

REVIEW OF RESEARCH INTO SALMONELLA INFECTIONS IN PIGS

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FOREWORD

The report reviews *Salmonella* research in pigs and the associated public health aspects with special reference to the Northern European situation. It should be remembered that some of the studies were performed under experimental conditions and that field investigations can only of necessity examine a small number of farms.

During the period 1990-2000, the number of scientific papers on *Salmonella* in pork has grown dramatically. The number of CAB abstracts has increased from 16 in 1999 to c.50 per annum, the more frequent topics are production food safety, including epidemiology and intervention, antimicrobial resistance and abattoir studies and interventions (Bahnson, 2001). I attended the 4th International Symposium on the Epidemiology and Control of *Salmonella* and other Food-borne Pathogens in Leipzig, Germany (2-5Sept.) and the 2nd OIE International Conference on Antimicrobial Resistance in Paris (2-4 Oct) to learn of recent developments and to liaise with active workers in the many different fields. I also visited Copenhagen to consult with staff at the following: the Danish Bacon and Meat Council, the Danish Veterinary Laboratory and the Veterinary School.

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HISTORICAL

The organism, now known as *Salmonella Choleraesuis* was first isolated from pigs by Salmon and Smith (1886), when they considered it to be the cause of swine fever (hog cholera). The importance of the organism as a cause of disease in pigs was neglected when the viral aetiology of swine fever was discovered, and a number of years elapsed before *S. Choleraesuis* was recognised as a primary pathogen that was capable of causing several different disease syndromes.

Pigs are also susceptible to *S. Typhimurium*, which is the more important pathogen in a number of countries, not only from the animal disease aspects but also from the public health point of view. A wide variety of other serovars have been isolated from pigs and although they may occasionally cause disease, in general, infected swine remain healthy carriers, but as a consequence are of public health importance. In addition to the economic impact of salmonellosis on the human population, it is also a major cause of economic loss in swine production, resulting in millions of dollars in lost income to the pork industry (Schwartz, 1990). *Salmonella Choleraesuis* although an important cause of disease in pigs in many countries is considered to be a host adapted serovar and seldom causes disease in man, though when human infection does occur it often causes a life threatening disease (Cherubin, 1980).

However the purpose of this review will be to consider the occurrence in pigs of *S. Typhimurium* and other food-borne *Salmonella* because of their major public health concern.

EPIDEMIOLOGY

Fuller information will be found in tables 1-13 of the annex on the different serotypes and phage types in pigs and in humans in the UK, France, The Netherlands and Denmark.

Prevalent serovars

In the UK, *S. Choleraesuis* was the predominant serovar in pigs during the 1950s-60s and in 1958 and 1968 it constituted 90% and 74.2%, respectively, of all *Salmonella* isolates from pigs (Sojka *et al.*, 1977). Subsequently its prevalence has declined in the UK and only 2 incidents of *S. Choleraesuis* were reported in pigs in 1999. It is now regarded as an infrequent isolate usually causing 1-2 incidents per annum. This seems to be the current situation in a number of other European countries (Laval *et al.*, 1992; Helmuth *et al.*, 1997; Baggesen *et al.*, 1997a) and also in Australia where the organism was last isolated in 1987 (Murray, C, personal communication). However, *S. Choleraesuis* is of importance in Poland and, perhaps other Central European countries, and it must always pose a threat to those countries where it is either absent or its prevalence is low. Interestingly, the first outbreak of *S. Choleraesuis* in Denmark since 1972 was reported recently by Baggesen *et al.*, (2000). Likewise, in North America, *S. Choleraesuis* remains a major problem for the pig industry (Wilcock and Schwartz, 1992). The reasons for the differences between North America and Europe are not known but may be related to husbandry practices.

In Europe, the predominant *Salmonella* serovars in a number of different countries are *S. Typhimurium* and *S. Derby*. In the UK in 1999, of the 262 *Salmonella* incidents reported in pigs, 67.9% were caused by *S. Typhimurium* and 11.5% by *S. Derby*; some of the other more common serovars were Goldcoast, Kedougou and Panama (Report, 1999). Also, in France, the Netherlands and Denmark, the most common isolates from pigs were *S. Typhimurium* and *Derby*. However, regional differences are apparent and in Germany and Denmark, incidents of *S. Agona* and *S. Infantis*

respectively are frequent.

In the USA, *S. Typhimurium* (including var. Copenhagen), *S. Derby* and *S. Choleraesuis* (var. Kunzendorf) are the 3 commonest serovars recovered from clinical submissions to the National Veterinary Services Laboratory (Ferris and Miller, 1998). The top 5 serovars recovered during a national prevalence study conducted in 1995 were *S. Derby* (33.5%), *S. Agona* (13.0%), *S. Typhimurium* (including var. Copenhagen) (14.7%), *S. Brandenburg* (8.0%), *S. Mbandaka* (7.7%) (Bush, E, personal communication). However, the recovery of serovar varies by source as well as geographic location (Davies *et al.*, 1997; Currier *et al.*, 1986). The number of reports of some other serovars has increased during the last decade but it is not known whether this is the result of better monitoring or whether it indicates an increased disease or environmental prevalence.

Phage Types of *S. Typhimurium* and antibacterial resistance

Of the different phage types of *S. Typhimurium*, the most frequent in pigs in the UK are DTs 104, 193 and 208, all of which are resistant to a number of antibiotics (Wray *et al.*, 1997). In 1999, only 15.1% of the *Salmonella* isolates from predominantly diseased pigs were fully susceptible to the antibiotics used for monitoring; resistance to tetracycline being the most frequent, ranging from 77–83% of the cultures tested during the period 1995-99. Resistance to other antibacterial drugs was also frequent and in the case of streptomycin, sulphonamides, ampicillin, trimethoprim/sulphonamide and chloramphenicol usually exceeded 30% of the cultures tested. Resistance to nalidixic acid was also detected, which indicates a reduced susceptibility to fluoroquinolone drugs such as enro- and ciprofloxacin (Davies *et al.*, 1999a). Resistance to the aminoglycosides; neomycin and apramycin, was usually less than 5% of the cultures tested. Likewise, in The Netherlands, the penta-resistant *S. Typhimurium* DT 104 was a frequent isolate but overall of the 115 *Salmonella* isolates

tested only 19 were resistant to 2 or 3 antibacterials (van der Wolf *et al.*, 1999a). An increasing prevalence of resistance in *Salmonella* to quinolones has also been detected in Germany (Malorny *et al.*, 1990) and in France (Corre *et al.*, 1999). In Denmark, *S. enterica* isolated from 247 of 317 (81%) herds investigated were fully susceptible to the antimicrobial agents tested. In 48 (16%) the isolates were resistant to 1-3 antibacterial drugs and in 10 herds multiple resistant isolates were detected (Baggesen *et al.*, 1999a). Multiple resistance was detected in a single isolate of DT 104 as well as other serovars and phage types. Phage typing of veterinary isolates of *S. Typhimurium* in the USA was initiated in 1996 and *S. Typhimurium* DT 104 has been isolated from a number of species including swine. In 1997, 19% of the diagnostic and 44.2% of the slaughter isolates were fully susceptible to all antimicrobials. Multiple resistance to 5 or more antimicrobials was observed in 23.3% of diagnostic and 16.8% of slaughter isolates (NARMS-EB, 1997). Gray *et al.*, (2001) reported an increasing prevalence of resistance to expanded spectrum cephalosporins (ceftriaxone) among a number of different swine *Salmonella* isolates; many of the isolates were resistant to eight or more antibacterial drugs.

In the UK, a new phage type of *S. Typhimurium*: U 310, which is resistant to tetracyclines, is increasingly being recognised in pigs and associated with human illness (Ward and Threlfall, 2001).

On farm studies

Surveys have been done in many countries to determine the number of pig farms on which *Salmonella* is present and its prevalence, but it is often unclear as to how representative the farms are, how they were selected and the sensitivity of the detection techniques. However, it must not be assumed that all farms are contaminated with *Salmonella* e.g. Stege *et al.*, (2000) using culture and ELISA detected 23/96 farms on which there were no positive pen samples and all pigs had negative serological results. In the UK, Davies and Wray, (1997a) detected *Salmonella* on all of the 23 farms investigated, whereas Pearce and Revitt, (1998)

found 14.8% (4/27) farms positive, with 12.5% of the finishers being infected. Data from Denmark show that the herd prevalence in 1993/4 was 22% and in 1998 it was 11.4%. Of the different herd types, the percentages positive for breeding/multiplying, farrow to grower, and finisher were 11.7%, 16.7% and 11.4% respectively (Christensen *et al.*, 1999). In The Netherlands, *Salmonella* were isolated from the faeces of pigs in 71 of the 306 herds that were tested (van der Wolf *et al.*, 1999a). In Germany 27% of herds were positive (Felhaber *et al.*, 1996) and a small survey in Austria detected *Salmonella* on 4.3% of farms (Kofer *et al.*, 2000). In France, Beloeil *et al.*, (1999) isolated *Salmonella* on 40 of the 69 finishing farms, which belonged to 5 companies; the most prevalent serovars were *S. Typhimurium* and *S. Derby*. A similar survey in the USA (Bush *et al.*, 1999) detected *Salmonella* on 58 of 152 farms, where the most common serovars were *S. Typhimurium* and *Derby*, the prevalence being higher in the SE states (65.5%) than in mid-Western states (29.9%). In the northern states of the USA, 96 of 142 (67%) herds were positive for *Salmonella* (Damman *et al.*, 1999). In Canada, *Salmonella* were isolated on 16/28 herds examined (Quessy *et al.*, 1999). In Greece, a serological survey by Grafanakis *et al.*, (2001) detected seropositive pigs on 21 of 59 farrow-finish herds that were tested but only one of the 5 multiplying herds.

