disease dynamics are likely to differ according to the type of farm system. Although intended as a strength in this study, to challenge the feasibility of the approach under diverse conditions, in a future study it may be worthwhile focusing on only one production system or analysing and interpreting each production system separately, and ensuring that each production system is represented more equally. A larger sample size is also recommended, not least because of the difficulties fitting mixed-effects logistic regression models, which likely require a larger sample size at the highest level of analysis (eg a greater number of batches). A larger sample size would also enable the fitting of multivariable models, with the ability to account for confounding factors, which we were unable to do in this study. Given the lack of previous studies on animal-based measures linked to food safety indicators, appropriate sample sizes for exploring these relationships could not be calculated *a priori*. These data in this study can be used for such calculations in the future.

The observations and measurements were taken during mild weather months and the strength of the relationships might vary according to the weather or variation in the organisation of the pork production chain over the year. Although we are confident of the strongest results in this study, we resist from placing too much emphasis on the precise magnitude of the association since that would need a larger study and studies from other countries where the epidemiology of zoonotic agents is sufficiently high to investigate the association. Again, we prefer to emphasise the potential of this approach for a more systematic evaluation of the links between animal welfare and food safety in the food-chain.

Animal welfare implications

This study has indicated that improving health (reducing the mortality on the farm) and welfare (decreasing stress and fear from humans) could also reduce the presence of *Y. enterocolitica* in finishing pigs. Establishing the association between animal-based welfare outcome measures and the presence of specific infection could further motivate farmers to avoid practices associated with these poor welfare outcomes. Animal welfare could benefit from the association with food safety and public health.

Acknowledgements

The authors gratefully acknowledge the Italian Veterinary Services, specifically Dr Pier Ugo Cavallini, and the participating farmers and slaughter plants for their assistance in data collection. The final stages of this work were supported by the Centre of Excellence in Animal Welfare Science, a collaborative research environment funded by the Swedish Research Council FORMAS.

References

Bahnson PB, Fedorka-Cray PJ, Ladely SR and Mateus-Pinilla NE 2006 Herd-level risk factors for *Salmonella enterica* subsp. *enterica* in US market pigs. *Preventive Veterinary Medicine 76*: 249-262. http://dx.doi.org/10.1016/j.prevetmed.2006.05.009

Bonardi S, Alpigiani I, Pongolini S, Morganti M, Tagliabue S, Bacci C and Brindani F 2014 Detection, enumeration and characterization of *Yersinia enterocolitica* 4/O:3 in pig tonsils at slaughter in Northern Italy. *International Journal of Food Microbiology 177*: 9-15. http://dx.doi.org/10.1016/j.ijfoodmicro.2014.02.005

Bonardi S, Bassi L, Brindani F, D'Incau M, Barco L, Carra E and Pongolini S 2013 Prevalence, characterization and antimicrobial susceptibility of *Salmonella enterica* and *Yersinia enterocolitica* in pigs at slaughter in Italy. *International Journal of Food Microbiology 163*: 248-257. http://dx.doi.org/10.1016/j.ijfoodmicro.2013.02.012

Bottone EJ 2015 *Yersinia enterocolitica*: Revisitation of an enduring human pathogen. *Clinical Microbiology Newsletter 37*: 1-8. http://dx.doi.org/10.1016/j.clinmicnews.2014.12.003

Bull SA, Humphrey TA, Ellis-Iversen J, Cook AJ, Lovell R and Jorgensen F 2008 Flock health indicators and *Campylobacter* spp in commercial housed broilers reared in Great Britain. *Applied Environmental Microbiology 74*: 5408-5413. http://dx.doi.org/10.1128/AEM.00462-08

Callaway TR, Morrow JL, Edrington TS, Genovese KJ, Dowd S, Carroll J, Dailey JW, Harvey RB, Poole TL, Anderson RC and Nisbet DJ 2006 Social stress increases fecal shedding of *Salmonella typhimurium* by early weaned piglets. *Current Issues in Intestinal Microbiology 7*: 65-71

Cogan TA, Thomas AO, Rees LEN, Taylor AH, Jepson MA, Williams PH, Ketley J and Humphrey TJ 2007 Norepinephrine increases the pathogenic potential of *Campylobacter jejuni*. *Gut 56*: 1060-1065. http://dx.doi.org/10.1136/gut.2006.114926

De Passillé AM and Rushen J 2005 Food safety and environmental issues in animal welfare. *Revue scientifique et technique (International Office of Epizootics) 24*: 757-766

Dowd SE, Callaway TR and Morrow-Tesch J 2007 Handling may cause increased shedding of *Escherichia coli* and total coliforms in pigs. *Foodborne Pathogenes and Disease 4*: 99-102. http://dx.doi.org/10.1089/fpd.2006.53

EFSA (European Food Safety Authority) 2012 Statement on the use of animal-based measures to assess the welfare of animals. *EFSA Journal 10 (2767)*: 29

EFSA and ECDC 2013 The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2011. *EFSA Journal 11 (3129)*: 250

EFSA and ECDC 2014 The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2012. *EFSA Journal 12 (3547)*: 312

