5

Salmonella Control Measures at Farm in Swine Production

Héctor Argüello, Pedro Rubio and Ana Carvajal
Animal Health Department
University of León
Spain

1. Introduction

Salmonella is one of the most important food-borne pathogens. Each year a high number of cases as well as outbreaks of *Salmonella* in humans are reported (EFSA, 2011). Despite the fact that *Salmonella* can cause disease in pigs mainly associated to infections by *Salmonella enterica* subsp. *enterica* serovar Cholerasuis, the importance of swine salmonellosis is related to human infections caused by pork products. During the nineties and the first years of the present century it was estimated that 10% of the human salmonellosis cases were attributed to pork products (Hald *et al.,* 2004), classically categorized as the third most common source of human infections after poultry and turkey meat. Nevertheless with the implementation of control programs in avian production with the subsequent prevalence reduction, the role of pork products have been enhanced and nowadays it is the second most common source of human salmonellosis after laying hens (Pires *et al.,* 2011). Regarding swine salmonellosis, control programs are not compulsory at the moment but the EU Regulation 2160/2003 has established the need for developing proper and effective measures to detect and control *Salmonella* at all relevant stages of pork production chain and particularly at the primary production level in order to reduce the prevalence and the risk that *Salmonella* poses to public health (EU Regulation 2160/2003). From our point of view, *Salmonella* control should start at the end of the pork production chain (slaughterhouses and finishing farms) and go back to the first steps of the production system (breeding herds and feed suppliers). The compulsory or voluntary *Salmonella* control programmes that have already been established in several European countries base their *Salmonella* evaluation on serological and carcass microbiological contamination results principally (Alban et al., 2002; Nielsen et al., 2001). At the farm level, these control programmes include the implementation of specific measures to reduce the *Salmonella* prevalence in those herds identified as highly contaminated according to their serological results.

The present chapter aims to give a view of the most relevant control measures that can be used to reduce *Salmonella* prevalence in swine farms including a deep review of scientific research as well as our personal experience with control strategies at swine finishing farms. They will be presented into three different categories: 1) measures related to feeding practices, 2) vaccination and 3) generic measures of hygiene and biosecurity.

2. *Salmonella* control in the pork production chain

Focused in food safety, the control of *Salmonella* must be carried out taking into account the philosophy "*from farm to fork*" which implies the participation of all sectors involved throughout the food chain. Due to the complexity of its epidemiology and ecology, *Salmonella* control is a great challenge. As it has been proposed by Davies *et al.* (2004) the food supply should be seen as a linear series of sectors engaged in production, harvest, distribution and consumption and the goal of control programmes is to define the optimal combination of interventions at each sector that delivers the maximum risk reduction at minimal cost. It has been demonstrated that the risk of *Salmonella* contamination increases across the pork production chain and reaches its maximum in the slaughterhouse (Duggan *et al*., 2010; Argüello *et al*., 2011a; Visscher *et al*., 2011). Therefore, the slaughter process seems to be the main target to implement control measures and its importance has been pointed by many studies (Hurd *et al*., 2002, Argüello *et al*., 2011a). According to this, several countries, led by Denmark with more than twenty years of experience with a national swine *Salmonella* control program are pointing nowadays their reduction strategies towards slaughterhouse interventions (Alban *et al*., 2005; Goldbach *et al*., 2006). Nevertheless there are some aspects that make us to include pig finishing farms, together with the slaughterhouses, as the primary control points in the pork production chain. On the one hand sometimes it is difficult to implement slaughterhouse strategies due to policy restrictions at this level. On the other hand, it has been clearly demonstrated that infected pigs are the main source of *Salmonella* at the slaughterhouse (Argüello *et al*., 2011a; Visscher *et al*., 2011), thus in order to reduce the risk of *Salmonella* transmission in the food chain including feasible cost and success, finishing farms should be taken into consideration.

Although sows have been implicated as the primary source of infection to finishers (Lettelier *et al*., 1999; Kranker *et al*., 2003), other studies have demonstrated that this transmission can be controlled or interrupted by proper handling practices (Dahl *et al*., 1997). Several surveys have also supported that vertical transmission is not the main source of *Salmonella* in finishers (Berends *et al*., 1996; Stege *et al*., 2000; Funk *et al*., 2001; Argüello *et al*., 2010a). According to this, finishing farms should be the main target of *Salmonella* control programmes at the primary level.

3. Feeding practices for *Salmonella* control in swine farms

The role of feed in the control of *Salmonella* in swine farms includes two different views. On the one hand, feed can be a source of *Salmonella* contamination while on the other hand there are several feeding practices that are useful tools to be used in the control of *Salmonella*. Several studies have demonstrated the relative low importance of feed as a primary source of infection to pigs (Harris *et al*., 1997). *Salmonella* is rarely detected after feed processing at the feed mills due to the thermal treatment coupled with good manufacturing practices, and moreover *Salmonella* serotypes sporadically isolated from feed are not related to those usually identified at the farm level (Harris *et al*., 1997; Davies *et al*., 2004; Torres *et al*., 2011). However, most researchers agree that feed can be easily contaminated at the farm level.

Feeding practices include many strategies for the control of *Salmonella*. Most of them are based on the same principle: the modification of the intestinal environment and the promotion of the normal microbial flora within the gastrointestinal tract, creating a healthier

environment. Even when the feeding practices that are exposed in this chapter are very different, the main mechanisms elicited to reduce or prevent *Salmonella* contamination are shared. Briefly, these feeding control strategies reduce directly or indirectly the pH within the intestinal tract and create an environment which is adverse to *Salmonella* and favours the growth of other bacteria. In a second step, this beneficial gut microflora contributes to maintain a hostile environment to *Salmonella* by lowering the pH and/or producing several anti-*Salmonella* compounds and metabolites.

3.1 Feed composition and feed physical structure

It is well documented that feed presentation, pelletized or not, coupled with the milling type, coarse or fine, has an influence on the gut microflora and therefore determines the success in the establishment and multiplication of *Salmonella* in the intestinal tract of swine. Although, pelleting and thermal-treatment processes can reduce the *Salmonella* contamination in compound feed, it has been demonstrated that non-pelleted feed has a clear protective effect against *Salmonella* compared to the use of pelleted feed (Jørgensen *et al.,* 1999; Kjeldsen & Dahl, 1999; Kranker *et al.,* 2001; Leontides *et al.,* 2003; Lo Fo Wong *et al.,* 2004; Rajik *et al.,* 2007; García-Feliz *et al.,* 2009). In a similar way, coarsely ground meal has been demonstrated to have a protective effect compared to fine grounded meal (Jørgensen *et al.,* 1999; Kjeldsen & Dahl, 1999; Jørgensen *et al.,* 2001; Mikkelsen *et al.,* 2004). It is important to remark that more than defining pelleted or fined ground meal as risk factors that promote the presence of *Salmonella* at farm level, we should define non-pelleted feed or coarsely meal as efficient protective elements against *Salmonella* in swine farms.

As we have already indicated, the anti-*Salmonella* activity seems to be related to the changes in the intestinal microflora that are associated with these types of feed. The effect of feed grinding and feed processing on physicochemical properties and microbial populations in the gastrointestinal tract of pigs were evaluated by Mikkesen *et al.* (2004). Those pigs fed a coarse non-pelleted feed showed a significant increase in the number of total anaerobic bacteria within the stomach as well as higher concentrations of various organic acids and lower pH compared to those pigs fed other diets suggesting a higher microbial fermentation in the stomach, fact that was also asserted by a slower gastric passage rate. These environmental conditions in the stomach would reduce the population of *Salmonella* populations by 1000-fold (Mikkesen *et al*., 2004). Other effects were also observed, to a leser extent, in other parts of the gastrointestinal tract with a lower number of coliform bacteria in the distal small intestine, in the colon and in the caecum and higher concentrations of butyric acid (Mikkesen *et al.*, 2004). Apart from these findings, it is well known that the digestibility of non-pelleted and coarse feed is lower than that of fine pelleted feed. Consequently, higher amounts of carbohydrates reach the last part of the small intestine and the large intestine providing a source of energy for anaerobic bacteria settled there.

3.2 Dry or liquid feed

It is well documented that liquid feed has a protective effect against *Salmonella* as compared to dry feed (Van der Wolf *et al*., 2001a; Højberg *et al*., 2003). Basically, this feeding strategy can be accomplished by using non-fermented liquid feed or fermented feed. In the first case, water or food industry derivates such as serum from dairy industry are added to the mixed feed immediately before its administration while when using

fermentation, the feed and the water are mixed and stored at a certain temperature for a period of time, prior to its use. Traditionally, liquid feeding systems are much extended in areas where liquid co-products from the human food industry are abundant and cheap. Industries involved in potato, vegetable, milk and fish processing, starch and sugar manufacture, baking, brewing and bio-ethanol production generate co-products that can be valuable and cost-saving inclusions in liquid diets.

The beneficial effects of liquid diets in the gastrointestinal tract are related to the stimulation of epithelial cells growth, the reduction of the intestinal pH and the increase in the lactic acid microbial flora. Its anti-*Salmonella* activity is based on the effect of the fermented feed against *Salmonella* itself since fermented liquid feed contains high concentrations of acids including lactic acid and short chain fatty acids and decreases the pH level in the gastrointestinal tract which in turn influence the ecology of the gastrointestinal microflora. In a study carried out in Canada, Farzan *et al.* (2006) compared *Salmonella* infection between 20 liquid-feeding farms and 61 dry-feeding farms. The use of liquid feed was associated to a lower number of *Salmonella* positive farms by both serological and bacteriological analysis. Moreover, a reduced usage of antimicrobials and consequently an improved pig health status was reported in those farms using liquid feed. Winsen *et al.* (2001) carried out a clinical trial comparing two groups of pigs, one fed with a dry-diet and the other with a *Lactobacillus plantarum* supplemented fermented liquid diet. A reduction in the total counts of *Enterobacteriaceae* within the gastrointestinal tract was reported in those animals receiving the supplemented fermented liquid food and was associated with an increase in the concentration of undissociated lactic acid and short chain fatty acids in the stomach content. According to these results, many risk factor studies have also described the protective effect of the liquid feed in *Salmonella* infection in swine farms (Beloeil *et al.,* 2004; Lo Fo Wong *et al.,* 2004; Poljak *et al*., 2008; Farzan *et al*., 2010; Hotes *et al*., 2010).