Molecular genetic techniques are being increasingly used in a number of countries to study the epidemiology, especially on-farm, of *Salmonella* in pigs (Sandvang *et al.*, 2000; Skov *et al.*, 2001; Davies, R personal communication). Weigel *et al.*, (2001) compared 2 different methods (pulsed field gel electrophoresis and repetitive sequence polymerase chain reaction) to identify patterns of transmission on pig farms and considered the latter method had the greater precision in differentiating some *Salmonella* resident on farms.

On farm variation

Even on farms where *Salmonella* has been detected there does seem to be variation in its distribution, not only with regards to the age of the pigs but also

to the buildings and pens. Carlson and Blaha, (1999a) found wide variation in different shipments from the same farm and also in different sites. Van der Wolf *et al.*, (1999b) did a longitudinal study on 5 sero-positive and 5 sero-negative farms, all the positive farms remained contaminated but even on these farms some groups of pigs and pens were negative. The negative farms remained free from *Salmonella* for long periods but were always at risk of infection.

Age of Pigs

There seems to be variation in which age group of pigs *Salmonella* is most prevalent. Van Schie and Overgoor (1987) found a high *Salmonella* prevalence in sows with the boars' areas being the most contaminated, with a constant cycle with the non-immune gilts. However, Barber *et al.*, (1999) found the highest prevalence in finishers, but commented that the organism was present in all age groups, whereas Weigel *et al.*, (1999) considered that prevalence increased with age on the 6 farrow to finish farms that they investigated, being 1.4% in suckled pigs and 6.2% in sows. Funk *et al.*, (1999a) found wide variation in the age of pigs in which the prevalence of *Salmonella* was highest. In Canada, Letellier *et al.*, (1999a), found that 15.9% of the replacement sows were positive and 21.9% of the gilts and they suggested that vertical transmission was occurring throughout all the different production stages. In the UK, Davies and Wray (1997a) found that on Breeder farms, gilts and boars were more heavily infected with *S. Typhimurium* than adult sows, and on a breeder/ rearer farm the prevalence for weaners, growers and fatteners ranged from 32-44%. In Ireland, Rowe *et al.*, (2001) isolated *Salmonella* from 30 of the 59 farrow-to-finish farms sampled. Herds classified as low risk were as likely to be positive as high risk farms. The prevalence of infection was 2.3% in lactating sows, 5.1% in dry sows, 4.6% in gilts, 5.9% in fatteners, 4.8% in second stage weaners and 8.0% in first stage weaners. The predominant serotypes were *S. Typhimurium* and Derby. However, the role of vertical transmission has been questioned

because of the success of strategic removal of weaners to clean premises (Dahl *et al.*, 1997a). Davies, *et al.*, (1998) examined faeces obtained from 792 pigs on swine housed on 7 farms: 1 gilt development farm, 2 breeding farms, 1 nursery farm and 3 finishing farms, *Salmonella* was isolated from all farms and from 12% of faeces. Prevalence of *Salmonella* ranged from 3.4% at the gilt development farm to 18 and 22% respectively at the breeding farms. The most prevalent serovar on the finishing farms was *S. Typhimurium*, which was not isolated on the breeding or nursery farms. They consequently concluded vertical transmission was unimportant as source of *Salmonella* for finishers and high prevalence in breeders implicated feed. This is agreement with the findings of Berends *et al.*, (1996). However earlier studies of farrow to finish farms indicated that boars and sows play an important role in maintaining *Salmonella* infection on farms and more recent publications indicate that though breeding herds may be a minor source of infection for finishers they can play an important role in the transmission of *Salmonella* to other farms (Davies *et al.*, 2000).

A six year study by Davies *et al.* (2001a) on a 400 sow farrow to finish farm initially detected *Salmonella Typhimurium* DT 104 in breeding stock and in weaner and finishing pigs, and 3 other serovars in the breeding stock. In the second year, a different genotype of DT 104 was detected and in the third year 2 different phage types of *S. Typhimurium* appeared and also another different serovar in the breeding stock. A study was performed by Lo Fo Wong *et al.*, (2001) to assess the stability of an assigned *Salmonella* status of finishing pig herds over a two year period. Of the finishing herds studied, 62% changed from their initial status at least once and steady status periods varied from one month to more than two years. The odds for finishers being positive were ten times higher if growers were positive in the previous round of sampling than if they were seronegative. When *Salmonella* was detected in pen faecal samples in the same sampling round, the herd was 4 times more likely to be

seropositive, as compared with the absence of *Salmonella*.

A strong association between the seroprevalence in sows and the occurrence of *S. Typhimurium* among weaners was observed by Kranker and Dahl (2000), who commented that ready-mixed feed and herd health status were risk factors for sows.

The *Salmonella* seroprevalence in finishing pigs and sows for regular, free range and biologic-dynamic (certified system with extra controls over free range) pig herds in The Netherlands was determined by van der Wolf, (2000). (See below).

***Salmonella* seroprevalence for finishing pigs and sows in the Dutch pig population (van der Wolf, 2000).**

| | No of Samples | Percentage positive |
|------------------------|---------------|---------------------|
| Regular finishers | 1760 | 11.1 |
| Regular sows | 1902 | 9.9 |
| Free range finishers | 56 | 25 |
| Free range sows | 66 | 10.6 |
| Bio.-dynamic finishers | 16 | 12.5 |
| Bio.-dynamic sows | 42 | 4.8 |

The *Salmonella* seroprevalence in the free range finishers is significantly higher than in the other 2 systems, though the sows do not differ significantly from each other. The *Salmonella* prevalence was studied on organic, free range and conventional pig farms by Wingstrand *et al.*, 1999, who found that the relative risk for free range as opposed to conventional farms was 1.7 with an apparent increase in risk for organic farms, although the number of farms was too small to make valid conclusions. They suggested that because the number of alternative systems are likely to increase in the future more studies should focus on the identification of risk factors for these systems.

Transmission on Farms

Historically, transmission of *Salmonella* between pigs is thought to occur via the faecal-oral route of exposure. Since *Salmonella* are often shed in large numbers in the faeces, it is not improbable to expect that this is a major route for transmission of the organism. A number of studies have reproduced experimental infection by the oral route and during acute disease pigs will shed up to 10^6 *S. Choleraesuis* (Smith and Jones, 1967) or 10^7 *S. Typhimurium* (Gutzmann *et al.*, 1976) g^{-1} faeces. Generally high doses have to be used and disease is frequently difficult to reproduce. Dawe and Troutt (1976) produced moderate disease following oral inoculation of 10^6 cells, but most authors report successful disease reproduction with doses of 10^8 - 10^{11} *Salmonella* (Gray *et al.*, 1995) which are artificially high and unlikely to occur under natural conditions.

However, as early as 1965, de Jong and Ekdahl (1965), after oesophgectomizing calves and giving an oral challenge, proposed that both hematogenous and lymphatogenous routes of infection are important in the dissemination of *Salmonella*, particularly *S. Typhimurium*. Hardman *et al.*, (1991) further demonstrated that calves penned individually were susceptible to infection with *Salmonella*. Aerosol experiments in chickens and mice have shown that infections with *Salmonella* species can be achieved more regularly via the lungs than by oral inoculation (Clemmer *et al.*, 1960, Darlow *et al.*, 1961). This gave support to the role that aerosols may also play in transmission and dissemination of *Salmonella*.

Further studies in swine using oesophagotomy indicated that the upper respiratory tract may be equally important in transmission (Fedorka-Cray *et al.*, 1995) and that the tonsils and lungs may be important sites for invasion and dissemination of *Salmonella* species. Pneumonia associated with *S. Choleraesuis* infection has been previously described (Baskerville and Dow, 1973) and a recent increase in *S. Choleraesuis* associated pneumonia has been

reported in the USA (Turk *et al.*, 1992). It is unclear whether this predilection for the lung is due solely to the pathogen, poor ventilation in large confinement buildings or some combination of these and other factors. Experimental infection models have not provided good answers because positive lung samples have been regarded as an artifact of intranasal or *per os* inoculation. However, in addition to the oesophagotomy work by Fedorka-Cray *et al.*, (1995), Gray *et al.*, (1996b) also demonstrated that the lung is colonized in swine that are naturally exposed to pigs infected with *S. Choleraesuis* indicating that lung colonization is not an artifact of experimental inoculation. More recent experiments, (Proux, K, personal communication) in which pigs that were kept separate with their only contact via an airspace, showed that infection could be transmitted by aerosol. Collectively, these studies indicate that the traditional paradigm of faecal-oral transmission is no longer valid and that other possibilities, such as aerosol or dust-borne transmission, need to be considered.