European Commission 2008 Commission Decision (EC) 55/2008 concerning a financial contribution from the Community towards a survey on the prevalence of *Salmonella* spp and Methicillin-resistant *Staphylococcus* aureus in herds of breeding pigs to be carried out in the Member States, Annex I Part B. *Official Journal of the European Union L14*: 10-25

Fosse J, Seegers H and Magras C 2009 Prevalence and risk factors for bacterial food-borne zoonotic hazards in slaughtered pigs: A review. *Zoonoses and Public Health 56*: 429-454. http://dx.doi.org/10.1111/j.1863-2378.2008.01185.x

Fredriksson-Ahomaa M, Stolle A and Stephan R 2007 Prevalence of pathogenic *Yersinia enterocolitica* in pigs slaughtered at a Swiss abattoir. *International Journal of Food Microbiology 119*: 207-212. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.07.050

Garcıa-Feliz C, Carvajal Angel Collazos J and Rubio P 2009 Herd-level risk factors for faecal shedding of *Salmonella enterica* in Spanish fattening pigs. *Preventive Veterinary Medicine 91*: 130-136. http://dx.doi.org/10.1016/j.prevetmed.2009.05.011

Hemsworth PH 2003 Human-animal interaction in livestock production. *Applied Animal Behaviour Science 81*: 185-198. http://dx.doi.org/10.1016/S0168-1591(02)00280-0

Kopinski JS and McKenzie RA 2007 Oesophagogastric ulceration in pigs: a visual morphological scoring guide. *Australian Veterinary Journal 85*: 356-361. http://dx.doi.org/10.1111/j.1751-0813.2007.196_1.x

Nesbakken T, Iversen T, Eckner K and Lium B 2006 Testing of pathogenic *Yersinia enterocolitica* in pig herds based on the natural dynamic of infection. *International Journal of Food Microbiology 111*: 99-104. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.04.019

Noordhuizen JPTM and Frankena K 1999 Epidemiology and quality assurance: applications at farm level. *Preventive Veterinary Medicine 39*: 93-110. http://dx.doi.org/10.1016/S0167-5877(98)00151-2

Petersen B, Knura-Desczka S, Pönsgen-Schmidt E and Gymnich S 2002 Computerised food safety monitoring in animal production. *Livestock Production Science 76*: 207-213. http://dx.doi.org/10.1016/S0301-6226(02)00120-3

Pilon J, Higgins H and Quessy S 2000 Epidemiological study of *Yersinia enterocolitica* in swine herds in Quebec. *Canadian Veterinary Journal 41*: 383-387

Rostagno MH 2009 Can stress in farm animals increase food safety risk? *Foodborne Pathogens and Disease 6*: 767-776. http://dx.doi.org/10.1089/fpd.2009.0315

Rostagno MH, Eicher SD and Lay DC Jr 2011 Immunological, physiological, and behavioral effects of *Salmonella enterica* carriage and shedding in experimentally infected finishing pigs. *Foodborne Pathogens and Disease 8*: 623-630. http://dx.doi.org/10.1089/fpd.2010.0735

Smith RP, Sanchez-Vazquez MJ, Cook AJC and Edwards SA 2011 Abattoir-based study investigating the association between gross pathological lesions and serological tests for Salmonella infection in pigs. *Veterinary Record 168*: 240. http://dx.doi.org/10.1136/vr.c6823

Temple D, Dalmau A, Ruiz de la Torrea JL, Manteca X and Velarde A 2011 Application of the Welfare Quality® protocol to assess growing pigs kept under intensive conditions in Spain. *Journal Veterinary Behavior 6*: 138-149. http://dx.doi.org/10.1016/j.jveb.2010.10.003

Verbrugghe E, Boyen F, Gaastra W, Bekhuis L, Leyman B, Van Parys A, Haesebrouck F and Pasmans F 2011 The complex interplay between stress and bacterial infections in animals. *Veterinary Microbiology 23*: 115-127

Von-Borell E, Bockisch FJ, Büscherc W, Hoy S, Krieter J, Müller C, Parvizi N, Richter T, Rudovsky A, Sundrum A and Van den Weghe H 2001 Critical control points for on-farm assessment of pig housing. *Livestock Production Science 72*: 177-184. http://dx.doi.org/ 10.1016/S0301-6226(01)00278-0

Welfare Quality® 2009 *Welfare Quality*® *Assessment Protocol for Pigs.* Welfare Quality® Consortium: Lelystad, The Netherlands

Whay HR, Main DCJ, Green LE and Webster AJF 2003 Animal-based measures for the assessment of welfare state of dairy cattle, pigs and laying hens: consensus of expert opinion. *Animal Welfare 12*: 205-217

Zheng DM, Bonde M and Sørensen JT 2007 Associations between the proportion of *Salmonella* seropositive slaughter pigs and the presence of herd level risk factors for introduction and transmission of *Salmonella* in 34 Danish organic, outdoor (non-organic) and indoor finishing-pig farms. *Livestock Science 106*: 189-199. http://dx.doi.org/10.1016/j.livsci.2006.08.003