It is important to remark that in contrast to other feeding practices, the use of liquid feed and particularly of fermented liquid feed has been associated with an improvement in the growth performance. However, this feed system is not feasible economically in all herds due to the investment needed for storage capacity, mixers, pumps, pipelines and computers (Van der Wolf *et al.,* 2001a).

3.3 Probiotics

Feeding antibiotics is one of the most effective strategies of prophylactically controlling gastrointestinal infections but this practice is in decline because of the concern with antibiotic resistance in human medicine (Fairbrother *et al*., 2005). Even more, the European Union banned the use of antibiotics as growth promoters in food animals in 1999, on the basis of the "*precautionary principle*". One of the most promising and attractive alternatives to in-feed antibiotics is the use of probiotics and according to this, several researchers have also proposed their utility in the control of *Salmonella* infections in swine farms.

Probiotic treatment is based on the oral administration of viable bacteria, generally non-pathogenic anaerobic bacteria, with the objective to establish the first indigenous flora in newborn piglets or remove the pathogenic flora already established in growers or finishers. The two main actions of probiotics include the nutritional effect and the sanitary effect (Anadon *et al.,* 2006). The nutritional effect is attributed to a reduction of the metabolic

reactions that produce toxic substances, a stimulation of the indigenous enzymes and a production of vitamins. The sanitary effect of probiotics is linked to several actions including the creation of a restrictive environment by reducing the pH at the intestinal tract, the competition for gut surface adhesion, the production of anti-bacterial substances such as bacteriocins, the competition for the nutrients, the improvement of the epithelial gut cells health and the stimulation of the immune system acting as bio-regulators of the gut microflora and reinforcing the host natural defences.

At the moment most of the probiotics that are in use consist in a well-defined mix of microorganisms. The main bacterial genera used in these probiotics include *Clostridium, Enterococcus, Bacteroides, Streptococcus, Pediococcus, Bifidobacterium* or *Lactobacillus* as well as yeast such as *Saccharomyces (S. cerevesiae)* or *Kluyveromyces*. According to the guidelines from the EFSA, the identification of all the bacteria included in the mixture and the determination of the absence of antimicrobial resistance genes or plasmids and toxic metabolites are recommended for all probiotic products in the market (Anadon *et al.,* 2006).

Despite the fact that anti-*Salmonella* activity of several lactic acid bacteria has been already demonstrated using *in vitro* procedures (Hume *et al*., 2001; Harvey *et al*., 2002; Casey *et al*., 2004), the literature regarding the efficacy of probiotics in clinical trials is scarce, above all in pig surveys. According to the idea that the efficiency of probiotics is strongly related to the host animal where they have been developed (Ozawa *et al.,* 1983), we will focus the discussion on trials performed in pigs even when there is not too much data available. Genovese *et al.* (2000 and 2003) evaluated the effect of an undefined mixture of lactic acid bacteria of porcine origin previously developed by Harvey *et al.* (2002) on caecal colonization and faecal shedding of *S.* Cholerasuis in neonatal and weaned pigs. Their results showed a significant decrease in colonization as well as a reduced shedding after experimental infection with *S.* Cholerasuis in treated animals as compared with the control group. In a similar way, Fedorka-Cray *et al.* (1999) demonstrated the usefulness of a mixed and undefined culture from caecal mucosa of a 6-week-old healthy pig for the control of *Salmonella* infection. A 2- to 5-log reduction of *Salmonella* in the caecal content or ileocolic junction was observed in the pigs that received this probiotic mixture when compared with the controls. Moreover, 28% of the gut tissues from the treated pigs were positive versus 79% from the control pigs. More recently, the effect of a defined mixture of lactic acid bacteria of porcine origin containing *Lactobacillus murinus*, *L. pentosus, L. salivarus* and *Pediococcus pentosaceus* developed by Casey *et al.* (2004) was evaluated in weaned pigs (Casey *et al.,* 2007). The study design included three groups of five pigs: two treated groups that were administered the probiotic directly or fermented prior to its use and a control group in which milk was used as a placebo. All the animals from the treated groups were administered $4x10^9$ colony forming units (CFU) of the probiotic bacteria during 6 days. On day 7, all the pigs were challenged with 10^9 CFUs of *S.* Typhimurium and were monitored for 23 days. Probiotic treated animals showed reduced incidence, severity and duration of diarrhoea as well as a lower concentration of *Salmonella* in faeces. In contrast Zsabo *et al.* (2009) did not find differences in clinical symptoms after a probiotic treatment based on *Enterococcus faecium* and subsequent challenge with *S.* Typhimurium DT104. Moreover the invasiveness was greater in the treated group than in the control one, showing that not all the potential probiotic bacteria offer protection against *Salmonella.*

Our research group has evaluated hundreds of lactic acid bacteria recovered from faeces, intestinal content or intestinal mucosa of healthy pigs and selected according to their potential probiotic properties including their anti-*Salmonella* effect. Among those with high anti-bacterial activity against *Salmonella* we found isolates from the *Streptococcus* and *Lactobacillus* genera, including *L. reuteri, S. gallolyticus* subsp. *gallolyticus, L. delbrueckii, S. alactolyticus, L. animalis, L. salivarius*, *L ruminis* and *L. murinus* (Collazos *et al*., 2008a). In general, *L. reuteri* and *L. animalis* isolates are particularly resistant to the gastrointestinal environment of swine. Although *L. delbrueckii* isolates exhibited a strong anti-*Salmonella* activity, they were particularly sensitive to gastric conditions. When a defined probiotic mixture of five lactobacilli containing *L. reuteri, L. delbrueckii, L. animalis, L. murinus* and *L. ruminis* was administered to 5-weeks old piglets for 7 days before the challenge with *S.* Typhimurium (10^9 CFU) a significant reduction in the pathogen shedding and its dissemination to different organs and tissues as well as an alleviation of the clinical signs of the infection as compared with the pigs from the control group was demonstrated (Collazos *et al*., 2008b). Similar results were previously reported by Casey *et al.* (2007).

In spite of all these promising results from experimental trials, there is very little experience regarding the effect of such probiotic treatments in *Salmonella* infected swine farms. Moreover, at least two relevant questions regarding the probiotic use in the real practice still arise: (1) how can or should be administered the probiotic and (2) at which growth stage should it be used in order to reduce *Salmonella* contamination at the time of the slaughtering. Regarding the first question, two main possibilities should be considered. On the one hand, direct administration of the probiotic bacteria should be very effective and consequently high ratios of viable bacteria would reach the gastrointestinal tract. However it is almost impossible to use this administration in field conditions at farm level, particularly if the product is going to be used in growers or finishers. On the other hand, probiotic bacteria could be mixed with feed or drinking water allowing a very easy administration that could be extended for large periods of time. According to this, De Angelis *et al.* (2006) proposed that one of the main prerequisites for the selection of probiotic bacteria in swine is that these bacteria should be able to survive and maintain their health-promoting properties during feed manufacturing and storage. Our research group have evaluated the survival of five lactic acid bacteria of porcine origin incorporated into pelleted feed and stored for 24 days at farm conditions. Although one of the evaluated isolates was not included because its performance in the previous steps of fermentation and lyophilisation, was not satisfactory, stable numbers of the other four bacteria were recovered from pelleted feed stored in the farm until the end of the experiment allowing us to conclude that pelleted feed can apparently be used as a vehicle to administer probiotics in swine. Regarding the second question elicited, the moment of administration of the probiotic in a *Salmonella* control strategy in a swine farm, to our knowledge there is no field study in a *Salmonella* infected farm that can be used to give a well-grounded answer. In general, probiotics can be used to establish the flora in a newborn piglet, strengthen colonization resistance to pathogenic bacteria, or to compete with potential pathogenic bacteria already established in the gastrointestinal tract. Hence, the administration of probiotics is recommended during critical periods such as weaning (3 or 4 age-weeks) or at the beginning of the fattening period, when intestinal disorders are common. Focusing on the control of *Salmonella* infection in swine farms, both periods seem to be also suitable to establish a health intestinal status which would increase the resistance to *Salmonella* colonization. However, special attention should be paid in

order to avoid infections by *Salmonella* during the fattening period. Other option would be the administration of the probiotic during this fattening period or even at the end of the fattening period to reduce the risk of *Salmonella* transmission in the food chain.

3.4 Acids

A well studied strategy for the control of *Salmonella* infection on swine farms is the addition of acidic compounds to feed or drinking water. The main idea is not new and acids have been evaluated to replace growth promoters and to improve the hygiene and quality of the gut microflora since the 1980's (Giesting & Easter, 1985). It has been demonstrated that the un-dissociated form of various acids can freely cross the bacterial cell membrane and enter the bacterial cell, causing cell death (Van Immersel *et al*., 2006). Moreover, acids decrease the pH at the gastrointestinal tract and they could serve as carbon source, taking part in several bacterial metabolic routes.