Environmental considerations

Although transmission of *Salmonella* typically occurs through faecal-oral or aerogenous transmission, other vectors must be considered when discussing dissemination of the organism. In pigs, observed sources of contamination include rodents, insects, humans and contaminated feed and feedstuffs (McChesney *et al.*, 1995). Mice faecal pellets have been shown to contain up to 10⁵ cfu *Salmonella* (Henzler and Opitz, 1992). During an investigation of *Salmonella* contamination, which involved 23 pig farms, Davies and Wray (1997a) found a wide range of wild animals, that included rats, mice, cats, and birds to be infected. Cats and birds were associated with contamination of feed and grain stores, and rodents were involved in the perpetuation of infection in specific buildings on the farm. Infected foxes were most common on out-door breeding farms. Flies and dust can also act as mechanical vectors that spread *Salmonella* throughout the facility or environment (Edel *et al.*, 1967; 1970).

Salmonella may persist in the environment for long periods and Linton *et al.*, (1970) considered such persistence an important risk factor. Berends *et al.*, (1996) suggested that contamination of endemic flora in finishing sites was the predominant source of infection of finished pigs, rather than infection originating from breeding farms or other sources, though the latter is more important when pigs from various sources are mixed on finishing farms. Baggesen *et al.*, (1997b) isolated *Salmonella* from faeces, pens, dust, equipment, ventilation equipment and slurry during their studies on pig farms. Gray and Fedorka-Cray (1995) showed that *S. Choleraesuis* survives in dry faeces for at least 13 months post-shedding demonstrating the importance in clearing organic matter from the environment. Davies and Wray (1997a) found a high level of *Salmonella* persisting in pig pens after disinfection and the organism persisted in soil for at least 6 months on outdoor farms and mice (Davies *et al.*, 2001a). In porcine slurry *Salmonella* Typhimurium DT 104 was isolated from 16 of the 18 herds studied with most probable number estimates from less than 0.02-23 cfu/g slurry. The prevalence of seropositive meat juice samples in the last month of filling the slurry tank was found to be a significant predictor of the slurry contamination level (Bagger *et al.*, 2001).

Animal Feed

It is well known that animal feeds frequently contain *Salmonella* and that animals fed contaminated feed often become infected (Linton and Jennet, 1970) and a detailed account of the differing aspects and control of *Salmonella* contamination of animal feed is given by Davies and Hinton (2000). The rate of contamination of animal protein delivered to a large feed-mill in the South-Eastern part of the USA was reported over a 10 month period (Williams *et al.*, 1969). Of 311 samples, 68% contained one or more of the 68 *Salmonella* serovars identified in the study. Eighty-six percent of the meat-meal and 18% of the fish-meal sampled were found to be contaminated. Davies and Wray (1997) carried out detailed sampling of spillage and dust from milling equipment in 9 animal feed mills. The *Salmonella* isolation rate ranged from 1.1%-41.7% of the

samples and the most contaminated mills were from those where the inside of the cooling systems for pellet or mash had been colonised by *Salmonella*. A wide range of *Salmonella* serovars were found and included *S. Typhimurium* and *S. Enteritidis*. In the UK, tests were performed on 62470 samples of feeding stuff and ingredients and 1.9% of the samples were *Salmonella* positive (Report, 1999). In another study to determine the prevalence of *Salmonella* in swine feeds, 2.8% of the feeds and feed ingredients taken from farm environments were positive for *Salmonella* (Harris *et al.*, 1997). Feed trucks have also been implicated as a source for feed and feedstuff contamination (Fedorka-Cray *et al.*, 1997a). Jorgensen *et al.*, (1999) compared the effect of feeding pellets and meal on the prevalence of *Salmonella* in pigs, they found that meal reduced the risk but it also reduced the feed conversion. Similar findings were also made by Hansen *et al.*, (2001a), who also found that dried sugar beet pulp reduced *Salmonella* prevalence significantly and did not affect production. Likewise finely ground meal presented a greater risk than coarsely ground meal. Dahl *et al.*, (1999) found that adding non-heat treated wheat or barley to pelleted feed reduced the prevalence of *Salmonella* shedding. They suggested that the use of coarsely ground barley improved the microbial ecosystem by increasing the numbers of *Lactobacilli* and producing more acidic conditions in the gastro-intestinal tract (Prohaszka *et al.*, 1990). Barley made the intestinal contents firmer as compared with wheat and they concluded that 25% of the ration should be barley. Field studies in Denmark found a lower prevalence of *Salmonella* on farms mixing their own feed and feeding liquid feed (Bager, 1994).

In The Netherlands, it was found that the prevalence of *Salmonella* infection was 10 times lower in pigs fed “porridge” than those fed dry feed (Tielen *et al.*, 1997). A study of 40 fattening farms in The

Netherlands isolated *Salmonella* from 19.4% of the samples from farms using whey as compared with 64.4% of farms using water (van Schie and Overgoor, 1987). Many recent studies have shown that liquid feed especially fermented liquid feed (FLF) reduce the risk of *Salmonella* infection in pigs (Dahl, 1998; van der Wolf *et al.*, 1998; Lo Fo Wong *et al.*, 1999), though van Winsen *et al.* (2001) and McLaren *et al.* (2001) were unable to find a reduced *Salmonella* prevalence. It has been suggested that FLF reduces the stomach pH, and reduces the bacterial numbers, especially enterobacteria, in the small intestine by the production of fatty acids (Mikkelsen and Jensen, 2000). As a consequence different acids have been added to drinking water and feed. Hansen *et al.*, (1999, 2001b) used formic acid, and found that it affected the microbial composition in the intestine and suggested that it may reduce *Salmonella* prevalence. Preliminary experiments by van der Wolf *et al.*, (1999c) used a mixture of acids including formic and lactic acid and found 17.8% of the treated positive as compared with 24.7% of the controls. The effect of feed’s form and acidification with lactic acid was studied in weaners by Jorgensen *et al.* (2001) who found that the addition of 2.8% lactic acid reduced the number of *Salmonella* positive pens and improved productivity. In *in vitro* experiments, van Winsen *et al.*, (1999a) found fermentation produced lactic and acetic acid and when the acids were used alone, acetic acid was bacteriostatic and lactic acid bactericidal. The bactericidal activity was stronger when the 2 acids were used together. A word of caution is necessary, however, as addition of yeast to liquid feed on a farm produced alcohol which caused the death of a number of pigs.

RISK FACTORS FOR SALMONELLA INFECTION OF PIGS

Various surveys have been undertaken, to determine the factors which predispose to *Salmonella* infection in pigs, but these are often by questionnaire and it is not known what the controls were built into the questionnaire to ensure the validity of answers.

Feed

Proprietary rations increased the risk (1998). Meal reduces risk as compared with pelleted feed but the risk was three times greater when finely ground as compared with coarsely ground. [But reduced feed conversion] (Jorgensen *et al.*, 1999).

Use of liquid feeding reduces risk (Dahl, 1998; van der Wolf *et al.*, 1998).

(Re)contamination of feed, odds ratio (OR) 1.6 (Berends *et al.*, 1996).

Dry feed (Beloeil *et al.*, 1999; Lo Fo Wong *et al.*, 1999).

No use of whey, OR 5.6, (Lo Fo Wong *et al.*, 1999).

Husbandry

Concrete walls between pens and a central slurry collection reduced the risk.

Transfer of piglets at a younger age gives better lactogenic immunity (van der Wolf, 1998).

Poor hygiene *e.g.* ineffective C and D, dirty boots (Barber *et al.*, 1999) OR of 39.7 (Berends *et al.*,

1996).

Poor biosecurity, more than 2 people present on the site each day and hygiene. *e.g.* absence of a toilet for the workers (Funk *et al.*, 2001).

Rodents, wild birds, *etc.* (Davies and Wray, 1997a; Weigel *et al.*, 1999). Presence of other domestic animals on the farm (Funk *et al.*, 2001).

Duration of time between batches of finishing pigs, [<1 day increased risk] (Beloeil *et al.*, 1999; Lo Fo Wong *et al.*, 1999).

Concurrent Disease and production performance (Beloeil *et al.*, 1999; Bush *et al.*, 1999; Funk *et al.*, 2001).

Single sex pens (Bush *et al.*, 1999). Amount of floor space, above a median density increased the risk (Funk *et al.*, 2001).

Management: relative risk for free range v. conventional housing = 1.7; possible increased risk with organic farms (Wingstrand *et al.*, 1999).

Negative association between faecal accumulation in pen and *Salmonella* shedding by pigs (Funk *et al.*, 1999b).

Mixing of pigs from different sources (Bush *et al.*, 1999).

