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XXV Ciclo

Associations between animal-based welfare measures and the presence of *Yersinia enterocolitica* and *Salmonella* spp. as indicators of food safety in finishing pigs at slaughter plants in Northern Italy

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Alla mia famiglia

Abstract

The major current challenges for animal production are animal welfare and food safety (Rostagno, 2009). Animal welfare, which is a relevant part of the EU hygiene package, is usually dealt with as a separate issue and rarely directly connected to food safety hazards (Kijlstra and Bos, 2008). Stressful housing and management affect animals which, depending on their characteristics, may increase their receptiveness to pathogens. Since some pathogens do not lead to clinical signs of sickness, asymptomatic pigs could enter the food chain, contaminating carcasses and offal at slaughter and representing a threat to human health. The aim of this project was to assess the animal welfare status of finishing pigs on farm and its association with the occurrence of *Yersinia enterocolitica* and *Salmonella* spp. at slaughter plants in Northern Italy. Thirty batches of finishing pigs were assessed for animal-based measures according to the Welfare Quality® protocol for pigs on farm and at slaughter. A representative sample of five individuals per batch was tested for *Y. enterocolitica* and *Salmonella* spp. in tonsils and in mesenteric lymph nodes and gross pathological changes in these carcasses were recorded. Environmental faecal samples were collected from the same farms and tested for the same pathogens. The sum of positive batches to pen welfare measures were analysed by individual logistic regression against the sum of the *Salmonella* and *Y. enterocolitica* positive batches. Panic response to humans, pleuritis, pericarditis, space allowance (0.3-0.9m^2/100Kg), mortality (2.6-4.5%), slatted floor, absence of enrichment material and absence of outdoor access all tended to be associated to *Y. enterocolitica.* White spot liver tended to be associated to *Salmonella* spp. Identifying strengths and weaknesses in animal husbandry systems serves to guide future actions which may address animal welfare (De Passillé and Rushen, 2005) and food safety legislative initiatives. Thus establishing the association between animal-based welfare measures and food safety hazards could support farmers in avoiding those practices likely to be associated with the occurrence and/or recrudescence of diseases. Further research in this field is needed.

KEYWORDS: Animal welfare, animal-based measures, food safety, finishing pigs, *Yersinia enterocolitica*, *Salmonella* spp.

Riassunto

Le maggiori sfide correnti della produzione animale sono il benessere animale e la sicurezza alimentare (Rostagno, 2009). Il benessere animale, che è una parte rilevante del pacchetto igiene, è di solito trattato come argomento separatamente e raramente è direttamente connesso in letteratura a pericoli di sicurezza alimentare (Kijlstra and Bos, 2008). Stressanti sistemi di produzione influenzano l'animale, il quale secondo le proprie caratteristiche può aumentare la sua recettività ai patogeni; dunque suini asintomatici potrebbero entrare nella catena alimentare contaminando carcasse e frattaglie al macello e rappresentare una minaccia per la salute umana. L'obiettivo del progetto è di valutare lo stato di benessere animale di suini all'ingrasso in azienda e la sua associazione con lo stato di portatore di *Yersinia enterocolitica* e *Salmonella* spp. al macello nel Nord Italia. Le misure basate sull'animale di suini all'ingrasso appartenenti a 30 lotti sono state valutate secondo il protocollo Welfare Quality® per suini in azienda e al macello. Un campione rappresentativo di cinque individui per lotto è stato testato per *Y. enterocolitica* and *Salmonella* spp. rispettivamente nelle tonsille e nei linfonodi mesenterici. Campioni ambientali di feci appartenenti allo stesso lotto sono stati testati per gli stessi patogeni. La somma dei lotti positivi alle misure di benessere che riguardavano ciascun lotto, è stata analizzate per mezzo di una regressione logistica individuale contro la somma dei lotti positivi a *Y. enterocolitica* and *Salmonella* spp. Risposta di panico alla presenza dell'uomo, pleurite, pericardite, superficie disponibile (0,3-0,9 m^2/100Kg), mortalità (2,6-4,5%), tipo di pavimento (fessurato verso pieno), assenza di materiale di arricchimento ed assenza di spazio all'aperto tendono ad essere associati alla presenza di *Y. enterocolitica.* L'infestazione da *Ascaris suum* e la conseguente lesione epatica (white spot liver) tende alla associazione con *Salmonella* spp. Identificare i punti di forza e di debolezza dei sistemi di allevamento può servire a guidare ed a monitorare future correzioni che potrebbero indirizzare iniziative legislative sia relative al benessere animale sia alla sicurezza alimentare (De Passillé e Rushen, 2005). Pertanto stabilire un'associazione tra le misure di benessere basate sull'animale e sui pericoli di sicurezza alimentare potrebbe essere d'aiuto agli allevatori nell'evitare quelle pratiche associate al manifestarsi e alla recrudescenza di malattie. Ulteriori ricerche in questo settore sono necessarie.

PAROLE CHIAVE: Benessere animale, indicatori di benessere animale, sicurezza alimentare, suini all'ingrasso, *Yersinia enterocolitica, Salmonella* spp.

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Chapter 1: Introduction

Animal welfare is an increasingly sensitive issue for the whole society and especially for the national competent authorities in their work to fulfil the European legislation.

Legislation requirements concerning animal welfare are often mentioned in the hygiene package (European Parliament and of the Council Regulation (EC) No 852/2004 on the hygiene of foodstuffs and European Parliament and of the Council Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin). Nevertheless in literature animal welfare is usually dealt with as a separate issue and rarely directly connected to food safety hazards (Kijlstra and Bos, 2008).

Food business operators for the entire food chain have to comply with appropriate community and national legislative provisions related to the control of hazards in primary production, including programmes for the monitoring and control of zoonoses and zoonotic agents (European Parliament and of the Council Regulation (EC) No 852/2004 on the hygiene of foodstuffs and European Parliament and Council Directive 2003/99/ EC on the monitoring of zoonoses and zoonotic agents).

It has been proposed that the major current challenges for animal production are animal welfare and food safety (Rostagno, 2009). Regarding animal welfare the main concern of animal welfare scientists is that welfare assessment should be based on quantitative observations regarding health and behaviour which reflect the effects of resource and management input factors on the animal. Animal production systems and practices affect animal health and welfare with consequences derived not only from animals interacting with wildlife but also from risks arising at the human-animal-environment interface. The main concerns arising from food safety issues in fact are the carrier animals infected with foodborne pathogens pass undetected at meat inspection process, thus entailing risks of contamination for the entire meat production chain.

Based on surveillance system the main threats in developed countries were identified among bacterial zoonoses, such as thermotolerant *Campylobacter*, *Salmonella* spp., *Yersinia enterocolitica* and verocytotoxin producing *Escherichia coli* mainly excreted via faeces from asymptomatic animals (EFSA, 2011b). The contamination of carcasses at slaughter is a major public health and an economic concern for the food industries (Rostagno, 2009). However this economic implication for the food industries connected with loss due to the foodborne diseases (Rostagno, 2009) was not be dealt with in this study.

Despite the decreasing trend of the last five years, yersiniosis is still the third most numerously reported zoonosis in the EU, with an overall notification rate of 1.58 per 100,000 persons in 2010 (EFSA and ECDC, 2012) after campylobacteriosis and salmonellosis. *Yersinia* spp. is a food-borne pathogen that can cause serious illness in humans. Among others, raw or undercooked pig meat has also been suspected sources of *Yersinia enterocolitica* infections, which is frequently found in tonsils and intestinal contents of clinically healthy pigs at slaughter plants.

In 2010, the number of reported human *Salmonella* cases continued to decrease, and 99,020 confirmed cases (notification rate 21.5 cases per 100,000 population) were reported by 27 EU Member States (EFSA and ECDC, 2012). Finishing pigs carriers of *Salmonella enterica* are believed to be main source of carcass and pork contamination at the beginning of the meat process.

Meat inspection, both *ante* and *post-mortem* is a key factor of the overall surveillance system for pig health and welfare (EFSA, 2011a). As part of the meat inspection process, the *ante mortem* examination aims to detect diseased animals entering in the food chain. The *post mortem* is a pathological examination which poses the objective of identifying gross pathology irregularities along the slaughter line.

Since asymptomatic animals often pass undetected through the meat inspection visit, it is of paramount importance to enforce the measures to control the risk factors affecting the occurrence of human pathogenic *Y. enterocolitica* in pigs on farm and at the slaughter plant in order to reduce the pathogen prevalence in the contaminated carcasses and pluck set (Nollet *et al*., 2004). In addition, surveillance programs aim to improve the understanding of the source and the amount of contamination of *Salmonella* spp. starting from the primary production. Reducing the pathogen load by identifying which factors influence the animal carriage status, could help in reducing the risk to human health as part of an integrated food chain strategy (Milnes *et al*., 2009).

The aim of this project was to assess animal welfare status of finishing pigs on farm and its association with the occurrence of *Y. enterocolitica* and *Salmonella* spp. carriage at slaughter plants in the North East of Italy.

To our knowledge this study represents the first attempt to quantitatively and statistically investigate and estimate the possible association between animal welfare and health and food safety indicators. The link between animal welfare and animal health and implicitly food safety may appear self-evident, but it is also supported by scientific evidence. Improvements in animal welfare standards can potentially reduce on-farm risks to food safety; for example the reduction of stress-induced immunosuppression determines a decreased incidence of infectious diseases on farms and a reduced shedding of human pathogens by farm animals, as well as a reduced antibiotic use and the risk of antibiotic resistance (De Passillé and Rushen 2005).

The link between animal and human health and by implication food safety needs to be measured because the accurate study of this relationship could support stakeholders and risk managers in accurate policy-making (Hurd *et al.*, 2008). Identifying strengths and weaknesses in animal husbandry systems serves to guide and monitor future corrections which address animal welfare legislative initiatives (De Passillé and Rushen 2005). Thus establishing the association between animal-based welfare measures could support the farmers in avoiding those practices supposed to be likely associated with the occurrence and/or recrudescence of diseases, such as some welfare indicators.

The use of welfare-outcome indicators at slaughter plants is a valuable tool for monitoring welfare on-farm and during transport and pre-slaughter management (EFSA, 2011a).

One of the most accepted approaches for assessing welfare outcomes indicators to be used in commercial practice came out from a large European research project called Welfare Quality®. Welfare Quality® was a European funded project based on the integration of animal welfare in the food chain (Blokhuis *et al*., 2005). This large project, started in 2004 and was completed in 2009, involved many academics and animal welfare scientists throughout Europe. Welfare Quality's ® challenge was to develop a standardised methodology to be applied throughout Europe to assess animal welfare in an objective way (Botreau *et al*., 2007). Since animal welfare aims to assess the actual state of the animal when coping with its environment (Temple *et al.*, 2012) the Welfare Quality® scientists focused on setting up protocols based on direct observations (animal-based measures) of the animals trying to cope with the environment which include both behavioural, physiological and immune measures and incidence of health problems at production level (De Passillé and Rushen, 2005).

The validation of the Welfare Quality® measures required an extensive work by the animal welfare scientist trying to obtain valid, reliable and feasible measures (Temple *et al*., 2011a).

The Welfare Quality® investigation validated the approach in the field in the attempt to determine how animal welfare measures on farm and/or at slaughter are valuable as means to assess animal welfare. In conclusion the use of animal-based measures demonstrates the actual level of animal welfare and allows for corrective actions in case of welfare problems; consequently these measures could be used to improve different resource and management input factors in different countries applying different animal production systems (De Passillé and Rushen, 2005). This valuable and practical methodology convinced us to evaluate animal welfare on farm according to the Welfare Quality® protocol for pigs and few other aspects of the Welfare Quality® protocol for pigs at slaughter.

Animal welfare is not often dealt with in association with food safety indicators in the literature (Kijlstra and Bos, 2008); there are instead some studies which aim to associate the prevalence of *Y. enterocolitica* or *Salmonella* spp. to welfare resource and management-based measures, which are considered risk factors to biological hazards (Jensen *et al*., 2004, Zheng *et al*., 2007, Virtanen *et al*., 2011), but no measures based on the specific animal welfare outcomes were associated with food safety indicators. These measurable observations based on animals were linked to food safety indicators with the aim to benefit both animal welfare and food safety. De Passillé and Rushen (2005) confirmed that animal welfare standards determined on animal-based measures provide more flexibility in ensuring improvements in animal welfare as well as in food safety and environmental protection.

The innovation and motivation for this integrated approach is first of all just the application of a protocol based on the application of welfare outcome indicators to be linked to food safety indicators; secondly, this new and unused approach focuses to statistically associate animal welfare and food safety indicators with the aim to address the risk managers in identifying those animal production practices whose animal welfare is impaired and which are possibly considered to be at higher risks of spreading foodborne pathogens. In fact stressful housing and management affect animals which, depending on their characteristics, may increase the receptiveness to pathogens; thus clinically healthy pigs could enter the food chain contaminating carcasses and offal at slaughter chain, thus representing a threat to human health.

Among the studies based on risk factors linked to biological hazards, in Finland Virtanen *et al.* (2011) investigated the presence of specific factors affecting management on farm with the association of within farm prevalence of *Y. enterocolitica.* Factors linked to high on farm prevalence for *Y. enterocolitica* were identified in high production capacity, wet feeding, faecally contaminated feed, presence of cats in the farm, use of straw bedding or absence of bedding; low prevalence of *Y. enterocolitica* was observed in association with the use of bedding, limited use of antibiotics and lower animal density (Virtanen *et al*., 2011). The prevalence of *Y. enterocolitica* was found to be higher in conventional intensive farms rather than in organic production (Laukkanen *et al*., 2009).

Stress has implications on the susceptibility to infectious disease in animals (Rostagno, 2009). Since farm animals experience stress in their lives, therefore, animal welfare has indirect implications to food safety, entailing risks to human health; it has been known in fact that stress may cause recrudescence of some bacterial infections in food-producing animals, such as poultry and pigs (Rostagno, 2009). Moreover fight-flight stress response and the conservation-withdrawal response differ in susceptibility to develop cardiovascular pathology, ulcer development, stereotypies and infectious diseases (Koolhaas *et al*., 1999).
Stress in defined by Dhabhar and McEwen (1997) as “a constellation of events, consisting of a stimulus (stressor) that precipitates a reaction in the brain (stress perception), which activates physiological fight-or-flight systems in the body (stress response)’’. Psychophysiological stress response is one of nature’s fundamental mechanisms for the survival of the organisms (Dhabhar, 2009). The severity of the stressor depends on how the animal is able to cope with that condition. A stressor can affect the brain or body by producing biological changes in the organism. Dhabhar (2009) suggested that acute stress response may serve as an endogenous psychophysiological support in increasing the response of the immune system (Fig. 1); however it may also exacerbate immunopathology if the

immune reaction is directed against innocuous or self-antigens, or may lead to dysregulation following prolonged activation as seen during chronic stress. Chronic stress has been shown to dysregulate immune system and to suppress immunity. Therefore, the physiological stress response is critical for the stress consequence results on health (Dhabhar, 2009).

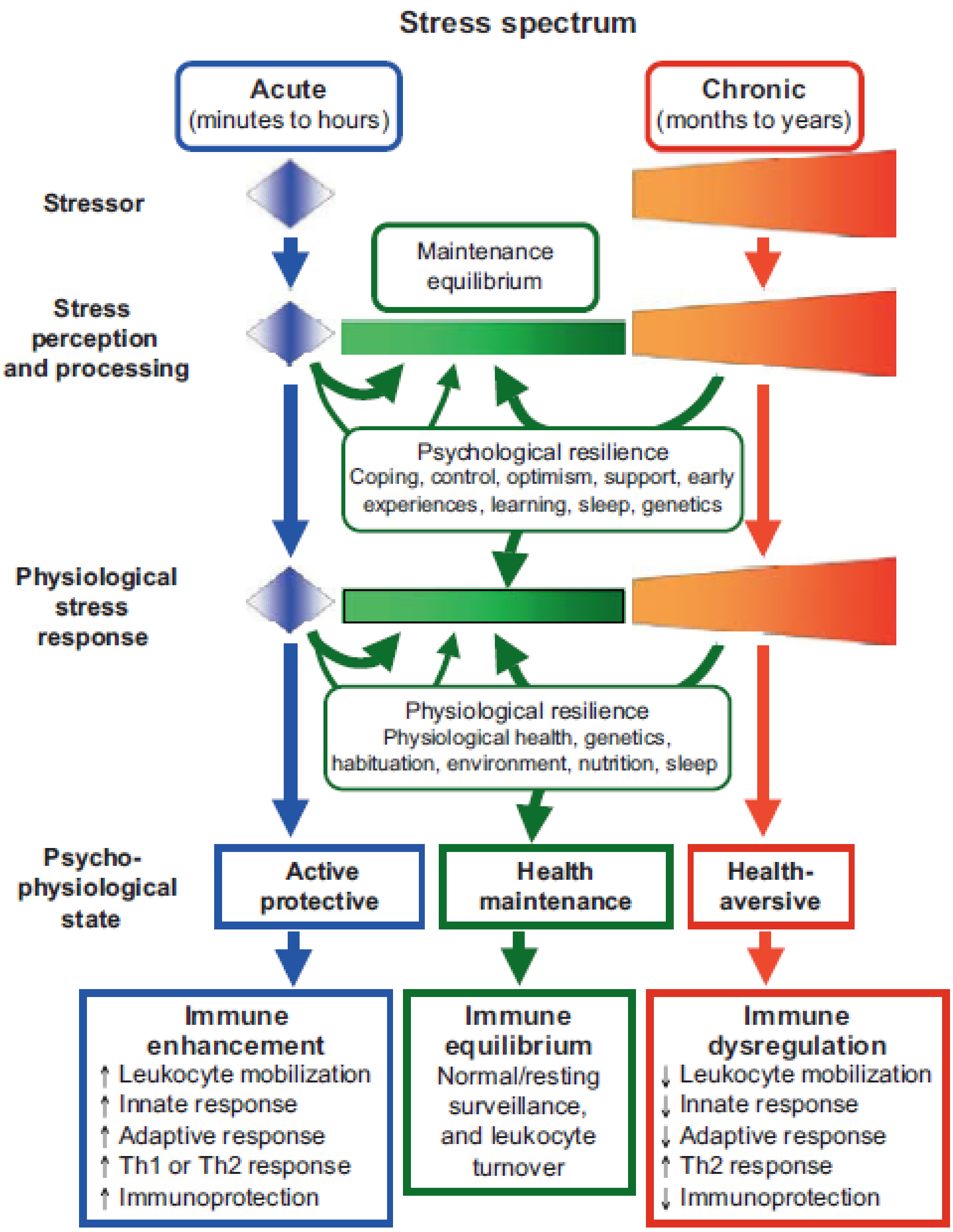

Figure 1: Stress response and its effects on health through the immune function (source: Dhabhar, 2009).

Stress is responsible of decreasing the immune function and increasing susceptibility to disease (Dhabar and McEwen, 1997). Many efforts were also committed to the study of the potential effect of stress on the gastrointestinal tract in laboratory animals. Stress in fact could not only determine functional disorders, but also favour the infection and susceptibility to disease of the gastrointestinal tract. The functional change occurring in the permeability of the intestinal mucosa leads to an increased invasion of the pathogens in the gastrointestinal tract. As a consequence animals become more susceptible to new diseases and/or excrete more microorganisms for the increased defecation due to the augmented intestinal motility (Rostagno, 2009). In the gastrointestinal tract pathogens interact with commensal bacteria. This microbial population is fundamental for the gastrointestinal microbial homeostasis which contrasts the infection by pathogens. Stress driven changes can affect the gastrointestinal tract and influence the survival/density of commensal bacteria. This new sector of science, called microbial endocrinology, investigates the effects of neuroendocrine hormones such as catecholamine on microbial growth; catecholamines in fact stimulate growth and virulence of many Gram-negative and Gram-positive bacteria; the concentration of these neuroendocrine hormones in the gut is detected by the pathogens which react with growth and increase the possibility to cause disease (Verbrugghe *et al*., 2012; Rostagno, 2009). This is true for many bacteria as well as for *Salmonella enterica*, as demonstrated by *in vitro* studies. Moreover the duration of the stress situation affect not only the host immune system, but also infectious microorganisms; during acute stress the rapid bacterial response to stress hormones is by enhancing the cell-mediate immunity; in chronic situations instead cell-mediated immunity is suppressed, therefore the outcome of bacterial infection is influenced by the chronic exposures to stress (Verbrugghe *et al*., 2012).

Animal welfare challenges experienced by the animals in the modern intensive farming systems are sufficient to cause chronic physiological stress responses and may thus contribute to a higher incidence of animal disease (De Passillé and Rushen, 2005). Dowd *et al*. (2007) demonstrated that a routine animal management practice, such as daily weighing or transport and handling of pigs, considered by humans to have a low stress impact could be perceived differently by livestock, causing stress-induced changes in the gastrointestinal tract that would affect shedding of any pathogen subsequently acquired by the animals.

Verbrugghe *et al*. (2012) explored the mechanism of stress related recrudescence of *Salmonella* infection. Some of the factors responsible of stress on the animal production are lack or inadequate provision of feed and water, inadequate thermal comfort, insufficient space allowance, and animal handling i.e. a poor human animal relationship which is one of the animal-based measures belonging to the principle of appropriate behaviour in the Welfare quality protocol (Verbrugghe *et al*., 2012; Rostagno, 2009). These factors have been linked to disease susceptibility; moreover transport was associated to the increased *Salmonella* shedding in swine (Berends, 1997; Verbrugghe *et al*., 2012). Callaway *et al*. (2006) indicated that social stress such as social mixing can influence the intestinal population of *Salmonella* in weaned pigs, with an effect on increased susceptibility to and/or faecal shedding of *Salmonella*.

In conclusion stressed animals can be a risk for food safety through various mechanisms. Increased susceptibility to diseases not only affects the health status of animals, but also influences public health through the increased pathogen shedding and the possible increased level of contamination of other animals and the environment, which could lead to cross-contamination during transport and lairage, and cross-contamination of carcasses. Impaired animal welfare could thus enhance the susceptibility to disease with microorganisms responsible for human foodborne diseases.

Understanding pathogen load in correspondence to specific animal welfare indicators and in correspondence to the time when animals are more susceptible to infection, would help to identify the times when preventive and control measures should be successfully applied. Intervention strategy could include changes in management practice in order to reduce stress situation and improve animal welfare standards; the stable presence of commensal bacteria in fact inhibits the colonisation of pathogens in the gastrointestinal tract; therefore it is recommended to avoid as much as possible broad spectrum antibiotics treatments (Rostagno, 2009).

In the last decade some attempts were made to establish an integrated approach based on the interface between environmental conditions, animal health and welfare and quality of animal products through the entire food chain from breeding to slaughter (Petersen *et al*., 2002); others aimed at developing a Hazard Analysis of Critical Control Points (HACCP) - based approach to animal welfare (Grandin, 2011), or to animal health (Noordhuizen and Frankena, 1999). Much effort should be addressed to integrating animal health and welfare with food safety hazards in the implementation of a platform linking together all these aspects through the use of an integrated approach.

Reducing stress by improving welfare in animal production and limiting risk factors associated to a higher prevalence of foodborne pathogens could directly influence food quality and safety Blokhuis *et al*. (2005).

In the North East of Italy the domestic animal density (especially pigs) is high because of its “Protected Designation of Origin” mark (PDO); in other Southern European countries as France and Spain, several large companies are orientated towards PDO and regional specialties production which are a quality assurance label for food products. Special attention is given in these countries to the development of consortia regulations and standards to protect the brand names of these products. Another clear trend is towards caution in the use of drugs and to increased organic production. Although, organic pig production is moving forward quite slowly and currently only accounts for a niche market share of 0-2% in most countries (Trienekens *et al*., 2008).

The quality of products of animal origin is perceived by the consumers not only as a food safety issue, but also as the welfare of the animals from which the products derive (Blokhuis et al., 2008). This has led to the development of a number of niche marketing schemes for farms that meet high standards of animal welfare (De Passillé and Rushen, 2005).

In conclusion this study aimed at establishes the approach which focuses to determine if it is possible to associate animal welfare and food safety indicators. *Y. enterocolitica* and *Salmonella* spp. were selected as indicators of food safety. They were investigated respectively in tonsils and mesenteric lymph nodes of finishing pigs during commercial procedures at slaughter plants. In addition, at slaughter line viscera were also assessed and scored as part of the animal-based welfare protocol.

In the next chapters more information on pig production in Northern Italy were presented; moreover some background to animal welfare indicators and also on food safety indicators were dealt with before the research project itself was described.

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Chapter 2: Pig population production systems in Northern Italy

2.1. Introduction

Data from Eurostat point out a decrease of the 1.7% of the European swine holdings in 2011, which counted 148,545,200 thousands heads. The reasons for this decrease are probably related to the financial worldwide crisis, which had repercussion on the price of raw material such as feed and cereals, and on the implementation of the Council Directive 2008/120/EC laying down minimum standards for the protection of pigs and compliance to the obligation of the sows group housing for the close cycle pig farms starting from January 2013 (Montanari, 2012).

German pig production continued to increase of the 1.9% in 2011, while in Denmark and Italy the swine holdings remained at the levels of the previous years. Decreases below 1.0% were registered in Spain and The Netherlands. France, Belgium and United Kingdom registered decreased value from 1.3% to 4.1%. European Eastern countries registered reductions from 8% to 19% with Czech Republic, Bulgaria, Lithuania and Estonia registering the higher falls (Eurostat, 2011) (Table 1).

In 2011 the Italian pork meat production decreased of the 1.7% for the reduction in the slaughtered heads (Eurostat, 2011). According to the Parma Quality Institute (IPQ) heavy pig slaughtered for PDO products decreased of 2.0% compared to 2010. In 2011 the import of pigs and pork meat in Italy increased by 1.1% compared to 2010. The production costs taken into account in the analysis of Montanari (2012), showed an increase of the production costs in 2011 of 10.2% compared to 2010; this was explained by the increase prices of cereals and fuel in 2011.

geo\time	2009	2010		2011		Variation 2011/2010
	No. heads	No. heads	%		%	%
EU (27 countries)*	**151,569,3**	**151,130,1***	**100.0**	**148,545,2***	**100.0**	**-1.7**
Germany	2,6841,0	26900,8	17.8	27402,5	18.4	1.9
Spain*	25,342,6	25,704,0	17.0	25634,9	17.3	-0.3
France	14,552,0	14,279,0	9.4	13,967,0	9.4	-2.2
Denmark	12,873,0	12,293,0	8.1	12,348,0	8.3	0.4
Netherlands*	12,108.0	12,206,0	8.1	12,103,0	8.1	-0.8
Italy	**9,157,1**	**9,321,1**	**6.2**	**9,350,8**	**6.3**	**0.3**
Belgium	6,227,9	6,176,3	4.1	6,327,9	4.3	2.5
United Kingdom	2,300,4	4,385,0	2.9	4,326	2.9	-1.3
Austria	3,137,0	3,134,2	2.1	3004,9	2.0	-4.1
Portugal	1,944,6	1,917,3	1.3	1,985,0	1.3	3.
Ireland	1,501,9	1,500,4	1.0	1,552,9	1.0	3.5
Sweden	2,551,9	1,607,0	1.1	1,567,7	1.1	-2.4
Finland	1,353,3	1,339,9	0.9	1,289,7	0.9	-3.7
Greece*	1,112,0	1,087,0*	0.7	1,109,0*	0.7	2.0
Luxembourg	88,6	89,4	0.1	91,3	0.1	2.1
Poland	14,252,5	14,775,7	9.8	13,056,4	8.8	-11.6
Romania	5,793,4	5,428,3	3.6	5,363,8	3.6	-1.2
Hungary	3,247,0	3,169,0	2.1	3,025,0	2.0	-4.5
Czech Republic	1,913,7	1,846,0	1.2	1,487,2	1.0	-19.4
Lithuania	928,2	929,4	0.6	790,3	0.5	-15.0
Slovakia	740,9	687,3	0.5	580,4	0.4	-15.6
Bulgaria	729,8	664,0	0.4	608,3	0.4	-8.4
Cyprus	463,3	463,7	0.3	438,9	0.3	-5.3
Slovenia*	415,2	395,6	0.3	347.3*	0.2	-12.2
Latvia	376,5	389,7	0.3	375.0	0.3	-3.8
Estonia	365,1	371,7	0.2	365,7	0.2	-1.6
Malta	65,9	69,3	0.0	46,3	0.0	-33.2

*provisional data

Table 1: Pig population in Europe (thousands heads), source: elaborated on Eurostat data, 2011, accessed on the 28th December 2012).

The increase of the price of the live pigs at slaughter, however, improved the profitability of the close cycle rearing system especially in the second part of 2011, despite the increase of the production costs.

Danish and French farmers maintained the lower production costs, while in Italy costs are obviously more elevated for the heavy breed pig rearing system which has a longer duration (Montanari, 2012). In 2011 the growth of the global pork meat demand led to an increase of the European exports. Farmers' income increased by 15.2% in 2011 compared to 2010 (Table 2); slaughter plants turnover registered a rise of the 9.0% for the economic recovery of the fresh loins and thighs (Figure 2). Loss of pig production together with the consumers' demand stimulated the economic recovery in 2011 of the heavy pig market.