The anti-*Salmonella* effect of many acids have been tested and evaluated in several experimental and field studies. The fact that short chain volatile fatty acids are produced by anaerobic bacteria of the gut microflora has focused many studies on their effectiveness against *Salmonella.* Propionic acid has shown satisfactory results against *Salmonella* in poultry (Hume *et al.,* 1993). To our knowledge, there is no reported clinical trial based on the use of butyric acid, nevertheless its activity against *Salmonella* has been documented *in vitro.* The increase of butyric acid concentration in the gut has been associated with a decrease in *Enterobacteriacea*e and *Salmonella* populations (Van Immerseel *et al.,* 2006) and an inhibition of the pathogenicity island I of *Salmonella*, involved in the gut cells invasion, after exposure to butyric acid has been reported (Gantois *et al.,* 2005). Acetic acid is probably the most evaluated short chain volatile fatty acid in clinical trials. However, several studies have concluded that this acid does not show a relevant anti-*Salmonella* activity (Dahl *et al.,* 1996; Van Immerseel *et al.,* 2006) and further, it increases the development of resistance against acids by the mechanisms defined as acid tolerance response (Known *et al*., 1998). The anti-*Salmonella* effect of lactic and propionic acids have also been evaluated in several studies with promising results (Tsiloyiannis *et al.,* 2001; Wingstrand *et al.,* 1996; Creus *et al.,* 2007). Apart from these five acids described here, many other studies have been carried out using other products such as citric acid, fumaric acid, malic acid and many other acid products that can be found in the market. At the same time, some of these acids have been coated in an attempt to avoid an early absorption in the small intestine. The most relevant results of clinical trials evaluating the use of acids in the control of *Salmonella* infection in swine farms are summarized in Table 1 (Dahl *et al*., 1996; Wolf *et al*., 2001b; Tsiloyiannis *et al*., 2001; Anderson *et al*., 2004; Creus *et al*., 2007; Boyen *et al*., 2008; De Busser *et al*., 2008; Argüello *et al*., 2010b).

Our research group carried out an interventional study in a pig fattening unit infected *by Salmonella* to assess the effectiveness of an acid treatment administered in drinking water for the control of salmonellosis (Argüello *et al*., 2011b). Animals from the experimental group were administered a commercial acid, composed of lactic acid (56%), formic acid (23%), propionic acid (13%) and acetic acid (5%), that was added to drinking water during the last 40 days of the fattening period at a concentration of 0.035%. This treatment was able to reduce the number of *Salmonella* shedders as well as the number of *Salmonella* seropositive animals at the end of the fattening period.

Study	Trial type	Production Stage	Acid selected	Vehicle	Concentration used	Treatment duration	Results and discussion
Anderson, 2004	Clinical trial	Weaning and fattening	Sodium chlorate	Water	30-80 mg/kg bw	36 h.	24 h. of administration in weaned pigs are enough to reduce the qualitatively recovery of *Salmonella* from gut and rectum. Proportions of *Salmonella* positive pigs were not significant reduced in finishers
Arguello, 2011	Field trial	Fattening	Mixture of Lactic (56 %), formic (23 %) propionic (13 %) and acetic acid (5 %).	Water	0.035 %[1]	60 days	Reduction at farm in bacteriological (faces) and serological results at the end of the fattening period. Reduced positive lymph nodes and cecal samples at the slaughterhouse (no reach significance).
Boyen, 2008	Clinical trial	6-week old piglets	1-. Coated butyric 2-. Coated caprilyc 3- Uncoated butyric 4-. Uncoated caprilyc	Feed	1-. Butyric 0.02% 2-. Caprylic 0.03% 3-. Butyric 0.01% 4-. Caprylic 0.017%	12 days	Treatment with coated butyric acid decreased the intestinal *Salmonella* load and shedding. (the concentration of butyric acid used in the uncoated treatment was half the coated).
Creus, 2007	Field trial	Finishers	Formic-propionic (50:50)	Feed	a) 1.2 % b) 0.8 %	a) 14 weeks b) 8 weeks	a) Reduction of percentage of *Salmonella* carriage in lymph nodes. b) Clear serological reduction and partial reduction of carriers in lymph nodes or cecal content.
Dahl, 1996	Field trial	Finishers	Formic, propionic, ammoniumformiate ammoniumpropionate	Feed	0.4%	14 days	No differences in shedding or serological prevalence. The treatment was not effective in previously infected pigs.
De Busser, 2008	Field trial	Finishers	-	Water	-	14 days	No beneficial effect in samples collected (carcass, lymph nodes or rectum)
Tsiloyiannis, 2001	Field trial	Weaners	Separately diets of: - Propionic acid (1 %) / Malic acid (1.2 %) / Formic acid (1.2 %) / Lactic acid (1.6 %) / Citric acid (1.5 %) / Fumaric acid (1.5 %)	Feed	Cited in acids colums	14 days	Thse study was carried out in a famr with clinical post-weaning diarrhoea syndrome caused by ECET. All the treatments reduced the numbers of ECET and showed ain improved growing specially the lactic acid group.
Wolf, 2000	Field trial	Finishers	Acid mixture: Lactic (8 %), formic (23 %), ammonium formiat (28 %), acetic (4 %), propionic (3 %) sorbic (1 %).	Water	0.2 %	12 weeks	The overall prevalence in control group was three times the treated groups, but just in a situation with clinical problems would justify the use of acids to the authors.

Table 1. Summary of experimental and field trials carried out using acid treatments to control *Salmonella.*

In summary and taking into account all the information provided by the different studies, it seems that the success in the control of *Salmonella* infection by using acids is related to several factors. The concentration given must be related to the pH value (Boyen *et al.*,

2008) and the duration of the treatment should be higher than a few weeks. No differences have been demonstrated between their administration in the feed or water. While the incorporation of the acids in the drinking water allows an easy regulation of the concentration and duration of the treatment, it has been associated with damages in the supply water circuits (Van der Wolf *et al.,* 2001b). Moreover, it has been proposed that the success of these acid treatments administered at the end of the fattening period is related to the establishment of the *Salmonella* infection before the acid addition (Dahl *et al*, 1996; Creus *et al*., 2007).

3.5 Other feed strategies

Other products such as prebiotics, mainly fructo-oligosacharides that cannot be digested by the animal but serve as carbon source for intestinal bacteria, or herbal extracts with significant anti-*Salmonella* activities have been proposed as potential options in the control of *Salmonella* infection in swine farms. However, further studies evaluating their usefulness in *Salmonella* infected swine units are required.

4. Vaccination

Immune response stimulation by vaccines has been a useful mechanism to battle against pathogens. In this subheading of the chapter, vaccinology to control *Salmonella* in pigs will be reviewed including the different types of vaccines tested against *Salmonella* in swine, discussing their efficacy, advantages and disadvantages. In order to develop a useful vaccine against *Salmonella,* the mechanisms involved in the defence of the host as well as those by which the bacteria is able to establish the infection in the host have to be taken into consideration. Hence, a brief revision of the *Salmonella* transmission, pathogenesis and host immune response will be included to improve the reader comprehension about vaccination theories.

4.1 *Salmonella* transmission, pathogenesis and immune response

The fecal-oral route is the typical mode of transmission of *Salmonella.* Once it is ingested, *Salmonella* is able to resist the acid environment in the stomach and the bactericidal effect of compounds such as bile salts in the first part of the small intestine (Fedorka-Cray *et al*., 1994). In the ileum, the peristalsis together with the indigenous microflora are the main difficulties that *Salmonella* has to overcome to reach its main target, the gut associated lymphoid tissue forming the Peyer's patches in the ileum wall and more exactly the microfold or 'M' cells of this tissue. The virulence genes encoded in the *Salmonella* pathogenicity island I allow the bacteria to trigger macropinocytosis (a form of endocytosis of large particles such as bacteria) in these M cells and also in enterocytes and goblet cells (Frances *et al*., 1993; Ginocchio *et al.,* 1994). After intestinal wall colonization *Salmonella* is presented to macrophages where it is able to survive by inhibiting the endosome-lysosome fusion through virulence genes encoded in the *Salmonella* pathogenicity island II (Hensel, 2004; Gal-Mor & Finlay, 2006). This allows the bacteria to reach the reticulloendothelial system as previous stage prior to systemic infection. Most of the swine infections caused by *Salmonella* serotypes different from the host-specific serotype *S.* Cholerasuis are restricted to

the follicle associated epithelium that surrounds the intestine. That is why we will focus this description of the immune response elicited by the host in this first stage of the infection to the gut and lymphoid tissue associated thereof.

Immediately after entering in the gastrointestinal tract, a complex and concerted immune response involving epithelial cells and both innate and adaptive immune response is mounted against pathogenic *Salmonella.* Although the microfold or M cells aforementioned are the main target to cross the intestinal wall, it has been described that *Salmonella* is also able to disrupt tight junctions between epithelial cells allowing paracellular transit into gastrointestinal tract tissue (Boile *et al.,* 2006). The approach that *Salmonella* uses to cross the epithelial cell barrier is a critical step in the immune responses generated. Those invasive bacteria that cross through the M cells activate particularly the secretion of IgA in the lamina propia; in contrast those non-invasive *Salmonella* that use mainly the paracellular transport do not induce the secretion of IgA.

Once the epithelial barrier is breached, the innate immune cells stimulate the pattern recognition receptors. Macrophages localized in the interfollicular region, neutrophils and monocytes (which will be differentiated in dendritic cells or macrophages) accumulated in the gut associated lymphoid tissue induce the classic T helper 1 immune response and provide the first cellular defence against invasion. Interleukins such as IL-12, IL-1 or those more recently found participating in the immune response to *Salmonella* such as IL-23, IL-22 (Schulz *et al*., 2008; Godinez *et al.,* 2009) and IL-17 (Raffatelu *et al*., 2008) as well as TNFα and IFNγ take part in the organism defence.