Risk Factors for *Salmonella* infection of pigs (Lo Fo Wong *et al.*, 1999).

| Variable | Parameter | Odds Ratio |
|----------|-----------------------|------------|
| FEED | Pelleted and dry | 8.2 |
| | Pelleted and Wet | 10.4 |
| | Non-pelleted and dry | 4.2 |
| | Non-pelleted and wet | 1.0 |
| BATCH | Continuous production | 2.0 |
| | Batch Production | 1.0 |
| WHEY | No use of whey | 5.6 |
| | Use of whey | 1.0 |

DISEASE IN SWINE

Associated serovars

Clinical swine salmonellosis can be separated into two syndromes, one involving *S. Typhimurium* which is associated with enterocolitis, while the other involves *S. Choleraesuis* and is usually associated with septicaemia. Clinical porcine salmonellosis is almost solely due to infection with *S. Typhimurium* or *S. Choleraesuis*. Clinical disease has also been associated with *S. Typhisuis*. This serotype is difficult to isolate and because of this difficulty may be responsible for more outbreaks than it is directly associated with by culture (Wilcock and Schwartz, 1992). In addition, there have been reports of both *S. Dublin* (Lawson and Dow, 1966) and *S. Enteritidis* (Reynolds *et al.*, 1967) causing disease in swine. A survey of the literature shows that clinical disease has been associated with many other serovars but the fact that they are reported suggests that they may be considered an uncommon occurrence.

Populations affected

Intensively reared weaned pigs are most often affected by *Salmonella* infection. In general, *S. Typhimurium* tends to cause disease in young pigs from six to twelve weeks of age. Disease from this serovar is rare in adult animals but infection is frequent. *Salmonella Choleraesuis* causes disease among a wider range of ages. Mortality tends to be higher in younger rather than older pigs, while morbidity is often equal regardless of age.

The occurrence of salmonellosis in sucking pigs is rare, presumably because of lactogenic immunity, but infection is not uncommon (Gooch and Haddock, 1969; Wilcock *et al.*, 1976). However, neonatal swine are susceptible to oral challenge with *Salmonella* and develop disease similar to that observed in weaned pigs (Wilcock and Olander, 1978).

Septicaemia

The septicaemic form of porcine salmonellosis is usually caused by *S. Choleraesuis* although other serovars may occasionally cause acute disease. The clinical signs and post-mortem findings are well described in the standard text books and will not be considered further (see Wilcock and Schwartz, 1992; Wray and Wray, 2000).

Enterocolitis

Enterocolitis in pigs is typically associated with *S. Typhimurium* infection and occasionally with infection caused by *S. Choleraesuis* and other serovars. In contrast to the septicaemic disease, the initial sign of infection is often watery, yellow diarrhoea. Infected pigs are inappetent, febrile and lethargic. Mortality is usually low. However, morbidity can be high within a few days after infection (Wilcock and Schwartz, 1992). The economic cost of an outbreak of *S. Typhimurium* infection, in which 40/505 of the pigs developed diarrhoea was calculated by Neumann and Kniffen, (1999) to be \$2.15-3.35 per head (See below).

Estimated costs associated with *Salmonella enteritis* outbreak (Neumann and Kniffen, 1999).

| Parameter | Effect | Cost/ head (\$) |
|--|--|-----------------|
| Average daily gain (g) | Reduced by 45 | 0.60 |
| Antibiotic therapy | Mass medication and individual therapy | 0.30 |
| Substandard pigs | Increased by 25% | 1.00 |
| Mortality | Increased by 3% | up to 1.20 |
| Quarantine and biosecurity | Various | 0.25 |
| Total cost on sites related to mortality | | 2.15-3.35 |

The major gross lesion at necropsy is focal or diffuse necrotic colitis and typhlitis. Mesenteric lymph nodes are greatly enlarged. Intestinal lesions develop as red, rough mucosal surfaces that may also have grey-yellow debris. Colon and caecal contents are bile stained and scant, often with black or sand-like gritty material on the surface. Intestinal necrosis may be seen as sharply delineated button ulcers often associated with resolving lesions (Wood and Rose, 1992; Wilcock and Olander, 1978; Wilcock and Schwartz, 1992). In cases of *S. Typhimurium* enterocolitis, the liver and spleen are not enlarged except by terminal congestion (Wilcock and Schwartz, 1992).

Histopathological examination reveals necrosis of cryptic and surface enterocytes, which may be local or diffuse. The lamina propria and submucosa contain macrophages and lymphocytes with neutrophils observed only in the very early stages of disease. It is not uncommon to see lymphoid atrophy or regenerative hyperplasia associated with this disease (Wilcock *et al.*, 1976; Jubb *et al.*, 1985; Reed *et al.*, 1986).

Virulence factors/ Pathogenesis

Many potential virulence factors have been identified for *Salmonella* species but few have been tested critically for their contribution to virulence and the subject is reviewed in Wray and Wray, (2000). It has been estimated that *Salmonella* species possess over 200 virulence factors, of which only a fraction have been characterized. Many studies have relied on *in vitro* data to draw their conclusions. This makes it difficult to develop meaningful extrapolations for human and animal disease. In addition many studies utilize mice as a model for disease and these results often cannot be repeated in other hosts and few studies have been done in pigs.

Pospischil *et al.*, (1990) used immuno-labelling techniques (peroxidase and immunogold) to detect and locate *Salmonella* in the tissues of experimentally infected pigs. *Salmonella*

Typhimurium had a low tendency to invade the enteric mucosa and did not reveal any predilection for a specific intestinal location. In contrast, *S. Choleraesuis* was located preferentially in the colon and on the luminal surface of the ileal M cells of the Peyer's Patches from where it had a tendency to invade. However, Bolton *et al.*, (1999) found no evidence to support Peyer's Patches especially M cells, as the major route of entry of *Salmonella* because the numbers of *Salmonella* were similar in the absorptive mucosa to that of the Peyer's Patches. Studies *in vitro* suggest that *S. Typhimurium* may adhere by means of mannose-resistant adhesins (Jones *et al.*, 1981; Isaacson *et al.*, 1992), and invasion of Madin Darby canine kidney cells by *S. Choleraesuis* has been shown to be an active process requiring bacterial RNA and protein synthesis (Finlay *et al.*, 1988). These findings suggest that it may be possible to develop vaccines which prevent the adherence of the *Salmonella* to the intestinal mucosa.

Several serovars have been shown to produce enterotoxins, specifically cholera-like toxin (Prasad *et al.*, 1990). Very little is known about this toxin as it relates to the pathogenesis of *Salmonella* species. A common feature of *Salmonella* species induced enteritis is severe damage to intestinal epithelial cells, likely to be the result of a cytotoxin. At least three cytotoxins have been identified. A wide variety of serovars possess a heat-labile cytotoxin described by Ashkenazi *et al.*, (1988). Another cytotoxin is a low molecular weight membrane associated toxin, which has not been characterized (Reitmeyer *et al.*, 1986). A third toxin, described by Libby *et al.*, (1990), appears to be present in nearly all *Salmonella* species, *Shigella* and enteroinvasive *E. coli*. This cloned protein is a 26 kDa cell-associated hemolysin and its role in virulence is under study.

Roof and Kramer (1989) showed that virulent *S. Choleraesuis* were able to survive within porcine neutrophils by inhibiting superoxide anion production and resisting the bactericidal activity of the cells. Heat shock proteins have been shown to be produced by *S. Typhimurium* inside murine

macrophages. Mutants that are defective in this ability to produce these proteins are less virulent in mice and do not survive well in macrophages (Falkow and Mekalanos, 1990). Watson *et al.*, (2000) studied the interaction of different *Salmonella* serotypes with porcine macrophages *in vitro*, *S. Typhimurium* persisted longest and there was little or no significant difference in induction of pro-inflammatory cytokines by macrophages. *Salmonellas* Typhimurium and Dublin damaged macrophages but not *S. Choleraesuis* and they concluded that there was no correlation with virulence. Riber and Lind (1999) found a variation in the bactericidal activity of phagocytes from different pigs against *S. Typhimurium*. The phagocytosis of *S. Choleraesuis* in the lungs of pigs was studied by Baskerville *et al.*, (1972) during the period 6 h-14 days after intranasal infection. All bacteria were phagocytosed soon after arrival by polymorphonuclear leucocytes and macrophages; many were destroyed but some survived and multiplied within the cells. Between days 5-7, the *Salmonella* caused necrosis of the phagocytes and were liberated in large numbers and caused damage of the lung tissues. Later studies showed that the free bacteria did not attach to or penetrate pulmonary cells and they suggested the damage was caused by toxin (Baskerville *et al.*, 1973). Matalova *et al.*, (2000) suggested that the presence of living *Salmonella* in pig leucocytes delayed the onset of apoptosis. This would lead to the hypothesis that infection may be prolonged in this situation.

The LPS of *Salmonella* species is a major determinant of host specificity and virulence (see Wray and Wray, 2000). The intact LPS affords

resistance to phagocytosis and killing by macrophages and complement-mediated killing (Saxen *et al.*, 1987; Robbins *et al.*, 1992). In addition it has been shown that LPS is a major contributor to survival of *Salmonella* species in the intestinal tract (Nnalue and Lindberg, 1990). The LPS component of *Salmonella* species also contributes to vascular damage and thrombosis. Endotoxic properties result in fever, disseminated intravascular coagulation, circulatory collapse and endotoxic shock associated with salmonellosis (Takeuchi and Sprinz, 1967).