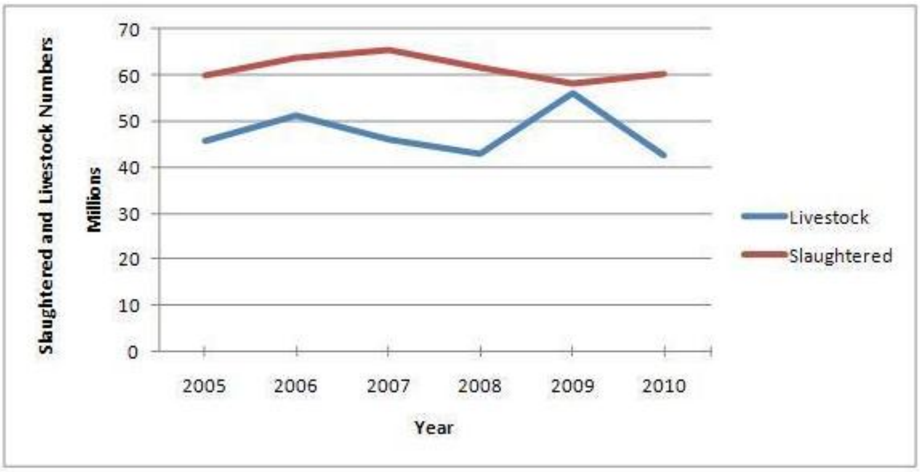

Figure 2: Trends for slaughtered and livestock number of pigs in reporting Members States in 2005-2010 (Source: EFSA, ECDC, 2012).

geo\time	2009		2010		2011		Variation 2011/2010
	No. tons	%		%		%	%
EU (27 countries)*	**21,279,46***	**100.0**	**22,010,78***	**100.0**	**22,387,6***	**100.0**	**1.7**
Germany	5,241,355	24.6	5,443,166	24.7	5,598	25.0	2.8
Spain*	3,290,571	15.5	3,368,921	15.3	3,469,345	15.5	3.0
France	2,004,185*	9.4	2,010,326*	9.1	1,998,317*	8.9	-0.6
Denmark	1583,2	7.4	1,666,3	7.6	1,718,4	7.7	3.1
Netherlands*	1274,98	6.0	1,288,274*	5.9	1347,165	6.0	4.6
Italy	**1,588,444**	**7.5**	**1,632,715**	**7.4**	**1570,225**	**7.0**	**-3.8**
Belgium	1,082,036	5.1	1,123,769	5.1	1108,255	5.0	-1.4
United Kingdom	720,253	3.4	774,466	3.5	806,021	3.6	4.1
Austria	533,436	2.5	542,131	2.5	543.771	2.4	0.3
Portugal	373,42	1.8	384,201	1.7	383,75	1.7	-0.1
Ireland	195,575	0.9	214.129	1.0	233,708	1.0	9.1
Sweden	260,748	1.2	263,478	1.2	256,085	1.1	-2.8
Finland	205,655	1.0	203,068	0.9	201,755	0.9	-0.6
Greece	117,583	0.6	113,717	0.5	115,121	0.5	1.2
Luxembourg	9,409	0.0	9,509	0.0	9,504	0.0	-0.1
Poland*	1,608,238*	7.6	1,741,425*	7.9	1,810,778	8.1	4.0
Romania	222 07	1.0	234,195	1.1	263,329	1.2	12.4
Hungary	388,717	1.8	416,146	1.9	387,304	1.7	-6.9
Czech Republic	284,572	1.3	275,905	1.3	262,944	1.2	-4.7
Lithuania	41,428	0.2	54,814	0.2	58,856	0.3	7.4
Slovakia	70,145	0.3	68,599	0.3	56,908	0.3	-17.0
Bulgaria	38,287	0.2	37,346	0.2	48,222	0.2	29.1
Cyprus	58,102	0.3	57,059	0.3	55,213	0.2	-3.2
Slovenia	24,115	0.1	24.902	0.1	22,954	0.1	-7.8
Latvia	24,757	0.1	23,327*	0.1	23,451*	0.1	0.5
Estonia	30,808	0.1	31,93	0.1	30,961	0.1	-3.0
Malta	7,369	0.0	6,96	0.0	7,262	0.0	4.3

Table 2: European pork meat production (Tons) (source Eurostat, 2011, accessed on the 28th December 2012).

2.2. Intensive Italian heavy breed system

Italy mainly focuses its pig production on heavy breed swine (85.0% of the whole Italian slaughtered pigs are from 130 to 180 Kg) in order to produce protected designation of origin (PDO) ham and other processed meat food. This particular type of production implies higher costs for the farmer due to the longer production cycle and to the increasing costs of feeding (ASS.ICA, 2011). The Italian pig market also reflects the action of complex factors shown by Montanari (2012) in the analysis described above for the year 2011.

Pig farms that produce under these PDO standards undergo regular inspections by external quality controls certification bodies which state the fulfilment of the requirements of the consortia procedural guidelines. The system also foresees a range of possible penalties including warnings, fines, or in the case of repetitive non-compliance by the farm, exclusion from the quality system.

The farms visited in this research project were located in the Emilia Romagna and Lombardy regions of the Northern Italy which accounted for 1,247,460 and 4,758,963 pigs respectively in 2010 (Eurostat, 2010) (Table 3).

In 2011data from the Parma Quality Institute (IPQ) reports 172 processing plants and two slaughter plants registered in the PDO certification in the province of Parma, while 26,740 pig farms are distributed all through Italy (data Istat, 2010) (Table 4).

Council Regulation No 208/1992 ruling the protection of designations of origin and geographical indications of agricultural products and food address the new European policy for the enhancement of food quality and protection and for a market oriented agricultural production, aimed to favour both consumers and producers.

Regions of Italy	Pigs no.
Piedmont	1,112,083
Valle d'Aosta	212
Liguria	972
Lombardy	**4,758,963**
Trentino Alto Adige	10,119
Independent Province of Bolzano	4,703
Independent Province of Trento	5,416
Veneto	798,242
Friuli-Venezia Giulia	216,430
Emilia-Romagna	**1,247,460**
Tuscany	119,230
Umbria	190,174
Marche	200,579
Lazio	77,183
Abruzzo	94,894
Molise	25,192
Campania	85,705
Puglia	41,780
Basilicata	84,838
Calabria	51,214
Sicily	46,292
Sardinia	169,752

Table 3: Italian pig distribution at regional level (source Istat, 2010, accessed on the 28th December 2012).

The typical production area of Parma ham, as identified by Law No 26 dated 13th February 1990, includes the territory of the province of Parma only (Emilia-Romagna region, Italy). The processing plants (ham factories) and the slicing and packaging plants must be located within the territory where all raw material processing phases must take place, as envisaged by the specifications. The raw material comes from a larger geographical area than the processing one, and which includes the following Italian Regions: Emilia-Romagna, Veneto, Lombardy, Piedmont, Molise, Umbria, Tuscany, Marche, Abruzzo and Lazio (Italy).

Heavy breed pig production systems are ruled by the requirements of the consortia in order to guarantee the high quality of dry-cured ham. Consortia requirements are based on the experience of the producers and on scientific data. Consortia prescribe the rules related to the characteristics of the raw meat (for example, the minimum weight of the thighs), but also the allowed genotypes (breed and crosses), the age and slaughter weight of the pigs, and the feeding routines (Bosi and Russo, 2004).

Regions of Italy	Farms no.
Piedmont	1197
Valle d'Aosta	27
Liguria	131
Lombardy	**2642**
Trentino Alto Adige	543
Independent province Bolzano	427
Independent province Trento	116
Veneto	1793
Friuli-Venezia Giulia	586
Emilia-Romagna	**1179**
Tuscany	1293
Umbria	759
Marche	1741
Lazio	901
Abruzzo	1961
Molise	583
Campania	1844
Puglia	744
Basilicata	479
Calabria	2193
Sicily	741
Sardinia	4860
Total	26740

Table 4: Italian distribution of the pig farms at regional level (source Istat, 2010, accessed on the 28th December 2012)

For the production of the Italian heavy pig, only purebred subjects coming from the Italian Large White and Italian Landrace breeds and Italian Large White x Landrace crosses are admitted. Slaughter weight should be around 160.0 Kg in order to obtain fresh thighs of the expected weight (Table 5). Moreover Consortia introduced a minimum age at slaughtering of nine months.

Characteristics of the PDO Parma ham Production	
Average live weight	160Kg ± 10%
Minimum age	At least 9 months
Minimum weight fresh thighs	12-14 Kg, but not <10Kg
Used breed	Large White, Landrace

Table 5: Characteristics of PDO production according to Parma ham procedural guidelines.

According to the procedural guidelines for the production of Parma ham the pig breeding policy identifies four categories of pig:

- Suckling: first four weeks by the sow;
- Weaning: from the 5th to the 12th week;
- Piglet fattening: from 30 to 80 Kg of weight;
- Fattening: from 80 to 160 Kg of weight and above (this category of pigs was the focus of the research project). There are two phases of rearing:
    1. Growing phase, with administration of a feed ration which aims to decrease the growth, allow muscles to form and limit the subcutaneous fat deposition.
    2. Finishing phase, which aims to increase the intramuscular fat deposition.

For heavy breed production the expected slaughtering weight is 160 Kg ± 10% for PDO products and 125-135Kg for the production of fresh sausages and cooked hams.

In order to achieve this objective, feed must be distributed in meals, preferably in liquid form or as a mash, with the addition of whey according to the procedural guidelines of the Parma ham.

2.3. Organic and extensive rearing system

Organic pig rearing system is still a limited scale market share. It is driven by the increased consumer demand for higher standards of animal health and welfare, for elevated food safety standards and by concerns about conventional production systems.

Territory	**Farms No.**
Italy	1059
Piedmont	42
Valle d'Aosta	3
Liguria	8
Lombardy	38
Trentino Alto Adige	29
Independent province of Bolzano	25
Independent province of Trento	4
Veneto	29
Friuli-Venezia Giulia	7
Emilia-Romagna	**66**
Tuscany	49
Umbria	14
Marche	54
Lazio	57
Abruzzo	72
Molise	3
Campania	33
Puglia	60
Basilicata	48
Calabria	118
Sicily	91
Sardinia	209

Table 6: Number of the certified organic pig farms in 2010 (source: censagri.Stat, accessed on the 28th December 2012).

The number of certified organic farms in Italy has increased considerably from 2005, when the number of herds was about 300 (Edwards, 2011). Organic pork market is about 0.3 % of conventional pork meat market (Table, 6).

Although the EU Regulation EC 834/2007 on organic agriculture provides a clear framework for the housing of fattening pigs, the practical implementation varies between countries. The pigs may be housed indoors with access to a concrete outrun or in an outdoor system with access to areas with soil/grass. In general, two types of outdoor systems can be identified. One system consists of a permanent building, e.g. a barn, with permanent outdoor areas and sometimes connected to two or three rotated pasture areas. The other system is more mobile; the pigs and their huts are more or less regularly moved to new areas, which can be fields included in the crop rotation or woodland.

Sometimes animal welfare is just the first driven issue which pushes the market to satisfy the public perception of animal welfare and address animal holdings to produce animals bred outdoor or according to quality assurance schemes in which animal welfare is considerably fundamental.

In a review by Saltalamacchia *et al*. (2004) which described the situation for Italian organic pig herds, the author concluded that breeds suitable for organic farming included Siena Belted, Casertana, Romagnola, Calabrian, Black Madonie, Duroc, Large White ×Duroc, and Large White × Siena Belted pigs.

In Italy there are different rearing systems according to geographical location. Most of the organic pig herds are outdoors and are located in fringe areas, although a small percentage of herds are situated in flat country. Fattening is about 60% outdoors, with the rest indoors with an outrun. In Italy fattening pigs are slaughtered from 120 kg (other productions) to 160 Kg (DPO) live weight, so in the legislation a minimum surface area for fattening pigs over 110 Kg live weight has been added, which is indoor 1,6 m^2/head and outdoor 2,0 m^2/head. The earliest weaning age is 40 days, but the mean is 45 days and often herds wean later (until 60 days) (Edwards, 2011). The main health problems for fattening pigs are leg problems, injuries and abscesses. Certified organic farms must be totally organic and outdoors: only one deworming per year is allowed; only feeding of organic components is permitted, and 50% of the feed must be produced on the farm or purchased in the farm district.

Keep pigs outdoors, however, is not synonymous with organic farming. In these cases outdoor farming must comply with a suitable range between farms' square meters and total live weight bred. In addition many farmers recently experimented the outdoor farming to allow the exploitation of such fields/forests and the breeding/rearing of endangered autochthonous pig breeds, such as Parma Black pig breed, which is protected by a certified breed register and its farming follows a specific procedural guidelines. In addition such autochthones breeds are preferred for their robustness in the outdoor systems and for their resistance to diseases, but they require longer production cycles, reaching lower weights compared to conventional breeds (FIBL, technical guide Core organic, 2011). Such practices characterise markets with short food chains that offer consumers particular local products from these specific breeds. These products are appreciated not only at regional and national levels but also abroad. The outcome production is profitable incomes for farmers and a more conscious choice for consumers who are able to decide to purchase animal friendly labels.

Figure 3: Parma Black pigs in extensive rearing system, Northern Italy, 2012.

Disadvantages of the extensive rearing systems include that the management logistics can be laborious during the cold and wet seasons. Rigorous parasite control is necessary as there is reduced biosecurity concerning contact with wildlife disease reservoirs and the soil may be a risk for health due to infection with a range of parasites. It is also more difficult to identify and treat sick animals as well as to supervise animals generally. Advantages include that there are little or no building costs, it better meets consumer expectations, there is more space and a greater environmental diversity which permits a better expression of the pigs' natural behaviour which has a positive influence on health and welfare. The low animal density and good air quality positively contribute to pigs' health. There is efficient use of the manure, if pig husbandry is integrated into the farm's crop rotation and the huts and feed area are moved regularly. This provides nutrients for the following crops and prevents major losses through nutrient leaching; vegetation and soil provide significant quantities of vitamins and minerals to the animals at pasture.

Figure 4: Parma Black foraging pigs in extensive rearing system, Northern Italy, 2012.

In conventional housing systems it is sometimes impossible to provide ways to increase locomotion in indoor pens with limited space, while it is more straightforward to control parasites by a preventive deworming strategy. Moreover Millet *et al.* (2005) demonstrated that alternative production farms can lead to acceptable production performance and meat quality characteristics when compared to conventional systems. Taking into account that these production systems aim to enhance animal welfare standards, which are also foreseen by the general public, the absence of negative effects on the performance and meat quality for pigs further encourages this preference for extensive production systems.

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Chapter 3: Animal welfare indicators

3.1. Introduction

Animal welfare is often dealt with by people other than scientists in the attempt to define what humans should do to protect their animals. As scientists, understanding animal welfare implies understanding animal welfare in the context of animal welfare science (Keeling *et al*., 2011). Animal welfare is a necessarily driven expression of people concerns about specific animal welfare issues, such as intensive farming practices and experimental animals. Public animal welfare awareness inspired the approaches on legislation of many countries. Some pioneering countries have taken into account public concern when adopting animal welfare legislation which prohibited certain practices and such legislation becomes today part of our current European law on animal welfare. However, sometimes legislation concepts may cause comprehension problems for science in the explanation of their definition on ethical issues (Keeling *et al*., 2011).The most influential writings on animal welfare, the Brambell Committee already in 1965 acknowledged that feelings or the emotional state of the animals were an important characteristic of welfare. In its actual concepts it stated: 'Welfare is a wide term that embraces both the physical and mental well-being of the animal. Any attempt to evaluate welfare, therefore, must take into account the scientific evidence available concerning the feelings of animals that can be derived from their structure and functions and also from their behaviour'. The Brambell Committee thus anticipated what nowadays animal welfare researchers are addressing, i.e. the emotional state of the animal is accessible to scientific investigation (Keeling *et al*., 2011). The concept of animal welfare has been updated and complemented over the years and it is still developing. The basic perception of good welfare as being free from disease and injuries has being integrated with the conclusion that behaviour is a fundamental reflection of what animals want and should be the essential part of the whole animal welfare concept (Dawkins, 2004). As a scientific convention 'animal welfare' concerns the actual state of an animal rather than to the human ethical implications on animal protection. Welfare is thus considered as a characteristic of the animal, which describe the quality of its life (Broom, 1986).

The World Organisation of Animal Health (OIE) gives a more extended definition on animal welfare, which 'means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/killing. Animal welfare refers to the state of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment.'

Recent literature has a more integrated perception of animal welfare as a multidimensional concept (Botreau *et al*., 2007) in which science play an essential role beyond ethical, socio-economic, cultural and religious aspects, as well as sustainability and quality. Thus animal welfare must be dealt with according to a multi-criteria evaluation (Botreau *et al*., 2007).

The factors influencing the response of the animal includes the environment and resources available to the animal (resource-based measures), such as space allocation, housing facilities, provision of bedding material, etc. and the management practices of the farm (management-based measures), which indicate if enrichment material is provided, if anaesthetics and analgesics are used in mutilations, cleaning and disinfection procedure etc.

Animal-based examination provides the most direct indication of the response of the animal trying to cope with the surrounding environment (Botreau *et al*., 2007).

Factors may interact with each other having a synergic, antagonistic or additive effect on the way they act on the animal. Relevant resource-based and management-based measures, which describe the environment and conditions in which the animal is living, are considered complementary to the evaluation of animal-based measures. Collectively, these measures help to assess the animals' welfare status and to identify causes of poor welfare in order to propose advice for farmers for possible improvements.

Research is directed toward the adoption of validated measures of animal welfare, which can be reliably and feasibly used on farm, during transport or at slaughter.

The European founded project Welfare Quality® gathered animal welfare scientists from all over European different institutions and research centres to develop standardised practical strategies/measures to improve animal welfare standards through welfare assessment. Many studies were carried out in order to determine in a scientific way whether or not measures were valid, feasible and repeatable. The measures were thus evaluated on validity (does it measure what we think it does), repeatability (do different observers generate the same outcome), and feasibility (is it possible to use the measure given the constraints of a practical assessment system) (from Welfare Quality® website).

After a long validating process the assessment systems allowed the evaluation the quality of animal welfare on farms or at slaughter. These systems for three livestock species (and seven animal categories) were founded upon animal-based measures. The systems combine a science-based methodology for assessing farm animal welfare with a standardised way of integrating this information to assign farms and slaughterhouses to one of four categories (from poor to excellent animal welfare).

Researchers acknowledged that the best animal welfare assessments derived from a direct observation of the animals. Based on existing scientific literature or carrying out *ad hoc* research projects, animal-based measures were tested to determine whether or not they accurately reflected the actual welfare of the animal. The assessment procedures have been designed to reflect the multidimensional aspect of welfare (Botreau *et al*., 2007).

Because animals are kept in so many different environments and they are bred in many different ways, the measures can be applied to all systems. For each livestock species, around 40 different animal-based measures were identified in order to verify compliance with the 12 different criteria for farms or slaughterhouses.

The assessment protocols provide a mean that makes possible the assessment of farms through Europe in a standardized way by observers who received an identical training.

3.2. Welfare Quality®

In this framework the European research project Welfare Quality® focused on the integration of animal welfare in the food quality chain (Blokhius *et al*., 2003). One of its primary aims was to develop standardised practical strategies/measures to improve animal welfare standards through on-farm welfare assessment with consequences on the consumers and the market (Welfare Quality® website).

The welfare of an animal is a result of its attempts to cope with the environments in which it lives. The Welfare Quality® assessment scheme focuses on the animal’s point of view introducing measures directly taken on individuals of different animals’ species (cattle, pigs and poultry).

Welfare Quality® contemplates four main principles of animal welfare (Table 7).

Welfare Principles	Welfare Criteria	Measures
Good feeding	1 Absence of prolonged hunger	Body condition score
	2 Absence of prolonged thirst	Water supply
Good housing	3 Comfort around resting	Bursitis, absence of manure on the body
	4 Thermal comfort	Shivering, panting and huddling
	5 Ease of movement	Space allowance
Good health	6 Absence of injuries	Lameness, wounds on the body, tail biting
	7 Absence of disease	Coughing, sneezing, pumping, twisted snouts, rectal prolapse, scouring, skin condition, ruptures and hernias
	8 Absence of pain induced by management procedures	Castration, tail docking
Appropriate behaviour	9 Expression of social behaviours	Social behaviour
	10 Expression of other behaviours	Exploratory behaviour
	11 Good human-animal relationship	Fear from human beings
	12 Positive emotional state	Qualitative behaviour assessment

Table 7: Welfare Quality® principles and criteria and related animal welfare indicator applied protocol (source Welfare Quality® website).

These welfare principles were developed from the five freedom concepts: 1) freedom from hunger and thirst, 2) freedom from discomfort, 3) freedom from pain, injury and disease, 4) freedom to express normal behaviour, and 5) freedom from fear and distress (Farm Animal Welfare Council 1992). Each of these four principles comprises several independent but complementary criteria, which are in turn characterised by one or several measures (Botreau *et al*., 2007) (Fig. 7).

For each criterion several animal-based measures were established in order to evaluate the extent to which the criterion is fulfilled. Welfare Quality® proposed that animal-based measures are used during a single short farm visit by an independent assessor to measure welfare outcomes at that particular point in time. Once all the measures are taken a bottom-up approach is carried out combining measures into criteria-scores and then in to principles-scores to provide an overall assessment.

The practicability of the protocol as a tool would enable farmers and scientists to easily carry out the welfare assessment in the field in a relatively short time. The purpose of the assessment is to identify the farms that are likely to present welfare deficiencies. Some authors have applied the Welfare Quality® protocol on farms throughout Europe in order to analyse the assessment and check for feasibility. Temple *et al*. (2011) reported an average time of six hours and 20 minutes to assess pig farms and concluded that it may be considered excessively long compared to the procedures applied by stakeholders such as quality assurance inspectors, national veterinary services official officers and farmers. The direction of the research is moving toward reducing the time required to run the assessment on farm.
The Welfare Quality® assessment for pigs on farm will be described in Chapter 5.

3.3. Animal-based measures

The main concern of animal welfare scientists is to explain to the society that animal welfare is not just subjective issue. It can instead be measured objectively through the evaluation of animal-based measures combined with resource and management-based measures. Thus, the overall status of the welfare of the farming animals can be objectively determined. The application of the Welfare Quality® project in the field demonstrates that it is possible to integrate different animal welfare measurements in an unbiased and scientific way (Temple *et al*., 2011). Following on the four Welfare Quality® principles, quantitative observations of behavioural indicators address the animal welfare scientist toward an objective indication of the welfare status of the animal. Recent literature applied the Welfare Quality® protocols on different housing systems and in different categories of animals throughout Europe.

The first study assessing welfare outcomes started examining already existing quality assurance protocols to prove the potential added value provided by the study of outcomes such as direct observations of the behaviour and health of animals rather than that of husbandry resources (Whay *et al*., 2007, Courboulay *et al*., 2009; Mullan *et al*., 2009). These voluntary schemes allow higher standards of animal welfare met the need of the consumer to make a more conscious choice when buying products of animal origin. But they differed one from another and the animal welfare was not a standardised assessment as it became after the application of Welfare Quality® protocols (Botreau *et al*. 2007). Standardisation permitted the comparison of welfare outputs and had clearer overview between farms.

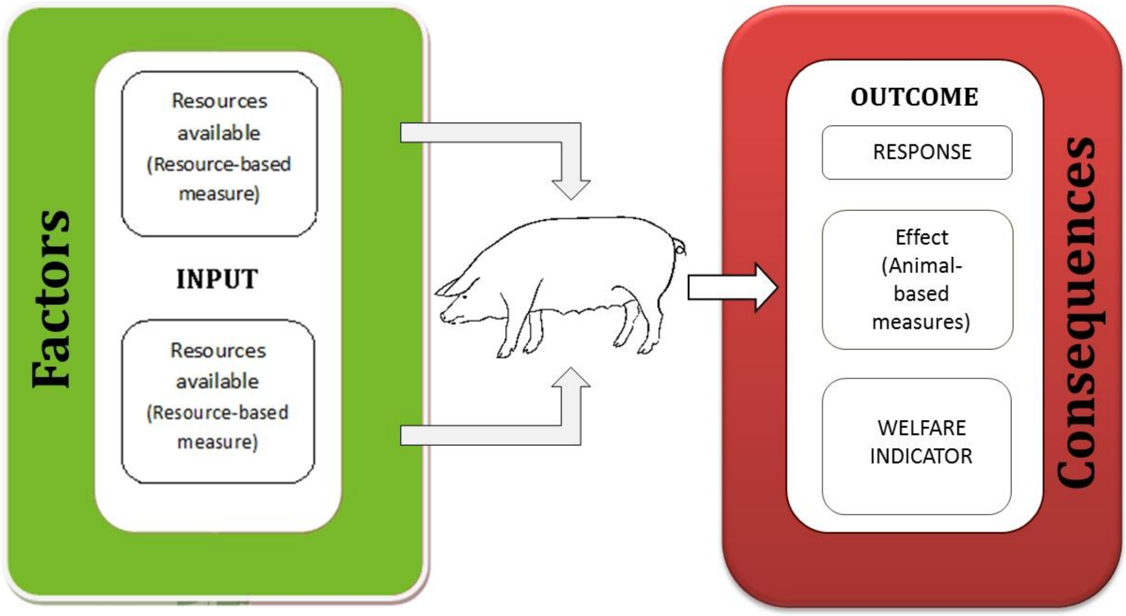

Figure 5: Animal welfare application of input and outcomes and their relationship (elaborated from EFSA, 2012).

Temple *et al*. (2011) applied the Welfare Quality® protocols to the growing pigs in Spain. The protocol is divided into animal-based measures of good feeding, housing and health together with behavioural measures. Measures were also compared in terms of housing systems of commercial farms in France and Spain. For example in the work of Temple *et al.* (2012a), pigs kept under straw-bedded systems were compared with outdoor production systems and intensive pigs kept under indoor slatted pens. Animal, resource and management-based measures were combined and analysed together in this study to obtain the most valid assessment (Temple *et al*., 2012a). Animal-based measures can be observations and measures made during the welfare assessment directly on the individuals or on the herd on farm or at slaughter, and they are not only considered as direct indicators of the animals' welfare, but are also evaluated for their potential risk to their welfare (Blokhuis *et al*., 2003). The records of animal breeding, growth, health, culling rate and abattoir condemnations, which may also include records of animal-based measures obtained using automated devices, as the records of the feeding formula or of the water consumption, are considered as indirect indicators of welfare. Non-animal-based measures includes resource and management-based measures, which influence the response to the animal. They are observations and measures of the type of housing, such as floor type, space allowance or of management used such as mutilation procedures. Documentation is an indirect management measure to record data on food provision strategies, staff training records, use of drugs etc. Animal-based measures can be determined at the individual level or at the group level depending on their value and meaning. Animals may be inspected either on the farm or during *ante-mortem* or *post-mortem* inspection in the slaughterhouse. Animal-based measures taken at slaughter plant *ante-mortem* and *post mortem* inspection give many indications of the welfare of animals either on-farm and during transport, lairage and pre-slaughter handling. Currently many animal welfare assessments applied by stakeholders are non-animal-based, since they are determined on the resource factors and on management only; these procedures have to comply with quality assurance requirements or with the legislation in force (Velarde and Dalmau, 2012). Such assessments based on inputs measures are easier to carry out but they do not reflect the exact status of the welfare of the animal; it is known that different animals in fact may respond to the same environment or to management practices in different ways depending on their specific characteristics such as breed and history; the responses are assessed using animal-based measures. Thus animal-based measures describe the outcome of the actual welfare state of the animal. Depending on the purpose of the assessment, the most appropriate measure for a particular situation could be selected and used. The objective of European risk assessment and management bodies for the near future is to monitor animal welfare through a systematic collection of these indicators and further recording them through a database tool, as part of an animal welfare surveillance scheme as it is applied for animal or human diseases. In these surveillance systems such as the National Notifiable Diseases Surveillance System, the public health disease surveillance system gives public health officials the capabilities to monitor the occurrence and spread of diseases. Such system applied to animal welfare would permit the observation of specific parameters within a farm over the time; the consequent analysis would lead to improvements and the implementation of a new farm strategy. However, these analyses of recorded data could serve the farmers to avoid situation of poor welfare near to come by means of the predictive role of some animal-based measures. Comparison of animal-based measures within the same farm over time or between farms in association with resource and management measures, enables the users of such a ‘tool’ to identify the farms outside of the normal distribution of variation and to establish thresholds for specific measures (Mullan *et al*., 2009). Moreover, EFSA in its opinion on the inspection of swine (2011) recommended that standards such as indicators of welfare outcomes and major endemic diseases should be developed and applied to support the surveillance of pig health and welfare during meat inspection. They also proposed the exploitation of existing data on pig health and welfare from slaughter plants should be implemented. Thus, animal-based measures could serve as welfare outcomes to help in the assessment of pig health and welfare during the inspection of swine.

Recently, different companies dealing with animal product chains are implementing monitoring and certification schemes, in order to communicate associated information to the consumers via branding and labelling on the animal friendly husbandry systems (Blokhuis, 2008). The implementation of the monitoring and information systems regarding animal welfare in a systematic and standardised way would give other opportunities to the producers to use internationally validated measures to assess animal welfare and to communicate this to the consumers demanding for information on animal welfare when purchasing products of animal origin as part of a labelling information.

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Chapter 4: Food safety indicators

4.1. Introduction

According to EFSA specification on epidemiological indicators in swine meat inspection (2011a) a harmonised epidemiological indicator is specified as the prevalence or incidence of the hazard at a certain stage of the food chain that correlates to a human health risk caused by the hazard. The epidemiological indicators provide information to be used in the swine meat chain. The indicators, either alone or in combination, may be used by competent national, regional authorities, slaughter plants or farmers depending on their purpose. The Scientific Opinion on the public health hazards to be covered by inspection of swine meat (EFSA, 2011a) identified *Salmonella*, *Yersinia enterocolitica* and *Toxoplasma gondii* as such hazards to be covered by the generic framework for swine meat carcasses safety assurance.

This opinion influenced the choice of the selection of the epidemiological indicators (outcome variables) in pigs and the stages of the food chain to be used in this study, but the type of samples to be collected was modified to avoid interference with the slaughter plants' commercial operations.

The selected food safety indicators for *Y. enterocolitica* considered in the research were adapted from EFSA Opinion on the specification of the epidemiological indicators in swine meat inspection (2011b) and were the following,

1. *Yersinia enterocolitica* in fattening pigs prior to slaughter on farm from environmental faecal samples.
2. *Yersinia enterocolitica* in fattening pigs in-coming to slaughter process (evisceration stage) from tonsils.

Currently there is no suitable harmonized indicator for *Y. enterocolitica* which may be used on farm level. Tonsil samples would help to define the infection status of pigs on farm level;

however, routine swine tonsil swab sampling is not feasible and cannot be justified for animal welfare reasons. On the other hand, examination of the faecal material leads to significant underestimation of the number of positive pigs on farm level (Nesbakken *et al*., 2006).

In pigs slaughtered over 135 days tonsils may be a more significant source of human pathogenic *Y. enterocolitica* than faeces (Nesbakken *et al*., 2006), thus at slaughter age, *Y. enterocolitica* is mostly found in the tonsils and to a lesser extent in the faecal content of pigs of heavy breed with a long production cycle.

The selected epidemiological food safety indicators for *Salmonella* spp. were:

1. *Salmonell*a in fattening pigs prior to slaughter on farm from environmental faecal samplings.
2. *Salmonella* in fattening pigs in-coming to the slaughter process (after the evisceration stage) from mesenteric lymph nodes.

Analytical methods for microbiology detection and serotyping from pooled faecal samples, ileal content and/or carcass swabs were provided in the opinion on the epidemiological specifications (EFSA, 2011b). Testing of mesenteric lymph nodes for *Salmonella* was not included in the opinion because sampling of ileal content is easier in practice than sampling the mesenteric lymph nodes (De Busser *et al*., 2011). Isolation and serotyping of *Salmonella* spp. strains provide data on new zoonotic serovars such as the monophasic variant of *S.* Typhimurium and new emerging ones.