In later stages of the infection, *Salmonella* clearance is mediated by the specific immune response. Humoral response can be detected one week after the infection of the pigs (Gray *et al*., 1996a; 1996b), firstly represented by IgM and followed by IgG and IgA (Hasan *et al.,* 1991). The levels of IgM and IgA decrease gradually while IgG persist during extended periods of time, being detected at the time of slaughtering in finisher pigs. Cell immune response is principally represented by $CD4^+$ T lymphocytes (Hess *et al.,* 1996). The exact mechanism by which $CD4^+$ T-cells are able to control bacterial growth is unknown and seems not to be related to the production of TNFα and IFNγ. The antibody production against various *Salmonella* antigens also plays a role in the *Salmonella* clearance from systemic sites. This antibody production is stimulated by T-cells and the $CD8^+$ cytotoxic lymphocytes (Mastroeni *et al.,* 2009) and is directed against antigens such as the lipopolysaccharides, the capsular Vi polysaccharide or flagelins.

Salmonella is a potential intra-cellular pathogen. Its ability to survive and replicate in the macrophages and the reticulloendothelial system let it to avoid, at least partially, the immune response (Hormaeche *et al.,* 1993). Nevertheless it has been described that control and clearance of *Salmonella* rely in the cell immune response mediated by $CD4^+$ and $CD8^+$. (Mastroeni *et al.,* 1997). The specific humoral immune response, except for the IgA presented in the intestinal mucosa, is at least partially avoided by the fact that *Salmonella* is "protected" by the cells which infects and also the innate response (mediated by neutrophils and macrophages) even being the first barrier and also participating in antigens presentation, does not offer protection against *Salmonella.* So to summarize it seems that vaccines should stimulate the cell immune response to protect pigs against *Salmonella* infection.

4.2 Vaccination in pigs against *Salmonella*

The stimulation of the immune system by vaccines against *Salmonella* in swine aims to prevent gut colonization and faecal shedding as well as the development of a carrier state; in a word, bring to end the infection cycle at the farm level (Haesebrouck *et al.,* 2004). The disappearance of clinical symptoms is not the goal of this vaccination since most of the infections by *Salmonella* are not associated with clinical disease in pigs.

Several vaccines have been tested against *Salmonella* including live vaccines, attenuated or genetically modified, inactivated vaccines and also subunit vaccines. Live vaccines have the ability to arouse the best immune response; they stimulate the production of IgA in the intestinal mucosa since they can be used by oral administration and on the other hand they are theoretically able to produce a strong cell-mediated immune response (Lindberg & Robbertson, 1983). Besides, antibody titres seem to be lower than those induced by inactivated vaccines (Springer *et al*., 2001; Husa *et al.,* 2008) and this fact is relevant if the vaccine is going to be used in the course of control programmes based on serological detection and quantification of the infection. Live vaccines against *Salmonella* included (i) attenuated vaccines obtained by the dwindling of at least one of the virulence mechanisms of the bacteria without localizing or characterizing the molecular basis of attenuation; and (ii) genetically modified vaccines which in contrast to attenuated vaccines are those in which identified genes for the bacterial metabolism such as *aroA* (Lumsden *et al.,* 1991), global regulator genes or virulence genes such as *spv* genes located in *Salmonella* virulence plasmid (Kramer *et al.,* 1992) have suffered induced mutations to attenuate the bacteria.

Many studies have tested live vaccines in both challenge and clinical trials. We will focus our attention on those studies that have reported bacteriological results and therefore have measured the impact of vaccination in the *Salmonella* shedding and *Salmonella* infection in the gut or the associated lymphoid tissue. Several live vaccines including those based on modifications of their genome such as *aroA* mutants, *Δcya-Δcrp, gyrA-cpxA-rpoB* or *adenine-histidine* auxotrophy organisms (Lumsden *et al.,* 1991; Lumsden *et al.,* 1992; Springer *et al.,* 2001; Denagamage *et al.,* 2007; Selke *et al.,* 2007; Husa *et al.,* 2008) have demonstrated a reduction in the faecal shedding and isolation of *Salmonella* from the gut and lymphoid tissues. When piglets were vaccinated with these vaccines and challenged with the bacteria, a diminution in the infection pressure based on a reduction of the *Salmonella* faecal shedding and isolation from the gut and the lymphoid tissue associated was demonstrated. Nevertheless in most of these challenge experiments, the monitoring of the piglets was only carried out during the subsequent days or weeks after the experimental infection and therefore there are doubts regarding the duration of this protection. The experience from field trials has provided scarce but very interesting data; an *adenine-histidine* auxotrophy *S*. Typhimurium vaccine was tested for a period of six months in a farrow-to-finish farm. The prevalence of *Salmonella* infection in the unit decreased from 65% to 23% in 6 weeks. Unfortunately, this study does not include a control group and comparison was made using historical data. More recently, Farzan & Frienship (2009) have evaluated a commercial *S.* Cholerasuis live vaccine in a clinical field trial. The prevalence of *Salmonella* shedding animals decreased as immunized pigs aged but the results were not conclusive since this fact was also reported to a lesser degree in the control pigs. The point that the pigs were probably infected before their vaccination

together with the coexistence of three different serotypes of *Salmonella* involved in the infection at the farm could explain at least part of the low efficacy of vaccination against *Salmonella* found in this study. Finally another study using a *S.* Cholerasuis live vaccine (Maes *et al.,* 2001) showed a reduction in the positive ileocaecal lymph nodes (ILN) in the vaccinated group, 0.6%, compared to the control 7.2% while 24% and 9% of the vaccinated and control animals were positive in serology at 24 weeks (cut-off > 10).

Although theoretically live vaccines offer the best protection, they have also several disadvantages; firstly they are not as secure as inactivated vaccines since reversion to virulence can theoretically occur. Besides, transport and storage conditions are more demanding and finally if they are going to be administered orally several factors such as handling, withdrawal of antimicrobial treatments during administration or negative effects such pyrexia or reduced daily gain have to be taken into consideration (Husa *et al.,* 2008). For these reasons, there is still interest in *Salmonella* inactivated vaccines, which are easier to administer, more secure and also cheaper than attenuated life vaccines. In general, inactivated vaccines are useful against extracellular or toxin producer bacteria because humoral immune response can easily and effectively protects the host. It could be expected that no protective or a very limited effect would be seen with intracellular bacteria since the cell-mediated response is not stimulated directly. However, it is important to take into account that at least part of the infection cycle of *Salmonella* takes place in the extracellular space being vulnerable to the action of specific antibodies.

Inactivated vaccines are easy to produce and there are a number of clinical field and experimental trials to evaluate their effectiveness against *Salmonella* in different stages of the swine production including breeding herds, nursery pigs and finishers. A homologous inactivated *S.* Typhimurium vaccine was applied to sows in a research performed by Roesler *et al.* (2006). The results of this vaccination were measured in the offspring and revealed a decreased in the prevalence of *Salmonella* shedders as well as in the prevalence of seropositive piglets. According to these results, vaccination with an inactivated vaccine could be a proper tool to control *Salmonella* transmission from the sows to their progeny, easy to apply and cheap. On the contrary, Farzan & Friendship (2009) failed to demonstrate a clear protection in piglets after vaccination with an autogenous *S.* Typhimurium bacterin probably because the vaccine failed to elicit cross-protection against other serovars and piglets were suffering a multiple-serovar infection. The effectiveness of vaccination of finishers at the beginning of the fattening period with a whole-cell inactivated *S.* Typhimurium bacterin was tested in a field trial carried out by our research group (Argüello *et al.,* 2010c). Vaccinated pigs showed lower faecal shedding throughout the fattening period as well as lower serological response at the slaughter time. Moreover *Salmonella* prevalence in caecal content and mesenteric lymph nodes were also lower in vaccinated pigs as compared with control animals. However, the undesirable effect of vaccination was the strong humoral immune response which would interfere with a serological surveillance on the farm since more than a forty percent of the vaccinated pigs were seropositive (OD cut-off >40%) at the end of the fattening period.

In spite of their limitations, inactivated vaccines as well as subunit vaccines can increase in usefulness by taking advantage of the improvements in DIVA vaccines (Differenciating Infected from Vaccinated Animals) which have already been tested (Selke *et al.,* 2006; Leyman *et al.,* 2011) and also in adjuvants which should be able to increase the

immunostimulation boost of these vaccines (Leclerc, 2003). The application of such technology in conjunction with the ongoing developments in identifying new virulence determinants such as purified recombinant proteins, synthetic peptides or plasmid DNA could induce protective immunity by the selective activation of immune effectors mechanisms. The next generation of *Salmonella* vaccines could be based on these premises, to overcome the problems discussed above and improve the protections elicited by vaccines against *Salmonella.*

Despite the fact that the vaccine field has been the target of many surveys since decades, there are still many gaps. Most of the investigations regarding *Salmonella* immunity have been done in a murine model without taking account that *S.* Typhimurium is the host-specific pathogen for this specie. Moreover, most of the challenge trials carried out in piglets do not perform an extended monitoring of the animals until the market-weigh. Further research should be done to increase the knowledge in the immune response against *Salmonella* in production animal species and to non-host specific serotypes as well as in vaccine field trials in both finishers and sows (transmission of the immunity to the piglet).