Motility provided by flagella appears to be important for invasion for some, but not all, serovars of *Salmonella*. Regardless of the other contributions the flagella may make, their presence increases the probability that the organism will come in contact with an epithelial cell. It has been shown that strains with polar rather than peritrichous flagella have increased ability to come in contact with, and potentially invade, epithelial cells (Jones *et al.*, 1992).

A siderophore has been identified in *S. Typhimurium* called enterobactin (Benjamin *et al.*, 1985). This protein does not appear to be necessary for full virulence and the importance of the protein may be relative to the amount of extracellular growth, which occurs. Interestingly, pigs infected with *S. Choleraesuis* have a reduction in serum iron, total-iron binding capacity and transferrin. The intracellular environment is low in iron and it has been suggesting that *S. Choleraesuis* has a non-siderophore mechanism for scavenging iron (Clarke and Gyles, 1993).

TREATMENT

Various antibiotics have been used to treat severe *Salmonella* infections in pigs, but actual controlled trials in the field to judge their efficacy are few. In experimental infections, sub-therapeutic levels of tetracyclines have been shown to reduce *Salmonella* shedding when the organism was sensitive but had no effect when it was resistant (Clausen *et al.*, 1998). Apramycin/ oxytetracycline were used to treat experimental *Salmonella* infections in pigs and shedding of *Salmonella* was reduced in those pigs on antibiotics (Ebner and Mathaw, 2000). Likewise, Schwartz and Lucas (1994) found that pigs receiving Aureo sp250 medicated feed and subsequently challenged with *S. Choleraesuis* had lower mortalities, less severe clinical illness, better growth rates and feed conversion than those which received no medication. Wilcock and Schwartz (1992), mention a number of trials, but the general conclusion was that therapy was equivocal or of little merit under field conditions. Antimicrobials have also been used to reduce the shedding of *Salmonella* by sick or recovered pigs, and once again there is no evidence as to their efficacy. However, anecdotal information from practitioners suggests that severe *Salmonella* infections will respond to appropriate antimicrobial therapy. Ancillary therapy such as the use of fluids to replace lost electrolytes and to prevent dehydration will assist recovery. The association between antimicrobial resistance and usage was investigated by Bahnson and Fedorka-Cray (1999) who found that there was a relationship between tetracycline resistance and usage but not between any of the other antibiotics. Further studies, Fedorka-Cray *et al.*, (1999) suggested that *Salmonella* isolates from lymph nodes were more likely to be resistant than those from other sources. However, Blaha *et al.*, (1999a) could find no difference in the frequency of resistance between strains from lymph nodes and those from the environment. They suggested that

antimicrobial resistance in *Salmonella* is much more complex than just a consequence of antimicrobial usage in animals and further research is necessary.

The use of antibacterial drugs for growth promotion is controversial but Baggesen *et al.*, (1999c) found that tylosin did not promote the shedding of *Salmonella* in pigs, and Shryock *et al.*, (1998) found that prolonged feeding of tylosin reduced the duration of *Salmonella* shedding in pigs. The use of Flavophospholipol (Bambermycin) has been shown to reduce *Salmonella* shedding in pigs and to reduce the level of resistance in enteric micro-organisms (Dealy and Mueller, 1976; Oostenbach, 2001). Studies on the productivity and economic impacts of feedgrade antimicrobial use in pork production (Algozin *et al.*, 2001) indicated that the average daily weight gain and feed conversion ratio improved by 0.9% and 2.3% respectively and finisher mortality was reduced by 0.29%. Though they considered that pork producers might be reluctant to produce pigs without the use of antimicrobials they also suggested that there is a need for carefully controlled field trials to assess their benefit and an assessment of the risk for human health. In countries where the use of antibiotic growth promoters has been phased out, disease in pigs increased in prevalence and necessitated higher usage of antibacterial drugs such as tetracyclines.

In the UK the use of antibiotics in pig husbandry has declined from 121 tonnes in 1997 to 89 tonnes in 1999 (Report, 2001). Dunlop *et al.*, (1998) assessed antibiotic usage on 34 farms, of which none used antibiotics in pigs after weaning, they found that treatment records underestimated usage by at least 35%. The rates varied from 0-24.1 pig/ 1000 pig days, median 5.29. Penicillin was the most common antibiotic used and most treatment was given to sows and piglets at parturition.

DETECTION OF *SALMONELLA*

Culture

A great interest has developed in the animal production and food processing industries to create and evaluate new methods to detect, either directly or indirectly, the presence of *Salmonella* species. Traditional culture methods may take 3-5 days to complete and much effort has been directed towards finding more rapid methods. However, the culture of *Salmonella* is the standard by which all other methods are measured. Recovery of the organism is the only means by which definitive serotyping, and other techniques which may be of value epidemiologically, can be achieved. In addition, the isolation of the organism serves as an invaluable source of epidemiological data, which cannot be overlooked.

Most of the original methods were developed for the diagnosis of clinical salmonellosis in man and other animals. In swine, clinical salmonellosis is, with the exception of *S. Choleraesuis* infection, uncommon. The sensitivity of the culture method may also be affected by the phase of the infection. In acute salmonellosis, large numbers of *Salmonella* are shed in the faeces, whereas a chronically infected pig or a carrier may only excrete low numbers of *Salmonella* intermittently. Thus for clinical samples direct culture may suffice, whereas samples from chronically infected pigs or from the environment will almost certainly require pre-enrichment and selective enrichment.

Many different culture media and methods have been developed and used for *Salmonella* detection and it is well known that for the isolation of *S. Choleraesuis* some of the more common enrichment media e.g. tetrathionate broth and selenite F broth, may be toxic for the organism (Smith, 1952). It has been suggested that this may explain the infrequent isolation of *S. Choleraesuis* in swine during epidemiological surveys (Ewing, 1986). More recently, Davies *et al.*, (1997) failed to isolate *S. Choleraesuis* on XLT4 medium, though the organism grew on modified brilliant

green agar. It should also be remembered that the classical *S. Choleraesuis* does not produce H₂S and may be missed on media such as XLD, which incorporates a H₂S indicator.

From the literature it is often difficult to evaluate isolation techniques because of the lack of an agreed standard method against which other techniques can be compared; the problem being exacerbated by the wide variety of different samples/ materials examined. When possible, a combination of enrichment media should be employed and may include GN-Hajna broth and tetrathionate broth for the isolation of host adapted serovars as well as broad host range *Salmonella* species (Ewing, 1986; Fedorka-Cray *et al.*, 1996).

Many plating media have been devised for the isolation and differentiation of *Salmonella* species (Wray and Wray, 2000) and the choice of the media will be governed by the operator's experience and requirements. A recent study compared Hektoen enteric (HE) agar, Rambach agar, *Salmonella* identification (SM-ID) medium, xylose-lysine-Tergitol 4 agar (XLT4), novobiocin-brilliant green-glycerol-lactose agar (NBGL) and modified semisolid Rappaport Vassiliadis medium (MSRV) for the isolation of *Salmonella* species. The test of these relatively new media found MSRV to be the most sensitive and specific but it was also the most difficult to use. The XLT4 plates were found to be as sensitive as HE with improved specificity. The other media did not perform as well (Dusch and Altwegg, 1995). Other comparison of different isolation techniques for *Salmonella* were those of Harvey *et al.*, (1999a) and Michael *et al.*, (1999). In all cases, pooled faecal samples are preferred over rectal swabs for the detection of *Salmonella*-carrier pigs (McCall *et al.*, 1966; Hurd *et al.*, (1999). Davies *et al.*, (1999) found that immediate processing of faeces gave the best results though storage for 6 days at 4°C was not significantly different, whereas freezing of

faeces had a deleterious effect on *Salmonella* isolation. Kim *et al.*, (1999) considered that pre-enrichment was less sensitive and not necessary when examining porcine faeces for the presence of *Salmonella* although the ratio of their pre-enrichment: RV broth was 1:10, rather than the standard 1:100 and the temperature of incubation was 37°C instead of 42°C. Using the ISO 6579 standard method, van Winsen *et al.*, (1999) found that when different *Salmonella* serovars were added to faeces the recovery rate of the different serovars differed. Fravelo *et al.*, (1999) considered that environmental samples were as discriminating as individual faeces samples.

A rapid isolation technique (SPRINT, Oxoid, UK), in which a short 5-7 h pre-enrichment is combined with overnight selective enrichment by delaying the release of selective ingredients from gelatine capsules when the media temperature reaches 41°C, was compared with ISO 6579 and found to provide a faster alternative for the isolation of *Salmonella* from swine faeces (Hoofar and Mortensen, 2000).

Enzyme linked immunosorbent assays

Enzyme linked Immunosorbent Assays (ELISA) can be used to detect either the organism or a humoral immune response to the organism.