4.2. General information on *Yersinia enterocolitica*

The genus *Yersinia* is currently composed of 12 species but only *Yersinia pseudotuberculosis* and the pathogenic biotypes of *Y. enterocolitica* cause food-borne enteric infections in humans; *Yersinia pestis* is not believed to exist anymore in Europe.

A total of 6,776 confirmed cases of yersiniosis were reported in the EU in 2010, with a decreasing trend (10.0 %) compared with 2009. Yersiniosis is still the third most numerously reported zoonosis in the EU, with an overall notification rate of 1.58 per 100,000 population in 2010 (Table 8) after *Campylobacter* and *Salmonella* human infections.

Diarrhoea, at times haemorrhagic, is the most common symptom of yersiniosis caused by *Y. enterocolitica*, and it occurs mostly in young children. Sometimes erythema, joint pain with or without bacteraemia may also follow. Infection is most often acquired by eating contaminated food, particularly raw or undercooked pig meat.

Biotyping of the isolates is essential to determine pathogenic human isolates, and this method could be complemented by serotyping. Pathogenicity can also be determined using PCR methods, targeting plasmid-borne and chromosomal virulence genes.

Y. enterocolitica is subdivided into 6 biotypes (1A, 1B, 2, 3, 4 and 5), and into more than 50 serotypes. Its strains can be grouped into three main groups of pathogenicity (EFSA, 2007):

- High pathogenicity: biotype 1B.
- Moderate pathogenicity: biotypes from 2 to 5.
- No pathogenicity: biotype 1A.

The highly pathogenic biotype 1B isolates are extremely rare in Europe and may be found in North America or Japan (EFSA, 2007).

Country	2010				2009	2008	2007	2006
	Report Type[1]	Cases	Confirmed cases	Confirmed cases/ 100,000	Confirmed cases			
Austria	C	84	84	1.00	140	93	142	158
Belgium	C	216	216	1.99	238	273	248	264
Bulgaria	A	5	5	0.07	8	10	8	5
Cyprus	U	0	0	0	0	0	0	0
Czech Republic	C	447	447	4.25	463	557	576	535
Denmark	C	193	193	3.49	238	331	274	215
Estonia	C	58	58	4.33	54	42	76	42
Finland	C	522	522	9.75	633	608	480	795
France	A	238	238	0.37	208	213	-	0
Germany	C	3,368	3,346	4.09	3,731	4,352	4,987	5,161
Greece	-	-	-	-	-	-	-	-
Hungary	C	87	87	0.87	51	40	55	38
Ireland	C	3	3	0.07	3	3	6	1
Italy	C	15	15	0.02	11	-	-	0
Latvia	C	23	23	1.02	45	50	41	94
Lithuania	C	428	428	12.86	438	536	569	411
Luxembourg	C	35	35	6.97	36	17	22	5
Malta	C	1	1	0	0	0	0	0
Netherlands	-	-	-	-	-	-	-	-
Poland	C	206	205	0.54	288	214	182	111
Portugal	-[2]	-	-	-	-	-	-	-
Romania	C	27	27	0.13	5	9	0	-
Slovakia	C	168	166	3.06	167	70	71	83
Slovenia	C	16	16	0.78	27	31	32	79
Spain[3]	C	325	325	2.83	291	315	381	375
Sweden	C	281	281	3.01	397	546	567	558
United Kingdom	C	55	55	0.09	61	48	86	65
EU Totals		**6,801**	**6,776**	**1.58**	**7,533**	**8,358**	**8,803**	**8,995**
Iceland	-[2]	-	-	-	-	-	-	-
Liechtenstein	-	-	-	-	0	-	-	-
Norway	C	52	52	1.07	60	50	71	86

1. A: aggregated data report; C: case-based report; U: unspecified; –: no report. 2. No surveillance system exists. 3. Surveillance system covers only 25 % of the total population.

Table 8: Reported cases of yersiniosis in humans in 2006-2010, and notification rates in 2010 (source EFSA and ECDC, 2012).

Country	Unit	2010				2009				2008			
		N	*Y. spp.*[1] % pos	*Y. e.*[2] % pos	*Y. e.*[3] isolates (N)	N	*Y. spp.* % pos	*Y. e.* % pos	*Y. e.* isolates (N)	N	*Y. spp.* % pos	*Y. e.* % pos	*Y. e.* isolates (N)
Germany	Herd	339	2.7	2.7	O:3 (4); O:9 (3)	525	1.0	1.0	O:3 (1)	5,450	1.0	1.0	O:3 (7); O:9 (32)
Ireland	Animal	-	-	-		391	0	0		480	0	0	
Italy	Animal	125	0	0		34	0	0		98	12.2	10.2	O:3 (1)
Latvia	Animal	68	0	0		-	-	-		-	-	-	
Netherlands	Animal	-	-	-		-	-	-		3,721	0.4	0.4	
Slovenia	Slaughter batch	-	-	-		131	19.8	19.8		384	19.3	19.3	
Spain	Slaughter batch	213	39.0	39.0	O:3 (83)	277	48.4	48.4	O:3 (134)	145	20.0	20.0	Biotype 4 (29)
Total (4 MSs in 2010)		**745**	**12.3**	**12.3**		**1,358**	**12.2**	**12.2**		**10,278**	**1.8**	**1.8**	

Note: Data are presented only for sample sizes ≥25. 1. *Yersinia* spp. 2. *Yersinia enterocolitica*. 3. *Yersinia enterocolitica* serotypes/biotypes (number of isolates).

Table 9: *Yersinia* spp. in pigs in 2008-2010 (source EFSA and ECDC, 2012).

The results of the reported data for *Yersinia* spp. from pigs in 2008-2010 are shown in Table 9 (only 1.3 % of the isolates were biotyped and only few Member States reported data).

In Europe, pigs are often asymptomatic carriers of human pathogenic *Y. enterocolitica*, in particular strains of biotype 4 (serotype O:3) and less frequently biotype 2 (serotype O:9 and O:5,27). The microorganisms are present in the oral cavity of pigs, especially in the tonsils, sub-maxillary lymph nodes, in the intestine and faeces.

Strains of bio-serotype 4/O:3 have been found frequently on the surface of slaughtered pig carcasses as a result of spread of the pathogens through contact with faeces, intestinal contents and tonsils during the slaughter and dressing processes. Since tonsils might be an important source of *Y. enterocolitica* bio-serotype 4/O:3 during slaughter, pork carcasses and edible offal can become contaminated with this pathogen. Slaughter practices and hygiene may influence the contamination rate of pork carcasses (Borch *et al.*, 1996). Since the oral cavity is frequently contaminated, handling the head and its parts during slaughter (removal of the tongue, splitting of the carcass and *post mortem* inspection on the viscera) may bring to spread the microorganisms. Since tonsils or a part of them are removed along the slaughter line with the pluck set and then suspended on viscera line, contamination of the rest of the pluck set by the tonsils is unavoidable. As a consequence, edible offal such as tongues, hearts and livers are more frequently and to a greater extent contaminated with *Y. enterocolitica* than pig carcasses (EFSA, 2007).

At herd level young pigs become healthy carriers of *Y. enterocolitica* in tonsils and faeces when they are about 60 to 80 days old, and become seropositive shortly thereafter (Nesbakken *et al*., 2006). High production capacity, water wet-feeding, feed distributors and quality of feed, absence coarse feed or bedding (Laukken *et al*., 2009), presence of cats in the farm, use of farm vehicles for transport of pigs to abattoirs, straw bedding for finishing pigs were all risk factors identified for *Y. enterocolitica* (Virtanen *et al*., 2011). Moreover antibiotic treatments were considered to affect the shedding of this zoonotic agent.

4.3. Methods for detection and identification of *Y. enterocolitica*

Different cultural methods for the isolation of *Y. enterocolitica* from foods have been described. These methods are also used for samples other than food, as animal and the environmental ones. Many of these methods result in the isolation of non-pathogenic *Yersinia* strains. Currently no single isolation procedure is considered ideal for the recovery of human-pathogenic strains of *Y. enterocolitica* in foods (De Boer, 2003) and many studies are still comparing feasible and practicable methods for the detection and enumeration of *Y. enterocolitica* i.e. Van Damme *et al*. (2010).

The International Standard Organization method for the detection of pathogenic *Y. enterocolitica* in foods can also be applied to lymphatic tissues such as tonsils (EFSA, 2007).

The enrichment and plating media which are currently used are not particularly selective for *Y. enterocolitica* as they support the growth of several other members of the Enterobacteriaceae family to which the microorganism belongs. This makes the isolation of low numbers of *Yersinia* among other contaminants rather difficult and may lead to false-negative results. Moreover, non-pathogenic strains of *Yersinia* are very common in many raw foods and may greatly interfere with the isolation of pathogenic *Yersinia* strains from these products. For a more rapid and sensitive detection, future research is committed to improve the analytical methods for human pathogenic *Y. enterocolitica* and to the parallel use of DNA-based methods (EFSA, 2011b).

4.4. General information on *Salmonella* spp.

Salmonella has long been recognised as an important zoonotic pathogen with economic implication in animals and humans. In 2010 the number of reported human Salmonellosis cases continued to decrease and 99,020 confirmed cases (notification rate 21.5 cases per 100,000 population) were reported by 27 EU Member States. The reduction was 8.8 % (9,598 cases) in 2010. In 2010 salmonellosis was the second most common foodborne zoonotic disease in the EU after campylobacteriosis (EFSA and ECDC, 2012).

Human salmonellosis is usually characterised by the acute onset of fever, abdominal pain, nausea, and sometimes vomiting. Symptoms are often mild and most infections are self-limiting; however, in some patients, they can be life-threatening. The common reservoir of *Salmonella* is the intestinal tract of domestic and wild animals. The pathogen can be transmitted through the consumption of contaminated food of animal and/or plant origin, mainly meat, poultry, eggs and milk beyond vegetables. Faeces contaminated environments can be the source of food contamination.

Country	2010				2009	2008	2007	2006
	Report Type[1]	Cases	Confirmed cases	Confirmed cases/ 100,000	Confirmed cases			
Austria	C	2,179	2,179	26.0	2,775	2,312	3,386	4,787
Belgium	C	3,169	3,169	29.2	3,113	3,831	3,915	3,630
Bulgaria	A	1,217	1,153	15.2	1,247	1,516	1,136	1,056
Cyprus	C	137	136	16.9	134	169	158	99
Czech Republic	C	8,456	8,209	78.1	10,480	10,707	17,655	24,186
Denmark	C	1,608	1,608	29.1	2,130	3,669	1,648	1,662
Estonia	C	414	381	28.4	261	647	428	453
Finland	C	2,422	2,422	45.3	2,329	3,126	2,738	2,576
France	C	7,184	7,184	11.1	7,153	7,186	5,313	6,008
Germany	C	25,306	24,833	30.4	31,395	42,885	55,399	52,575
Greece	C	300	299	2.6	403	792	706	890
Hungary	C	6,246	5,953	59.4	5,873	6,637	6,578	9,389
Ireland	C	356	349	7.8	335	447	440	420
Italy	C	2,730	2,730	4.5	4,156	6,662	6,731	6,272
Latvia	C	951	881	39.2	798	1,229	619	781
Lithuania	C	1,962	1,962	58.9	2,063	3,308	2,270	3,479
Luxembourg	C	211	211	42.0	162	153	163	308
Malta	C	160	160	38.7	125	161	85	63
Netherlands[2]	C	1,447	1,447	13.6	1,205	1,627	1,224	1,644
Poland	A	9,732	9,257	24.3	8,521	9,148	11,155	12,502
Portugal	C	207	205	1.9	220	332	438	387
Romania	C	1,291	1,285	6.0	1,105	624	620	645
Slovakia	C	5,171	4,942	91.1	4,182	6,849	8,367	8,191
Slovenia	C	363	363	17.7	616	1,033	1,336	1,519
Spain[3]	C	4,420	4,420	38.4	4,304	3,833	3,842	5,117
Sweden	C	3,612	3,612	38.7	3,054	4,185	3,930	4,056
United Kingdom	C	9,670	9,670	15.6	10,479	11,511	13,557	14,124
EU Total		**100,921**	**99,020**	**21.5**	**108,618**	**134,579**	**153,837**	**166,819**
Iceland	C	34	34	11.0	35	134	93	114
Liechtenstein	C	-	-	-	-	-	1	14
Norway	C	1,370	1,370	25.7	1,235	1,941	1,649	1,813
Switzerland[4]	C	1,179	1,179	15.1	1,298	2,031	1,778	1,768

1. A: aggregated data report; C: case-based report. 2. Sentinel system; notification rates calculated with estimated population coverage of 64 %. 3. Notification rates calculated with estimated population coverage of 25 %. 4. Switzerland provided data directly to EFSA.

Table 10: Reported human salmonellosis cases and notification rates for 2010 (source EFSA and ECDC, 2012).

In animals, sub-clinical infections are frequent. Pigs infected can shed the bacterium asymptomatically in their tonsils, gut and gut-associated lymphoid tissue for months. The pathogen may easily spread among animals in a herd without detection of clinical signs and animals may become intermittent or persistent carriers. This also occurs in pigs, which can be classified as:

- Actively excreting animals: Infected animals excrete *Salmonella* for months or even years following a clinical infection (Verbrugghe et al., 2012).
- Passive carriers: The animals take in *Salmonella* and excrete them again without being infected at all.
- Latent carriers: The infected animals carrier the pathogen in mesenteric lymph nodes resulting in fluctuating excretion (Verbrugghe et al., 2012).

Pigs ingesting *Salmonella* spp. excrete the pathogen after 24 hours and eight hours later it is found in lymph nodes (Berends *et al*., 1996). During periods of stress, like transport to the slaughter plant, recrudescence of *Salmonella* may occur.

Berends *et al*. (1996) recognised that within 2-6 hours of transport and lairage the number of animals excreting *Salmonella* can more than double, because of the new infections which reactive the latent ones (in which *Salmonella* was already in lymph nodes) and the presence of already excreting *Salmonella* animals.

Salmonella infections are evident in the pig sector across Europe but there is large variation. European Regulation (EC) No 2160/2003 requires Member States to set up national control programmes for *Salmonella* serovars deemed to be of particular public health significance in animal species presenting a high potential risk of transmitting *Salmonella*, such as poultry and pigs. The regulation aims at reducing the level of *Salmonella* in primary production. Some Member States which already implemented control programmes decreased the prevalence of the pathogen in slaughter pigs or even removed completely the infection, e.g. Finland. However, important pig producing countries such as France, Germany, Italy, Spain and the UK have levels of infection in slaughter pigs that would entail risks to human health, thus highlighting the need for a more efficient control and management of the disease. Assessments using carcass swabs also indicate that *Salmonella* risk can be reduced by good and hygienic slaughter processes.

The *Salmonella* criteria laying down by Regulation (EC) No 2073/2005 and modified by Regulation (EC) No 1441/2007 prescribe rules for sampling and testing. It also sets limits for the presence of *Salmonella* in specific food categories and in samples from food processing. The food safety criteria for *Salmonella* apply to products placed on the market during their entire shelf life, stating that *Salmonella* must be absent in the selected food categories (it should be absent in 25 grams of products). Minced pork meat and pork meat preparation are food categories which have to be tested for *Salmonella* spp.

In the most recent zoonosis report (EFSA and ECDC, 2012) the percentage of *Salmonella*-positive samples taken at pig slaughter plants ranged from 0.3% to 8.9%, with Belgium reporting the highest proportion of positives. Finland, Sweden and Norway reported no positive samples at slaughter, but there were discrepancies in the procedures used in the different Members states and not all of them reported their results. At processing and cutting plants, *Salmonella* was found in up to 10.4 % of fresh pig meat samples, with Spain reporting the highest proportion of positive samples, followed by Portugal (10.3 %) (Table 11) (EFSA and ECDC, 2012).

Country	Sample level	Sample unit	2010		2009		2008	
			N	% pos	N	% pos	N	% pos
Estonia	Farm	Animal, faeces	1,095	3.1	1,372	0.9	810	0
Finland	Farm	Herd (breeding), faeces	840	0	-	-	45	0
Italy[1]	Farm	Animal	1,272	0.6	-	-	-	-
		Herd	37	0	-	-	-	-
Estonia	Slaughter	Animal (fattening), lymph nodes	146	8.2	146	8.2	146	8.2
Finland	Slaughter	Animal (breeding), lymph nodes	3,207	<0.1	3,143	0.1	3,040	<0.1
		Animal (fattening), lymph nodes	3,332	<0.1	3,344	<0.1	3,112	<0.1
Slovakia	Slaughter	Animal (breeding)	-	-	122	5.7	-	-
		Animal (fattening)	98	3.1	-	-	-	-
Slovenia	Slaughter	Animal (fattening), lymph nodes	384	4.7	-	-	-	-
		Animal (fattening), faeces	384	5.5	-	-	-	-
Spain[1]	Slaughter	Slaughter batch	217	35.9	-	-	-	-
Sweden	Slaughter	Animal (breeding), lymph nodes	2,396	0.1	2,739	0.1	2,625	0.3
		Animal (fattening), lymph nodes	3,562	0.2	3,415	<0.1	3,187	0.3
Czech Republic	Unspecified	Animal (breeding)	-	-	87	1.1	-	-
		Animal (fattening)	-	-	837	2.6	-	-
		Animal (piglets)	-	-	635	1.3	-	-
Italy	Unspecified	Animal[1]	69	14.5	44	6.8	-	-
		Herd	31	0	-	-	-	-
Total (8 MSs)		**Animal**	**15,945**	**0.7**	**15,884**	**0.5**	**12,920**	**0.2**
		Batch/Herd/ Holding	**1,125**	**6.9**	**-**	**-**	**45**	**0**
Norway	Farm	Herd (breeding), faeces	117	0	116	0	-	-
	Slaughter	Animal (unspecified)	2,226	<0.1	-	-	-	-
		Animal (breeding), lymph nodes	-	-	859	0	651	0
		Animal (fattening), lymph nodes	-	-	1,620	0	1,475	0

[1] Samples are from a national survey

Table 11: Salmonella in pigs from bacteriological monitoring programmes in 2008-2010 (source EFSA and ECDC, 2012).

S. Typhimurium was the most frequently reported serovar (28.6 %) in pigs (Table 12). It was the prevailing serovar in all the Member States except Estonia and Romania. The second most common serovar was the monophasic *S*. Typhimurium (9.3 %), which was reported by three Member States.

Country	No of isolates serotyped	% positive										
		S. Typhimurium	Monophasic S. Typhimurium	S. Derby	S. Choleraesuis	S. Brandenberg	S. London	S. Rissen	S. Infantis	S. Enteritidis	S. Anatum	Other serovars, non-typeable, and unspecified
Total no of isolates	**3,301**	**945**	**307**	**187**	**172**	**52**	**46**	**43**	**35**	**16**	**16**	**1,482**
Austria	31	74.2	-	-	19.4	-	-	-	-	-	-	6.5
Estonia	78	15.4	-	17.9	1.3	-	-	-	5.1	2.6	-	57.7
Germany	2,263	30.3	11.2	4.5	-	1.9	0.4	0.1	1.1	0.3	0.6	49.6
Italy	421	12.6	-	8.6	11.9	-	6.9	5.7	-	-	-	54.4
Romania	129	6.2	1.6	-	85.3	3.1	-	1.6	-	-	-	2.3
Slovakia	15	26.7	-	6.7	20.0	6.7	-	-	6.7	6.7	-	26.7
Slovenia	40	32.5	-	12.5	-	-	-	-	5.0	7.5	-	42.5
Spain	78	25.6	-	11.5	-	5.1	1.3	14.1	1.3	2.6	1.3	37.2
Sweden	12	33.3	-	16.7	-	-		-	-	16.7	-	33.3
United Kingdom	234	52.1	21.8	8.1	0.9	-	2.6	1.3	1.3	-	0.4	11.5
Proportion of serotyped isolates		**28.6**	**9.3**	**5.7**	**5.2**	**1.6**	**1.4**	**1.3**	**1.1**	**0.5**	**0.5**	**44.9**

Table 12: Distribution of the 10 most common Salmonella serovars in pigs, 2010 (source EFSA and ECDC, 2012).

S. Typhimurium is the predominant serotype isolated from humans in Europe and pigs are an important reservoir of this serotype (Boyen *et al*., 2008). With the exception of infections with *S*. Choleraesuis and some strains of *S*. Typhimurium, *Salmonella* infections usually produce no severe disease in pigs. *S. enterica* ser. Typhimurium is frequently associated with *Salmonella* infections of pigs (Daube *et al*. 1998; van der Wolf *et al*. 1999). The major contamination sources of pig carcasses are pig faeces, pharynx and stomach and environment related to contact surfaces and handling by workers.

4.5. Methods for detection and identification of *Salmonella* spp.

The method for Salmonella spp. detection used is the ISO 6579:2002. It is applicable to products intended for human consumption or feeding of animals. The procedures for isolation of *Salmonella* from food and animal faeces given in this protocol follow the ISO-6579: 2002 standard. An overview of the procedure of isolating *Salmonella* according to other standards is given in Section 5.2.7.2. Many authors applied the ISO method on lymph nodes samples

(Nollet *et al*., 2004; Botteldoorn *et al*., 2003; Methner *et al.*, 2011; De Busser *et al*., 2011 etc.).

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Chapter 5: Research project

5.1. Introduction

The aim of the study is to explore the association between on farm collected animal-based welfare measures to the contamination by *Y. enterocolitica* and *Salmonella* spp. as food safety indicators in finishing pigs at slaughter plant.

5.2. Material and methods

5.2.1. Study sample

Managers of slaughter plants were contacted through the national veterinary services official officers. The managers were interviewed to select the farms to be included in the project. These farmers were contacted through the slaughter plant managers and subsequently farms were recruited according to their willingness to contribute to the research project. The selection criteria of the study were:

- Only finishing pigs were included in the study
- Clients' farms of the slaughter plant were selected (for a successful follow up).
- Slaughter batches of at least five finishing pigs were selected.

The entire research project was carried out according to the routine commercial activity of each slaughter plant without interfering with their operations either on the farm or at the slaughter plant facility. Confidentiality was maintained and the results of the study were only communicated to farmers so that they could benefit from them.

5.2.2. Study design

Data collection and sampling activity were carried out from April to mid-November 2012.

Three pig slaughter plants were selected in the North East of Italy upon their willingness to cooperate and taking into account the variability of the distribution of the types of farming systems. The slaughter plants were named as 1, 2, and 3. Slaughter plants 1 and 2 collected pigs only from intensive systems of farming whereas slaughter plant 3 collected pigs from both organic and intensive types of farming. From each slaughter plant five farms were selected, and two batches of pigs per farm were included in the study. As a whole, 14 different farms were visited at the end of the study, because one farm was sample four times instead of twice. All the farms supplying pigs to slaughter plants 1 (five farms, 10 visits) and 2 (five farms, 10 visits) and two farms from slaughter plant 3 had intensive farming systems; two farms were organic (four visits) and two were semi free range (four visits). The two semi free range farms were extensive Parma Black pigs rearing farms. Once the farm was selected the assessment of the slaughter batch on farm started. The effects of animal welfare factors potentially associated with food safety indicators were analysed at batch-level, although either farms or batches can be used as epidemiological unit depending on the analysis. This was considered to be the epidemiological unit, which corresponded to the farm (individual animals were representatives of the entire batch). One batch was made up of several pens usually coming from the same room in a building on farm.

5.2.3. On farm assessment

On the day of the assessment at the beginning of the visit the assessor met the farm unit manager. The Unit manager provided general information of the farm and the slaughter batch, soon confirmed by direct inspection. General information regarded the number of finishing pigs in the farm, in the room and in the pen respectively; the initial and final age and weight of the pigs belonging to the slaughter batch, the characteristics of the building; if mixing of animals was carried out; what was the number of meals and the times of feeding and how the feed was distributed. The indirect measures comprised the mortality rate, and other data recorded by the farm unit manager. The selected batch for slaughter was visited on farm from one to five days before the day of slaughter. Each batch was evaluated on farm for the animal welfare assessment and at the same time environmental faecal samples were collected in the pens of the slaughter batch. The visit consisted of behavioural observations through the use of a qualitative assessment and scan samplings of social exploratory behaviour. Then the visit was concluded evaluating the animal welfare indicators (section 5.2.4.) related to good feeding, housing, and health principles.

Animals of the slaughter-batch were assessed and animal-based measures related to the absence of injury and disease; mortality and symptoms such as respiratory disorders (coughing and sneezing and laboured breathing) and enteric disorders (scouring and rectal prolapse) were registered, measured and scored. Moreover, resource and management based measures (section 5.2.4.) were collected. The measures were taken according to Welfare Quality® protocol for finishing pigs at farm and slaughter.

Data recording of animal-based measures were evaluated at individual and pen level using a three point scale. The assessment scale was defined giving to score 0 a meaning of good welfare to score 2 a poor level of welfare; for stomach lesions the worst score is 3 and 4. In some cases a binary scale (absence: 0 or presence: 2) was used.

5.2.4. Animal welfare assessment according to the Welfare Quality® protocol (source: elaborated from Welfare Quality® Assessment protocol for pigs)

Animal management and resource- based measures were grouped according the welfare principle and criteria which were indicated in the Welfare Quality® protocol. All the measures in this phase were taken on farm except for the lameness assessment that was carried out during the unloading at slaughter houses 1 and 2 in order to have a better view of all the animals walking out from the truck. Qualitative behaviour assessment whereas part of the protocol, was not carried out.

After entering in the room the pen(s) in which the slaughter batch was located, were identified. For each pen both individual and pen measures were taken. The assessor was provided with assessment sheets (which were previously prepared), a camera, a clock and a tape measure. The assessment sheets served to document all data that were then recorded electronically. Pictures of each batch were taken for a better follow up in the data recording. The clock was used to measure the time of specific assessment and the meter tape was used to measure the pens.

5.2.4.1. Good feeding

5.2.4.1.1. Absence of prolonged hunger

5.2.4.1.1.1. Body condition score

The animals in the pen/pens were observed to assess their body condition. The spine, hip and pin bones of pigs in each pen were visually inspected. Animals with visible spine, hip, and pin bones were counted and scored as lean pigs.

At individual level the animal-based measure was scored as follows:

0- Animal with good body condition

2- Lean animals

At batch level, the percentage of lean pigs with poor condition (i.e. score 2) was determined. Data were recorded.

5.2.4.1.2. Absence of prolonged thirst

5.2.4.1.2.1. Water supply

There were three aspects that were taken into consideration in each pen of pigs observed for this resource-based measure:

- The number of drinking places
- The functioning of the drinkers
- The cleanliness of drinkers: drinkers were considered hygienic when without faeces and without mould

A drinking place was considered as the space occupied for one pig while it was drinking without being disturbed. The number of places could be one place per drinker for individual drinkers, but could also be several “places” per “long” drinker. The information provided by the manager was corroborated by the assessor during the course of the visit. Doing so the assessor assessed the type of drinker (pipe, bowl or trough), and also its length, cleanliness and whether the drinkers were functioning correctly or not. At batch level the number of drinking places and the functioning of the drinkers were scored as follows:

0- The drinkers function correctly

2- The drinkers do not function correctly

And regarding the cleanliness of drinkers the score was:

0- Clean

2- Dirty

5.2.4.2. Good Housing

5.2.4.2.1. Comfort around resting

5.2.4.2.1.1. Bursitis

By observing the animals in the pen/pens the assessor evaluated the animal at a distance of nearly one meter and examined the animal from one side. The side with the better view for observation of eventual bursitis was chosen. Each pig was scored according to the following categorisation for the animal-based measure:

Score 0: No evidence of bursitis

Score 1: One or several small (1.5-2cm) bursae on the same legs or one large bursa

Score 2: Several large bursae on the same leg or one extremely large bursa or any eroded

At batch level the percentage of pigs scored 0, 1 and 2 was determined. Data were recorded.

5.2.4.2.1.2. Manure on the body/pig dirtiness

The animals in each pen/pens were assessed to evaluate the dirtiness of their body. Only the presence of manure was assessed, since an outdoor pig soiled with mud was considered normal, and not indicating a welfare problem. The presence of manure/faeces on the body was visually assessed on one side of the body. The assessor stayed within a pen and had an unobstructed view of one side of the body. The side with better view for observation was chosen. Each pig was scored according to the following categorisation for the animal-based measure:

Score 0: Less than 20% of the body surface was soiled

Score 1: More than 20% but less than 50% of the body surface was soiled

Score 2: Over 50% of the body surface was soiled

At batch level the percentage of pigs scored 0, 1 and 2 was determined. Data were recorded.

Figure 6: Finishing pigs with absence of manure on the body (score 0, less than 20% of the body surface is soiled).

Figure 7: Finishing pigs with manure on the body (score 1, more than 20% and less than 50% of the body surface is soiled).

Figure 8: Finishing pigs with manure on the body (score 2, over 50% of the body surface is soiled).

5.2.4.2.2. Thermal comfort

5.2.4.2.2.1. Shivering

In order to evaluate shivering pigs the assessor stayed outside the pen. Shivering was defined as the slow and irregular vibration of any body part, or of the body as a whole. The selected group of animals were visually examined and the number of pigs that were shivering was estimated. At batch level pigs were scored according to the following categorisation for the animal-based measure:

0- No pigs in the pen/batch observed were shivering
1- Up to 20% of pigs in the pen observed were shivering
2- More than 20% of pigs in the pen observed were shivering

5.2.4.2.2.2. Panting

Since panting behaviour is best observed in resting animals, the assessor carried pot the evaluation from the corridor of the room. This is before they stood up as a response of her entering in the room. The assessor stayed outside the pen. Panting was defined as breathing rapidly in short gasps and carried out by breathing through the mouth. The selected group of animals were visually examined and the number of pigs that were panting was estimated. At batch level pigs were scored according to the following categorisation for the animal-based measure:

1- No pigs in the pen/batch observed were panting
3- Up to 20% of pigs in the pen observed were panting
4- More than 20% of pigs in the pen observed were panting

5.2.4.2.2.3. Huddling

Since huddling behaviour is best observed in resting animals, the assessor carried out the assessment from the corridor of the room. This is before they stood up as response of her entering in the room.

The definition of huddling was when a pig was lying with more than half of its body in contact with another pig (i.e. virtually lying on top of another pig). It was not considered huddling when an individual was just side by side i.e. alongside another animal.