5. Hygiene, handling practices and biosecurity

At farm level there are many factors that can modify the epidemiology of the infection determining the success of *Salmonella* colonization. Throughout this chapter we have mentioned that *Salmonella* needs to overcome the hostile environment of the gastrointestinal tract of the host as well as the immune response mechanisms elicited in order to establish an infection. In the pig, *Salmonella* can survive in the gut associated lymphoid tissue with reactivation of infection and shedding in favourable conditions, a fact which implies that infected animals are always a risk of infecting other animals throughout their lives. Moreover, *Salmonella* is perfectly adapted to the external environment and is able to survive outside the host for extended periods of time. These two premises, the carriage of *Salmonella* by apparently healthy animals and the ability of these bacteria to survive in the environment, determine the importance of hygiene and biosecurity practices in the control of the infection. None of the control measures aforementioned will be successful if they are not accompanied by adequate hygiene and biosecurity practices on the farm.

Hygiene standards are based on cleaning and disinfection procedures. All-in/all-out systems, where each room or building is completely emptied and sanitized between groups of pigs, are used frequently in finishing units in swine production and it is during the period of time comprised between two consecutive batches when the effort must be paid in order to prevent the infection of the incoming pigs. *Salmonella* can survive in the environment for long periods of time, for instance 14 days on smooth metallic surfaces, one year in wet soil or even up to two and four years in dry excrements and dust respectively (Murray, 2000). Its ability to persist in the environment enhances its transmission capacity. Apart from the direct transmission from pig to pig, the environment is the most important source of *Salmonella* infection in finishing units, being more relevant than contaminated sows at breeding herds (Berends *et al.,* 1996). As was demonstrated by Dahl *et al.* (1997) pigs coming from infected breeding herds, allocated in an environment perfectly cleaned and free of *Salmonella,* can arrive at the slaughterhouse without any positivity in bacteriological or serological samples. Hence, special attention should be paid to avoid the presence of *Salmonella* in the environment.

An effective cleaning protocol should cover the following premises: (i) clean the facilities with pressured water to remove the organic matter with special attention to holes and corners where it can be accumulated, (ii) apply detergents together with the pressured water to enhance the organic matter removal and finally (iii) apply a disinfectant after the proper cleaning protocol. Regarding useful disinfectants, it can be said that *Salmonella* is susceptible to most of the disinfectants used, such chlorine, iodine derivates, phenols, peroxides or quaternary ammonium compounds. However it is surprising that being susceptible to most disinfectants it can be found after cleaning and disinfection protocols routinely applied at farm level (Argüello *et al.,* 2011b).

We have evaluated the effectiveness of routinely cleaning and disinfection procedures against *Salmonella* in swine farms (Argüello *et al.,* 2011). A total number of thirty-six pig finishing farms performing a strict all-in/all-out management (AI/AO) were studied by collecting twelve samples within each farm including samples from pen floors (5 samples), pen walls (5 samples), corridors (1 sample) and dust (1 sample). All the farms were studied after cleaning and disinfection procedures, just before the entrance of a new batch of animals. Despite the fact that cleaning procedures were classified as satisfactory by clinicians and a phenol derivate disinfectant was used, *Salmonella* was still detected in one of each five investigated farms (22.2%). *Salmonella* was recovered mainly from floor samples (6 out of 8 positive farms were positive in floor samples) followed by pen walls (three farms). It is remarkable that in two of the positive farms the contamination was only detected in corridors. In contrast, *Salmonella* was not isolated from dust samples in any of the farms. In a similar farm environmental study performed in Germany, Gotter *et al.* (2011) reported *Salmonella* positive results in 22% of the pens floors, 28% of the pen walls and 32% of the central hallway. Gebrelles *et al.* (1999) also found that 80% of the pens were contaminated after cleaning and disinfection procedures in swine farms. Moreover, *Salmonella* serotypes isolated were related to new infections in the incoming pigs. Regarding these results, it is important to note that it has been described that holes in floors and walls make difficult the penetration of disinfectant solutions along with the biofilm formation by *Salmonella* can make the action of the disinfectants difficult (Marin *et al.*, 2009). Surprisingly, it has been reported that farms using cleaning protocols without disinfectants had lower *Salmonella* levels than those using disinfectants (Van der Wolf *et al.,* 2001a). This fact indicates that disinfection protocols are sometimes not carried out properly and points towards the importance of performing adequate cleaning protocols if we want to achieve an effective disinfection. Moreover, particular attention should be paid not only to pens but also to corridors in order to prevent infections between batches and also to the instruments employed at farm level since they can constitute a source of *Salmonella* contamination. Gotter *et al.* (2011) found that elements such as driving boards, pig toys or boots, presented the higher contamination values, showing that the farm equipment can be a source of contamination that sometimes is underestimated. These results together with the risk factors studies in which not beneficial effects were found in AI/AO systems (Nollet *et al.,* 2004; Rajic *et al,.* 2007; García-Feliz *et al.*, 2010) show that cleaning protocols carried out routinely at farm sometimes do not reach their goal and so special attention should be paid in the cleaning and disinfection carried out between batches removing the organic matter present, cleaning not only the surfaces visible to the naked eye but also equipments, corners, and other surfaces in which dust and contamination can be stored.

Regarding management and handling by farmers the main premises that should be taken into consideration are as follows; large facilities are usually supplied by several breeding origins and then *Salmonella*-free pigs can be mixed with infected pigs at the fattening unit. Thus, in order to avoid the risk of contamination by potentially infected pigs, the origin status of the piglets should be confirmed, above all in low *Salmonella* contaminated farms included in control programmes. Moreover, it is believed that mixing animals with different ages increases the risk of *Salmonella* transmission, so pig handling is also important in avoiding or minimizing *Salmonella* infections at the farm. Adequate handling and caring of the animals is also necessary to diminish the stress, which is related to an increase in pig susceptibility to *Salmonella* infection as well as to an increase in faecal shedding by carriers (Verbrugghe *et al.,* 2011).

Biosecurity is essential at farm level to avoid the entrance of infectious diseases and most of the swine farms fulfil the basic biosecurity measures. General biosecurity measures such as double external fence, footbaths, changing rooms with showers and farm clothes to staff and visitors, external access to feed and dead animal trucks are essential in farms and several risk factor studies have associated them to a lower *Salmonella* prevalence (Amass *et al.,* 2000; Lo Fo Wong *et al.,* 2004).

Apart from infected animals, which constitute the main source of *Salmonella* infection, the indirect transmission of disease by feed or wild animals present at the farm can be also relevant. The importance of feed as *Salmonella* vehicle has been already discussed in the feeding strategies subheading. As it was pointed out if feed transport or storage in the farm are not carried out under strict isolation conditions, feed can be easily contaminated by *Salmonella*. Water supply can also be a significant vehicle to indirect *Salmonella* transmission. The ability of *Salmonella* to survive in water supply depends on the nature of the water and factors such as the presence of protozoa, the concentration of organic matter, toxins, heavy metals, and several physicochemical properties. Fish & Petiborne (1995) estimated that *Salmonella* can survive at least 56 days in water. Farmers should pay attention to water quality and also to guarantee a supply of potable water on their farms. At the same time, wild birds or rodents can also contaminate the feed if they can access to the places where it is stored. Feed and water are effective vehicles to *Salmonella* transmission because they are supplied to all the animals and bring *Salmonella* directly to the gastrointestinal tract. So appropriate production and feed handling as well as water treatment has to be done in order to avoid contamination by *Salmonella* from these two sources.

Probably one of the main factors implied in the spreading of *Salmonella* is its ability to colonize a wide range of animal species including warm or cold blooded animals; this fact implies that most of the animals, birds or insects present in an environment with *Salmonella* will be infected or will carry *Salmonella.* This fact implies that all domestic and wild animals that get in touch with the farm can constitute a source of *Salmonella* for pigs. *Salmonella* has been isolated from rodents in several studies (Healing, 1991) and their faecal pellets can contain up to 10^5 CFU of *Salmonella* (Henzler & Opitz, 1992). Although wild birds have been recognised as carriers of *Salmonella,* evidence suggests that infected birds are rarely identified. It seems that birds are infected by their feeding environment with a short term carriage (Murray, 2000). *Salmonella* has been also isolated from insects including cockroaches, flies, and beetles (Benett, 1993; Davies & Wray 1995; Olsen & Hammack, 2000).

Other wild animals are more related with the maintenance and perpetuation of the infection in the farm more than with the introduction of *Salmonella* thereof; finding positive mice or rats or cats for instance in the farm proves that *Salmonella* is distributed in the environment and the elimination of these animals is crucial if other efforts are taken at the same time to reduce the *Salmonella* prevalence.

Hygiene, handling practices or biosecurity are not sometimes taken into account to battle against a pathogen but in facultative environmental pathogens such *Salmonella,* they can play a crucial role in its maintenance and perpetuation and must be included in the practices to reduce the prevalence at farm level if practitioners want to have success reducing *Salmonella.*

6. Conclusions

The main objective of this chapter was to identify the main potential control strategies applicable in swine production to reduce *Salmonella* prevalence. Fortunately there is enough background to discern which measures seem to be most efficient in general, but we must stress that *Salmonella* epidemiology is not completely understood and that there are many factors that can influence its presence at farm level. The achievement of success in a reduction programme will depend in which measures, of those described here, may be feasible applied taking into account the serotype involved in the infection, its prevalence, type of farm etc, and also factors such economical resources.

7. References

Alban, L.; Stege H., Dahl J., (2002). The new classification system for slaughter-pig herds in the Danish *Salmonella* surveillance-and-control program. *Preventive Veterinary Medicine*, No. 14 pp 133-146.

Alban, L. & Stärk, K.D. (2005). Where should the effort be put to reduce the *Salmonella* prevalence in the slaughtered swine carcass effectively? Preventive Veterinary Medicine., No. 68 pp 63-79.