Salmonella (antigen) detection

Antigen-capture ELISA to detect microorganisms in food and feed stuffs are gaining widespread use in the industry. Whereas culture may take 3-7 days to identify the organism, ELISA can detect the organism in a much shorter period of time, usually 1 day or less. However the reliability of some of these assays is questionable. In general, the cleaner the sample the better the assay will perform. Usually faeces, or faeces contaminated samples, do not test as well as food and feedstuffs. Feng (1992) listed and described several commercial rapid screening assays. Several antigen capture immunoassays have been utilized to detect

Salmonella species in swine faeces (Araj and Chugh, 1987; Lambiri *et al.*, 1990; van Poucke, 1990). They have the same disadvantage of many ELISA tests in that they require 10^4 - 10^5 cfu *Salmonella* ml⁻¹ to detect the organism (Dziezak, 1987). In order to achieve these numbers, a time consuming and expensive concentration protocol or a lengthy pre-enrichment must be employed. Harvey *et al.*, (1999b) compared 2 commercially available ELISAs for the detection of *Salmonella* in swine lymph nodes and caecal contents and faeces. One gave an 83% agreement with culture for lymph nodes, but it was not suitable for faeces because of cross reactions with other enterobacteriaceae. The other gave an 93% agreement with culture and worked well with faeces and caecal contents with results available in 24-48 h, but it was expensive to use. Some investigators have had success utilising rapid enrichment protocols to detect *Salmonella* species in swine faeces (Cherrington and Huis in't Veld, 1993).

Salmonella (antibody) detection

The second use of ELISA is to detect animals which have been, or are currently, infected with *Salmonella* species. The detection of antibodies to the O antigen of *Salmonella* has been utilised successfully in swine (Nielsen *et al.*, 1994). The mix ELISA utilises LPS produced from both *S. Typhimurium* and *S. Choleraesuis*. The majority of swine related *Salmonella* serovars produce high titres to the O-antigens that are present. Although the test can be utilised at the herd level, it is not suited as an individual pig test (Nielsen *et al.*, 1995). The mix-ELISA has been used for routine screening of breeding, multiplying and slaughter swine- herds in Denmark since 1993. The screening of breeding and multiplying herds is performed on serum samples whereas meat juice is used for slaughter pigs. The meat juice is obtained by freezing a 10g sample of muscle tissue at -20°C overnight and then allowing it to thaw, thereby releasing antibody containing tissue fluid. On the basis of the ELISA results further farm investigations may be undertaken using culture methods. Another ELISA has been used to detect

antibodies in *Salmonella* carrier swine employing a heat-extracted antigen (Kramer *et al.*, 1994). The results from this study indicate that most pigs infected with *S. Typhimurium* or *S. Choleraesuis* have an antibody response to this antigen. This assay shows a correlation between prevalence and severity of infection and the magnitude of the antibody response. A second ELISA utilising a different type of heat-extract antigen in a mixed-ELISA format has also been developed and shows good sensitivity and specificity (Gray and Fedorka-Cray, 1999). Similar ELISAs, of which some are now available commercially have been developed in a number of countries. These have used different methods of antigen preparation, coupling and choice of antigens to reflect the *Salmonella* serovars prevalent in the country and are listed below

USA: Baum *et al.*, (1999); Gray and Fedorka-Cray, (1999).

Germany: Blaha *et al.*, (1999b); Gabert *et al.*, (1999).

France: Proux *et al.*, (1999; 2000).

UK: Clouting, (2001, Clouting and Davies, 2001)

The Netherlands: van der Wolf *et al.*, (1999b).

An international ring trial for *Salmonella*-ELISAs for swine serology was organised by Van der Heijden (2001). Twelve laboratories participated and either used “in-house” ELISAs or commercially available kits. In total 47 well-defined sera were tested. The specificity of most ELISAs was satisfactory, but relatively large differences were found between the sensitivities of the tests. It was concluded that international reference samples should be available to guarantee a minimum level of sensitivity.

While some have shown a good association between serological results and *Salmonella* shedding or the presence of positive caecal samples (Dahl, 1999), others have found that bacteriological and serological results correlated well at the extremes, the correlation was not as good for intermediate herds (Proux *et al.*, 1999). Nielsen *et al.* (2001) found that the seroprevalence increased with age and that 40.4% of sows were positive as compared with 7.2% in 4-7 month old

animals. Unfortunately, experimentally and naturally infected swine have been shown to have a titre to LPS for at least 12 weeks after exposure to *S. Choleraesuis* even after clearing the bacteria (Gray *et al.*, 1996a). This may result in a number of ELISA positive pigs that are no longer infected. It is unclear what effect vaccination has on the outcome of this assay. However, data indicates swine vaccinated with a commercially available modified live plasmid-free *S. Choleraesuis* vaccine do not initiate a humoral immune response to *Salmonella* group C antigens, which would suggest that swine vaccinated with this strain would appear as non-infected pigs (Gray and Fedorka-Cray, 1999). However, further work with other vaccines is clearly desirable.

Polymerase chain reaction

The extraordinary ability of the polymerase chain reaction (PCR) to exponentially replicate a target DNA sequence has made it a very powerful tool in the armamentarium of the diagnostician, epidemiologist and molecular biologist. This assay is based on the ability of target (organism) specific primers which, through complementary DNA base-pairing, anneal only to the target sequence. Thermostable DNA polymerase recognizes the template primer complex as a substrate which results in the simultaneous copying of both strands of the segment of DNA between the two annealed primers. The denaturation annealing and elongation steps take place in a cyclical fashion relying on the thermostability of the *Taq*-polymerase until the target sequence is amplified to detectable amounts (Ehrlich and Sirko, 1994).

The PCR assay has been used to identify *Salmonella* species in food and clinical samples (Araj and Das Chugh, 1987; Rahn *et al.*, 1992; Cohen *et al.*, 1993). However, obstacles in the detection of organisms include the presence of substances inhibitory to PCR (Rossen *et al.*, 1992; Wilde *et al.*, 1990) and the inability to detect $<10^3$ cfu g⁻¹ of sample without pre-enrichment (Ehrlich and Sirko, 1994). Investigators have improved detection methods in PCR assays by combining it

with immunomagnetic separation (Widjoatmodjo *et al.*, 1991; 1992) or by enrichment culture (Stone *et al.*, 1994). Helmuth *et al.*, (1997) compared a pre-enrichment PCR with culture for *Salmonella* detection in 1200 samples and found a sensitivity of 93% and a specificity of 99%, the method providing a fast and reliable screening method for *Salmonella* detection. An *invA*-based PCR was evaluated for the detection of *S. Typhimurium* in the organs of experimentally infected pigs by Scholtz *et al.*, (2001) The results correlated with culture and enabled *Salmonella* to be detected within 48h. In contrast, Korsak *et al.*, (2001) compared the use of a PCR method and semi-solid

agar, (Diasalm) for the detection of *Salmonella* for a variety of pigs samples including pork. Though the specificity was good the sensitivity of the PCR ranged from 0-66.7% for faecal material and the technique was only reliable for pork meat and animal feeds. Schmid and Bauer (2001) using a combination of four hours pre-enrichment, immunomagnetic separation and PCR were able to detect 1 cfu *Salmonella*/ g spiked pork sample. However, a number of different PCR assays have been developed to detect *Salmonella* in faeces but further work is necessary to produce a standard PCR assay because results in comparative trials have been poor.

VACCINATION

It is generally accepted that live attenuated, orally-administered *Salmonella* vaccines provide the best protection against *Salmonella* infection. The superior protection achieved in comparison to killed *Salmonella* bacterins and subunit vaccines is generally attributed to the ability of live vaccines to stimulate a more effective cell-mediated immune response. Oral administration allows the attenuated mutant to utilize natural routes of infection, which facilitates the crucial step of antigen presentation to lymphocytes in the gut-associated lymphoid tissue. These events induce the production of secretory IgA on mucosal surfaces (Clarke and Gyles, 1993).

In many countries, however, inactivated vaccines are the only products available and their efficacy is equivocal. Linton *et al.*, (1970) considered that immunisation with a killed vaccine conferred only a weak protection against *Salmonella* infection in general. Davies and Wray (1997a) showed that vaccination of breeding stock on a farm with an inactivated *S. Typhimurium*/*S. Dublin* vaccine was associated with a reduction of *Salmonella* from 67% to 12% in weaned pigs and from 52% to 5% in the adult pigs. In Denmark, Dahl *et al.*, (1997) demonstrated that the use of killed vaccines reduced the clinical impact of *S. Typhimurium* infection in pigs but did not reduce subclinical infection.

Recently the development of specific non-reverting mutations to construct both homologous and heterologous vaccine vehicles, with multiple attenuating mutations, has been achieved (Chatfield *et al.*, 1992). A mutation in the *galE* region in *S. Typhi* results in a deficiency in UDP-glucose-4-epimerase, the enzyme which converts UDP-glucose to UDP-galactose, an essential component of *Salmonella* species smooth LPS (Levine *et al.*, 1989). In several large trials on humans this mutant has appeared to be efficacious although a number of doses have to be administered and some patients develop mild diarrhoea. Because of this success, this mutation has been employed for many *Salmonella* serovars including *S. Typhimurium* (Nnalue and Lindberg, 1990). However, a *galE* mutation in *S. Choleraesuis* does not reduce virulence in swine and

one assumes that this could be the case with other *Salmonella* of serogroup C. This is due to the fact that galactose is missing from the oligosaccharide repeating unit of the O antigen side chain of *S. Choleraesuis* (Nnalue and Stocker, 1986). The somatic antigens of *Salmonella* serogroups are the main component of host specificity and facilitate survival in the gastrointestinal tract and entry onto deeper tissues (Nnalue and Lindberg, 1990).