At batch level pigs were scored according to the following categorisation for the animal-based measure:

0- No pigs in the pen/batch displaying huddling behaviour
1- Up to 20% of resting pigs in the pen/batch displaying huddling behaviour
2- More than 20% of resting pigs in the pen/batch displaying huddling behaviour

5.2.4.2.3. Ease of movement

5.2.4.2.3.1. Space allowance

The animal unit manager was asked about the number of pigs in every pen/room/ building. After the health measure, the assessor evaluated the length and width of the area provided to the animals. Before the health measures were assessed, the assessor counted the total number of animals in the pens/batch of animals. The assessor asked the animal unit manager about the average weight of pigs. The live average weight of the batch was also provided in the slaughter plant records.

Space allowance was calculated measuring the area (length per width of the pen or paddock) provided to animals divided by the number of animals. At batch level the space allowance which is a resource and management-based measure, was expressed in m^2/100 Kg animal. The average live weight of the pigs was provided by data records of the slaughter plant.

5.2.4.3. Good health

5.2.4.3.1. Absence of injuries

5.2.4.3.1.1. Lameness

Lameness was assessed at the unloading phase of the transport for each batch belonging to slaughter plants 1 and 2. In this case the animals belonging to the selected slaughter batch on arrival to the slaughter plant were observed during the unloading phase of the transport. In slaughter plant 3 lameness was assessed on farm. For an optimal assessment the animal was evaluated from a minimum distance of 3 to 10 meters while it was walking. Lameness is the inability to use one or more limbs in a normal manner. It can vary in severity from reduced ability or inability to bear weight, to total recumbency. At individual level the gait of the animal was scored as follows. At individual level each pig was scored according to the following categorisation of the animal-based measure:

Score 0: Normal gate or difficulty in walking, but still using all legs; swagger of caudal body while walking, shortened stride

Score 1: Severely lame, minimum weight-bearing on affected limb

Score 2: No weight-bearing on affected limb, or not able to walk (score as sick animal)

At batch level the percentage of animals affected with lameness score 0, the percentage of animals affected with lameness score 1 and the percentage of animals affected with lameness score 2 were registered. Data were recorded.

5.2.4.3.1.2. Wounds on the body

Wounds on the body were visually assessed by inspecting one side of the pig's body. The side with the optimal view for observation was chosen. The tail zone was not considered here. Each body region was assigned with a score. Wounds on the body is an animal-based measure and can be in the form of superficial scratches, lesions (surface penetration of the epidermis) or wounds (penetration of the muscle tissue). The considered zones were: ears, front, middle, hind-quarters and legs.

Each zone was considered separately according to this standardisation:

- A group of small scratches were considered as 1 lesion
- Scratches greater than 2 cm were considered as 1 lesion
- 2 parallel scratches with up to 0.5 cm space between them were considered as 1 lesion
- A round lesion smaller than 2 cm was considered as 1 lesion

- A round lesion from 2 to 5 cm of diameter or more than 5 cm and healed were considered as 5 lesions
- A round lesion of more than 5 cm, deep and opened were considered as a cumulative score of 16 lesions

At individual level each pig was scored according to the following categorisation:

0- If all region of its body had up to 4 lesions

1- When from 5 to 10 lesions were observed on up to 5 zones of the animal or one zone had more than 15 lesions

2- When more than 10 lesions were observed on at least two zones of the body or if any zone had more than 15 lesions

At batch level the percentage of pigs scored 0, 1 and 2 were registered.

5.2.4.3.1.3. Assessment of tail biting lesion

The animal was assessed standing up and the assessor had a clear view of the pig's tail. Tail biting is a parameter related to damage to the tail, ranging from superficial bites along the length of the tail to absence of the tail. The animal-based measure was scored as follows. At individual level each pig was scored according to the following categorisation:

a: No evidence of tail biting

b: Superficial biting, but no evidence of fresh blood or swelling

c: Fresh blood visible on the tail; evidence of swelling and infection; part of tail tissue are missing and crust is formed

At batch level the following categorisation scores were used:

Percentage of pigs with no tail biting (score a) or score b.

Percentage of pigs with a bleeding tail and/or swollen infected tail lesion, and/or part of tail tissue missing and presence of crust (score c).

5.2.4.3.3. Absence of disease

5.2.4.3.2.1. Mortality

According to the protocol, as management-based measure mortality is defined as the "uncontrolled" death of animals (as distinct from culling/euthanasia). The animals may die from for example: septicaemia, respiratory disease, acute infection or dehydration. Any animal which was "found dead" on the floor in the house, or out on the field was considered a mortality.

The animal unit manager was asked about mortality management on the farm according to data collected from farms records. Using house records of animal numbers placed on the farm, the percentage mortality was calculated as the total number of animals which died (M) and were found dead (but were not actively culled) (A) during 2011 using the following equation:

Percentage of mortality = (M/A) x 100

5.2.4.3.2.2. Coughing and sneezing

Directly after getting the animals to stand up, coughing and sneezing, animal-based measures, were assessed. Coughing and sneezing were assessed from a total of six observation points inside the farm and from each point of observation at least two pens were observed (which normally corresponded to approximately 20-40 animals for each point of observation). Coughing and sneezing were assessed for five minutes at each observation point. The total number of pigs observed coughing and sneezing was counted. At batch level the average frequency of coughing and sneezing per animal per five minutes was recorded.

5.2.4.3.2.3. Pumping (laboured breathing)

Pumping is identified as when the pig's breathing is heavy and laboured with the chest rising and falling with each breath. At individual level pigs were scored according to the following categorisation for the animal-based measure:

0- Percentage of pigs with no evidence of laboured breathing

2- Percentage of pigs with evidence of laboured breathing

5.2.4.3.2.4. Twisted snouts

Twisted snouts are characteristic of atrophic rhinitis, and can vary in severity from a slight deformity of the snout to severe nasal distortion. The assessor scored pigs at individual level. At individual level the animal-based measure was scored according to the following categorisation:

0- Percentage of pigs with no evidence of twisted snouts

2- Percentage of pigs with evidence of twisted snouts

5.2.4.3.2.5. Rectal prolapse

A rectal prolapse is when internal tissue extrudes from the rectum. As rectal prolapsed was either present or absent, the number of pigs presenting this problem were scored. Often the first visible sign of a rectal prolapsed is blood on the faeces. The assessor scored pigs at individual level. At individual level the animal-based measure was scored on each pig according to the following categorisation:

0- Percentage of pigs with no evidence of rectal prolapse

2- Percentage of pigs with evidence of rectal prolapse

5.2.4.3.2.6. Scouring

The animal-based measure for scouring could not be carried out at the individual animal level, so the assessor identified areas in the pen where the dung was visible and fresh and then made the assessment. The number of animals in the pen was recorded.

Scouring is considered to occur when faeces become more fluid in consistency than normal. Scouring was assessed based on the visible and fresh dung on the floor in the pen, or from the surroundings of the area where pigs were kept in extensive conditions. At batch level pigs were scored according to the following categorisation:

0- No liquid manure visible
1- Some liquid manure visible
2- All faeces visible was liquid manure

5.2.4.3.2.7. Skin condition

One side of the body was assessed choosing the flank with the optimal view for observation. Certain diseases can cause characteristic inflammation or discolouration of the skin. The pig was visually examined while looking for evidence of inflammation or discolouration. At individual level the animal-based measure was scored on each pig according to the following categorisation:

0- No evidence of skin inflammation or discolouration
1- Some, but less than 10% of the skin inflamed, discoloured or spotted
2- More than 10% the skin is inflamed, discoloured or spotted

At herd level the percentage of pigs with score 2 was determined.

5.2.4.3.3.1. Rupture and hernias

The pig was observed from the front, back and side. Hernias and ruptures occur when there is protrusion of a bodily structure or an organ through the wall that normally contains it, resulting in a lump under the skin in the umbilical or inguinal area which is recorded. At individual level the animal-based measure was scored on each pig according to the following categorisation:

0- No hernia/rupture
1- Hernia/rupture present but not bleeding, not touching the floor or affecting locomotion
2- Hernia/rupture was bleeding lesion and touching the floor when the animal was standing up, or affecting its behaviour.

At batch level the percentage of pigs scored 0, 1 and 2 was registered.

5.2.4.3.3. Absence of pain induced by management procedures

5.2.4.3.3.1. Castration (mutilations)

Although the category of the pig implies that mutilations are done at a previous stage, this kind of information was recorded to characterise the farms according to animal welfare criteria. Many of the farms analysed in the project were specialised in fattening pigs so they used to buy weaned pigs already castrated and tail docked. The animal unit manager was asked if pigs were bought castrated or not. According to the law anaesthetic and analgesics were not used during the mutilation procedure in piglets, when these are performed before one week of age. Thus in some cases they did not know the answer to this question. The scores of the management-based measure were registered as follows:

0- No castration
1- Castration with use of anaesthetics
2- Castration without use of anaesthetics or analgesics

5.2.4.4.1.1. Tail docking (mutilations)

The animal unit manager was asked what proportion of the piglets were tail docked, at what age the procedure was performed, and whether anaesthetic and analgesics were used during the procedure. The scores of the management-based measure were registered as follows:

0. No tail docking:
1- Tail docking with use of anaesthetics
2- Tail docking without use of anaesthetics or analgesics

5.2.4.4. Appropriate behaviour

5.2.4.4.1. Expression of social and exploratory behaviour

5.2.4.4.1.1. Social behaviour (negative or positive)

Observations of the animal-based measure should take place in the morning when animals are more active. If animals are not fed ad libitum, observations are made outside the feeding period, at least one hour after the morning meal. Before starting the assessment, the assessor should enter the room, record the number of animals per pen/batch and ensure that all the animals are standing up. If necessary, clap the hands and disturb the pigs by touching them 5-10 minutes later make the observations from the passageways.

The behaviours recorded are:

Negative social behaviour (N), defined as an aggressive behaviour including biting, or aggressive social behaviour with a response from the disturbed animals.

Positive social behaviour (P), defined as sniffing, nosing, licking, and moving gently away from the animal without aggressive or flight reaction from this individual.

Animals not showing positive or negative social behaviour or exploratory behaviour were recorded as resting (R) or other (O), which is defined “other active behaviours”, such as eating, drinking or air sniffing.

From the passage way, the behaviour of all the active animals should be ideally recorded using five scan samples made at two minute intervals. A summary is calculated on the scoring sheet for each behaviour. However due to shortage of personnel one scan sample was carried out.

Batch level

Number of animals observed during which a negative social behaviour was observed

Number of sample p of animals observed oints during which a positive social behaviour was observed

Batch level

Proportion of animal observed during which a social behaviour was observed when an active behaviour was observed and

Proportion of animal observed during which a negative behaviour was observed from the total sample points when an active behaviour was observed

5.2.4.4.1.2. Exploratory behaviour

The assessment of the animal-based measure should be made in the morning when the animals are more active; however the assessor should avoid the period around feeding i.e. at least one hour after the morning meal if pigs are ration fed. Before starting the assessment the assessor should enter the room, record the number of animals per per/group and ensure that all animals stand up. If necessary, clap the hands and disturb the pigs by touching them. 5-10 minutes later make the observations from the passageways. It is important not to move during the observation in order to avoid a reaction from the animals.

The behaviours recorded were:

- Investigation of the pen (S) is defined as sniffing, nosing, licking or chewing any features within the pen.

- Exploring material (E) is defined as play/investigation towards straw or other enrichment material. These parameters are assessed at the same time as social behaviours.
- Animals not showing exploratory or positive and negative social behaviour should be recorded as resting (R) or “other” (O), which is defined as “other active behaviour” as eating, drinking or air sniffing.

From the passageway, the behaviour of all the active animals was recorded using five scan samples made at two minutes interval. A summary was calculated on the scoring sheet.

Batch level

Number of animal observed when exploration of pen features was observed

Number of animal observed when exploration of enrichment material was observed

Batch level

Proportion of animal observed when exploration of pen features and enrichment material was observed from the total sample points when an active behaviour was observed and

Proportion of sample points when exploration of pen features and enrichment material was observed from the total sample points when an active behaviour was observed

5.2.4.4.2. Fear of humans

Within this animal-based measure was considered whether the animals showed a panic response towards a human or not. Panic is defined as animals fleeing, or facing away from the assessor or huddling in the corner of the pen.

Firstly, the assessor entered the pen, or stood next to the group of animals either in intensive and extensive conditions, and then walked around the group very slowly. On returning to the starting point, the assessor stopped, waited for 30 seconds, and then walked around the pen/group of animals very slowly in the opposite direction. The assessor considered the response of the animals to this second contact. When walking through the group the assessor did not initiate any physical interaction or talk to the animals, although limited physical contact occurred during walking, such as gentle touching pigs ahead of the assessor and therefore very close. At batch level pigs were scored according to the following categorisation:

0- Up to 60% of the animals showing a panic response

2- More than 60% of the animals showing a panic response

At batch level the percentage of pens with panic score 2 were registered.

5.2.5. Environmental faecal sampling

After the welfare assessment, environmental faecal samples belonging to the batches of pigs soon to be slaughtered were collected in the same day. From each slaughter batch one sample of faeces of nearly 30 gr was collected in each corresponding pen according to the 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 1 Part B. The material collected for bacteriological analysis was freshly voided faeces representing the whole batch, which was the unit of interest. Environmental faecal sampling was carried out by using sterile equipment. Where there was no faecal accumulation, for example in a field, large yard, or pens then individual pinches were collected from individual fresh faecal masses or places so that a minimum of 10 individuals contributed to a total sample weight of at least 25 gr. The sites from which these pinches were collected were distributed in a representative manner across pens belonging to the slaughter batch.

5.2.6 Samples collection at slaughter plant

For each slaughter batch of pigs, information concerning the origin of the animal was collected from the *ante-mortem* records sheets of the slaughter plant. Moreover other information such as date of arrival, day of slaughter, live body weight per batch was recorded.

On the day of slaughter the selected batch was sampled along the slaughter line. Five individual carcasses per batch were randomly selected for viscera inspection and sample collection. Viscera assessment and sample collection were carried out at the evisceration stage of the slaughter line just after the cutting of the pigs' carcasses.

5.2.6.1. Sampling of the tonsils and of the mesenteric lymph nodes

Five tonsils (*tonsilla veli palatini*) and five mesenteric lymph nodes from the five randomly selected pigs from each of the 30 slaughter-batches were collected during 30 sampling visits at the three slaughter plants (10 visits per plant). The animals originated from the 14 different farms visited previously. From each identified animal, the tonsils were aseptically removed immediately after evisceration at the slaughter line, placed into sterile plastic bags and transported to the laboratory under chilled conditions where they were tested on the day of collection.

At the same time as the viscera inspection was carried out, mesenteric lymph nodes belonging to the same carcasses were aseptically removed, and collected into a disposable sterile plastic sampling bag.

5.2.6.2. Gross pathology

From the same five randomly chosen animals, stomachs were opened by puncturing the cardiac region and cutting along the greater curvature, the contents were expelled and the gastric surface was gently rinsed with water. The *pars oesophagea* was everted to facilitate easier morphological comparisons. The condition of the *pars oesophagea* of each stomach was photographed and ranked according to a modified version of the Kopinski and McKenzie (2007) scale; the scale was modified by extending it with one grade for the gastritis gross lesion. The photographs were collated to produce a morphological guide of the different changes ranging from normal epithelium to ulceration or stenosis. The appearance of the *pars* *oesophagea* of each stomach was ranked from 0 to 4 according to the following categorisations:

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 epitelium),

4 = developed ulcers, haemorrhage and stenosis present.

Evaluation of viscera was also carried out to check for pneumonia, pleurisy, pericarditis and white spot liver according to the Welfare Quality® protocol for pigs at the time of slaughter. This assessment was carried out before any further manipulation of these organs was executed.

According to Welfare Quality ® protocol for finishing pigs at slaughter, pneumonia is defined as lungs with inflammatory process on the surface, and with consolidation of the lung. Pleurisy is defined as an inflammation of the pleurae. It can lead to adhesions of the lungs with the pleura. When pleurisy is present the lungs appear partially or totally destroyed (i.e. a part of the lung is fixed to the carcass). Pericarditis is defined as adhesions between the heart and the pericardium.

According to Welfare Quality ® protocol pneumonia, pleurisy and pericarditis evaluation were individually assessed on the identified animals by visual inspection and palpation after evisceration and scored as shown below.

Score 0: No evidence of pleurisy

Score 2: Evidence of pleurisy

Score 0: No evidence of pneumonia

Score 2: Evidence of pneumonia

Score 0: No evidence of pericarditis

Score 2: Evidence of pericarditis

Score 0: No evidence of white spot liver

Score 2: Evidence of white spot liver

5.2.7 Microbiology

5.2.7.1. Enumeration and isolation of **Yersinia enterocolitica**

Tonsil samples were analysed for the presence of pathogenic *Y. enterocolitica* according to the ISO 10273:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of presumptive pathogenic *Y. enterocolitica* by direct plating and different enrichment protocols following Van Damme *et al*. (2010). In addition, using the direct plating method, enumeration of *Y. enterocolitica* in pig tonsils was performed. Six different methods were used.

Tonsil samples were washed with sterile water and aseptically cut into small pieces, and 10 g were transferred into a sterile filter bag. Samples were homogenized with 90 ml of Peptone-Sorbitol-Bile (PSB) (Biolife, Italy) broth for 4 min in a stomacher. From this initial homogenate, testing was carried out in parallel as follows. For isolation and enumeration by direct plating (Method 1), 100 µl were spread over two Cefsulodin Irgasan Novobiocin (CIN) (Oxoid, UK) agar plates (50 µl per plate).

All agar plates were incubated at 30°C for 48 h and examined for characteristic *Yersinia enterocolitica* colonies. The number of presumptive *Yersinia* colonies was counted and five characteristic colonies were biochemically and serologically confirmed as follows. Prior to biochemical confirmation, colonies were subcultured on Tryptone Soya Agar (TSA) (Oxoid, United Kingdom) and preliminary identification was carried out by seeding colonies in and Kligler Iron agar (Oxoid, United Kingdom) slants and Christensen urea agar (Oxoid, United

Kingdom). Colonies that produced typical reaction patterns (fermentation of glucose, no fementation of lactose and hydrolysis of urea) were further confirmed as *Y. enterocolitica* and biotyped following the revised scheme of Wauters *et al*. (1987). Confirmation tests were represented by individual biochemical tests (fermentation of saccharose, rhamnose, raffinose and melibiose) followed by biochemical species identification with API 20E system (bioMérieux, France). *Y. enterocolitica* isolates belonging to biotype 4 were serotyped by slide agglutination with a commercial O:3 antiserum (Denka Seiken, Japan).

For enrichment procedures (methods 2–4), 10 ml of the same initial homogenate was transferred to 90 ml Irgasan-Ticarcillin-potassium Chlorate (Biolife, Italy) broth supplemented with ticarcillin (Biolife, Italy) and $KClO_3$ (Biolife, Italy) and incubated at 25°C for two days. After enrichment, 10 µl were streaked onto *Salmonella*-*Shigella*-desoxycholate-calcium chloride (Oxoid, United Kingdom) agar plate and CIN agar (method 2). The remaining PSB homogenate was incubated at 25°C. After two (method 3) and five days (method 4), 10 µl were streaked on CIN agar plates. In parallel to that, the enriched PSB culture was plated on CIN agar after alkali treatment (methods 3K and 4K). Before plating, 0.5 ml of the enriched PSB cultures were transferred to 4.5 ml of 0.5% KOH (J.T. Baker, The Netherlands) solution and mixed for 20 s. All agar plates were incubated at 30°C for 48 h and *Yersinia* colonies were confirmed as described before.

Environmental faecal samples belonging to each batch were analysed following the same detection methods.

5.2.7.2. Isolation of **Salmonella enterica**

Mesenteric lymph nodes from each randomly selected pig were aseptically collected and placed in a sterile bag prior to processing. Mesenteric lymph nodes were processed according to the ISO method 6579:2002- Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp.

Environmental faecal samples belonging to each batch were analysed following the same detection method.

Detection of *Salmonella* spp. was performed according to ISO 6579:2002. The pre-enrichment step was performed by suspending 10 g of lymph nodes in 90 ml Buffered Peptone Water (BPW) (Oxoid, United Kingdom) and 10 g of faceces in 90 ml of BPW. Lymph nodes and faecal samples were homogenized in a Stomacher blender for two minutes BPW suspensions were incubated at 37 °C for 18-20 h. The enrichment step was performed by using Rappaport Vassiliadis Soya Broth (Oxoid, United Kingdom) and Mueller-Kauffman Tetrathionate broth (Oxoid, United Kingdom) added with novobiocin, incubated for 24 h at 41.5 °C and 37 °C, respectively. After incubation, selective broths were plated onto Xylose-Lysine-Desoxycholate (XLD) agar plates and Brilliant Green Agar plates, incubated at 37 °C for 24 h. Suspect colonies were confirmed by biochemical tests by seeding pure cultures in Triple Sugar Iron (TSI) agar, Lysine Iron Agar (LIA) agar and Urea Agar (Oxoid, United Kingdom). Cultures showing typical Salmonella reaction were further tested with API® 20E micro-substrate system (bioMérieux, France) for *Salmonella* genus identification. *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). *Salmonella* spp. isolates were finally serotyped following the White- Kauffmann-Le Minoir scheme by a national reference laboratory.

5.2.8 Data description

Observations and records from individual animals and pens were combined into their corresponding batches as the epidemiological units.

Data from the five individual pigs tested for the detection of the food safety indicators were presented in a descriptive mode (Annex I Table 1).

Welfare indicators (animal, resource and management-based measures) were linked to the corresponding food safety indicators with the aim to explore the relationship among these two characters of the study batches. The number of individuals observed and scored according to the Welfare Quality® protocol, was expressed as a percentage so allowing comparison between individuals affected by some measures with the number of total pigs per batch and grouped in ranges. These percentages were then related to the food safety indicators to determine the association using Chi-square statistical method and the magnitude of the association using odds ratios and their confidence intervals (Lison, 1961).

Individual animal-based measures were also summarised in a descriptive mode as a mean of batches with the calculation of confidence limits.

Although Welfare Quality® protocol allows results to be expressed as a mean of the specific scores or the mean percentage of animal affected at the present time the use of ranges was preferred to have a better overview of data.

5.2.9 Data analysis

A batch was considered positive if *Y. enterocolitica* was detected either in the environmental faecal samples or in tonsils collected at slaughter from five pigs originating from the batch. A batch was considered positive if *Salmonella* was detected either in the environmental faecal samples or from lymph nodes originating from the batch.

Only the pen animal-based measures were analysed statistically. The animal-based measures were considered to have a positive score the one with the worst score even though the percentage of the individuals having poor welfare indicators was minimal. The sum of positive batches of pen measures were analysed by individual logistic regression against the sum of the *Salmonella* and *Yersinia* positive batches. Odds ratio (OR) is used in epidemiology studies and also in cross sectional studies, in which sampling is carried out without regard to the exposure status. Odds ratio is a ratio of the odds of exposure/non-exposure in disease-specific groups or the ratio of the odds of disease/no disease in exposure-specific groups. The evaluation of significance of the OR includes Chi-Square Test (Yates corrected chi square *p*-value: 2-tail) and Confidence intervals (CI). Analysing a significance test for the OR, the true neutral value (indicating equal odds for both conditions or in this case no association) is one. Values above one indicate a strong association in as much as they distance from one. Values from 0 to 1 are protective toward the variable.

Confidence Intervals serve to quantify the precision of the estimate and consist of a lower and upper limit on either side of the point estimate. On the interpretation of the CI, 95% CI means that in repeated sets of samples, 95% of such intervals would be expected to contain the true value of the population mean. The *p* value quantifies exactly how unusual the observed result from our experiment would be if the null hypothesis was true (there is no difference between groups: the observed differences are accidental). The definition of a *p* value is the probability of obtaining a value of the test statistic at least as large as the one observed, given that the null hypothesis is true (Salman, 1998).

Statistical analysis was conducted through the on-line Open Source Epidemiologic Statistics for Public Health, Open Epi software[1]by two by two tables.

[1] http://www.openepi.com/OE2.3/Menu/OpenEpiMenu.htm

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5.3. Results

5.3.1. Introduction

First data description was indicated. Then individual welfare measures were presented. Results were presented following the Welfare Quality® criteria and principles. Gross pathology was shown. Finally statistical analysis results were provided.

5.3.2. Data description

Thirty batches of finishing pigs were included in this study. For each farm two slaughter batches were evaluated. The average size of the farms was 6351.7 (SD 5020.8) and ranged from 13 to 16000 pigs. The total number of pens that were included during the entire period of the study was 159 and the average number of pens per farm was 5.3 ranged and ranged from 1 to 11 pens. The total number of animals scored according to Welfare Quality® protocol was 3749 with an average of 125 (SD 51.2) animals per batch/farm (Table 1, Annex I).

5.3.3. Food safety indicators

The total number of animals tested for *Yersinia enterocolitica* was 150. The 30 batches were also tested for *Y. enterocolitica* in the faeces by environmental sampling. A total of 23 tonsils out of 150 (15.3%) were positive for *Y. enterocolitica.* The number of batches with *Y. enterocolitica* in tonsils was 15 out of 30 (50.0%). The number of *Y. enterocolitica*-positive faecal sample was 1 out of 30 (3.3%). There was only one batch (3.3%) which was positive to *Y. enterocolitica* on both tonsils and faeces. Twenty-two *Y. enterocolitica* isolates (95.7%) belonged to the human pathogenic bio-serotype 4/O:3 and one belonged to the avirulent 1A/ONT.

Results from the various methods used for the isolation of *Y. enterocolitica* were not presented as they were not within the objective of the thesis.

The total number of animals tested for *Salmonella* spp. was 150. The 30 batches were also tested for *Salmonella* spp. in the faeces by environmental faecal sampling. A total number of 16 lymph nodes out of 150 (10.6%) was positive for *Salmonella* spp. The number of batches with *Salmonella* spp. in faecal material was seven out of 30 (23.3%). There were four batches (13.3%) which were positive to *Salmonella* both in lymph nodes and in faeces. The number of batches considered positive to *Salmonella* spp. either in lymph nodes or in the faeces was 13 out of 30 (43.3%). The serovars isolated from lymph nodes were the following: *S*. Derby (26.8%), *S*. London (13.3%), *S*. Give (13.3%), *S*. Rissen (13.3%), *S*. Typhimurium (13.3%), *S*. Typhimurium monophasic variant 1, 4,[5],12:i:- (13.3%) and *S.* Braenderup (6.7%). The serovars isolated from faecal material were: *S.* Typhimurium monophasic variant 1, 4,[5],12:i:- (42.8%), followed by *S*. London (28.6%), *S*. Derby and *S*. Give (14.3%, respectively).

5.3.4. Individual animal-based measures

In this section the individual animal-based measures were presented. Most of the individual animal-based measures were score as 0, 1, or 2, with the greater score reflecting poorer welfare. For the purpose of this analysis only scored 2 measures were considered

The mean of the scores for each measure between the batches and 95% CI was provided in Table 13.

Individual animal-based measures	**Mean**	**SD**	**Median**	**CI 95%**	
				Lower	**Upper**
Poor body condition	0.3	0.4	0.0	-0.1	0.1
Moderate manure on the body	0.1	0.3	0.0	-0.0	0.0
Severe manure on the body	0.6	0.5	1.0	0.9	1.1
Moderate lameness	0.2	0.4	0.0	-0.1	0.1
Severe lameness	0.2	0.4	0.0	-0.1	0.1
Moderate wounds on the body	0.2	0.4	0.0	-0.1	0.1
Severe wounds on the body	0.0	0.2	0.0	-0.0	0.0
Moderate ruptures and hernias	0.2	0.4	0.0	-0.1	0.1
Severe ruptures and hernias	0.0	0.0	0.0	0.0	0.0
Severe tail biting lesions	0.1	0.3	0.0	-0.0	0.0
Twisted snouts	0.0	0.0	0.0	0.0	0.0
Rectal prolapse	0.0	0.0	0.0	0.0	0.0
Bursitis	n.a	n.a	n.a	n.a	n.a
Skin condition	n.a	n.a	n.a	n.a	n.a

Table 13: Batch score positivity for pen animal-based measures in Northern Italian finishing production, 2012.

Poor body condition had a mean value of 0.3, severe manure on the body (score 2) had the higher score (0.6), moderate and severe lameness and moderate wounds on the body gave a mean score of 0.2 (Table 13).

Although the unit of the study is the batch, we also provided in Annex I table 3 and 4, which shows the results of the individual animal-based and behavioural measures in order to make results comparable with other published articles applying the same protocol, despite it might not be the better analysis. According to that additional analysis, the highest prevalence was reported for severe (score 2) (36.1%) and moderate (score 1) (23.3%) pig dirtiness. The prevalence of poor body condition (score 2) was 3.7% among the 30 batches. Moderate wounds on the body (score 1) was 1.7%, while moderate and severe lameness, moderate ruptures and hernias, and moderate and severe tail biting lesions were below 0.7%.

Descriptive mode of the individual behavioural measures is described in Table 4, Annex I. Nearly 8.0% of finishing pigs at least nine months old showed social (positive and negative) behaviour. Their negative social interactions (1.4%) ranged from 0.0% to 5.4% in the batches.

Their exploration behaviour included either the “investigation of the pen” (S, in section 5.2.4.4.1.2.), defined as sniffing, nosing, licking or chewing any features within the pen and the “exploring material” (E, in section 5.2.4.4.4.1.2.), defined as play/investigation towards straw or other enrichment material) had an average value of 27.7% and ranged 0 to 85.7%; but enrichment material was absent in 73.3% of the batches.

5.3.4. Animal welfare indicators

5.3.4.1. Good feeding

5.3.4.1.1. Absence of prolonged hunger

Nearly all the batches had good individual body condition for the finishing pigs assessed: seven batches (23.3%) out of 30 had lean animals (score 2) at a range from 0-20% and only one batch (3.4%) at 25.0% of individual lean pigs per batch (Table 14).

	Score 1			**Score 2**		
	Ranges (%)	**Frequency**	**Relative Frequency (%)**	**Ranges (%)**	**Frequency**	**Relative Frequency (%)**
Body condition	100	22	73.3	0	22	73.3
	100- 90	4	13.3	0-20	7	23.3
	<90	4	13.4	>20	1	3.4
Total		30	100.0		30	100.0

Table 14: Frequency distribution of a discrete quantitative variable individual (body condition) in Northern Italy, 2012.

Poor body condition, visible hipbones and backbones (score 2) was detected in seven batches out of 30 (23.3%) and only one batch (27.7%) belonging to a semi free range farm had the highest percentage range (>20.0%). Three of the batches that scored 2 batches for body condition came from farms that had a concrete floor (two were from intensive organic farms straw bedded and having outdoor access and one was from conventional rearing system), three batches scored 2 for body condition came from semi free range production system and two batches came from conventional slatted floor farms (Table 15).