Amass, S.F.; Vyverberg, B.D., Ragland, D., Dowell, C.A., Anederson, C.D., Stover, J.H., & Beaudry, D.J. (2000). Evaluating the efficacy of boot baths in biosecurity protocols. *Swine Health and Production*, Vol. 4 No. 8 pp. 169-173.

Anadón, A.; Martínez-Larrañaga, M.R. & Aranzazu Martínez, M. (2006). Probiotics for animal nutrition in the European Union. Regulation and safety assessment. *Regulatory, Toxicology and Pharmacology.,* No. 45 pp. 91-95.

Anderson, R.C.; Hume, M.E., Genovese, K.J., Callaway, T.R., Jung, Y.S., Edrington, T.S., Poole, T.L., Harvey, R.B., Bischoff, K.M. & Nisbet, D.J. (2004). Effect of drinking-water administration of experimental chlorate ion preparations on Salmonella enterica serovar Typhimurium colonization in weaned and finished pigs. *Veterinary. Research Communications*., No. 28 pp. 179-189.

Argüello, H.; Carvajal, A., Alvarez, S., Osorio, J., Hidalgo, A., & Rubio, P., (2010a). Salmonellosis in fattening period: relative importance of sows in two points multiple-site production. *Proceedings of* 2nd *ESPHM*, Hannover, Germany, 26-28 May 2010.

Argüello, H.; Carvajal, A., Osorio, J., Martin, D. & Rubio, P. (2010b). Evaluation of a commercial acid mixture administered in drinking water as a control measure in swine salmonellosis. Proccedings of International Symposium Salmonella and Salmonellosis, pp: 455-466. Saint Malo, France 28-30 June, 2010.

Argüello, H.; Carvajal, A., Osorio, J., Martin, D. & Rubio, P. (2010c). Efficacy of vaccination with an inactivated vaccine to reduce *Salmonella* prevalence in a pig fattening unit. Proccedings of International Symposium Salmonella and Salmonellosis, pp: 465-466. Saint Malo, France 28-30 June, 2010

Argüello, H.; Carvajal, A., Collazos, J.A., García-Feliz, C., Rubio, P., (2011a). Prevalence and serovars of *Salmonella enterica* on pig carcasses, slaughtered pigs and the environment of four Spanish slaughterhouses. *Journal Food Research.* (in press).

Argüello, H.; Rubio, P., Jaramillo, A., Barrios, V., García, M., & Carvajal, A., (2011b). Evaluation of cleaning and disinfection procedures against *Samonella enterica* at swine farms, transport and lairage facilities. *Procidings of International Safepork congress, pp: 254-256*. Maastrich, Netherlands, 18-20 June 2011.

Beloeil, P.A.; Fravalo, P., Fablet, C., Jolly, J.P., Eveno, E., Hascoet, Y., Chauvin, C., Salvat, G. & Madec, F. (2004). Risk factors for *Salmonella enterica* subsp. *enterica* shedding by market-age pigs in French farrow-to-finish herds. *Preventive Veterinary Medicine*, No. 63 pp. 103-120.

Bennett, G. (1993). Cockroaches as carriers of bacteria. *Lancet*, No. 341 pp. 732.

Berends, B.R.; Urlings, H.A., Snijders, J.M., & Van Knapen, F. (1996). Identification and quantification of risk factors in animal management and transport regarding *Salmonella spp*. in pigs. *International Journal Food Microbiology.* No. 30 pp. 37-53.

Boyle, E.C.; Bishop, J.L., Grassl, G.A., & Finlay, B.B., (2006). *Salmonella*: from pathogenesis to therapeutics. *Journal of Bacteriology. Vol. 5 No. 189 pp. 1489-1495.*

Boyen, F.; Haesebrouck, F., Vanparys, A., Volf, J., Mahu, M., Van Immerseel, F., Rychlik, I., Dewulf, J., Ducatelle, R. & Pasmans, F. (2008). Coated fatty acids alter virulence properties of *Salmonella* Typhimurium and decrease intestinal colonization of pigs. *Veterinary Microbiology*, No. 132 pp. 319-327.

Casey, P.G.; Gardiner, G.E., Casey, G., Bradshaw, B., Lawlor, P.G., Lynch, P.B., Leonard, F.C., Stanton, C., Ross, R.P., Fitzgerald, G.F. & Hill, C. (2007). A five-strain probiotic combination reduces pathogen shedding and alleviates disease signs in pigs challenged with *Salmonella enterica* Serovar Typhimurium. *Applied Environmental Microbiology*. No. 73 pp. 1858-1863.

Casey, P.G.; Casey, G.D., Gardiner, G.E., Tangney, M., Stanton, C., Ross, R.P., Hill, C. & Fitzgerald, G.F. (2004). Isolation and characterization of anti-*Salmonella* lactic acid bacteria from the porcine gastrointestinal tract. *Letters of Applied Microbiology*, No. 39 pp. 431-438.

Collazos, J.A.; Samaniego, L.M., García, C., de Castro, L., Carvajal, A. & Rubio, P. (2008a). Selection and characterization of anti-salmonella lactic acid bacteria of porcine origin. *Proccedings of 20th International Pig Veterinary Society Congress*, pp. 317, Durban, South Africa, 20.25 June, 2008.

Collazos, J.A.; Samaniego, L.M., García, C., de Castro, L., Carvajal, A. & Rubio, P. (2008b). Effect of a probiotic mixture of porcine origin on experimental salmonellosis in

swine. *Proccedings of 20th International Pig Veterinary Society Congress*, pp. 317, Durban, South Africa, 20.25 June, 2008.

Creus, E.; Pérez, J.F., Peralta, B., Baucells, F. & Mateu, E. (2007). Effect of acidified feed on the prevalence of *Salmonella* in market-age pigs. Zoonoses and Public Health, No. 54 pp. 314-319.

Dahl, J.; Wingstrand, A., Baggesen, D.L., Nielsen, B. & Thomsen, L.K. (1996). The effect of a comercial, organic acid preparation on seroprevalence and shedding of *Samonella* in finishing herds. *Proccedings of 14th International Pig Veterinary Society Congress*, pp. Bologna, Italy, 1996.

Dahl, J.; Wingstrand, A., Nielsen, B. & Baggesen, D.L. (1997). Eradication of *Salmonella* Typhimurium infection by the strategic movement of pigs. *The Veterinary Record*, No. 140 pp. 679-681.

Davies, P.R.; Scott Hurd, H., Funk, J.A., Fedorka-Cray, P.J. & Jones, F.T. (2004). The role of contaminated feed in the epidemiology and control of *Salmonella enterica* in pork production. Foodborne Pathogens and Disease. No. 1. pp. 202-215.

Davies, R.H. & Wray C. (1997). Distribution of *Salmonella* on 23 pig farms in the U.K. *Proceedings of the 2nd International Symposium on the Epidemiology and Control of Salmonella in Pork*. p.137-141. Copenhagen, Denmark, 1997.

De Angelis, M.; Siragusa, S., Berloco, M., Caputo, L., Settanni, L., Alfonsi, G., Amerio, M., Grandi, A., Ragni, A. & Gobbetti, M. (2006). Selection of potential probiotic lactobacilli from pig feces to be used as additives in pelleted feeding. *Research in Microbiology,* No. 157 pp. 792-801.

De Busser, E.V.; Dewulf, J., Nollet, N., Houf, K., Schwarzer, K., De Sadeleer, L., De Zutter, L. & Maes, D. (2008). Effect of organic acids in drinking water during the last 2 weeks prior to slaughter on *Salmonella* shedding by slaughter pigs and contamination of carcasses. Zoonoses and Public Health, No. 56 pp. 129-136.

Duggan, S.J.; Mannion, C., Prendergast, D.M., Leonard, N., Fanning, S., Gonzales-Barron, U., Egan, J., Butler, F. & Duffy, G. (2010). Tracking the Salmonella status of pigs and pork from lairage through the slaughter process in the Republic of Ireland. *Journal of Food Protection*, No. 73 pp. 2148-2160.

EFSA, 2008. Analysis of the baseline survey on the prevalence of *Salmonella* in slaughtered pigs in the European Union. Part A *Salmonella* prevalence estimates. *EFSA Journal*, No. 135: 1-111.

EFSA, 2009. Analysis of the baseline survey on the prevalence of *Salmonella* in holding with breeding pigs in the European Union, (2008). Part A *Salmonella* prevalence estimates. *EFSA Journal*, No. 7 pp. 1377.

EFSA, 2011. The European Union summary report on trends and sources of zoonosis, zoonotic agents and food-borne outbreaks in (2009). *EFSA Journal*, No. 9 pp. 2090.

Fairbrother, J.M.; Nadeau, E. & Gyles, C.L. (2005). Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. *Animal Health Reseseach Review*, No 6. pp. 17-39.

Farzan, A.; Friendship, R.M., Dewey, C.E., Warriner, K., Poppe, C. & Klotins, K. (2006). Prevalence of *Salmonella* spp. on Canadian pig farms using liquid or dry-feeding. *Preventive Veterinary Medicine*, No. 73 pp. 241-254.

Farzan, A.; Friendship, R.M., Dewey, C.E., Poppe, C. & Funk, J. (2010). Evaluation of the risk factors for shedding Salmonella with or without antimicrobial resistance in swine using multinomial regression method. *Zoonoses Public Health*, No. 57 pp. 85-93

Fedorka-Cray, P.J.; Whipp S.C., Isaacson R.E., Nord N., & Lager K. (1994). Transmission of *Salmonella* typhimurium to swine. Veterinary Microbiology, Vol. 4 No. 41 pp. 333-344.

Fedorka-Cray, P.J.; Bailey, J.S., Stern, N.J., Cox, N.A., Ladely, S.R. & Musgrove, M. (1999). Mucosal competitive exclusion to reduce *Salmonella* in swine. Journal of Food Protection, No. 62 pp. 1376-1380.