Another common attenuation involves the creation of auxotrophic mutants that require metabolites not available in animal tissues. Aromatic mutants, which have a complete block in the aromatic biosynthetic pathway have a requirement for aromatic metabolites such as para-aminobenzoate and 2,3-dihydroxybenzoate. Oral vaccination with *aroA*, *aroD* mutants in mice and calves has been effective in reducing disease and has been shown to be safe (Hook, 1990; Robertsson *et al.*, 1983; Smith *et al.*, 1984). Experiments using an *aroA* mutant of *S. Typhimurium* indicated that vaccinated pigs shed *Salmonella* significantly less frequently than non-vaccinated pigs (Lumsden *et al.*, 1991). Lumsden and Wilkie, (1992) vaccinated pigs and challenged them, a good antibody response to LPS and killed bacteria was obtained, the *aroA* mutant avirulent in pigs was not shed in the faeces and significantly reduced the severity of diarrhoea following challenge. Further studies by Lumsden, *et al.*, (1993) suggested that there may be a genetic basis for the immune response of pigs to *Salmonella* because some animal genotypes gave a much better response.

Mutations in global regulatory pathways have also been a popular means of attenuation. Several studies have utilized strains with deletions in the genes for adenylate cyclase (*cya*) and for cAMP-receptor protein (*crp*). Cyclic AMP and cAMP-receptor protein regulate at least 200 genes, many of which are required for breakdown of catabolites. *Salmonella* with deletion mutations in the *cya*, *crp* genes have been shown to be safe and effective in eliciting protective immunity in mice, chickens and pigs (Coe and Wood, 1992; Curtiss and Kelly, 1987; Stabel *et al.*, 1991; 1993). A large study evaluating

the safety and efficacy of a battery of *S. Choleraesuis* Δ *cya*, Δ *crp* isogenic mutants in mice indicates that several of these strains are protective and safe (Kelly *et al.*, 1992). Further studies in pigs of these mutants showed that they were safe, although in some cases some caused a slight temperature elevation and increased diarrhoea scores (Kennedy *et al.*, 1999). When pigs were immunised orally with a commercial Δ *cya* Δ *crp*-*cdt* *S. Choleraesuis* vaccine and challenged with *S. Typhimurium*, there was a reduction in the severity of clinical signs, the shedding of the challenge strain in the faeces and carriage in the internal organs (Charles *et al.*, 1999). Intra-nasal inoculation with a similar vaccine reduced carriage of *S. Typhimurium* in the lymph nodes but did not reduce shedding in the faeces (Letellier *et al.*, 1999b). Similarly Groninga *et al.*, (2000) found that a *S. Choleraesuis* vaccine provided some cross protection against experimental *S. Derby* infection. A recent field study used a live *Salmonella* *Choleraesuis* vaccine to reduce the number of infections with *Salmonella* (Maes *et al.*, 2001). Twelve groups of c. 380 pigs were randomly allocated to either vaccination at 3 and 16 weeks or no vaccination. In the vaccinates only 0.6% of the 334 lymph nodes were positive, whereas 7.2% of 321 of those of the controls were positive at slaughter.

A *S. Choleraesuis* strain which has been cured of the 50 Kb virulence plasmid has been shown to be safe and efficacious in swine (Kramer *et al.*, 1992). The non-specific mutation was obtained by repeated passage through porcine neutrophils. The plasmid-free variant lacks the ability to invade Vero cell monolayers and porcine neutrophils as well as having increased resistance to killing by H₂O₂ and phagocytic killing by porcine neutrophils (Roof *et al.*, 1992). Kramer and Vote (2000) found that the reduced virulence of granulocytes selected *S. Enteritidis* vaccine was limited to the species of animal in which the granulocyte selection was made.

In Germany, an adenine-deficient strain of *S. Choleraesuis* is used for immunization of pigs and is

commercially available (Meyer *et al.*, 1993) and more recently a live, double attenuated *Salmonella* Typhimurium vaccine has been shown to give some protection against *Salmonella* colonisation under experimental conditions (Springer *et al.*, 2001) and field conditions (Lindner *et al.*, 2001). Two stable rough mutants of *Salmonella* were studied as live oral vaccines by Trebichavsky *et al.*, (1997). The SF1591 mutant of *S. Typhimurium* (Ra chemotype) protected 8 germ-free piglets against subsequent infection with virulent smooth *S. Typhimurium* LT2, whereas a deep-rough mutant of *S. Minnesota* mR595 (Re chemotype) did not protect 7 experimentally infected piglets. Cytokine and leukocyte profiles in the ilea of gnotobiotic piglets colonized for 1 week with either rough mutants alone or with rough mutants followed by *S. Typhimurium* LT2 were also investigated. The ileal mucosae of piglets associated with strain SF1591 alone were not inflamed. Villi contained activated macrophages and enterocytes expressed transforming growth factor beta (TGF- β). Subsequent infection of piglets with *S. Typhimurium* LT2 resulted in immigration of $\alpha\beta$ T cells and immunoglobulin A (IgA) response. In contrast, the ileal mucosae of piglets associated with strain mR595 alone expressed heat shock proteins and inflammatory cytokines but not TGF- β . Acellular villi contained numerous $\gamma\delta$ T cells but not $\alpha\beta$ T cells.

The use of vaccines should be considered as part of an overall strategy to control *Salmonella* on the farm and their use should be in conjunction with other measures. Since many *Salmonella* infections are sub-clinical, the use of vaccines in carrier animals will be to reduce *Salmonella* shedding in finishers and thus reduce carcass contamination. Field trials are necessary to determine whether this can be achieved economically.

BREEDING *SALMONELLA* RESISTANT PIGS

In poultry it is well documented that some breeds show more resistance to *Salmonella* infections. Pevzner *et al.*, (1981) and Bumstead and Barrow (1993) found substantial differences to lethal challenge with several different serovars of *Salmonella* in inbred lines of chickens. Breeding programmes are now being used to develop chickens which are resistant to *Salmonella* infection. Performance testing of pigs led to genetic improvement of productivity and the UK was a world leader with a sizeable export market. Sellwood *et al.*, (1975) found that some pigs were genetically resistant to some types of *E. coli* infection. The resistance gene was autosomally recessive which gave rise to pigs that lacked the receptor in their intestine for the adhesive F4 (K88) antigen of *E. coli*, i.e. the bacterium could not stick to the intestinal mucosa and colonise the intestine. Because the gene was recessive, breeding resistant sows was difficult and counterproductive because their piglets received colostrum which gave no protection against F4 (K88) positive *E. coli*.

However, Lacey *et al.*, (1989) found that peripheral blood monocytes from certain breed of pigs exhibited higher phagocytic capacity and bactericidal activity against *S. Typhimurium*. Breed

differences were also observed regarding humoral and cell-mediated responses to a vaccine strain of *S. Typhimurium* (Lumsden *et al.*, 1993). Resistance to *Salmonella* infections is believed to be associated with the antimicrobial activity of macrophages. In mice, resistance is linked with polymorphism in the natural resistance associated macrophage protein (*Nramp 1*) gene. This gene has been identified in several species including the pig, but data is so far lacking for any association with resistance or susceptibility to *Salmonella* infections. So far selection for specific disease resistance or general immuno-competence has not been successfully implemented in pigs but in the long term it may be possible to breed pigs which will be more resistant to stressors such as weaning, transport or feed removal which can exert a suppressive effect on immunity and exacerbate *Salmonella* infection (Blecha *et al.*,1980). The rapid development of molecular genetics has provided detailed genome maps and tools to identify and study individual genes, which may enable the selection in the future of breeding animals of preferred genotype (Edfors-Lilja and Wallgren, 2000).

COMPETITIVE EXCLUSION

To prevent the *Salmonella* carrier-state in pigs, new intervention strategies need to be investigated. Competitive exclusion (CE) is one approach which has been used successfully with poultry (Bailey, 1987; Bailey *et al.*, 1992; Blankenship *et al.*, 1993) and further information on the subject will be found in Wray and Wray, (2000). A mucosal competitive exclusion culture from swine (MCES) was developed by Fedorka-Cray *et al.*, (2000). Following application in suckling pigs and subsequent challenge with *S. Choleraesuis*, 28% of the gut tissues were positive from the MCES treated pigs versus 79% positive tissues from the control pigs. A 2-5 log₁₀ reduction of *Salmonella* in the caecal contents or ileocolic junction was observed in the MCES treated pigs when compared with the controls. These data indicate that use of MCES may be a useful approach for control of *Salmonella* in swine. In contrast, Anderson *et al.*, (1999) found that CE did not reduce carriage of *S. Typhimurium* in lymph nodes, although there was a reduction of *Salmonella* shedding in the faeces.

Letellier *et al.*, (2000) assessed various treatments to reduce *S. Typhimurium* carriage in swine. They found that acidification of drinking water, egg yolk immunoglobulins and an endotoxin vaccine had no effect although a live attenuated *S. Choleraesuis* vaccine and the use of bambarmycin reduced the number of *Salmonella* in the mesenteric lymph nodes.