Severe Body condition		
Floor type	**Batch no.**	**Type of farm**
Slatted	2	Conventional
Concrete	2	Organic straw bedded
Concrete	1	Conventional
Soil/grass	3	Semi free range
Total	8	

Table 15: Description of a discrete quantitative variable (severe body condition) in relation to the type of floor and type of farms in Northern Italy, 2012.

5.3.4.1.2. Absence of prolonged thirst

All the animals were fed with correctly functioning troughs (score 1); in 80.0% of the batches the trough was clean, while in six batches the troughs were dirty.

Variable	Ranges	Frequency	Relative Frequency (%)
Working trough	Clean trough	24	80
	Dirty trough	6	20
Total		30	100

Table 16: Frequency distribution of a discrete quantitative variable (cleanliness of the trough) in Northern Italy, 2012.

All batches except five had a suction pipe as drinker. The five batches which administered water in a bowl were from three farms (one conventional, one organic and one semi free range). Eight batches out of 30 (26.0%) did not have working. Five (16.6%) batches had dirty drinkers. The average number of pigs per drinking places was 20 (SD 9.0) ranged from 7 to 48. Only two batches (8.0%) out of the 25 batches that used suction pipes as drinkers had the correct number of animals per drinker, which is 10 according to Welfare Quality® (Table 16). These were both from a semi free range farm. Fourteen batches (56.0%) out of 25 batches had one drinking place for >10 to < 20 pigs. Seven batches (28.0%) had one drinking places for the range >20 to <30 pigs. Two batches (8.0%) had one drinking place for the range 30-48 pigs (Table 17).

Variable	Ranges	Frequency	Relative Frequency (%)
No. of pigs per drinking place	< 10	2	8.0
	10-20	14	56.0
	20-30	7	28.0
	30-48	2	8.0
Total		25	100

Table 17: Frequency distribution of a discrete quantitative variable (number of pigs per drinking place) in Northern Italy, 2012.

5.3.4.2. Good Housing

5.3.4.2.1. Comfort around resting

Bursitis which is an animal-based measure of comfort around resting was not analysed because either space allowance or the environmental condition i.e. insufficient light, made difficult to assess them on farm.

Nine (30.0%) out of the 30 batches had over 60% of the pigs with manure on the body at score 2, that is over 50.0% of the body surface of the finishing pigs was soiled. Moreover 17 (56.7%) out of the 30 batches had up to 20 % of the animals per batch at score 2 for dirtiness. In seven batches (23.3%) over 60% of the individuals batch had a score 1, that is more than 20.0% and less than 50.0% of the body surface of the animal was soiled. In 10 batches out of 30 (33.3%) all animals had score 0, that is, less than 20% of the body surface was soiled Table 18).

	Score 0			**Score 1**			**Score 2**		
Variable	**Ranges (%)**	**Frequency**	**Relative Frequency (%)**	**Ranges (%)**	**Frequency**	**Relative Frequency (%)**	**Ranges (%)**	**Frequency**	**Relative Frequency**
Manure on the body	100	10	33.3	100-60	7	23.3	100-60	9	30.0
	100-50	2	6.7	60-20	3	10.0	60-20	4	13.3
	50-0	18	60.0	20-0	20	66.7	20-0	17	56.7
Total		30	100.0		30	100.0		30	100.0

Table 18: Frequency distribution of a discrete quantitative variable (individual manure on the body) in northern Italy, 2012.

	Manure on the body		
	Moderate	**Severe**	
Floor type	**Batch no.**	**Batch no.**	**Type of farm**
Slatted	4	5	Conventional
Concrete	1	3	Organic straw bedded
Concrete	7	9	Conventional
Soil/grass	0	0	Semi free range
Total	12	17	

Table 19: Description of two discrete quantitative variable (Moderate and severe level of manure on the body) in relation to the type of floor and the type of farm.

Twelve batches with severe (score 2) pig dirtiness had a concrete floor and of these, three were organic straw bedded systems; while five batches had a slatted floor. Moderate (score 1) pig dirtiness was found in eight concrete floors in seven conventional farms and one organic straw bedded farm, while four batches had slatted floor. The space allowance of the nine batches from conventional farms with moderate and severe manure on the body was comprised in the range from 0.6-0.8 m^2/100Kg (Table 19).

5.3.4.4.1. Thermal comfort

No pigs assessed were observed to be shivering or huddling.

5.3.4.4.1. Easy of movement

The average space allowance was 48.5 m^2/100Kg (SD 161.4) and ranged from 0.2 up to 842.1 in semi free range farms. According to Welfare Quality® 0.3 m^2/100 Kg is considered the very minimal space allowance and 10 m^2/100 Kg is considered the maximum. In the study two batches (6.7%) were below the minimum level proposed by Welfare Quality® and four batches (13.3%) coming from semi free range farms were above the Welfare Quality® maximum. On the whole 20 batches (66.7%) had a space allowance between 0.3 and 0.9 m^2/100 Kg (Table 20).

Variable	Ranges	Frequency	Relative Frequency (%)
Space allowance	<0.3	2	6.7
	0.3-0.9	20	66.7
	0.9-10	4	13.3
	≥ 10	4	13.3
Total		30	100

Table 20: Frequency distribution of a discrete quantitative variable (space allowance) in Northern Italy, 2012.

Other resource-based measures are treated below.

The distribution of concrete and slatted floors in the all batches was high; 46.7% concrete and 40.0% slatted floors, while only 4 batches (13.3%) belonging to two semi free range farms had soil and grass (Table 21).

Variable	Ranges	Frequency	Relative Frequency (%)
Floor	Concrete	14	46.7
	Slatted	12	40.0
	Soil	4	13.3
Total		30	100

Table 21: Frequency distribution of a discrete quantitative variable (floor type) on Northern Italy, 2012.

Twenty-two batches out of 30 (73.4%) did not have any enrichment material (straw, piece of wood, tyres, or chain), while eight batches (26.6%) belonging to four organic farms and four semi free range farms provided enrichment materials either with the addition of straw or naturally present in the environment (Table 22).

Variable	Ranges	Frequency	Relative Frequency (%)
Enrichment material	Yes	22	73.4
	No	8	26.6
Total		30	100

Table 22 Frequency distribution of a discrete quantitative variable (enrichment material) in Northern Italy, 2012.

Twenty-one batches out of 30 (70.0%) had an outdoor access, while nine of them did not have it. Among the 21 batches, 13 (62.0%) were intensive farms, four (19.0%) were organic and four (19.0%) were semi free range farms (Table 23).

Variable	Ranges	Frequency	Relative Frequency (%)
Outdoor access	Yes	21	70.0
	No	9	30.0
Total		30	100

Table 23: Frequency distribution of a discrete quantitative variable (outdoor access) in Northern Italy.

In 14 batches (46.6%) the animals arrived to the slaughter plant on the same day of slaughter, while in 12 (40.0%) batches animals arrived to the slaughter plant one day before slaughter. In four (13.3%) batches animals arrived two days before slaughter (Table 24).

Variable	Ranges	Frequency	Relative Frequency (%)
Arrival to slaughter plant	Same day of slaughter	14	46.7
	One day before	12	40.0
	Two days before	4	13.3
Total		30	100

Table 24: Frequency distribution of a discrete quantitative variable (arrival to slaughter plant) in Northern Italy, 2012.

The average weight of the finishing pigs was 167.0 Kg based on the slaughter plants' data records of the live weight.

5.3.4.4. Good health

5.3.4.4.1. Absence of injuries

Four batches out of 30 (13.4%) had any pig lame at score 2, that is the animal is not able to bear weight on the affected limb, or not able to walk. In six batches (20.0%) the range from 0 to 2% of the animals had a score 1 for lameness that is, the animals were severely lame with minimum weight bearing on the affected limb. In addition five batches (16.7%) had greater than two per cent of the animals with lameness score 1 (Table 25).

Variable	Score 0 Ranges (%)	Score 0 Frequency	Score 0 Relative Frequency (%)	Score 1 Ranges (%)	Score 1 Frequency	Score 1 Relative Frequency (%)	Score 2 Ranges (%)	Score 2 Frequency	Score 2 Relative Frequency (%)
Lameness	100	16	53.3	0	19	63.3	0	26	86.6
	100-90	14	46.7	0-2	6	20.0	0-2	2	6.7
	<90	0	0.0	>2	5	16.7	>2	2	6.7
Total		30	100.0		30	100.0		30	100.0

Table 25: Frequency distribution of a discrete quantitative variable (individual lameness) in Northern Italy, 2012.

The relationship between moderate and severe lameness and the type of floor and type of system is similar (six batches with slatted floor and five batches with concrete for the moderate lameness; two batches with slatted floor and two batches with concrete for severe lameness) (Table 26).

Floor type	**Lameness: Moderate Batch no.**	**Lameness: Severe Batch no.**	**Type of farm**
Slatted	6	2	Conventional
Concrete	1	1	Organic straw bedded
Concrete	4	1	Conventional
Soil/grass	0	0	Semi free range
Total	11	4	

Table 26: Description of two discrete quantitative variable (moderate and severe lameness) in relation to the type of floor and type of farms.

One batch (3.3%) out of 30 had a percentage range of individuals between 0 and 1% with score 2 for individual wound on the body, which is when more than 10 lesions were observed on at least two zones of the body or if any zone had more than 15 lesions. Five batches (16.7%) out of 30 had a percentage range of individuals above 0 and below 20 with regards to score 1, that is when from 5 to 10 lesions were observed on up to five zones of the animal or one zone had from 11 to 15 lesions (Table 27).

Variable	Score 0 Ranges (%)	Score 0 Frequency	Score 0 Relative Frequency (%)	Score 1 Ranges (%)	Score 1 Frequency	Score 1 Relative Frequency (%)	Score 2 Ranges (%)	Score 2 Frequency	Score 2 Relative Frequency (%)
Wounds on the body	100	24	80.0	0	25	83.3	0	29	96.7
	100-80	6	20.0	0-20	5	16.7	0-1	1	3.3
Total		30	100.0		30	100.0		30	100.0

Table 27: Frequency distribution of a discrete quantitative variable (wounds on the animal's body) in Northern Italy, 2012.

Three batches displayed wounds on the body of moderate and severe grade in conventional concrete farms (Table 28).

	Wounds on the body		
	Moderate	Severe	
Floor type	**Batch no.**	**Batch no.**	**Type of farm**
Slatted	0	0	Conventional
Concrete	0	0	Organic straw bedded
Concrete	2	1	Conventional
Soil/grass	0	0	Semi free range
Total	2	1	

Table 28: Description of two discrete quantitative variables (moderate and severe wounds on the animal's body) in relation to the type of floor and the type of farm.

Two batches (6.7%) out of 30 had a percentage range of individuals 0-2 having score 2 for tail biting lesions, which is when fresh blood was visible on the tail, there was evidence of some swelling and infection, and part of tail tissue was missing and crusts of wounds were present on the tail. Three batches (10.0%) had a percentage range of individuals 0-5 with score 1, which is superficial biting along the length of the tail, but no evidence of fresh blood or of any swelling (Table 29).

Variable	Score 0 Ranges (%)	Score 0 Frequency	Score 0 Relative Frequency (%)	Score 1 Ranges (%)	Score 1 Frequency	Score 1 Relative Frequency (%)	Score 2 Ranges (%)	Score 2 Frequency	Score 2 Relative Frequency (%)
Tail biting	100	27	90.0	0	27	90.0	0	28	93.3
lesions	100-90	3	10.0	0-5	3	10.0	0-2	2	6.7
Total		30	100.0		30	100.0		30	100.0

Table 29: Frequency distribution of a discrete quantitative variable (individual tail biting lesions) in Northern Italy, 2012.

Moderate and severe tail biting lesions were assessed in three batches (Table 30) from conventional farms with no enrichment.

	Tail biting		
	Moderate	**Severe**	
Floor type	**Batch no.**	**Batch no.**	**Type of farm**
Slatted	2	2	Conventional
Concrete	0	0	Organic straw bedded
Concrete	1	1	Conventional
Soil/grass	0	0	Semi free range
Total	3	3	

Table 30: Description of two discrete quantitative variables (moderate and severe tail biting lesions) in relation to the type of floor and type of farm.

5.3.4.4.1. Absence of disease

The average mortality within the 30 batches was 5.1% (SD 2.4) and ranged from 0.0 to 9.9% which was far above the Welfare Quality® warning threshold (2.6%) and alarm threshold (4.5%). Twenty-six batches (86.7%) had mortality below the Welfare Quality® warning threshold, four (13.3%) of which were below 2.6%. Eight batches (26.7%) were between 2.6% and 4.5% and 18 batches out of 30 (60.0%) had mortality above the alarm threshold (Table 31).

Variable	Ranges (%)	Frequency	Relative Frequency (%)
Mortality	0-2.6	4	13.3
	2.6-4.5	8	26.7
	>4.5	18	60.0
Total		30	100

Table 31: Frequency distribution of a discrete quantitative variable (mortality) in Northern Italy, 2012.

The prevalence of pigs coughing and sneezing and total number of coughs and sneezes was low and below the warning threshold established by Welfare Quality®, which was of 15 coughs and 27 sneezes (Tables 32-35 and Annex I, Table 5).

Variable	Ranges (%)	Frequency	Relative Frequency (%)
Number of pigs coughing	0	14	46.7
	0-2	11	36.6
	2-4	5	16.7
Total		30	100.0

Table 32: Frequency distribution of a discrete quantitative variable (number of pigs coughing) in Northern Italy, 2012.

Variable	Ranges (%)	Frequency	Relative Frequency (%)
Number of coughs	0	14	46.7
	0-2	9	30.0
	2-7	7	23.3
Total		30	100.0

Table 33: Frequency distribution of a discrete quantitative variable (the number of coughs) in Northern Italy, 2012.

Variable	Ranges (%)	Frequency	Relative Frequency (%)
Number of pigs sneezing	0	5	16.7
	0-10	23	76.6
	10-20	2	6.7
Total		30	100.0

Table 34: Frequency distribution of a discrete quantitative variable (number of pigs sneezing) in Northern Italy, 2012.

Variable	Ranges (%)	Frequency	Relative Frequency (%)
Number of sneezes	0	5	16.7
	0-10	23	76.6
	10-20	2	6.7
Total		30	100.0

Table 35: Frequency distribution of a discrete quantitative variable (number of sneezes) in Northern Italy, 2012.

Only one batch from a conventional slatted floor farming system had 3.0% of the pigs pumping during breathing with two pigs coughing and no pig sneezing, and two coughs and no sneeze. The same farm without outdoor access had also more than 20.0% of the pigs panting. Moreover the farm had dirty and not functioning drinkers. The welfare assessment in this farm was carried out in July with an outside temperature of almost 35°C. Another conventional slatted floor farm observed, had up to 20.0% of the pigs panting in the month of July.

Twisted snouts cases and rectal prolapse were never observed.

The pen measures, rectal prolapse was never observed in any batch. Scouring was found to be difficult to assess and so was not recorded.

Skin condition, was not analysed because either space allowance or the environmental condition i.e. insufficient light, made difficult to assess them on farm.

None of the individuals had a score 2 for the ruptures and hernias variable which is, when bleeding lesions hernias or rupture and touching the floor were present. Five batches (16.7%) out of 30 had a percentage range of individuals 0-5% having score 1 which is, when hernias or rupture were present, but the affected area was not bleeding, not touching the floor nor affecting locomotion (Table 37).

Variable	Score 0 Ranges (%)	Score 0 Frequency	Score 0 Relative Frequency (%)	Score 1 Ranges (%)	Score 1 Frequency	Score 1 Relative Frequency (%)	Score 2 Ranges (%)	Score 2 Frequency	Score 2 Relative Frequency (%)
Ruptures and hernias	100	25	83.3	0	25	83.3	-	-	-
	100-90	5	16.7	0-5	5	16.7	-	-	-
Total		30	100.0		30	100.0		-	-

Table 36: Frequency distribution of a discrete quantitative variable (individual ruptures and hernias) in Northern Italy, 2012.

Moderate hernias were assessed in five batches (Table 38) from conventional farms with no enrichment material and one organic straw bedded farm.

	Hernias and ruptures	
	Moderate	
Floor type	**Batch no.**	**Type of farm**
Slatted	2	Conventional
Concrete	2	Organic straw bedded
Concrete	1	Conventional
Soil/grass	0	Semi free range
Total	5	

Table 37: Description of a discrete quantitative variable (moderate ruptures and hernias) in relation to the type of floor and the type of farm.

5.3.4.4.1. Absence of pain induced by management procedures

All male pigs were bought by the farmers already castrated and all, except pigs from one semi free range farm, were tail docked.

5.3.4.4. Appropriate behaviour

5.3.4.4.1. Expression of social and exploratory behaviour

5.3.4.4.2. Fear from humans

Concerning behavioural measures, 21 batches out of 30 (70.0%) had more than 60% of the animals showing a panic response. All the batches except one organic farm came from farms with a conventional farming system.

5.3.5. Gross pathology

Regarding gross pathology, 15 (1.0%) individuals out of 150 had pleurisy and of these 10 cases (66.7%) also had mild extended pneumonia. Forty-two (28.0%) individuals out of 150 had mild extended pneumonia. The majority of the gross lesions were apical pneumonia with moderate extension of apical lobes of the lungs.

Twenty-eight individuals (18.6%) showed normal and shiny epithelium of the stomach (score 0); of these, 18 subjects (64.0%) came from organic and semi free range farming systems. Gastritis (score 1) was found in 54 individuals (36.0%) out of 150; parakeratosis of the *pars esophagea* and thickened epithelium with little or no sloughing (score 2) was detected in 45 individuals (30.0%) out of 150 inspected of which, 32 individuals (71.0%) came from intensive farming. Eleven individuals (7.4%) out of 150 displayed erosions and/or mild ulcers with extensive sloughing of the epithelium (score 3), and all of them came from intensive farming. Twelve subjects (8.0%) out of 150 had developed ulcers and haemorrhage and presence of stenosis (score 4), and all came from intensive farming. (Table 2, Annex I).

Pericarditis was detected in five (3.3%) individuals out of 150 and white spot liver was found in 25 (16.8%) subjects out of 149 (one liver not present at the slaughter chain), where 14 individuals (56.0%) came from organic farming.

Ulcer severity	Frequency	Relative Frequency (%)
Score 0	28	18.6
Score 1	54	36.0
Score 2	45	30.0
Score 3	11	7.4
Score 4	12	8.0
Total	150	100

Table 38: Frequency distribution of a discrete quantitative variable (oesophago-gastric ulcer) in Northern Italy, 2012.

Five individuals out of the 12 (41.7%) displaying stomach lesions with a score 4, arrived to the slaughter plant one day before slaughter. Six individuals out of 11 (54.5%) with a score 3 arrived one day before slaughter. Nineteen (42.2%) individuals out of 45 who scored 2, arrived one day before slaughter and 7 individuals out of 45 (15.6%) arrived two days before. Twenty-nine individuals out of 54 (53.7%) which scored 1 arrived one day before slaughter and 3 out of 54 (5.6%) arrived two days before slaughter (Fig. 5).

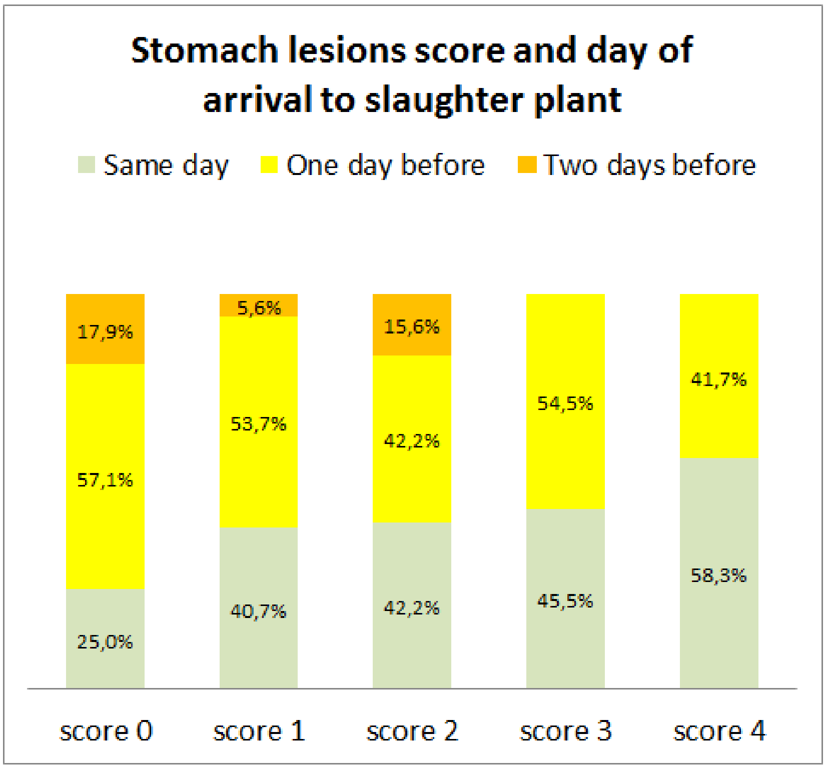

Figure 9: Stomach lesion scores in relation to day of arrival to slaughter plant in finishing pig production in Northern Italy, 2012.

In Figure 6 the individuals with score 4 for stomach lesions and had also different other gross pathology were presented. In Figure 7 individuals with score 4 for stomach lesions and had also different other gross pathology were presented.

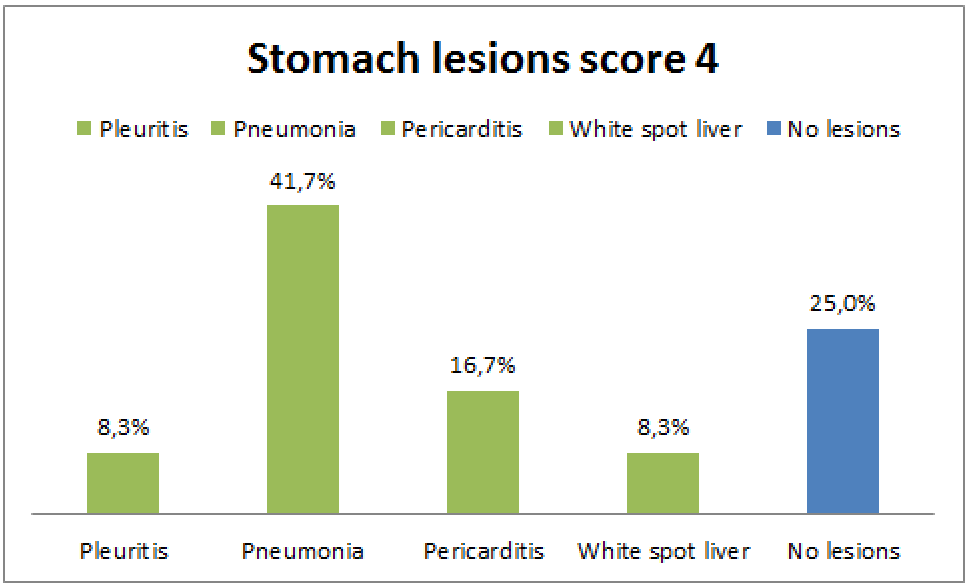

Figure 10: Stomach lesion score 4 combined with other lesion at viscera inspection in finishing pigs in Northern Italy, 2012.

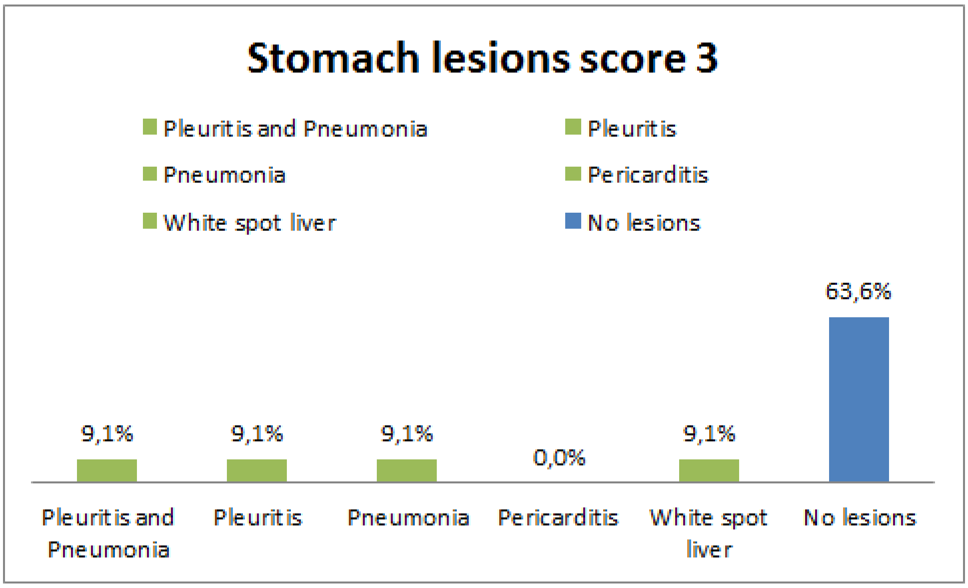

Figure 11: Stomach lesion score 4 combined with other lesion at viscera inspection in finishing pigs in Northern Italy, 2012.

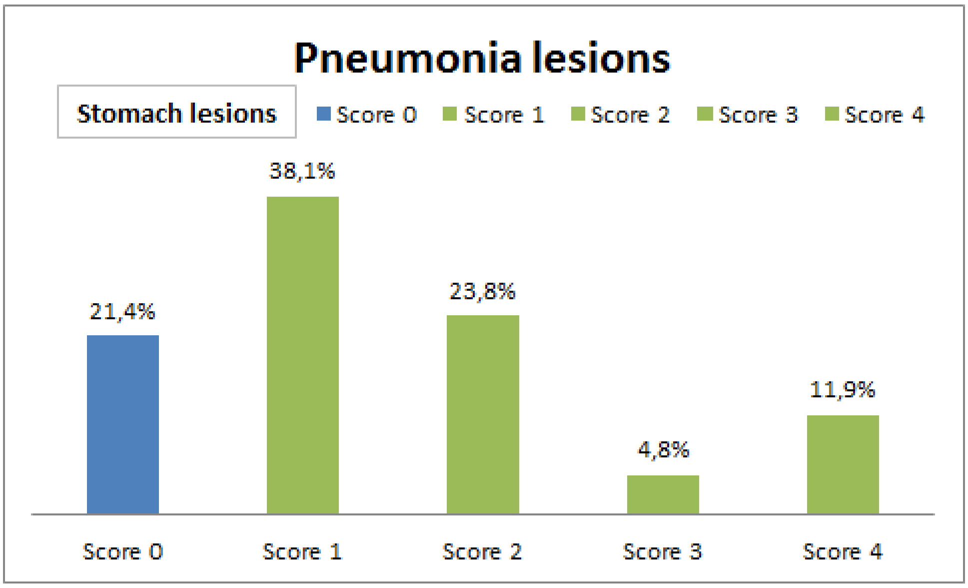

Figure 12: Pneumonia lesion and presence of stomach lesions (score 0 to score 4) in finishing pigs in Northern Italy, 2012.

In Figure 8 the individuals with pneumonia lesions (28.8%) were crossed with the different stomach lesions (from score 0 to score 4).

Batches positive to *Salmonella* spp. and *Y. enterocolitica*, type of floors and manure on the body

In Figure 9 the *Y. enterocolitica* positive batches, were analysed in relation to the type of floor and to the animal-based measure manure on the body. Sixty per cent of the *Y. enterocolitica* positive batches were on slatted floor and had 44.4% of manure on the body score 2. Nearly 34.0% of the *Y. enterocolitica* positive batches were on slatted floor and had 80.0% of the individuals with manure on the body score 2. No *Y. enterocolitica* positive batches had manure on the body scored 1.

In Figure 10 the *Salmonella* spp. positive batches were analysed in relation to the type of floor and the animal-based measure manure on the body.

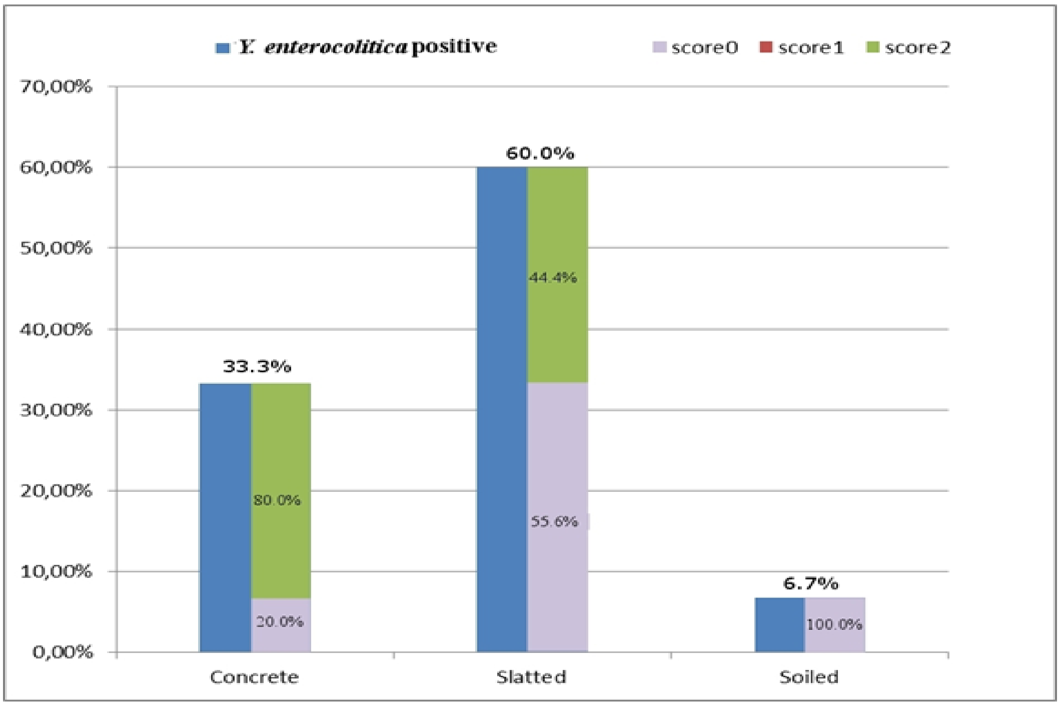

Figure 13: *Y. enterocolitica* positive batches and percentage of corresponding manure on the body (score 0, score 1, score 2) and floor types in finishing pigs of the Northern Italy, 2012.