Fish J.T.; & Pettibone G.W. (1995). Influence of freshwater sediment on the survival of Escherichia coli and Salmonella sp. as measured by three methods of enumeration. Letters of Applied Microbiology, Vol., 5 No 20 pp 277-281.

Funk, J.A.; Davies, P.R. & Nichols, M.A. (2001). Longitudinal study of *Salmonella enterica* in growing pigs reared in multiple-site swine production systems. Veterinary Microbiology, No. 83 pp. 45-60.

Gal-Mor, O., & Finlay, B.B. (2006). Pathogenicity islands: a molecular toolbox for bacterial virulence. Cell Microbiology. Vol. 8 No.11 pp. 1707-1719.

Gantois, I.; Ducatelle, R., Pasmans, F., Haesebrouck, F., Hautefort, I., Thompson, A., Hinton, J.C. & Van Immerseel, F. (2005). Butyrate specifically down-regulates Salmonella pathogenicity island 1 gene expression. *Applied Environmental Microbiology*, No 72 pp. 946-949.

García-Feliz, C.; Carvajal, A., Collazos, J.A. & Rubio, P. (2009). Herd-level risk factors for faecal shedding of *Salmonella enterica* in Spanish fattening pigs. *Preventive Veterinary. Medicine*, No. 91 pp. 130-136.

Gebreyes, W.A.; Turner M.B., Funk J.A., Altier C., & Davies P.R. (1999). *Salmonella* prevalence, serotypes, and patterns of antimicrobial resistance in cohorts of nursery and finishing pigs. *Proceedings of the 3rd International Symposium on the Epidemiology and Control of Salmonella in Pork*, p.250-251. Washington, D.C. USA.

Genovese, K.J.; Anderson, R.C., Harvey, R.B. & Nisbet, D.J. (2000). Competitive exclusion treatment reduces the mortality and fecal shedding associated with enterotoxigenic *Escherichia coli* infection in nursery-raised neonatal pigs. Canadian. Journal of Veterinary Research, No. 64 pp. 204-207.

Genovese, K.J.; Anderson, R.C., Harvey, R.B., Callaway, T.R., Poole, T.L., Edrington, T.S., Fedorka-Cray, P.J. & Nisbet, D.J. (2003). Competitive exclusion of *Salmonella* from the gut of neonatal and weaned pigs. Journal of Food Protection, No. 66 pp. 1353-1359.

Giesting, D.W. & Easter, R.A. (1985). Response of starter pigs to supplementation of corn-soybean meal diets with organic acids. Journal of Animal Science, No. 60 pp. 1288-1294.

Ginocchio, C.C.; Olmsted, S.B., Wells, C.L., & Galán, J.E. (1994). Contact with epithelial cells induces the formation of surface appendages on Salmonella typhimurium. Cell. Vol. 4 No. 76 pp. 717-724.

Goldbach, S.G., & Alban, L. (2006). A cost-benefit analysis of *Salmonella*-control strategies in Danish pork production. Preventive Veterinary Medicine. No 77pp. 1-14.

Godinez I.; Raffatellu M., Chu H., Paixão T.A., Haneda T., Santos R.L., Bevins C.L., Tsolis R.M., Bäumler A.J. (2009). Interleukin-23 orchestrates mucosal responses to Salmonella enterica serotype Typhimurium in the intestine. Infection and Immunity. Vol. 1 No. 77 pp. 387-398.

Gotter, V.; Blaha, T., & Klein, G. (2011). A case-control study on the occurrence of *Salmonella* spp. in the environment of pigs. Epidemiology and Infection. No. 16 pp. 1-7.

Gray, J.T.; Fedorka-Cray, P.J., Stabel, T.J., Kramer, T.T. (1996a). Natural transmission of *Salmonella* choleraesuis in swine. Applied Environmental Microbiology. Vol. 1 No. 62 pp. 141-146.

Gray, J.T.; Stabel, T.J., Fedorka-Cray, P.J. (1996b). Effect of dose on the immune response and persistence of Salmonella choleraesuis infection in swine. Amercan Journal Veterinary Research. Vol. 3 No. 57. pp 313-319.

Hald, T., & Wegener, H.C. (1999). Quantitative assesement of the sources of human salmonellosis attributable to pork. *Proceedinings of 3th International Symposium on the Epidemiology and Control of Salmonella in Pork*, pp. 200-205. Washington, D.C., USA.

Hald, T.; Vose D., Wegener H.C., Koupeev, T. (2004). A Bayesian approach to quantify the contribution of animal-food sources to human salmonellosis. *Risk Analysis* No. 24. pp 255-269.

Harris, I.T.; Fedorka-Cray, P.J., Gray, J.T., Thomas, L.A. & Ferris, K. (1997). Prevalence of *Salmonella* organisms in swine feed. Journal of the American. Veterinary Medical Association. No. 210 pp 382-385.

Harvey, R.B.; Droleskey, R.E., Hume, M.E., Anderson, R.C., Genovese, K.J., Andrews, K. & Nisbet, D.J. (2002). In vitro inhibition of *Salmonella enterica* serovars Choleraesuis and Typhimurium, *Escherichia coli* F-18, and *Escherichia coli* O157:H7 by a porcine continuous-flow competitive exclusion culture. *Current Microbiology*. No 45 pp 226–229.

Healing, T.D. (1991). *Salmonella* in rodents: a risk to man? *CDR (London, England Review*. Vol. 10 No.1 pp 114-116.

Hensel, M. (2004). Evolution of pathogenicity islands of *Salmonella enterica*. International Journal of Medicine Microbiology. Vol. 2-3 No. 294 pp. 95-102.

Henzler, D.J.; Opitz, M.H. (1992). The role of mice in the epizootiology of *Salmonella* Enteritidis infection in chicken layer farms. *Avian Diseases*, No 36 pp. 625-631.

Hess, J.; Ladel C., Miko, D., & Kaufmann, S.H. (1996). *Salmonella* typhimurium aroA-infection in gene-targeted immunodeficient mice: major role of CD4+ TCR-alpha beta cells and IFN-gamma in bacterial clearance independent of intracellular location. Journal of Immunology. Vol 9 No. 156 pp. 3321-3326.

Højberg, O.; Canibe, N., Knudsen, B. & Jensen, B.B. (2003). Potential rates of fermentation in digesta from the gastrointestinal tract of pigs: effect of feeding fermented liquid feed. *Applied Environmental Microbiology. No.* 69 pp. 408-418.

Hormaeche, C.E.; Villareal, N., Dougan, G., and Chatfield, S.N. (1993). Immunity mechanisms in experimental Salmonellosis. In: Cabello, F., Hormaeche ,C.E., MAstroeni , P., and Bonina, P.,(eds). *Biology of Salmonella*. Plenum press, New York, pp. 223-235.

Hotes, S.; Kemper, N., Traulsen, I., Rave, G. & Krieter, J. (2010). Risk factors for Salmonella infection in fattening pigs - an evaluation of blood and meat juice samples. *Zoonoses and Public Health*, No. 57 pp. 30-38.

Hume.; M.E., Corrier, D.E., Ambrus, S., Hinton, A. & DeLoach, J.R. (1993). Effectiveness of dietary propionic acid in controlling Salmonella typhimurium colonization in broiler chicks. *Avian Dissease*. No 37 pp. 1051-1056.

Hume, M.E.; Nisbet, D.J., Buckley, S.A., Ziprin, R.L., Anderson, R.C. & Stanker, L.H. (2001). Inhibition of in vitro *Salmonella* Typhimurium colonization in porcine cecal bacteria continuous-flow competitive exclusion cultures. *Journal of Food Protection* No 64 pp. 17–22.

Hurd, H.S.; McKean, J.D., Griffith, R.W., Wesley, I.V. & Rostagno, M.H. (2002). Salmonella enterica infections in market swine with and without transport and holding. *Applied Environmental Microbiology*. No 68 pp. 2376-2381.

Jørgensen, L.; Dahl, J. & Wingtrand, A. (1999). The effect of feeding pellets, meal and heat treatment on the *Salmonella*-prevalence of finishing pigs, *Procceedings 3th International. Symposium on Epidemiology and Control of Salmonella in Pork* pp. 308-312 Washington D.C., USA, 1999.

Jørgensen, L.; Kjærsgaard, H.D., Wachamann, H., Jensen, B. & Knudsen, B. (2001). Effect of pelleting and use of lactic acid in feed on *Salmonella* prevalence and productivity in weaners. P*rocceedings 4th International Symposium on the Epidemiology and Control of Salmonella in Pork*. Pp109-111 Leipzig, 2001.

Kjeldsen, N.J. & Dahl, J. (1999). The effect of feeding non-heat treated, non-pelleted feed compared to feeding pelleted, heat-treated feed on the *Salmonella*-prevalence of finishing pig. *Procceedings 3th International. Symposium on Epidemiology and Control of Salmonella in Pork* pp. 313-316 Washington D.C., USA, 1999.

Kranker, S.; Alban, L., Boes, J. & Dahl, J. (2003). Longitudinal study of *Salmonella enterica* serotype Typhimurium infection in three Danish farrow-to-finish swine herds. Journal of Clinical. *Microbiology*. No 41 pp. 2282-2288.

Kranker, S.; Dahl, J. & Wingstry, A. (2001). Bacteriological and serological examination and risk factor analysis of *Salmonella* occurrence in sow herds, including risk factors for high *Salmonella* seroprevalence in receiver finishing herds. *Berliner und Münchener Tierärztliche Wochenschrif*. No 114 pp. 350-352.