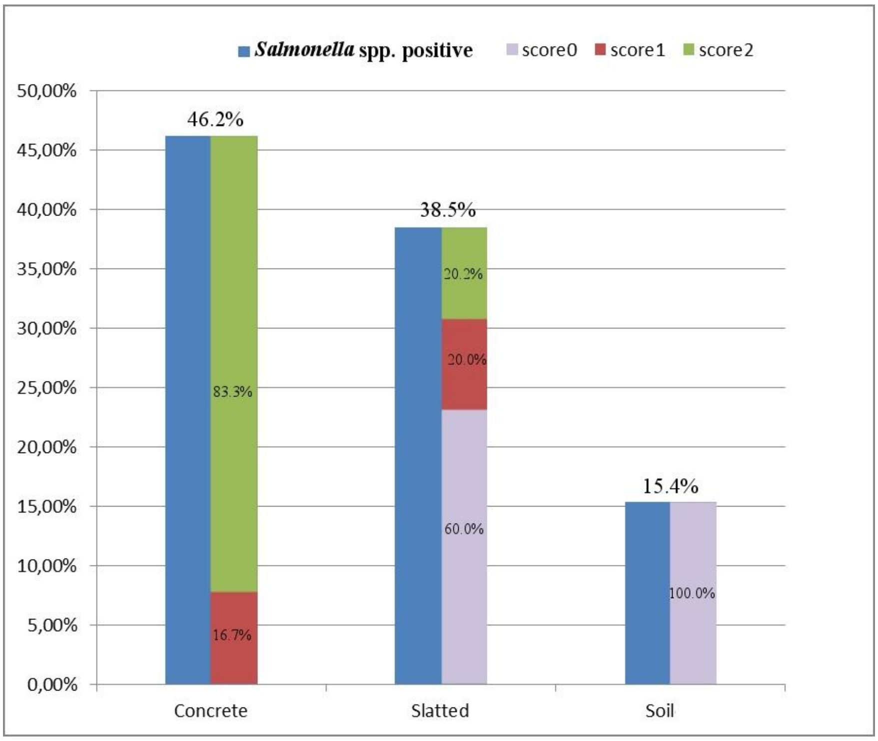

Figure 14: *Salmonella* spp. positive batches and percentage of corresponding manure on the body (score 0, score 1, score 2) and floor types in finishing pigs of the Northern Italy, 2012.

5.3.6. Statistical analysis of the association between animal-based welfare measures 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, mortality and human-animal relationship. Absence of enrichment material, absence of outdoor access, type of floor (soil and slatted vs concrete) and space allowance (<0.3 m^2/100Kg and 0.3-0.9m^2/100Kg) were the resource-based measures which were subjected to the statistical analysis. The food safety indicators were *Y. enterocolitica* and *Salmonella*. Frequency, *p*-value (Yates corrected chi square *p*-value 2-tail), Odds Ratio (OR), 95% Confidence Interval were reported in Table 40-41.

Bursitis, and skin condition were not applicable in the research. Rectal prolapse, twisted snouts, scouring, huddling and shivering animal-based measures were not included in the statistical analysis because the severity score assessed were 0 out for the 30 batches. Specifically for scouring, when water was used to wash concrete floors, it was difficult to distinguish diarrhoea from water mixed with manure.

5.3.6.1. *Yersinia enterocolitica*

Variable	OR	CI 95%	*p*-value*	Frequency (%)
Poor body condition	0.5	0.1-2.6	0.7	20.0
Severe manure on the body	0.6	0.1-2.9	0.8	53.3
Moderate manure on the body	0.3	0.0- 5.0	0.9	6.7
Severe panting	1.0	0.0-53.9	0.3	0.0
Moderate panting	3.0	0.1- 79.9	1.0	6.7
Space allowance <0.3 m^2/100Kg	3.0	0.1-73.6	0.9	6.7
Space allowance 0.3-0.9m^2/100Kg	4.5	0.7-28.1	0.2	80.0
Severe lameness	1.0	0.2- 5.8	0.7	20.0
Moderate lameness	3.2	0.5- 21.8	0.4	33.3
Severe wounds on the body	0.3	0.0- 9.0	1.0	0.0
Moderate wounds on the body	1.5	0.2-10.7	0.9	20.0
Severe tail biting	8.7	0.4- 184.2	0.3	20.0
Severe mortality >4.5%	1.9	0.2- 22.2	1.0	46.7
Moderate mortality 2.6-4.5%	21	1.0-458.8	0.1	46.7
Severe pumping	0.3	0.0-8.3	>1.0	0.0
Severe ruptures and hernias	1.3	0.0-68.5	0.4	0.0
Moderate ruptures and hernias	5.1	0.5-. 52.3	0.3	26.7
Poor human-animal relationship	5.7	0.9-34.5	0.1	86.7
Floor type (soil vs concrete)	0.6	0.0- 7.4	0.8	6.7
Floor type (slatted vs concrete)	5.4	1.0-29.7	0.1	60.0
Absence of enrichment material	4.3	0.7- 26.5	0.2	86.7
Absence of outdoor access	5.7	0.9-34.4	0.1	46.7

* Yates corrected chi square *p*-value (2-tail)

Table 39: Bivariate associations and their magnitude between *Y. enterocolitica* and welfare indicators in Northern Italy, 2012.

Neither animal-based measures nor resource based measures were statistically significantly related to *Y. enterocolitica* (Tables 40) but there were some measures (grey colour) for which the p-value tended towards significance. As far as concerns the animal-based measures the *p* value reached a rate close to <0.05 and odds ratio above 1.0 for the association with *Y. enterocolitica* with the following factors:

1. Panic response to human-animal relationship,

In relation to resource-based measures the *p* value reached a rate close to <0.05 and the odds separated from the 1 value, with 95% confidence intervals for the association with *Y. enterocolitica* with the following factors:

1. Space allowance (0.3-0.9 m^2/100Kg),
2. Mortality (2.6-4.5%),
3. Floor type (slatted vs soil),
4. Absence of enrichment material and
5. Absence of outdoor access.

Protective values were highlighted in the tables 41 (pink colour). This means that poor body condition, severe manure on the body, severe wounds on the body and severe pumping were protective against *Y. enterocolitica*.

Variable	OR	OR CI	*p*-value*
Pleuritis	8.7	0.4-184.2	0.3
Pneumonia	1.3	0.3-6.0	<0.9
Pericarditis	4.3	0.7-26.5	0.2
White spot liver	1.4	0.3-6.6	<0.9
Severe stomach lesion	0.6	0.1-2.5	0.7

* Yates corrected chi square p-value (2-tail)

Table 40: Bivariate associations and their magnitude between *Y. enterocolitica* and gross pathology in Northern Italy, 2012.

Pleuritis and pericarditis tended to be associated to *Y. enterocolitica*, since *p*-value is close to reach 0.05 and the OR is above one.

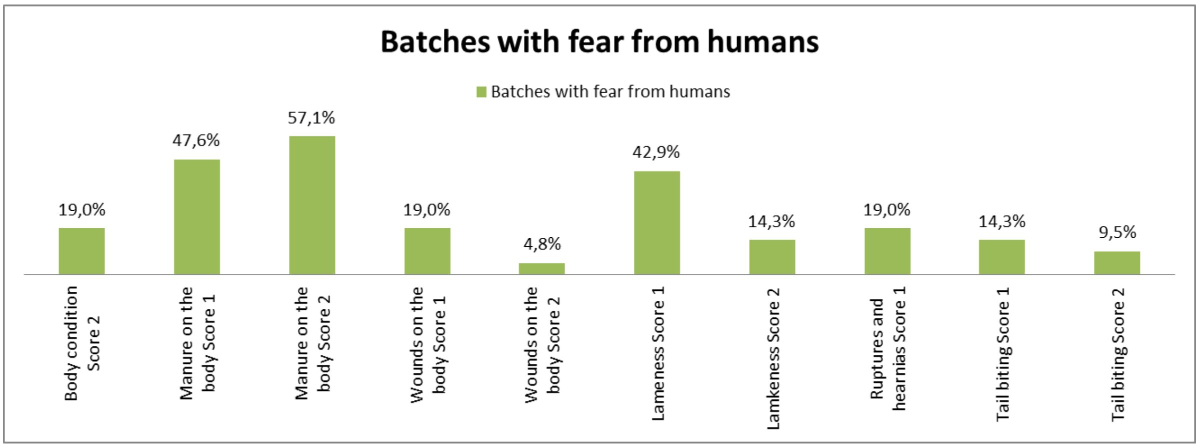

Figure 15: Analysis of the batches with fear from humans and the animal-based variables in finishing pigs in Northern Italy, 2012.

5.3.6.2. Salmonella spp.

Variable	OR	CI 95%	*p*-value*	Frequency (%)
Poor body condition	1.4	0.3-7.3	1.0	30.8
Severe manure on the body	0.5	0.1-2.7	0.7	46.2
Moderate manure on the body	2.0	0.1-29.8	0.9	15.4
Severe panting	0.4	0.0- 10.2	0.9	0.0
Moderate panting	0.4	0.0- 10.2	0.9	0.0
Space allowance <0.3 m^2/100Kg	0.1	0.0- 2.2	0.3	0.0
Space allowance 0.3-0.9 m^2/100Kg	0.8	0.0- 1.1	0.1	53.8
Severe lameness	0.8	0.1- 4.5	0.9	23.1
Moderate lameness	0.4	0.1 -2.7	0.6	15.4
Severe wounds on the body	4.9	0.2- 132.8	0.8	7.7
Moderate wounds on the body	2.5	0.3- 17.9	0.7	23.1
Severe tail biting	0.6	0.1-7.7	0.8	7.7
Severe mortality >4.5%	1.3	0.1- 10.9	0.7	76.9
Moderate mortality 2.6-4.5%	0.1	0.0-2.5	0.5	7.7
Severe pumping	0.4	0.0- 10.8	0.9	0.0
Severe ruptures and hernias	1.3	0.0- 68.5	0.4	0.0
Moderate ruptures and hernias	0.8	0.1- 6.0	0.7	15.4
Poor human-animal relationship	0.5	0.1. 2.4	0.6	61.5
Floor type (soil vs concrete)	1.3	0.1- 12.4	0.8	15.4
Floor type (slatted vs concrete)	1.0	0.2- 4.5	0.7	38.5
Absence of enrichment material	0.2	0.0- 1.0	0.1	53.8
Absence of outdoor access	0.6	0.1-2.8	0.7	23.1

* Yates corrected chi square p-value (2-tail)

Table 41: Bivariate associations and their magnitude between *Salmonella* spp. and welfare indicators in Northern Italy, 2012.

Neither animal-based measures nor resource based measures were statistically significantly related to *Salmonella* (Tables 42 and 43).

Protective values were highlighted in the tables 43 (pink colour). Severe manure on the body, moderate and severe lameness, severe tail biting lesions, moderate ruptures and hernias, severe and moderate panting, severe pumping, poor human-animal relationship, absence of enrichment material, moderate mortality, space allowance <0.3 m^2/100Kg were protective against *Salmonella* spp.

Variable	OR	OR CI	p-value*
Pleuritis	1.1	0.3-5.1	0.8
Pneumonia	0.7	0.1-3.5	1.0
Pericarditis	0.1	0.0-3.2	0.4
White spot liver	4.0	0.8-21.0	0.2
Severe stomach lesion	0.6	0.1-2.4	0.7

* Yates corrected chi square p-value (2-tail)

Table 42: Bivariate associations and their magnitude between *Salmonella* spp. and gross pathology in Northern Italy, 2012.

White spot liver tended to be associated to *Salmonella* spp., since *p*-value is close to reach 0.05 and the OR is above one.

5.4. Discussion

5.4.1. Introduction

The population was a representative sample of the Italian finishing pig population.

In Section 5.3.3. the main findings concerning food safety indicators are summarised. In section 5.4.3. the main findings concerning animal welfare indicators are presented and discussed according to the four principles of the Welfare Quality® approach; good housing, good feeding, good health and appropriate behaviour. At the end of each paragraph the objective of the study that is whether or not there was an association between that specific welfare indicator and any of the food safety indicators in finishing pigs is discussed.

The study reported in this thesis aimed at establishing a method for use under practical commercial conditions to determine the potential association between animal-based welfare indicators and food safety indicators. To my knowledge this approach has not been explored in literature in this way before. Several authors have focused on the level of prevalence of the pathogens and their association with resource and management-based welfare measures as risks factors observed on farm (Virtanen *et al*., 2011; Belœil *et al.*, 2004; Jensen *et al.*, 2004). However animal-based measures, which give a better insight of the status of the animal coping with the environment, were never associated to food safety indicators.

Odds Ratios (OR) provide information about the direction and magnitude of an association. There were no statistically significant results in this study, although some of the animal-based measures approached a significant level of $p<0.05$, with odds ratio above 1.0 (Section 5.3.6., Table 40 and 41) for their association with *Y. enterocolitica* for the following factors:

1. Human-animal relationship,
2. Pleuritis,
3. Pericarditis,

Since in comparison with many other epidemiological studies, this project is small, only addressing 30 batches of pigs, even these tendencies are of interest and worth discussing.

In relation to resource-based measures, the *p*-value approached a significance level of $p < 0.05$ and the odds ratios were above 1.0, for the association with *Y. enterocolitica* with the following factors:

1. Space allowance (0.3-0.9m^2/100Kg).
2. Mortality (2.6-4.5%).
3. Slatted floor type and
4. Absence of enrichment material,
5. Absence of outdoor access,

As far as the animal-based measures for the association with *Salmonella* spp were concerned, the *p*-value approached $p<0.05$ and odds ratio above 1.0. for the following factor

1. White spot liver tended to be associated to *Salmonella* spp.

I believe that considering the sample size of the study further research is needed to confirm that the sample population could influence the statistical analysis and give significant results.

Protective factors (Table 39 and 41) were not considered to have a biological meaning. Thus it seems likely that there are other non-measurable factors that lead to this spurious effect, such as the category of the animals under examination, farm management, individual characteristics of the subjects, health status, previous infections, the inclusion in the study of different farming systems etc.

These findings are better explained and compared to the variability of each animal-based measure observed in the following sections.

5.4.2. Food safety indicators

According to the EFSA opinion on meat inspection of swine (2011a) it was concluded that among the food safety indicators *Salmonella* spp. is considered of high relevance, while *Yersinia enterocolitica* is of medium relevance.

In the present study the prevalence of *Yersinia enterocolitica* was 15.3% from tonsils and 3.3% from environmental faecal samples. All but one of the isolates detected in either tonsils and environmental faecal samples (95.8%) belonged to the human pathogenic bio-serotype 4/O:3.

These findings confirmed that the finishing pigs in this area which are slaughtered at least at nine month of age were carrier *Y. enterocolitica* mostly in the tonsils. The prevalence in the environmental faecal samples was lower than the prevalence in tonsils. The positive batches mainly came from conventional farms with 13 batches out of 15 (86.7%) and only two batches from organic and extensive rearing systems, respectively. Stress probably plays a key role in the susceptibility to disease, since the majority (70.0%) of the pigs observed in the batches of conventional farms scored high for panic reaction during the assessment of the human-animal relationship. Only one conventional farm with outdoor access was positive on both for tonsils and the environmental faecal material. As a psychrophilic organism, *Yersinia* is able to grow at 4◦C, thus seasonality appears to play a key role with higher outbreaks of human diseases in cooler months (from December to May) (Milnes *et al*., 2009)

The findings of this study were similar to the ones of Nesbakken *et al*. (2006), who found that at older ages the percentage of contaminated pigs declines although the decrease in the number of positive faecal samples is more marked than in tonsils (Nesbakken *et al*., 2006). Similarly, in another study *Y. enterocolitica* was the most frequently isolated pathogen from the tonsils of fattening pigs at slaughter (62.0% of positive samples) even if the proportion of positive faecal samples was much lower (16.0%) (Fredriksson-Ahomaa *et al*., 2007). Fondrevez *et al*. (2010) observed a more similar prevalence (19.8%) in tonsil swabs from a single slaughter plant in France compared to the present study. Many authors found higher prevalence values of *Y. enterocolitica* in tonsils compared to our research, as 37.4% (Van Damme *et al*., 2010) and 44.0% (Ortiz Martinez *et al*., 2010); either because these two authors compared different methods for the detection and isolation of *Y. enterocolitica* or because Northern European countries have higher prevalence of the microorganism in pigs.

All the finishing pigs tested in this study entered in the food chain although being carrier of *Y. enterocolitica,* since they passed the *ante* and *post mortem* inspection visits. Thus it can be concluded that those pigs were asymptomatic carriers of *Y. enterocolitica*.

These findings indicated that pigs bred in Italy and entering the food chain can be carrier of human pathogenic *Y. enterocolitica* bio-serotype 4/O:3, in accordance with the results observed by Fredriksson-Ahomaa *et al*. (2007) who detected strains of the bio-serotype 4/O:3 in 96.0% of the culture-positive tonsils and concluded that pigs are the main source of sporadic human *Y. enterocolitica* bio-serotype 4/O:3 infections. In addition we can conclude that the methods used in the study were good epidemiologic indicators of the presence of *Y. enterocolitica.*

The prevalence of *Salmonella* spp. in mesenteric lymph nodes was 16.0%, while for the faecal environmental samples it was 7.0% in the 30 batches. In this study the most prevalent serovar isolated from the mesenteric lymph nodes was *S*. Derby (26.8%), followed by *S*. London, *S*. Give, *S*. Rissen, *S*. Typhimurium, *S*. Typhimurium monophasic variant 1, 4,[5],12:i:- (proportion of 13.3% for all the serovars). *Salmonella* serovars isolated from faeces were: *S*. Typhimurium monophasic variant 1, 4,[5],12:i:- (42.8%) followed by *S*. London (28.6%) and

S. Derby and *S*. Give (14.3%, respectively). There was correspondence between serovars isolated from swine lymph nodes and from environmental faecal samples.

Similarly to what was observed for *Y*. *enterocolitica* and *Salmonella*- positive, finishing pigs tested in this study entered in the food chain. They were asymptomatic carriers of *Salmonella* spp., since they were subjected to the *ante* and *post mortem* inspection visits and no symptoms of salmonellosis were observed. It can be concluded that *ante* and *post mortem* inspections are not accurate indicators of the *Salmonella* status on farm and at slaughter plant for finishing pigs.

Since sampling at lairage was not included in the study, the role of transport and lairage in the spread of the pathogens cannot be excluded; Berends *et al.* (1996) observed that eight hours are sufficient for *Salmonella* spp. to reach the lymph nodes in pigs after oral ingestion. However the transport times of the pigs to the three slaughter plants ranged from one to three hours and the majority (46.7%) of the batches were slaughtered after few hours on the same day of arrival to slaughter plant.

Botteldoorn *et al*. (2003) found that 21.0% of the animals carried *Salmonella* in the mesenteric lymph nodes and 19.0% in the faeces; however in this study faecal material was collected directly from the colon at the slaughter plant, while in the present research the faeces were collected from the floor of the animal pens. Metthner *et al*. (2011) recommended to examine not only ileocaecal lymph nodes but also caecal content to better identify *Salmonella* positive pigs at slaughter for future surveillance programmes. In addition environmental faecal samples are the most appropriate tool to assess the status of a herd since the ubiquitous *Salmonella* are resistant in pigs environment and can be representative of several individuals (12-20) living in a pen (Belœil *et al*., 2004).

Highly contaminated faecal material or lymph nodes could be a primary source of carcass contamination during evisceration at slaughter (Bottledoorn *et al*., 2003).
S. Typhimurium (28.6% in this study) and *S*. Derby (5.7%) are widespread and relevant in most Member States, while other serovars, such as *S*. London (1.4%), *S*. Infantis (1.1%) or *S*. Rissen (1.3%) are frequently isolated from pigs in some specific countries and their relevance cannot be generalised to the European Union as a whole (EFSA, 2011c). However *S*. London and *S*. Rissen were isolated from mesenteric lymph nodes in the current study.

Wilkins *et al*. (2010) found an association between *S*. Derby (OR= 10.2) and the production phase of the growing-finishing pigs in Canada. Similarly, Belœil *et al*. (2004) reported a higher prevalence of *S*. Derby (51.4%) and *S.* Typhimurium (37.8%) in finishing pigs in France; De Busser *et al*. (2011) identified as the predominant serotypes *S.* Typhimurium (58.7%) and *S.* Derby (17.4%) in pigs in Belgium.

Monophasic *S*. Typhimurium strains have rapidly increased in prevalence in human illness cases in the EU over a relatively short time period. They seemed to derive from the *S*. Typhimurium genetic lineages and had the same strain ability to infect and cause disease in both animals and humans of *S*. Typhimurium (EFSA, 2010). Monophasic variant *S*. *enterica* 1, 4,[5],12:i:- was already reported by Denmark, Germany, Ireland, Italy, Luxembourg, the Netherlands and Spain (EFSA, 2010).

We can conclude that the method used in the study was a good epidemiologic indicator of the presence of *Salmonella* spp. both on farm (environmental faecal sampling) and at slaughter plant (mesenteric lymph nodes).

The higher rate of positive match (four positive farms) between environmental faecal samples and lymph nodes for *Salmonella* spp. was observed in organic straw bedded and extensive farms. Jensen *et al*. (2006) were not able to demonstrate that organic rearing conditions were protective for pigs against *Salmonella* infections, as a high infection rate was found. In the same study the pathogen was able to survive in the paddock environment for several weeks, and even an estimated low level of *Salmonella* was able to pose an infection risk to newly introduced animals (Jensen *et al*., 2006). The high rate of positive match between environmental faecal samples and lymph nodes in the current study could be explained by the close contact between the pigs in the hut and by their rooting behaviour, which likely pose pigs at a high risk of ingestion of *Salmonella* from the contaminated environment. Similarly Smith *et al*. (2011) found a higher prevalence of *Salmonella* in outdoor farming systems rather than indoor ones.

Salmonella is able to survive in the soil, in water, and on a variety of surfaces which give the bacterium an increased likelihood of infecting new hosts; enhanced survival of *Salmonella* is favoured by a cyclic transmission from external environment to a new host (Winfield and Groissman, 2003).

Although the same individual was never positive to both pathogens in this study, five batches came positive to both *Salmonella* spp. and *Y. enterocolitica,* mainly from conventional intensive farms.

5.4.3 Animal welfare indicators

The Welfare Quality® project (Welfare Quality® website) together with EFSA Opinion on the Statement on the use of animal-based measures to assess the welfare of animals (EFSA, 2012) were the starting point for achieving a standardised collection and recording system of animal-based measures chosen *ad hoc* to be fit for purpose for a specific category of animal. The protocol could be used as part of an animal welfare surveillance scheme, which could be beneficial for the different users such as farmers, legislators, scientists and veterinary practitioners (EFSA, 2012). According to Welfare Quality® assessments, animal-based measures can be analysed for each of the four principles and criteria to give an indication of the response of the animal to certain inputs at a specific point in time of the production cycle, which is in this case, was the finishing operations in different pigs' rearing production systems. Animal-based measures in combination with resource and management-based measures can provide information on the current level of animal welfare as well as on the risk for future levels of welfare (EFSA, 2012). Measures are discussed in this section according to the Welfare Quality® principles and criteria scheme (Botreau *et al*., 2007) together with the description of the variability of the animal welfare indicators that were observed in this study on pig farms in Northern Italy.

5.4.3.1 Good feeding

5.2.4.1.1. Absence of prolonged hunger

The average prevalence of poor body condition among the 30 batches was 3.7%. This prevalence is higher than those reported by other studies (Temple *et al*., 2012a, Temple *et al*., 2012b, Temple *et al*., 2011). This could be explained by the inclusion in the present study of semi free range farms, since Temple *et al*. (2012a) demonstrated higher levels of poor body condition for extensive pigs. However we need to take into account that the range of scores for the same measures for different breeds within the same species may vary, as was already shown in dairy versus beef cattle, or boilers versus layer strain of poultry. Thus it is quite likely that heavy breed pigs could have different variation of the body condition compared light breed pigs. To my knowledge the Welfare Quality® protocol for growing pigs has never been applied to heavy breed pigs. The prevalence of lean animals nevertheless must be interpreted as a potential consequence of health deficiencies, thermal conditions and stress rather than toward poor feeding practices. Conventional finishing pigs were fed two/three times a day while pigs in semi free range productions can also count on natural foraging. Three batches out of eight (37.5%) with poor body condition had also *moderate* pig dirtiness and three batches out of eight (37.5%) with poor body condition had also *severe* pig dirtiness.

Moreover one batch out of the eight (12.5%) with poor body condition had also moderate lameness and ruptures and hernias. Beyond the health and thermal implication, half of the batches with poor body conditions had also poor scores for the human animal relationship with over 60% of pigs panicking at human presence.

Body condition is the only animal-based measure, indicator of 'Good Feeding' in the Welfare Quality® protocol. In the current research a soil/grass floor seemed to negatively affect body condition score, with the worst body condition score and range, even though the finishing pigs reared on soil/grass of the study, were provided with feed twice a day and they did not have to count only on natural foraging. The pigs reared on concrete and slatted floors achieved similar scores to each other. This trend is similar to what was described by Scott *et al*. (2006) and by Temple *et al*. (2012a), who reported poor body condition to be associated with extensive pig production and straw bedded systems.

In addition to the measure of the Welfare Quality® protocol, we recommend observing the animals at the time of feeding in order to detect those which cannot access the feed properly and so who have the potential consequence of a feed deficiency.

Poor body condition was not found to be statistically associated to both the food safety indicators in this study.

5.4.3.1.2. Absence of prolonged thirst

The prevalence of non-working drinkers was 26.0% and 16.6% of the drinkers were dirty. Only 8.0% of the suction pipes had the correct number of animals per drinking place. The recommended number of pigs per functioning drinking place according to Welfare Quality® is considered to be 10 pigs. If there are more pigs in the pen than recommended, then the number of drinking places is considered insufficient. The number of drinkers was not sufficient in the observed batches, which could lead to pigs having to fight for drinking space.

This is more likely than dehydration problems since conventional finishing pigs are usually liquid-fed, thus water deficiencies should not be a problem. Fighting for drinking space may however lead to stress which in turn, exposes pigs to increased susceptibility to disease (Rostagno, 2009).

Given the percentage of dirty drinkers, it is recommended that routine cleaning procedures of drinkers (and feeders) could be implemented on farms.

5.4.3.2. Good housing

5.4.3.2.1. Comfort around resting

Moderate and severe bursitis are indicators of poor comfort around resting according to Welfare Quality®; Temple *et al*. (2012) recorded the highest rates of moderate and severe bursitis in conventional farms. Unfortunately it was not possible to assess the level of bursitis in this current study due to the high stocking density, which made difficult to assess pigs individually and due to environmental condition (insufficient light).

The prevalence of moderate levels manure on the body in the present study was 23.3% and for severe levels of manure on the body was 36.1%.

Pig dirtiness (manure on the body) is of fundamental importance in reducing the risk of contamination with infectious pathogens from the faeces. Pigs can become dirty with manure for three main reasons: thermal, stress and previous housing. The first stimulus influencing pigs' excretory performance is temperature. Thus pigs would choose to lie in a warmer area and defecate in cooler area (Hacker *et al*., 1994). The second factors affecting pig cleanliness is space allowance. A lack of space can cause tension or aggression between pigs establishing a pen hierarchy. This hierarchy allow the dominant pigs to decide where to lie, which means that weaker individuals have to lie in the remaining spaces available and possibly in the dunging area. The third factor, previous housing, is a less commonly found cause of dirtiness in pigs in the finishing period, as farmers tend not to mix groups of pigs after they are placed in a pen. However this mixing practice is conducted during the ‘balancing’ phase, soon after being placed in the pen at the beginning of the growing phase, when pigs with low body condition and not gaining weight as the others, are taken from many different pens and grouped with others from other different pens of the same low weight.

Similarly to Temple *et al*. (2011, 2012a), the prevalence of moderate manure on the body in the current study was 23.3% in conventional farms. Temple *et al*., (2011, 2012a) however detected lower prevalence for severe manure on the body than in this study. Mullan *et al*. (2009) found ranges from 10.0% to 81.6% of pigs soiled, but this was using a different protocol so is not really comparable. Contrary to Temple *et al*. (2012a) who detected higher prevalence of moderate and severe manure on the body in pigs housed on straw bedding, the present research observed higher levels of severe and moderate pig dirtiness associated with conventional farms on concrete floors. No semi free range batches of pigs had moderate or severe manure on the body in the current study.

Space allowance is considered one of the factors associated with manure on the body and indicator of ease of movement (Hacker, 1994; Forkmann and Keeling, 2009). In the present research the higher prevalence of moderate and severe pig dirtiness could be explained by the reduced space allowance, which had values below the limits required by Council Directive 2008/120/EC laying down minimum standards for the protection of pigs. The stocking density in this study was converted according to the calculation in the was converted according to Welfare Quality® protocols to 0.9 m^2/100Kg corresponding to 1 m^2/110Kg in the above mentioned legislation.

Older pigs present a higher risk of poor hygiene than younger ones according to Temple *et al*. (2012a) due to their habit of spending more time lying. Although the present research did not differentiate on the age of individuals, the category of finishing pigs (at the end of the production cycle) and the longer heavy breed pig production cycle (older and heavier pigs) would imply these pigs were at higher risk of contamination with faeces. Despite this the statistical analysis did not detect any associations between dirtiness and the food safety indicators. Temple *et al*. (2012a) did however observe an association between severe manure on the body and liquid-fed pigs.

5.4.3.2.2. Thermal comfort

No finishing pigs were observed huddling or shivering and the prevalence of panting was low. In the period of observation, temperatures ranged from 10 to 30°C and so were not sufficiently cold to induce shivering, panting and huddling. These measures were assessed at batch level. Similarly Temple *et al*. (2012a) detected a low prevalence of animals huddling, shivering and panting and also noted that their assessment was carried out in mild weather conditions. The frequency of panting was also very low in this research although pigs were observed panting on two very warm days.

Statistical analysis gave no significant results in relation to panting.

5.4.3.2.3. Ease of movement

Nearly 67.0% of the batches had a space allowance above 0.3 m^2/100Kg and below 0.9 m^2/100Kg and 6.7% of the batches had a space allowance below 0.3 m^2/100Kg. The space allowance recorded in the present study was considered to be insufficient for the number of pigs in the pens, according to Council Directive 2008/120/EC laying down minimum standards for the protection of pigs. In a study conducted by Jensen *et al*. (2012) there was no evidence that productivity or pen hygiene were improved by increasing space allowance of finishing pigs from 0.67 m^2/pig to 0.79 m^2/pig in the weight range 32.01 kg to 91.25 kg. The pigs in the present study were much heavier, the average live weight of the pigs in the present study was 167.0 Kg, and thus a comparison cannot be justified. However, it is noteworthy that studies concerning pig stocking density and its influence on growth performance have been restricted to lighter-weight pigs (Rossi *et al*. 2008). Italian swine production mainly focuses on the use of heavy breed pigs destined to the Protected Denomination of Origin (PDO) products such as Parma ham (Rossi *et al*. 2008). Many authors (Hacker *et al*. 1994; Hyun *et al*., 1998; Rossi *et al*., 2008) have demonstrated that a reduced space allowance has a detrimental effect on the growth rate. The prevalence of poor body condition in this study could be explained by the high frequency (66.7%) of low space allowances. Moreover Rossi *et al*. (2008) conducted a study on heavy breed pigs in Northern Italy and concluded (in contract to the work of Jensen et al. 2012) that increased space allowance is associated with an increase of the average daily gain in pigs over 120 Kg of live weight. Thus heavy breed pigs may benefit from lower stocking densities.

In the statistical analysis space allowance at the range 0.3-0.9 m^2/100Kg tended to be associated the presence of *Y. enterocolitica* infection in pigs.

Virtanen *et al*. (2011) analysed many different risk factors, such as differences in space allowance as well as the use of antibiotics, organic production type, the presence of deep bedding, presence of manure in the pen, to evaluate the association with the shedding prevalence of *Y. enterocolitica.* The authors concluded that organic production was a significant protective factor against *Y. enterocolitica.* This supports the work of many authors who have investigated this issue and detected an association between the high prevalence of *Y. enterocolitica* in conventional intensive farms and a lower prevalence in organic production. For example, high stocking densities were found to be associated with *Y. enterocolitica* (Laukkanen *et al*., 2009). These findings could be explained by the reduced space which hampers pigs from performing sufficient locomotion. High stocking density increases the possibility to fight for satisfying basic animal needs, such as feeding, drinking, laying down etc. Satisfying physiological needs become for the animal source of stress which make it susceptible to infections, as by *Y. enterocolitica*. However a study demonstrated the association of the higher level of *Salmonella* seropositive animals and outdoor pig productions (Jensen *et al*., 2004).

Pigs reared in organic (straw bedded) and outdoor (non-organic and extensive) production systems benefit from a low stocking density, access to outdoor area, and good conditions for expressing normal behaviour, such as locomotion, rooting and exploration (Zheng *et al*., 2007). However Zheng *et al*. (2007) detected no significant differences in the number of *Salmonella* seropositive animals between organic, outdoor and indoor pig farms, even though the probability of outdoor reared pigs being infected at slaughter was lower than for pigs reared on conventional farms.

The rationale behind the association between *Y. enterocolitica* and outdoor production was according to Virtanen *et al*. (2011) was the use of deep bedding, the limited use of antibiotics and the lower animal density. It was demonstrated that indiscriminate use of broad spectrum antibiotics affected the commensal gastrointestinal bacteria, which are responsible of contrasting incoming pathogens in the gastrointestinal tract.

In my opinion the results from Virtanen and the result from the current study on the close link between the absence of enrichment material and the positivity to *Y. enterocolitica* support the results of Virtanen *et al*. (2011). In my study 13 batches out of the 15 (86.7%) positive ones which were infected with *Y. enterocolitica* belonged to conventional production farms and were provided neither with straw nor with enrichment material, such as piece of wood, tyres or toys. There are two possible interpretations of the results. The provision of straw and other piece of enrichment such as wood or tyres allows the animal to perform the ranges of innate behaviour such as rooting, exploring and locomotion and which can satisfy their needs, are also healthier animals. Secondly, the absence of straw bedding enhances the probability to be infected with *Y. enterocolitica*, because the animals are more exposed to faecal material. As it has been said, rooting is an instinctive need of the pig (Studnitz *et al*., 2007), and which is performed even in the absence of enrichment, or rather, in the presence of manure. Faecal material of asymptomatic carriers of *Y. enterocolitica* therefore becomes a risk for other animals rooting in the presence of manure. Pigs in fact can acquire *Y. enterocolitica* from contaminated faeces or pen floors (Virtanen *et al*., 2011).

5.4.3.3. Good health

5.4.3.3.1. Absence of injuries

In the current study the prevalence of moderate and severe lameness was 0.6% and 0.3% respectively, which is a similar to the 0.2% for both moderate and severe lameness that was found by Temple *et al*. (2011). Mullan *et al*. (2009) instead observed higher prevalence of lameness applying a different method.

Regarding moderate and severe lameness data, almost half of the batches had slatted and concrete floor and the majority of the farms were conventional. Data on lameness could have been underestimated because in the slaughter plant 3, lameness was assessed on farm and when stocking densities were high some cases could have been missed. In slaughter plants 1 and 2, where lameness was assessed at the unloading phase of the transport, lame animals might not have been included in the batch because of the penalties given by the abattoir to the farmer in case of un-healthy pigs on entrance to the abattoir.

Statistical analysis gave no significant result for lameness with the two pathogens.

The prevalence of moderate wounds on the animal’s body was 1.7% and no severe wounds were observed. These findings are similar to the results of Temple *et al*. (2011) and Courboulay *et al*. (2009) that used the same protocol for growing pigs.

Lesion score is a measure of the outcome of aggression (Turner *et al*., 2006). Mixing of non-familiar pigs should be minimised as this results in high levels of aggression, leading to physical injury and physiological changes. There have been contradictory results from mixing evenly and unevenly weighted pigs (Ekkel *et al*., 1995). This practice is commonly used in the early stages of the production cycle in Italian pig production to balance any unevenness between individuals in the pens after the placing in the house. It is also more reliable to carry out the protocol on individuals which had already reached a social stability (Courboulay *et al*., 2009). Due to the low prevalence it is reasonable to conclude that wounds on the animal's body resulting from aggression are less common at the finishing phase of the production cycle. This low variability would be the reason for the non-significant statistical results.

Although in many batches pig dirtiness made difficult to observe lesions on the animals' body, the frequency of the wounds on the body was very low either at moderate (score 1) and severe (score 2) grades. Mullan *et al*. (2009) recommended that assessments on farm should only be conducted on finishing pigs with a prevalence for dirtiness of less than 17.0%, in order to prevent potential bias when recording tail and body lesions. Statistical analysis on the measure 'wounds on the body' and its link with the two pathogens gave no significant results.

Mullan et al. (2009) detected higher prevalence from 13.0% to 55.9% between farms of finishing pigs in United Kingdom. Whereas in a study conducted in France and Spain in growing pigs (Temple *et al*., 2011) the prevalence of wounds on the animal's body was very low (2.0%). The assessment methodology used by Temple *et al*. (2011) is strictly defined on a rigid scale giving the exact thresholds. The strict parameters of evaluation such as number, severity and distribution of lesions may have created such discrepancy between these two studies. Low prevalence 0.3% for scratches and less than 0.5% for severe lesions were found by also by Courboulay *et al*. (2009) applying the same methodology.

In the current study the prevalence of moderate and severe tail biting lesions was 0.3% and 0.1%. The two batches affected by the severe tail biting lesions and the three batches with moderate biting lesions were all conventional intensive farms with low space allowance comprised in the range 0.3-0.9 m^2/100 Kg and with no provision of enrichment material.

Tail-biting seems to result from the pigs' natural inclination to root and chew on objects in their environment (Walker and Bilkei, 2006). Tail-biting causes considerable economic losses in pig production and has a negative effect on the welfare of the animals (Walker and Bilkei, 2006). The behaviour is mainly seen in commercial indoor environments, where more tail-biting is usually observed in pens with higher stocking densities, lack of enrichment substrate, poor ventilation, deficiencies in feed quality or accessibility, or poor health. However, moderate tail-biting (Courboulay *et al*., 2009) and severe tail biting lesions have also been recorded in outdoor herds and under organic conditions (Walker and Bilkei, 2006). The assessment of tail biting lesion in this research resulted in low prevalence both for the moderate (score 1) and severe (score 2) tail biting in this research. Courboulay *et al*. (2009) observed a higher prevalence of moderate tail biting lesions and a lower prevalence of severe tail biting lesions, but still higher than in the present study. Temple *et al*. (2011) observed a low prevalence for tail biting lesions (0.9%), while Mullan *et al*. (2009) found higher rates of tail lesions (1.93-14.27%). Pig age could explain the low levels of the tail biting lesions observed in the current research; tail biting measure is associated with a major incidence in previous pig age rather than the higher slaughter age reached by the heavy breed pigs used in the Italian PDO production. All the batches which were observed with tail biting lesions belonged to conventional intensive farms. It seems thus reasonable to presume that for pigs in the outdoor and organic farms of the present study, the greater space allowance, more objects to chew on, implicit in these types of rearing production systems contributed to fewer pigs developing tail biting behaviour. However many authors (Walker and Bilkey, 2006; Kritas *et al*., 2004) concluded that raising finishing pigs outdoors with higher space allowance, does not prevent tail-biting in pigs, even though factors as genetics, respiratory problems, possible dietary deficiencies, and general rooting in muddy pastures cannot be excluded.

5.4.3.3.2. Absence of disease

The average mortality rate observed in the 30 batches was 5.1%, and it ranged from 0.0% to 9.91%. The higher mortality rate was reached by a farm on its first organic production cycle. Farmers are allowed to purchase non organic pigs for the first cycle to be fattened. The lowest mortality rate was achieved by an extensive Parma Black pig farm. In the statistical analysis the *p* value reached a rate close to <0.05 and odds ratio above 1.0 for the association with *Y. enterocolitica* with mortality in the range 2.6-4.5%. It is quite inconceivable to justify, why just the middle range had a tendency to significance in the association with *Y. enterocolitica* but it may be because 26.7% of the batches in the middle range were outside the warning mortality threshold identified by Welfare Quality®. Moreover 60.0% of the batches were above the alarming mortality threshold.

In the study conducted in Hungary inspecting 1,319 dead or emergency-culled pigs, the authors found a mortality rate of 8.6% (Bauman and Bilkei, 2002). This is higher than the average mortality of the present research and this may be because of the exclusion in the current study of the emergency-culled animals. As Baumann and Bilkei (2002) suggested a routine and systemic *post-mortem* examination of all dead pigs that would allow the farmers to obtain a clear picture of the causes of mortality on their farm. It would also contribute to better understand the multifactorial conditions influencing animal health, welfare and production, as well as address a more controlled management of the vaccination programme, environmental conditions and genetic improvement (Baumann and Bilkei, 2002). In the same study the causes of mortality were diagnosed. Gastrointestinal problems represented the major cause of death and emergency-culled animals and 4.55% of the necropsied animals had gastric ulcer, which was the cause of death for the animals (Bauman and Bilkei, 2002). In the present study, eight per cent of the higher severity (score 4) for gastric ulcers and stenosis detected in finishing pigs at slaughter came from intensive conventional production batches.

Moreover the 7.4% of the pigs inspected that had score 3 i.e. that displayed erosions and/or mild ulcers with extensive sloughing of the epithelium, also belonged also to intensive rearing farms. The two findings can be explained bearing in mind that stress responses influence the susceptibility to develop cardiovascular pathology, ulcer development, stereotypies and infectious disease (Koolhaas *et al.*, 1999). Secondly, the statistical analysis showed a tendency for the fear from humans' measure to be associated with *Y. enterocolitica.* In the current study 70.0% of finishing pigs showed a panic reaction and were thus probably stressed by operations of the personnel, or other management procedures. These findings were confirmed by the gross pathology recordings which revealed severe ulcer lesions (score 3 and 4). We concluded that finishing pigs from conventional farms are stressed by management practices and are thus more susceptible to diseases.

In another study conducted in Belgium (Maes, 2004) the mortality rate (4.7%) was similar to the findings of the present study. The mortality rate was statistically associated to the longer duration of the fattening period, to the season of the placement in the fattening unit and to the origin of the piglets. Data by another study of Maes *et al*. (2001) considered the mortality from year 1996 to 1999 assessed on a weekly basis. This new approach of evaluating mortality could be a more sensitive method to reveal changes in the mortality pattern, so decreasing the costs of dead subjects.

Other individual animal-based measured such as bursitis and skin condition were not evaluated because either space allowance or environmental condition such as insufficient light, made it difficult to assess them on farm. However these measures were not detected at the unloading phase of the transport in the slaughter batches on arrival to slaughter plants 1 and 2 which implies that the levels of the lesions were low.

In this study only one batch (3.3%) out of 30 presented heavy and laboured breathing in 3.0% of the animals. Pumping is a symptomatology implicated in severe respiratory disorders and it is considered to a major health problem. These findings had low levels in the study and statistical analysis did not show significant results in the association with the food safety pathogens.

Atrophic rhinitis is characterized by sneezing, followed by atrophy of the turbinate bones, which may be accompanied by distortion of the nasal septum. The absence of twisted snout cases implies that this does not seem to be a major problem among the inspected batches. Similar results were observed by Temple *et al*. (2011) in growing pigs. The expected prevalence in herds is estimated to be around 0.1% according to a Danish study (Petersen *et al*., 2008).

Rectal prolapse was not observed in the study, which is similar to the findings of Temple *et al*. (2011) in growing pigs. Rectal prolapse is more common in growing than finishing pigs, and it is associated with a number of factors which increase abdominal pressure. Factors may include excessive coughing, or straining for episodes of enteritis, or huddling due to cold conditions. None of these factors were present in the observations, thus the conclusion is that this animal-based measure is more relevant to pigs of younger age. It must also be said that pigs are transferred into the sick pen as soon as ant pathologic sign of rectal prolapse occurs in the pen. Finishing pigs which are close to slaughter are mainly healthy pigs, since many slaughter plants give penalties to the farmers who bring unhealthy subjects, especially if the pigs are included in the PDO quality certification. This may have contributed to the low frequencies of such individual animal-based measures at the slaughter plants. For the reasons above mentioned, the high prevalence of these findings could be considered, not only as a health problem, but also a severe management practice (Temple *et al*., 2011) for not bringing the animals to the sick room.

The prevalence of moderate and severe ruptures and hernias was 0.3% and no severe ruptures were observed in the current research. Temple *et al*. (2011) observed a similar prevalence of

0.1%. Straw *et al.* (2008) observed a higher prevalence of mortality rates in finishing pigs with umbilical (0.86% prevalence) or scrotal hernias (0.70% prevalence) or kyphosis (0.42% prevalence) than in unaffected animals, and a slower growth rate in the pigs affected by these lesions compared to unaffected pigs. Nearly 15.0% of pigs with hernias died during the following 80 days from the observation, experiencing abdominal discomfort, and previous research suggesting that up to 50.0% of the survivors might have been condemned for peritonitis. Retaining pigs with hernias or kyphosis becomes for the farmers who have to take decisions concerning the care of these pigs. The author proposed euthanasia in some cases of affected animals rather than placing them in the finishing phase (Straw *et al*., 2008).
The percentages of pigs coughing (1.1%) and sneezing (2.5%) was quite low, implying that there were at least no major respiratory problems in these farms.

All the above aspects observed so far, decrease the growth rate and feed conversion efficiency, so impairing the productivity of the pigs as well as their welfare. Moreover mortality from gastric ulcers especially in intensive growing to finishing pig productions is known to cause relevant economic loss.

5.4.3.4. Appropriate behaviour

5.4.3.4.1. Expression of social and exploratory behaviour

The number of pen scan samples of social and exploratory behaviour was limited due to lack of time and personnel so the results should be treated cautiously. The proportion of active animals was 37.8%, which is quite low compared to the prevalence (67.7%) observed by Temple *et al*. (2011). This could be explained by the older age of the pigs investigated in this study and also by breed variation. Animals were quite inactive and exploration was mainly sniffing, nosing, licking or chewing features within the pen rather than to exploration and play directed towards straw or other enrichment material. In fact 73.3% of the batches were not provided with enrichment material. The prevalence of 'other behaviour', that is eating, drinking or air sniffing, was 4.5%. It was expected to be low because animals are fed two/three times a day and assessments were purposely made far away from meal times.

In our study the absence of enrichment material, which also meant absence of straw bedding, was a factor which tended to be associated with positivity to *Y. enterocolitica*. Although there are no studies in literature dealing with this precise type of association, there are some studies on the association between food safety indicators such as *Salmonella* spp. and *Y. enterocolitica,* and risk factors identified in primary production all the way through to the slaughter process. Many of these risk factors are input factors which relate to management and resource measures and thus affect the welfare of animals.

Milnes *et al*. (2009) found that pigs who were not fed, but who were provided with bedding had a higher risk of infection with *Salmonella* spp. They proposed that a stressful environment, such as that associated with the fasting practices used in finishing pigs when they are soon to be slaughtered, could increase the risk of ingestion of material in the pen that was contaminated with *Salmonella* spp. Forty per cent of pigs were brought to the abattoir one day before slaughter and 13.3% two days before slaughter. By implication these animals were fastened so increasing the risk of infection with pathogens and the risk of developing ulcers in the stomach. In fact Milnes *et al*. (2009) demonstrated that when pigs are not slaughtered on the day of arrival, the risk of *Salmonella* carriage increased significantly. The risk also was confirmed for *Y. enterocolitica* especially in the season from December to May (Milnes *et al*., 2009).

In the current study slatted floors tended to be associated to the positivity to *Y. enterocolitica.* However, many authors agree that both solid floors and partially slatted floors are associated with a higher risk for *Salmonella* spp. than fully slatted floor (Zheng *et al*., 2007, Davies *et al*., 1997, Nollet *et al*., 2004).

The absence of outdoor access was a factor in this study which tended to be associated with the positivity to *Y. enterocolitica.* It is known that the infection with *Y. enterocolitica* is more influenced by the animal itself rather than by the environment (Virtanen *et al*., 2011). On the other hand outdoor access could increase the risk of contact with *Salmonella* spp. contaminated soil or wildlife thus increasing the risk of infection with the pathogen (Jensen *et al*., 2004, Zheng *et al*., 2007).

In order to control *Salmonella* spp. in pigs the need to quantify possible risk factors and develop effective management strategies in pig herds is of paramount importance.

Many studies have investigated management and resource-based welfare factors (Berends *et al*., 1996; Verbrugghe *et al*., 2012) and concluded that a very strict hygiene in the pens and reduction of the use of broad spectrum antibiotics, which can impede the colonisation of the gut flora are linked to low prevalence in *Salmonella* spp.

According to the Council Directive EC 2008/120 laying down minimum standards for the protection of pigs, 'pigs must have permanent access to a sufficient quantity of material to enable proper investigation and manipulation activities, such as straw, hay, wood, sawdust, mushroom compost, peat or a mixture of such, which does not compromise the health of the animals'. Since 73.3% of the batches were not provided with any enrichment material, we can thus assume that the welfare of these pigs was impaired. It has been demonstrated that pigs would spend half of the day light period foraging (rooting and grazing) (Studnitz *et al.,* 2007).

5.4.3.4.2. Fear from humans

Fear from humans is defined as animals fleeing, or facing away from the assessor or huddling in the corner of the pen (Welfare Quality® protocol). Fear from humans is greatly influenced by stockmen management, breed and age (Temple *et al*., 2011). In this study the prevalence of a poor human animal relationship was very high (70.0% of the batches) especially in the conventional farms with space allowance ranged from 0.3 to 0.9 m^2/100Kg.

Stress can alter the outcome of infections in animals. *Salmonella* spp. is often shed by asymptomatic carriers and stress-induced pathogen shedding could result in an increased transmission of the bacterium (Verbrugghe *et al*., 2012). Stress is often related to animal management practice (Freestone *et al*., 2008). Considering the high prevalence of fear towards observed in the current study, we could suppose that the finishing pigs were exposed to stress by the routine farm management practices e.g. each time personnel enter the room, the pigs panic and run away. With the same situation repeating over the time, we could assume that the pigs were exposed to chronic stress (months in duration) and that they were thus more susceptible to disease. This is supported by the fact that the statistical analysis showed a tendency toward significance in the association between the test for fear from humans and *Y. enterocolitica.* One more time we should highlight that heavy breed pigs, which represented the majority of the animals observed in the study, are usually slaughtered at older ages compared to light breed pigs. This would mean that they have a longer fattening phase in which they are exposed to stress.

5.4.3.4. Gross pathology

In this study gross pathology information was collected according to the Welfare Quality® slaughterhouse protocol with the intention of obtaining information on the animal health and welfare on farm.

Respiratory diseases are a major problem in intensive pig farming with major economic losses due to increased mortality, morbidity and treatment costs reduced growth rates and carcass quality (Holt *et al*., 2011). In the present study the prevalence of pneumonia was 28.0%. This high prevalence of pneumonia could be regarded as being a problem at herd level, although the gross pathology lesions were low extended areas, in the cranial and cardiac lobes of the lungs, usually called Enzootic pneumonia-like lesions, imputable to *Mycoplasma hyopneumoniae* (Holt *et al*., 2011; The Merck Manual). In herds in which the disease is endemic, morbidity is high, but clinical signs may be minimal and mortality low. Coughing is the most common sign and is most obvious when pigs are disturbed. Individual pigs or groups sporadically develop severe pneumonia. Changing the predisposing input factors such as stress due to transient viral infections, parasitic migration, and mixing pigs, may also cause outbreaks (The Merck Manual). Control of enzootic pneumonia can be accomplished by optimising managing practices and housing conditions, strategic medication and vaccination programmes. These measures can decrease the infection level in a herd thereby improving pig health but they cannot prevent re-infection (Maes *et al*., 2007). In addition Liljegren *et al*. (2003) considered broncho-pneumonia in slaughter pigs to be primarily caused by bacteria, such as *Mycoplasma* spp., *Pasteurella multocida* and *Actinobacillus pleuropneumoniae*. Holt *et al.* (2011) used the routine inspection of pigs' lungs at slaughter, according to the Pig Health Scheme in the United Kingdom to monitor herd health in order to support producers and veterinarians in farm-level decision making. The authors aimed to assess whether information provided by monitoring system at slaughter regarding gross respiratory lesions were associated with respiratory pathogens in the farm. They concluded that lesion scores reported by monitoring system might reflect the presence of respiratory pathogens on the farms and that these lesions might be indicative of decreased productivity (Holt *et al*., 2011).

Holt *et al*. (2011) reported pleuritis as common respiratory diseases in the United Kingdom pig industry, with gross lesions frequently found in pig lungs at slaughter. The likely causal organism is *Actinobacillus pleuropneumoniae.* Lesions can vary from severe fibrinonecrotic and hemorrhagic pneumonia with accompanying fibrinous pleuritis and many recovered pigs are carriers (The Merck Manual). In the present research combined pleuritis and gross pneumonia lesions were detected in 66.7% of the cases.

Concerning stomach lesions, scoring gross lesions were carried out according to Kopinski and McKenzie (2007) who achieved consistency in the evaluation of oesophagogastric changes that may lead to ulcerations. Their ranking system was further elaborated in this study. It should be highlighted that examining gross lesions in the stomach could lead to an underestimation of the presence of oesophago-gastric ulcers, compared to histological examination.

The oesophago-gastric ulceration prevalence at slaughter plant has been reported by many authors to be 15.5-20% (Amory *et al*., 2006; Eisemann, 2002; Melnichouck, 2002). To compare those findings with the results of the current research, the more severe stomach's lesions (score 3 and 4) were summed together and the consequent prevalence was 15.4%. It is well recognised that severe stomach lesions are painful and they imply stress due to management and environmental factors together with poor health status. Only severe stomach lesions should be selected in a welfare measures assessment when monitoring pig welfare at slaughter. This is because mild stomach lesions in fact can develop in 24 hours or less of fastening or less or in case of stress induced by transportation (Swaby and Gregory, 2012). In the current research 18.6% of pigs had no lesions (score 0). This value is similar to 20.4% reported by Swaby and Gregory (2012).

In the present study data on the presence of developed ulcer with haemorrhage and stenosis were similar to those reported by Swaby and Gregory (2012). They registered a higher frequency of ulcer of a severe grade in pigs kept overnight in the lairage of the slaughter facility. In the present study the recordings of pigs staying at slaughter plant overnight was recorded in terms of days and not hours, which would have been more precise. Moreover systematic recording of data on the timing of the last meal was not registered. Eleven cases with severe stomach lesions (score 3 and 4) out of 23 (47.8%) were held overnight in the lairage of the slaughter plant and, since feed is not administered at the slaughter plant, they were thus fastened all the time. According to the Council Regulation (EC) 1099/2009 on the protection of animals at the time of killing, animals should not be fasted for more than 12 hours (Swaby and Gregory, 2012). Lawrence *et al*. (1998) observed that fasting increased the severity of *pars oesophageal* lesions at slaughter. Moreover Koolhaas *et al.* (1999) indicated that controllability of a stressor influenced the likelihood of gastric ulcers.

Despite the fact that stomach inspection is not part of the meat inspection procedures in Europe, a recommendation from this study is that the presence of severe grade oesophago-gastric ulcers should be included in a welfare-focused surveillance monitoring system. Many slaughter plants have a separate room for the emptying and cleaning of stomachs and intestines, unless the competent authority authorises the separation in time of these operations within a specific slaughterhouse on a case-by-case basis (European Parliament and Council Regulation EC 853/2004 laying down specific hygiene rules for on the hygiene of foodstuffs). Since these operations must be kept separated from the carcass line, there is no longer individual correspondence with the carcass although it is maintained for the batch identification. Batch traceability of the sever grade oesophago-gastric ulcers to the farm should be kept as a useful feedback when monitoring gross pathology measures.

In the current study the prevalence of white spot liver was 16.8%. Migration of larvae through the liver causes haemorrhage and fibrosis that appears as “white spots” under the capsule and leads to condemnation of the liver at slaughter (The Merck Manual). The majority of the white spot detected in this study (56.0%) belonged to outdoor and pigs reared in extensive conditions. As Millet *et al*. (2005) observed, alternative housing systems allow pigs to display their species-specific behavioural repertoire, although this may endanger aspects related to animal health. In these extensive and especially certified organic production systems, control of parasites can be carried out by a preventive deworming strategy. Health and welfare problems in pig production are also affected by the restrictions on the use of allopathic medicine and prophylactic treatments as well as the risk of wildlife contact by difficulties in the hygiene management of the outdoor access, including the management of the land rotation (Bonde and Sørensen, 2004).

In the present study the association between gross pathology and food safety indicators was carried out using the five inspected individuals per batch as representative of the entire batch. In the statistical analysis pleuritis and pericarditis tended to be associated with *Y. enterocolitica*, the *p*-value tended towards 0.05 and the OR was above one and white spot liver tended to be associated with *Salmonella* spp. No previous studies were found on the potential link between gross pathology with *Y. enterocolitica.* Smith *et al*. (2011) demonstrated with multivariable models the associations between Enzootic pneumonia-like lesions, peritonitis, pericarditis and white spot liver and *Salmonella* sero-prevalence, although the OR detected by Smith *et al*. were small (close to 1.00). Associations between pleuritis and pericarditis and *Salmonella* are even less investigated in literature (Smith *et al*., 2011). The justification for the possible link could be overcrowding and stressful management such as handling, transport and individual mixing. Callaway *et al*. (2006) reported similar stressors to be associated with the increased shedding of *Salmonella*. The same hypotheses could be proposed for the tendency to a significant association found in the current study for *Y. enterocolitica*.

The link between *Salmonella* and white spot liver could be attributed to a relationship with *Ascaris suum*, the causal agent of white spots. The implication is that the parasites penetrate the mucosa inducing intestinal mucosal lesions, which facilitates the invasion and persistence of *Salmonella* infections. The association between *Mycoplasma hyopneumoniae*, which is responsible for Enzootic pneumonia-like lesions and salmonellae, could be explained by the depression of the immune system caused by the pathogen, which is often found in tonsils and in the respiratory tract. Coughing and sneezing caused by respiratory diseases could favour the spread of *Salmonella* (Smith *et al*., 2011).

Gross pathology has detrimental effect on swine production (feed conversion, daily intake). Reducing the prevalence of *Salmonella* on farm may reduce gross pathology incidence and *vice versa*, both of which would have a beneficial effect on production.

New animal-based measures are likely to be developed in future and they could be included in the protocol. For example, Pineiro *et al*. (2011) analysed the combination of seven acute phase proteins in the detection of diseases in the serum of the pigs experimentally exposed to different bacteria and viruses. The combination of acute phase proteins allowed the detection of clinical or subclinical disease and of stress caused by poor management (temperature, high stocking density, mixing, and lack of ventilation). Using serum detection of acute phase proteins could become part of an animal-based measure monitoring system to be utilised on farm or at slaughter as part of an animal welfare surveillance scheme if future studies will support it scientifically. This aspect was not explored in the study.

According to the legislation, enrichment material such as straw, pieces of woods or other appropriate material should be introduced in the intensive conventional farms to satisfy the animal’s need to root, to chew on new objectives and to explore (Council Directive 2008/120/EC). According to Morgan *et al.* (1998) the provision of straw increased the growth rate and time pigs spent lying, probably in response to a combination of beneficial effects in a cold environment. However alternative enrichment material to straw should be further experimented to allow their use in countries which reach higher temperatures and where straw cannot be used.

5.4.4. Limitations

The results of the statistical analysis clearly indicate that *Y. enterocolitica* more than *Salmonella* spp. tends to be associated with the previously listed indicators of animal welfare. *Salmonella* spp. has in fact an ecological cycle which is more influenced by wildlife animal reservoirs (Winfield and Groisman, 2003) was demonstrated by the higher matches between individual and environmental faecal samples in extensive and organic productions in the current study. *Y. enterocolitica* on the other hand is more strictly dependent on the host rather than the environment. The environment is a less relevant source of *Y. enterocolitica,* while pig-to-pig transmission is considered most important in the spread of infection (Virtanen *et al*., 2011).

This study was conducted with the aim to explore the possibility for linking specific animal-based welfare indicators to the safety of the pork meat. A secondary aim of this study was to explore the possibility to obtain measurements and observations that can test the underlining hypotheses for the association. Several assumptions were made prior to conducting the study. One of these assumptions was that animal welfare animal-based measurements are reliable indicators of the animal welfare issues among livestock. Another assumption was to consider the presence of either *Salmonella* or *Y. enterocolitica* in lymphatic tissue is an indicator of the meat safety. Both of these assumptions were made in order to perform the study using commercial operations with limited interference to their routine daily practices.

Although there were some trends, none of the animal welfare measures were statistically significantly associated with the two food safety indicators (presence of *Salmonella* and *Y. enterocolitica* in lymphatic tissue). One or more of the following issues may lead to these non-statistical findings. There are also limitations associated with the generalisation of the results in this study:

1. The sample size was too small to provide evidence for these associations.

2. The use of these indicators (either or both of animal welfare and food safety) may not be a sufficiently good proxy to demonstrate the link between the animal welfare and food safety in commercial swine operations. Increasing the sample size may not eliminate this type of proxy limitation.
3. The observations and measurements were taken at specific times and so cannot reflect the entire year. Therefore the lack of associations cannot represent the entire production period of the swine operation.
4. The study may have selection or measurement biases that include some of the above or others.