Kwon, Y.M. & Ricke, S.C. (1998). Induction of acid resistance of *Salmonella* typhimurium by exposure to short-chain fatty acids. Applied Environmental Microbiology. 64: 3458-3463.

Leyman, B.; Boyen F., Parys, A., Verbrugghe, E., Haesebrouck, F., Pasmans, F. (2011). Application of the DIVA principle to *Salmonella* Typhimurium vaccines in pigs avoids interference with serosurveillance programmes. *Procedings in International Safepork congress*, pp: 254-256 Maastrich, The Netherlands, 18-20 June, 2011.

Leontides, L.S.; Grafanakis, E. & Genigeorgis, C. (2003). Factors associated with the serological prevalence of *Salmonella enterica* in Greek finishing swine herds. *Epidemiology and Infection*. No. 131 pp. 599-606.

Letellier, A.; Messier, S., Pare, J., Menard, J. & Quessy, S., 1999. Distribution of *Salmonella* in swine herds in Quebec. *Veterinary Microbiology*. No. 67 pp. 299-306.

Lo Fo Wong, D.M.A.; Dahl, J., Stege, H., van der Wolf, P.J, Leontides, L., von Altrock, A. & Thorberg, B.M. (2004). Herd-level risk factors for subclinical *Salmonella* infection in European finishing-pig herds. *Preventive Veterinary Medicine*. No 62 pp. 253-266.

Maes, D.; Gibson K., Trigo, E., Saszak, A., Grass, J., Carlson, A., & Blaha, T. (2001). Evaluation of cross-protection afforded by a *Salmonella* Choleraesuis vaccine against *Salmonella* infections in pigs under field conditions. Berliner und Münchener Tierärztliche Wochenschrif. Vol 9-10. No 114. pp. 339-41.

Marin, C.; Hernandiz, A., & Lainez, M. (2009). Biofilm development capacity of *Salmonella* strains isolated in poultry risk factors and their resistance against disinfectants. Poultry Science. Vol 2. No 88. pp 424-431.

Mastroeni, P.; Grant, A., Restif, O., & Maskell, D. (2009). A dynamic view of the spread and intracellular distribution of *Salmonella* enterica. Natural Reviews, Microbiology. Vol. 1 No 7. pp. 73-80.

Mikkelsen, L.L.; Naughton, P.J., Hedemann, M.S. & Jensen, B.B. (2004). Effects of physical properties of feed on microbial ecology and survival of *Salmonella enterica* serovar Typhimurium in the pig gastrointestinal tract. Applied Environmental Microbiology No. 70 pp. 3485-3492.

Murray, C.J. (2000). Environmental aspects of *Salmonella.* In: WRAY, C; WRAY, . *Salmonella in Domestic Animals.* CABI Publishing, Wallingford pp. 265-283.

Nielsen, B.; Alban, L., Stege, H., Sørensen, L.L., Møgelmose, V., Bagger, J., Dahl, J., & Baggesen, D.L. (2001). A new *Salmonella* surveillance and control programme in Danish pig herds and slaughterhouses. *Berliner und Münchener Tierärztliche Wochenschrif*, No. 114 pp. 323-326.

Nollet, N., Maes, D., De Zutter, L., Duchateau, L., Houf, K., Huysmans, K., Imberechts, H., Geers, R., de Kruif ,A., & Van Hoof, J. (2004). Risk factors for the herd-level bacteriologic prevalence of *Salmonella* in Belgian slaughter pigs. Preventive Veterinary Medicine. Vol 1,2 No 65 (1-2) pp. 63-75.

Olsen, A.R., & Hammack, T.S. (2000). Isolation of *Salmonella* spp. from the housefly, Musca domestica L., and the Dump fly, Hydrotaea aenescens (Wiedemann) (Diptera: Muscidae), at caged-layer houses. *Journal of Food Protection*, No.63 pp. 958-960.

Ozawa, K.; Yabuuchi, K., Yamanaka, K., Yamashita, Y., Nomura, S. & Oku, I. (1983). Effect of *Streptococcus faecalis* BIO-4R on intestinal flora of weaning piglets and calves. *Applied Environmental. Microbiology*. No. 45 pp. 1513-1518.

Pires, S.M.; Knegt, L., and Hald, T (2011). Estimation of the relative contribution of different food and animal sources to human Salmonella infections in the European Union. *EFSA-Q*-2010-00685.

Poljak, Z.; Dewey, C.E., Friendship, R.M., Martin, S.W. & Christensen, J., (2008). Multilevel analysis of risk factors for Salmonella shedding in Ontario finishing pigs. *Epidemiology and Infection*. No. 136 pp. 1388-1400.

Raffatellu, M.; Santos, R.L., Verhoeven, D.E., George, M.D., Wilson, R.P., Winter, S.E., Godinez, I., Sankaran, S., Paixao,T.A., Gordon, M.A., Kolls, J.K., Dandekar, S., & Bäumler, A.J., (2008). Simian immunodeficiency virus-induced mucosal interleukin-17 deficiency promotes Salmonella dissemination from the gut. *Nature Medicine*. Vol. 4 No. 14 pp. 421-428.

Rajic, A.; O'Connor, B.P., Deckert, A.E., Keenliside, J., McFall, M.E., Re-Smith, R.J., Dewey, C.E. & McEwen, S.A. (2007). Farm-level risk factors for the presence of *Salmonella* in 89 Alberta swine-finishing barns. *Canadian Journal of Veterinary Research*. No 71 pp. 264-270.

Stege, H.; Christensen, J., Nielsen, J.P., Baggesen, D.L., Enoe, C. & Willeberg, P. (2000). Prevalence of subclinical *Salmonella enterica* infection in Danish finishing pig herds. *Preventive Veterinary Medicine* No. 44 pp. 175-188.

Schulz, S.M.; Köhler, G., Schütze, N., Knauer, J., Straubinger, R.K., Chackerian, A.A., Witte, E., Wolk, K., Sabat, R., Iwakura, Y., Holscher, C., Müller, U., Kastelein, R.A., & Alber, G. (2008). Protective immunity to systemic infection with attenuated *Salmonella enterica* serovar enteritidis in the absence of IL-12 is associated with IL-23-dependent IL-22, but not IL-17. *Journal of Immunology*. Vol. 11 No. 181 pp. 7891-7901.

Szabó, I.; Wieler. L.H., Tedin, K., Scharek-Tedin, L., Taras, D., Hensel, A., Appel, B., Nöckler K. (2009). Influence of a probiotic strain of Enterococcus faecium on *Salmonella* enterica serovar Typhimurium DT104 infection in a porcine animal infection model. Applied Environmental Microbiology. Vo. 9 No. 75 pp: 2621-2628.

Torres, G.J.; Piquer, F.J.; Algarra, L., de Frutos, C. & Sobrino, O.J., (2011). The prevalence of *Salmonella enterica* in Spanish feed mills and potent*ial feed-related risk factors for contamination. Preventive Veterinary Medicine*. No 98 pp. 81-87.

Tsiloyiannis, V.K.; Kyriakis, S.C., Vlemmas, J. & Sarris, K., (2001). The effect of organic acids on the control of porcine post-weaning diarrhoea. *Research in Veterinary Science*. No 70 pp. 287-293.

Van der Wolf, P.J.; Wolbers, W.B., Elbers, A.R.W., van der Heijden, H.M.J.F., Koppen, J.M.C.C., Hunneman, W.A., van Schie, F.W. & Tielen, M.J.M., (2001a). Herd level husbandry factors associated with the serological *Salmonella* prevalence in finishing pig herds in The Netherlands. *Veterinary Microbiology* No 78 pp. 205-219.

Van der Wolf, P.J.; van Schie, F.W., Elbers, A.R., van der Heijden, H.M., Hunneman, W. A., Tielen, M.J. (2001b). Administration of acidified drinking water to finishing pigs in order to prevent *Salmonella* infections. *The Veterinary Quarterly* No. 23 pp. 121-125

Van Immerseel, F.; Russell, J.B., Flythe, M.D., Gantois, I., Timbermont, L., Pasmans, F., Haesebrouck, F. & Ducatelle, R. (2006). The use of organic acids to combat *Salmonella* in poultry: a mechanistic explanation of the efficacy. Avian Pathology. No 35 pp. 182-188.

Verbrugghe, E.; Haesebrouck, F., Boyen, F.L., Leyman, B., Van Deun, K., A. Thompson, A., Shearer, N., Van Parys, A., & Pasmans, F. (2011). Stress induced *Salmonella* Typhimurium re-excretion by pigs is associated with cortisol induced increased intracellular proliferation in porcine macrophages. *Proceedings of International Safepork congress, pp: 49-52*. Maastrich, Netherlands, 18-20 June 2011.

Visscher, C.F., Klein, G., Verspohl, J., Beyerbach, M., Stratmann-Selke, J. & Kamphues, J., 2011. Serodiversity and serological as well as cultural distribution of Salmonella on farms and in abattoirs in Lower Saxony, Germany. *International. Journal of Food Microbiology*. No. 146 pp. 44-51.

Wingstrand, A.; Jorgensen, L., Christensen, G., Thomsen, L.K., Dahl., J. & Jensen, B. (1996). Reduction of subclinical *Salmonella* infection by feeding coarse ground feed and adding formic acid to water. *Proceedings of 14th International Pig Veterinary Society Congress*. pp. 180 Bologna, Italy. 1996.

Van Winsen, R.L.; Lipman, L.J., Biesterveld, S., Urlings, B.A., Snijders, J.A., & van Knapen, F. (2001). Mechanism of *Salmonella* reduction in fermented pig feed. *Journal of the Science of Food and Agriculture*, No. 81 pp. 342-346.