Although the study did not demonstrate the significant associations, the design can be considered as practical approach to explore the relationship between animal welfare issues and food safety indicators. The tendencies that were found may give some useful indication of where attention may be directed in future studies.

It is necessary to underline that this study was conducted in a heavy pig breed farming system carried out in Italy mainly for the PDO production. While worthwhile and interesting for this pig sector, this fact makes it difficult to compare data from this study with the light breed pig production studies conducted throughout the world in literature.

The assessments and analysis were carried out only on potentially clinically healthy pigs on arrival to the slaughter plant since many slaughter plants give penalties to farmers who bring lean animals (below 160Kg) or non-healthy subjects, especially if the pigs are included in the PDO quality certification. The exclusion of sick pigs may have influenced the variability of the pig population with regard to the individual animal-based measures and of the prevalence of the pathogens.

The selection of the farms was based on the availability and willingness of the farmers and on the stratification of the batch samples in order to better represent the Northern Italian pig population. All the types of farm production systems in the area were examined.

Since the category of finishing pigs, the object of the study showed low animal-based measures variability, re-assessing the approach in other categories of pigs e.g. piglets, weaners, growing pigs or sows may give higher frequency of the animal-based measures. This consideration could be taken for future research.

Since sampling at lairage was not included in the study in order to not interfere with commercial operation of the slaughter plants, the role of transport and lairage in the cross-contamination cannot be excluded. However the transport times of the pigs to three slaughter plants were of one to three hours, and the majority of the batches were slaughtered after few hours on the same day of arrival to the slaughter plant.

Unfortunately food safety indicators were not tested at transport and lairage for fattening pigs, but it would have added more information to the entire pig food chain including specific aspects on transport time, mixing of pig batches and reuse of pens in the lairage.

This study was conducted with limited prior knowledge about the relationships between food safety and animal welfare parameters, since they had not been investigated before. Appropriate sample sizes therefore for exploring these relationships could not be calculated due to the limited or unavailable parameters for this calculation. Later analysis indicated that the power of this study was low, which means that findings should be interpreted cautiously. The results do however provide valuable information to explore the feasibility of further investigations to determine the relationship between food safety and animal welfare parameters.

5.4.5. Conclusions

Based on the observed prevalence, *Salmonella* spp. and *Y. enterocolitica* represented a high risk of contamination through the entire pork meat food safety chain. The methods used to detect and isolate the pathogens were valuable since the prevalence found in the different samples were in line with the available literature.

To our knowledge this study seems to be the first attempt to quantitatively associates animal welfare indicators to the positivity for food safety indicators in the swine production chain. The approach aimed to associate evidence of standardised welfare outcomes with susceptibility to infection to *Y. enterocolitica* and *Salmonella* spp. It is important to observe that the lack of significant results does not imply that factors are not related to the shedding of the pathogens. The small number of observed batches suggested that the sample size to detect associations was limited. In addition many of the analysed factors showed a lack of variation and frequency in the study population.

The frequency found of the animal-based measures of moderate and severe levels of manure on the body in finishing pigs in Northern Italy was high. Although there was not significant association with the food pathogens, this welfare aspect in swine conventional farming should be improved.

The high levels of fear towards humans suggest that finishing pigs are stressed by human handling. This tended to be associated with *Y. enterocolitica*, thus implying that pigs are susceptible to disease because of stress.

Mortality is a useful measure, but it should be supported by data from the *ante* and *post mortem* inspection which could give information on the causes of death of individuals on the farm.

Among the resource-based measures, space allowance is really critical for finishing pigs because it can have several other consequences for animal welfare. Small space allowances tended to be associated with *Y. enterocolitica.* Provision of straw bedding is still an unsolved problem in the Europe, but it seems also to be associated to *Y. enterocolitica*.

The study aimed at focusing on the establishment of the procedure for field application and assessing the relationship between animal welfare and food safety indicators. The results of this study indicate that future research is needed to further investigate this association further and that it is logically motivated.

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ANNEX I

Batch ID	No. of pigs/ farm	Pens' no.	No. of animals positive to *Y. enterocolitica*	Presence of *Y. enterocolitica* in faeces	No. of *Y. enterocolitica* positive batches	No. of pigs positive to *Salmonella* spp. in lymph nodes	Presence of *Salmonella* in faeces	No. of *Salmonella* spp. positive batches
1	4400	6	0	0	0	0	0	0
2	4400	6	0	0	0	1	0	1
3	4500	6	0	0	0	0	1	1
4	4500	9	1	0	1	0	0	0
5	6000	6	2	0	1	0	0	0
6	6000	7	1	1	1	0	0	0
7	3000	8	3	0	1	0	0	0
8	16000	5	1	0	1	0	0	0
9	16000	4	0	0	0	0	0	0
10	3000	6	2	0	1	0	0	0
11	8000	7	2	0	1	3	0	1
12	8000	11	1	0	1	0	0	0
13	10000	4	0	0	0	0	0	0
14	10000	9	0	0	0	0	0	0
15	15000	7	1	0	1	2	0	1
16	15000	8	1	0	1	0	0	0
17	11500	7	2	0	1	1	0	1
18	11500	8	0	0	0	0	0	0
19	8000	8	1	0	1	1	0	1
20	8000	8	2	0	1	0	1	1
21	100	1	2	0	1	0	1	1
22	1200	1	0	0	0	1	1	1
23	13	1	0	0	0	0	0	0
24	13	1	0	0	0	0	0	0
25	1200	2	0	0	0	1	0	1
26	100	1	0	0	1	3	1	1
27	1063	1	1	0	0	1	1	1
28	1063	1	0	0	0	0	1	1
29	6500	5	0	0	0	1	0	0
30	6500	5	0	0	0	1	0	0
Total		159	23	1	15	16	7	13

Table 1: Description of the batches and number of positive batches to *Salmonella* spp. in lymph nodes and faeces and *Yersinia enterocolitica* in tonsils and faeces of finishing pigs, 2012.

Batch	Pleuritis	Pneumonia	Stomach lesions					Pericarditis	White spot liver
	Score 2	Score 2	Score 0	Score 1	Score 2	Score 3	Score 4	Score 2	Score 2
	No. (%)								
1	0	1	1	1	2	0	1	0	0
2	0	0	1	0	2	1	1	0	0
3	0	0	2	1	2	0	0	0	0
4	0	0	0	3	2	0	0	0	0
5	0	3	1	4	0	0	0	0	0
6	0	3	2	2	1	0	0	0	0
7	0	1	0	5	0	0	0	2	1
8	0	2	0	2	2	0	1	2	0
9	0	2	1	1	1	0	2	0	1
10	0	2	0	2	3	0	0	0	3
11	1	1	0	2	2	1	0	0	0
12	1	2	0	0	5	0	0	0	0
13	1	4	2	0	0	1	2	0	0
14	1	1	0	1	2	1	1	0	0
15	0	1	0	2	1	1	1	0	0
16	1	1	0	1	2	1	1	1	0
17	0	1	0	4	0	1	0	0	0
18	0	4	0	3	1	1	0	0	0
19	2	1	0	5	0	0	0	0	4
20	1	0	0	2	0	3	0	0	2
21	0	2	0	0	5	0	0	0	0
22	0	2	1	4	0	0	0	0	3
23	0	0	1	1	3	0	0	0	0
24	0	0	3	0	2	0	0	0	n.a.
25	0	2	4	1	0	0	0	0	3
26	0	0	1	2	2	0	0	0	0
27	1	3	5	0	0	0	0	0	4
28	2	2	3	0	2	0	0	0	4
29	3	0	0	3	1	0	1	0	0
30	1	1	0	2	2	0	1	0	0
Total (%)	15 (1.0)	42 (28.0)	28 (18.6)	54(36.0)	45 (30.0)	11 (7.4)	12 (8.0)	5 (3.3)	25 (16.8)

n.a.= not applicable

Table 2: Gross pathology findings (each batch is composed of five individuals) at slaughter.

Individual Animal-based measures	**Mean**	**SD**	**Median**
Poor body condition	3.7	7.0	0.0
Moderate manure on the body	23.3	34.5	0.0
Severe manure on the body	36.1	43.8	14.9
Moderate lameness	0.6	0.9	0.0
Severe lameness	0.3	0.8	0.0
Moderate wounds on the body	1.7	5.1	0.0
Severe wounds on the body	0.0	0.1	0.0
Moderate ruptures and hernias	0.4	1.0	0.0
Severe ruptures and hernias	0.0	0.0	0.0
Moderate tail biting lesions	0.3	1.1	0.0
Severe tail biting lesions	0.1	0.5	0.0
Coughs	1.1	1.6	0.7
Sneezes	2.5	3.7	1.5
Twisted snouts	0.0	0.0	0.0
Rectal prolapse	0.0	0.0	0.0
Bursitis	n.a	n.a	n.a
Skin condition	n.a	n.a	n.a

Table 3: Descriptive mode of discrete quantitative variables (individual animal-based measures) in Northern Italy, 2012.

Individual behavioural measures	**Mean (%)**	**SD**	**Median**
Active	37.8	19.1	38.2
Positive social	6.9	20.3	0.0
Negative social	1.4	1.7	0.7
Exploration	27.4	17.4	25.5
Other	4.3	7.8	0.7

Table 4: Percentage of animals performing the behaviour observed in relation to the number of active finishing pigs for each batch, 2012.

Coughing pigs			**Coughs**			**Sneezing pigs**			**Sneezes**		
No. (%)	**Mean**	**SD**	**No. (%)**	**Mean**	**SD**	**No. (%)**	**Mean**	**SD**	**No. (%)**	**Mean**	**SD**
29 (0.8)	1.0	1.0	43 (1.2)	1.4	1.9	52 (1.4)	1.7	1.2	58 (1.6)	1.9	1.4

Table 43: Description of discrete quantitative variables (pigs coughing and sneezing and total number of coughs and sneezes) in Northern Italy, 2012.

Single Table Analysis

		Disease		
		Yersinia enterocolitica (+)	*Yersinia enterocolitica* (-)	
Huma-animal relationship Score 2	(+)	13	8	21
Exposure Score 0	(-)	2	7	9
		15	15	30

Chi Square and Exact Measures of Association

Test	Value	p-value(1-tail)	p-value(2-tail)
Uncorrected chi square	3.968	0.02318	0.04637
Yates corrected chi square	2.54	0.05553	0.1111
Mantel-Haenszel chi square	3.836	0.02508	0.05017
Fisher exact		0.05432	0.1086
Mid-P exact		0.03071	0.06142

At least one expected value (row total*column total/grand total) is < 5
Fisher or Mid-P exact tests are recommended rather than chi square.

Risk-Based* Estimates and 95% Confidence Intervals
(Not valid for Case-Control studies)

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
Risk in Exposed	61.9%	40.81, 79.32	Taylor series
Risk in Unexposed	22.22%	5.343, 55.72	Taylor series
Overall Risk	50%	33.16, 66.84	Taylor series
Risk Ratio	2.786	0.7844, 9.894[1]	Taylor series
Risk Difference	39.68%	5.492, 73.87°	Taylor series
Etiologic fraction in pop.(EFp)	55.56%	6.396, 100	
Etiologic fraction in exposed(EFe)	64.1%	-27.49, 89.89	

Odds-Based Estimates and Confidence Limits

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
CMLE Odds Ratio*	5.353	0.929, 45.69[1]	Mid-P Exact
		0.7611, 65.44[1]	Fisher Exact
Odds Ratio	5.688	0.9389, 34.45[1]	Taylor series
Etiologic fraction in pop.(EFp|OR)	71.43%	31.46, 100	
Etiologic fraction in exposed(EFe|OR)	82.42%	-6.509, 97.1	

*Conditional maximum likelihood estimate of Odds Ratio
(P)indicates a one-tail P-value for Protective or negative association; otherwise one-tailed exact P-values are for a positive association.

Figure 1. Bivariate associations and their magnitude between *Y. enterocolitica* and Human-animal relationship in finishing pig production in Northern Italy, 2012 (Open Epi: Open Source Epidemiologic Statistics for Public Health).

Single Table Analysis

		Disease: *Yersinia enterocolitica* (+)	*Yersinia enterocolitica* (-)	
Pleuritis	(+)	6	5	11
Exposure	(-)	9	10	19
		15	15	30

Chi Square and Exact Measures of Association

Test	Value	p-value(1-tail)	p-value(2-tail)
Uncorrected chi square	0.1435	0.3524	0.7048
Yates corrected chi square	0	0.5000	>0.9999999
Mantel-Haenszel chi square	0.1388	0.3548	0.7095
Fisher exact		0.5000	>0.9999999
Mid-P exact		0.3624	0.7249

All expected values (row total*column total/grand total) are >=5
OK to use chi square.

Risk-Based* Estimates and 95% Confidence Intervals
(Not valid for Case-Control studies)

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
Risk in Exposed	54.55%	27.99, 78.75	Taylor series
Risk in Unexposed	47.37%	27.33, 68.3	Taylor series
Overall Risk	50%	33.16, 66.84	Taylor series
Risk Ratio	1.152	0.5616, 2.361[1]	Taylor series
Risk Difference	7.177%	-29.83, 44.19°	Taylor series
Etiologic fraction in pop.(EFp)	5.263%	-22.05, 32.57	
Etiologic fraction in exposed(EFe)	13.16%	-78.06, 57.65	

Odds-Based Estimates and Confidence Limits

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
CMLE Odds Ratio*	1.321	0.2851, 6.301[1]	Mid-P Exact
		0.237, 7.669[1]	Fisher Exact
Odds Ratio	1.333	0.3006, 5.914[1]	Taylor series
Etiologic fraction in pop.(EFp|OR)	10%	-39.19, 59.19	
Etiologic fraction in exposed(EFe|OR)	25%	-100, 83.09	

*Conditional maximum likelihood estimate of Odds Ratio
(P)indicates a one-tail P-value for Protective or negative association; otherwise one-tailed exact P-values are for a positive association.
Martin,D; Austin,H (1991) An efficient program for computing conditional maximum likelihood estimates and exact confidence limits for a common odds ratio. Epidemiology 2, 359-362.

Figure 2: Bivariate associations and their magnitude between *Y. enterocolitica* and pleuritis in finishing pig production in Northern Italy, 2012 (Open Epi: Open Source Epidemiologic Statistics for Public Health).

Single Table Analysis

		Disease		
		Yersinia enterocolitica (+)	*Yersinia enterocolitica* (-)	
Pericarditis	(+)	3.5	0.5	4
Exposure	(-)	12.5	15.5	28
		16	16	32

Chi Square and Exact Measures of Association

Test	Value	p-value(1-tail)	p-value(2-tail)
Uncorrected chi square	2.571	0.05442	0.1088
Yates corrected chi square	1.143	0.1432	0.2864
Mantel-Haenszel chi square	2.491	0.05727	0.1145
Fisher exact		0.05061	0.1012
Mid-P exact		0.02531	0.05061

At least one expected value (row total*column total/grand total) is < 5
Fisher or Mid-P exact tests are recommended rather than chi square.

Risk-Based* Estimates and 95% Confidence Intervals
(Not valid for Case-Control studies)

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
Risk in Exposed	87.5%	36.8, 100	Taylor series
Risk in Unexposed	44.64%	28, 62.58	Taylor series
Overall Risk	50%	33.63, 66.37	Taylor series
Risk Ratio	1.96	1.126, 3.412[1]	Taylor series
Risk Difference	42.86%	5.584, 80.13°	Taylor series
Etiologic fraction in pop.(EFp)	10.71%	-3.218, 24.65	
Etiologic fraction in exposed(EFe)	48.98%	11.19, 70.69	

Odds-Based Estimates and Confidence Limits

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
CMLE Odds Ratio*	undefined	0.9939, 'undefined'[1]	Mid-P Exact
		0.7201, 'undefined'[1]	Fisher Exact
Odds Ratio	8.68	0.4089, 184.2[1]	Taylor series
Etiologic fraction in pop.(EFp|OR)	19.35%	-2.725, 41.43	
Etiologic fraction in exposed(EFe|OR)	88.48%	-100, 99.46	

*Conditional maximum likelihood estimate of Odds Ratio
(P)indicates a one-tail P-value for Protective or negative association; otherwise one-tailed exact P-values are for a positive association.

Figure 3: Bivariate associations and their magnitude between Y. enterocolitica and pericarditis in finishing pig production in Northern Italy, 2012 (Open Epi: Open Source Epidemiologic Statistics for Public Health).

Single Table Analysis

		Disease		
		Yersinia enterocolitica (+)	*Yersinia enterocolitica* (-)	
Space allowance (0.3.0.9m²/100Kg) Score 2	(+)	1	1	2
Exposure Score 0	(-)	2	6	8
		3	7	10

Chi Square and Exact Measures of Association

Test	Value	p-value(1-tail)	p-value(2-tail)
Uncorrected chi square	0.4762	0.2451	0.4902
Yates corrected chi square	0.02976	0.4315	0.8630
Mantel-Haenszel chi square	0.4286	0.2563	0.5127
Fisher exact		0.5333	>0.9999999
Mid-P exact		0.3000	0.6000

At least one expected value (row total*column total/grand total) is < 5
Fisher or Mid-P exact tests are recommended rather than chi square.

Risk-Based* Estimates and 95% Confidence Intervals
(Not valid for Case-Control studies)

Point Estimates		Confidence Limits	
Type	Value	Lower, Upper	Type
Risk in Exposed	50%	9.454, 90.55	Taylor series
Risk in Unexposed	25%	6.306, 59.91	Taylor series
Overall Risk	30%	10.33, 60.77	Taylor series
Risk Ratio	2	0.3198, 12.51¹	Taylor series
Risk Difference	25%	-50.51, 100.5°	Taylor series
Etiologic fraction in pop.(EFp)	16.67%	-37.09, 70.42	
Etiologic fraction in exposed(EFe)	50%	-100, 92.01	

Odds-Based Estimates and Confidence Limits

Point Estimates		Confidence Limits	
Type	Value	Lower, Upper	Type
CMLE Odds Ratio*	2.646	0.05184, 135¹	Mid-P Exact
		0.02555, 274¹	Fisher Exact
Odds Ratio	3	0.1222, 73.63¹	Taylor series
Etiologic fraction in pop.(EFp|OR)	22.22%	-44.3, 88.75	
Etiologic fraction in exposed(EFe|OR)	66.67%	-100, 98.64	

*Conditional maximum likelihood estimate of Odds Ratio
(P)indicates a one-tail P-value for Protective or negative association; otherwise one-tailed exact P-values are for a positive association.

Figure 4: Bivariate associations and their magnitude between *Y. enterocolitica* and space allowance (0.3-0.9m²/100Kg) in finishing pig production in Northern Italy, 2012 (Open Epi: Open Source Epidemiologic Statistics for Public Health).

Single Table Analysis

		Disease		
		***Yersinia enterocolitica* (+)**	***Yersinia enterocolitica* (-)**	
Mortality Score 1 (2.6-4.5%)	(+)	7	1	8
Exposure Score 0	(-)	1	3	4
		8	4	12

Chi Square and Exact Measures of Association

Test	Value	p-value(1-tail)	p-value(2-tail)
Uncorrected chi square	4.688	0.01519	0.03038
Yates corrected chi square	2.297	0.06485	0.1297
Mantel-Haenszel chi square	4.297	0.01909	0.03818
Fisher exact		0.06667	0.1333
Mid-P exact		0.03434	0.06869

At least one expected value (row total*column total/grand total) is < 5
Fisher or Mid-P exact tests are recommended rather than chi square.

Risk-Based* Estimates and 95% Confidence Intervals
(Not valid for Case-Control studies)

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
Risk in Exposed	87.5%	50.79, 99.89	Taylor series
Risk in Unexposed	25%	3.409, 71.08	Taylor series
Overall Risk	66.67%	38.8, 86.45	Taylor series
Risk Ratio	3.5	0.6284, 19.49[1]	Taylor series
Risk Difference	62.5%	14.28, 110.7°	Taylor series
Etiologic fraction in pop.(EFp)	62.5%	5.374, 100	
Etiologic fraction in exposed(EFe)	71.43%	-59.14, 94.87	

Odds-Based Estimates and Confidence Limits

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
CMLE Odds Ratio*	14.17	0.8365, 618.6[1]	Mid-P Exact
		0.5896, 1253[1]	Fisher Exact
Odds Ratio	21	0.9613, 458.8[1]	Taylor series
Etiologic fraction in pop.(EFp|OR)	83.33%	51.36, 100	
Etiologic fraction in exposed(EFe|OR)	95.24%	-4.027, 99.78	

*Conditional maximum likelihood estimate of Odds Ratio
(P)indicates a one-tail P-value for Protective or negative association; otherwise one-tailed exact P-values are for a positive association.
Martin,D; Austin,H (1991) An efficient program for computing conditional maximum likelihood estimates and exact confidence limits for a common odds ratio. Epidemiology 2, 359-362.

Figure 5: Bivariate associations and their magnitude between *Y. enterocolitica* and mortality (2.6-4.5%) in finishing pig production in Northern Italy, 2012 (Open Epi: Open Source Epidemiologic Statistics for Public Health).

Single Table Analysis

		Disease		
		Yersinia enterocolitica **(+)**	***Yersinia enterocolitica*** **(-)**	
Floor type (slatted vs concrete) Score 1	(+)	9	3	12
Exposure Score 0	(-)	5	9	14
		14	12	26

Chi Square and Exact Measures of Association

Test	Value	p-value(1-tail)	p-value(2-tail)
Uncorrected chi square	4.013	0.02258	0.04516
Yates corrected chi square	2.588	0.05387	0.1077
Mantel-Haenszel chi square	3.858	0.02475	0.04950
Fisher exact		0.05291	0.1058
Mid-P exact		0.03010	0.06021

All expected values (row total*column total/grand total) are >=5
OK to use chi square.

Risk-Based* Estimates and 95% Confidence Intervals
(Not valid for Case-Control studies)

Point Estimates		**Confidence Limits**	
Type	**Value**	**Lower, Upper**	**Type**
Risk in Exposed	75%	46.15, 91.72	Taylor series
Risk in Unexposed	35.71%	16.18, 61.4	Taylor series
Overall Risk	53.85%	35.45, 71.25	Taylor series
Risk Ratio	2.1	0.9675, 4.558[1]	Taylor series
Risk Difference	39.29%	4.214, 74.36°	Taylor series
Etiologic fraction in pop.(EFp)	33.67%	-0.6453, 67.99	
Etiologic fraction in exposed(EFe)	52.38%	-3.356, 78.06	

Odds-Based Estimates and Confidence Limits

Point Estimates		**Confidence Limits**	
Type	**Value**	**Lower, Upper**	**Type**
CMLE Odds Ratio*	5.026	0.9368, 33.03[1]	Mid-P Exact
		0.7712, 43.44[1]	Fisher Exact
Odds Ratio	5.4	0.983, 29.66[1]	Taylor series
Etiologic fraction in pop.(EFp|OR)	52.38%	15.48, 89.28	
Etiologic fraction in exposed(EFe|OR)	81.48%	-1.73, 96.63	

*Conditional maximum likelihood estimate of Odds Ratio
(P)indicates a one-tail P-value for Protective or negative association; otherwise one-tailed exact P-values are for a positive association.
Martin,D; Austin,H (1991) An efficient program for computing conditional maximum likelihood estimates and exact confidence limits for a common odds ratio. Epidemiology 2, 359-362.

Figure 6: Bivariate associations and their magnitude between *Y. enterocolitica* and floor type (slatted vs concrete) in finishing pig production in Northern Italy, 2012 (Open Epi: Open Source Epidemiologic Statistics for Public Health).

Single Table Analysis

		Disease		
		Yersinia enterocolitica **(+)**	***Yersinia enterocolitica*** **(-)**	
Absence of enrichment material Score 2	(+)	13	9	22
Exposure Score 0	(-)	2	6	8
		15	15	30

Chi Square and Exact Measures of Association

Test	Value	p-value(1-tail)	p-value(2-tail)
Uncorrected chi square	2.727	0.04934	0.09867
Yates corrected chi square	1.534	0.1080	0.2159
Mantel-Haenszel chi square	2.636	0.05224	0.1045
Fisher exact		0.1074	0.2148
Mid-P exact		0.06249	0.1250

At least one expected value (row total*column total/grand total) is < 5
Fisher or Mid-P exact tests are recommended rather than chi square.

Risk-Based* Estimates and 95% Confidence Intervals
(Not valid for Case-Control studies)

Point Estimates		**Confidence Limits**	
Type	**Value**	**Lower, Upper**	**Type**
Risk in Exposed	59.09%	38.7, 76.78	Taylor series
Risk in Unexposed	25%	6.306, 59.91	Taylor series
Overall Risk	50%	33.16, 66.84	Taylor series
Risk Ratio	2.364	0.6775, 8.246[1]	Taylor series
Risk Difference	34.09%	-2.272, 70.45°	Taylor series
Etiologic fraction in pop.(EFp)	50%	-4.413, 100	
Etiologic fraction in exposed(EFe)	57.69%	-47.59, 87.87	

Odds-Based Estimates and Confidence Limits

Point Estimates		**Confidence Limits**	
Type	**Value**	**Lower, Upper**	**Type**
CMLE Odds Ratio*	4.123	0.6964, 35.51[1]	Mid-P Exact
		0.5663, 50.88[1]	Fisher Exact
Odds Ratio	4.333	0.7078, 26.53[1]	Taylor series
Etiologic fraction in pop.(EFp|OR)	66.67%	18.96, 100	
Etiologic fraction in exposed(EFe|OR)	76.92%	-41.28, 96.23	

*Conditional maximum likelihood estimate of Odds Ratio
(P)indicates a one-tail P-value for Protective or negative association; otherwise one-tailed exact P-values are for a positive association.
Martin,D; Austin,H (1991) An efficient program for computing conditional maximum likelihood estimates and exact confidence limits for a common odds ratio. Epidemiology 2, 359-362.

Figure 7: Bivariate associations and their magnitude between *Y. enterocolitica* and absence of enrichment material in finishing pig production in Northern Italy, 2012 (Open Epi: Open Source Epidemiologic Statistics for Public Health).

Single Table Analysis

		Disease		
		***Yersinia enterocolitica* (+)**	**Yersinia enterocolitica (-)**	
Absence of Outdoor Score 2	(+)	7	2	9
Exposure Score 0	(-)	8	13	21
		15	15	30

Chi Square and Exact Measures of Association

Test	Value	p-value(1-tail)	p-value(2-tail)
Uncorrected chi square	3.968	0.02318	0.04637
Yates corrected chi square	2.54	0.05553	0.1111
Mantel-Haenszel chi square	3.836	0.02508	0.05017
Fisher exact		0.05432	0.1086
Mid-P exact		0.03071	0.06142

At least one expected value (row total*column total/grand total) is < 5
Fisher or Mid-P exact tests are recommended rather than chi square.

Risk-Based* Estimates and 95% Confidence Intervals
(Not valid for Case-Control studies)

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
Risk in Exposed	77.78%	44.28, 94.66	Taylor series
Risk in Unexposed	38.1%	20.68, 59.19	Taylor series
Overall Risk	50%	33.16, 66.84	Taylor series
Risk Ratio	2.042	1.069, 3.901[1]	Taylor series
Risk Difference	39.68%	5.492, 73.87°	Taylor series
Etiologic fraction in pop.(EFp)	23.81%	-1.255, 48.87	
Etiologic fraction in exposed(EFe)	51.02%	6.419, 74.36	

Odds-Based Estimates and Confidence Limits

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
CMLE Odds Ratio*	5.353	0.929, 45.69[1]	Mid-P Exact
		0.7611, 65.44[1]	Fisher Exact
Odds Ratio	5.688	0.9389, 34.45[1]	Taylor series
Etiologic fraction in pop.(EFp|OR)	38.46%	6.875, 70.05	
Etiologic fraction in exposed(EFe|OR)	82.42%	-6.509, 97.1	

*Conditional maximum likelihood estimate of Odds Ratio
(P)indicates a one-tail P-value for Protective or negative association; otherwise one-tailed exact P-values are for a positive association.
Martin,D; Austin,H (1991) An efficient program for computing conditional maximum likelihood estimates and exact confidence limits for a common odds ratio. Epidemiology 2, 359-362.

Figure 8: Bivariate associations and their magnitude between *Y. enterocolitica* and absence of outdoor in finishing pig production in Northern Italy, 2012 (Open Epi: Open Source Epidemiologic Statistics for Public Health).

Single Table Analysis

		Disease		
		Salmonella spp. (+)	*Salmonella* spp. (-)	
White spot liver	(+)	6	3	9
Exposure	(-)	7	14	21
		13	17	30

Chi Square and Exact Measures of Association

Test	Value	p-value(1-tail)	p-value(2-tail)
Uncorrected chi square	2.851	0.04568	0.09135
Yates corrected chi square	1.655	0.09931	0.1986
Mantel-Haenszel chi square	2.756	0.04847	0.09693
Fisher exact		0.09945	0.1989
Mid-P exact		0.05867	0.1173

At least one expected value (row total*column total/grand total) is < 5
Fisher or Mid-P exact tests are recommended rather than chi square.

Risk-Based* Estimates and 95% Confidence Intervals
(Not valid for Case-Control studies)

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
Risk in Exposed	66.67%	35.09, 88.27	Taylor series
Risk in Unexposed	33.33%	17.05, 54.77	Taylor series
Overall Risk	43.33%	27.36, 60.82	Taylor series
Risk Ratio	2	0.9344, 4.281[1]	Taylor series
Risk Difference	33.33%	-3.475, 70.14°	Taylor series
Etiologic fraction in pop.(EFp)	23.08%	-5.632, 51.79	
Etiologic fraction in exposed(EFe)	50%	-7.026, 76.64	

Odds-Based Estimates and Confidence Limits

Point Estimates		Confidence Limits	
Type	**Value**	**Lower, Upper**	**Type**
CMLE Odds Ratio*	3.805	0.7245, 23.77[1]	Mid-P Exact
		0.5954, 31[1]	Fisher Exact
Odds Ratio	4	0.7633, 20.96[1]	Taylor series
Etiologic fraction in pop.(EFp|OR)	34.62%	-1.297, 70.53	
Etiologic fraction in exposed(EFe|OR)	75%	-31.01, 95.23	

*Conditional maximum likelihood estimate of Odds Ratio
(P)indicates a one-tail P-value for Protective or negative association; otherwise one-tailed exact P-values are for a positive association.

Figure 9: Bivariate associations and their magnitude between *Salmonella* spp. and White spot liver in finishing pig production in Northern Italy, 2012 (Open Epi: Open Source Epidemiologic Statistics for Public Health).