Another form of competitive exclusion has been described by Lovell and Barrow (1999) who found that infection of gnotobiotic piglets with one strain of *Salmonella* prevented colonisation of the intestine 24 h later by a different *Salmonella* strain. This phenomenon was also observed with isogenic mutants but not all mutants were as inhibitory.

Bacteriophage lysate was used by Lee and Harris (2001) to reduce the dissemination of *Salmonella* in experimentally infected pigs, and its use reduced the numbers of *Salmonella* in the large intestine by two logs as compared with the controls. However much more extensive studies are necessary to determine whether its use under field conditions is practical.

PUBLIC HEALTH ASPECTS

Pigs are an important reservoir of *Salmonella* for man and there have been many reports on the isolation of the organism from pork and pork products (Bryan, 1980).

Relationship between *Salmonella* testing on farms and at the abattoir

The prevalence of *Salmonella* in the intestine of individual pigs from different sources is extremely variable (Gray *et al.*, 1995a; 1996a; b). Individual animals may remain as carriers for up to 36 weeks (Wood and Rose, 1992).

Proescholdt *et al.*, (1999) found a poor correlation between on farm sampling of pigs and culture of organs at the abattoir. They detected *Salmonella* in 4.3% of faeces and positive serological results in 1.5% of blood samples from pigs on farms in Iowa and North Carolina, yet when tissues were examined in the abattoir 39.4% of the samples (lymph nodes and caecal contents) yielded *Salmonella*. They commented that on farm sampling is either insensitive or that the pigs were infected during transport or in the abattoir. Carlson and Blaha (1999b) compared pigs from a high prevalence farm (30% samples positive) with those from a low prevalence farm (1% samples positive) at slaughter, when 2/181 lymph nodes and 1/107 lymph nodes respectively were positive for *Salmonella*. A similar survey in Ireland found a positive association between seropositivity of pigs from high risk herds and caecal carriage and a higher prevalence of *Salmonella* positive skin swabs from pigs of the high risk herds (Quirke *et al.*, 2001). However, Sorensen, *et al.*, (1999a) found a correlation between the meat-juice sero-prevalence in a heavily infected herd and the risk of a carcass being *Salmonella* positive. Clouting and Davies, (2001) found a poor correlation between the result of the meat-juice ELISA and the result of caecal culture for the detection of *Salmonella* at slaughter. These results indicate that only in heavily infected herds are on farm tests likely to indicate potential carcass contamination.

Salmonella shedding may be increased by any stress factor including transport and it was shown that the proportion of pigs, of all ages, in a herd that excreted *Salmonella* increased after transport (Williams and Newell, 1970; Isaacson *et al.*, 1999; Scheepens *et al.*, 1994, McKean *et al.*, 2001). Berends (1998) suggested that 90% of new infections during transport were caused by stress and were thus infections with types already present in the herd before transport and the other 10% was caused by cross contamination between herds. However, he did not consider the high prevalence of *Salmonella* in transport vehicles (Wray *et al.*, 1991; Swanenburg, 2000) which may also cause infection of animals being transported. It was also suggested that feed withdrawal 12-24 h before slaughter may reduce carcass contamination at evisceration caused by intestinal laceration (Miller *et al.*, 1997), but conversely the stress of feed withdrawal may cause *Salmonella* excretion. Morrow *et al.*, (1999) found that feed withdrawal 12-24 h before transport did not increase the percentage of caecal samples positive for *Salmonella*.

Studies at the Abattoir

The Lairage

The lairage is a location where pigs from many farms are gathered, which allows ample opportunity for cross contamination and infection of *Salmonella* free pigs. Stress of pigs within the lairage may also result in *Salmonella* excretion putting many pigs at risk. Experiments by Hurd *et al.*, (2001) showed that market swine can become infected during routine resting or holding periods when exposed to relatively low levels of *Salmonella* (10^3 cfu) and that exposure times as short as 30 min. are sufficient to produce contaminated gastro-intestinal tracts. Weber (1996) found 16-50% *Salmonella* positive samples in the lairage after the presence of pigs and 0-33% *Salmonella* positive samples after cleansing and disinfection. Lazaro *et al.*, (1997) found that 20% of the lairage samples were positive for *Salmonella* in Brazil. Swanenburg *et al.*, (2001) examined samples from the floor and walls of 2 lairages when pigs were present, and isolated *Salmonella* from 70-90%

of the samples. The usual cleaning and disinfection routine reduced the level of contamination to 25% positive samples and an improved cleaning and disinfection routine reduced this level to 10%. They considered that the *Salmonella* flora of lairages consists of a resident flora of genotypes that were consistently present and a transient flora that did not persist. Since cleaning and disinfection was unable to eliminate all the *Salmonella* they also suggested that the resident strains may be resistant to the disinfectant in use. Experiments have been done in which the effect of the waiting time in the lairage on the number of pigs infected with *Salmonella* was studied (Craven and Hurst, 1982; Morgan *et al.*, 1987; Weber, 1996) but the conclusions from these experiments were not consistent. It is commonly supposed that longer periods in the lairage will increase the chance of cross-infection and contamination of pigs (Morgan *et al.*, 1987; Weber, 1996), but stress levels, and possibly *Salmonella* excretion are reduced by overnight lairage (Warris *et al.*, 1998) or longer, 2-3 days (Craven and Hurst, 1982). There appears to be little difference in the magnitude of this effect in relation to the distance travelled (Rajkowski *et al.*, 1998). Davies *et al.* (1999b) found a reduced rate of intestinal carriage of *Salmonella* and carcass contamination in pigs that had been held overnight in lairage as compared with pigs slaughtered within 2-3 h of arrival. The effect of separate transport, lairage and slaughter on the occurrence of *Salmonella* in pigs from *Salmonella* positive and negative herds was studied by Boes *et al.*, (2001) but their results as yet are equivocal.

Prevalence of Salmonella in Pigs at the abattoir

Many surveys have been done in abattoirs and it is often difficult to compare results because different isolation techniques and tissues may be examined. Sampling methods are also important in these surveys because it has been shown that rectal swabs provide an underestimate of the level of infection as may carcass swabs.

A significant correlation was found between carcass contamination and the presence of *Salmonella* in the tonsils, although the serology of an individual pig/herd did not correlate with carcass contamination

(Swanenburg *et al.*, 1999). Fedorka-Cray *et al.*, (1995) found that *Salmonella* in contact with tonsils and nasal lymphoid tissue could disseminate rapidly by blood and lymph to organs sampled at the abattoir. Davies *et al.*, (1999b) investigated *Salmonella* contamination at a large abattoir and found that the rate of isolation of *Salmonella* within different batches of pigs ranged from 0-71.4% of intestinal contents, but overall of the 2211 pigs examined *Salmonella* was isolated from 7% of carcass swabs and from 11.6% of the 2205 large intestinal contents. *Salmonella* Typhimurium DT 104 was found in 3.2% of large intestinal contents and in 2.7% of the carcass swabs. The number of *Salmonella* in the intestinal contents ranged from $1.0 \cdot 10^5$ /g. A national survey for *Salmonella* in slaughter pigs was carried in 34 abattoirs when 2509 pigs were sampled (Davies *et al.*, 2001). Caecal carriage of *Salmonella* was identified in 23% of the pigs but only 5.3% of the carcass swabs were positive. The commonest serovars isolated were *S.* Typhimurium (11.1% caeca, 2.1% carcasses) and Derby (6.3% caeca, 1.6% carcasses). The main phage types of Typhimurium were DT 104 (21.9% caecal isolates), DT 193 (18.7%), DT 208 (13.3%) and U 302(13.3%). There was a poor correlation between positive ELISA results, or carcass contamination and the caecal carriage of *Salmonella*: 15.2% of pigs gave a positive result at the 40% optical cut off, and 35.7% at the 10% cut off.

In The Netherlands, Berends *et al.*, (1996) estimated that 5-30% of carcasses could be contaminated with *Salmonella*. In Germany, Kaesbohrer *et al.*, (2000) detected *Salmonella* in 3.7% of faeces, 3.3% of lymph nodes and 4.7% of surface swabs and estimated the prevalence of *Salmonella* in slaughter pigs to be 6.2%. In Denmark, 6.2 % of caecal samples were found to be *Salmonella* positive at the abattoir, usually with one serovar or phage type predominating from each farm source (Baggesen *et al.*, 1996). Sorensen and Bager, (1995) detected *Salmonella* contamination in 1.5-1.6% of fresh pork, 8% of offal, 3.1% of fore-quarters and 1.2% of hind-quarters and suggested that the removal of the pluck was responsible for the higher level of contamination in the fore-quarters. Sorensen *et al.*,

(1999a) found an association between the meat-juice ELISA seroprevalence and the risk of a carcass being contaminated with *Salmonella*. Further studies (Dahl and Sorensen, 2001), found a higher prevalence of *Salmonella* on carcasses from low seroprevalence herds than had been anticipated, which they suggested was a result of cross contamination from other herds. They also investigated the effect of different ways of rectum removal and found that the use of a plastic bag and knotting the rectum reduced carcass contamination (Sorensen *et al.*, 1999b). Swanenburg (2000) found that the prevalence of *Salmonella* in pig samples differed between abattoirs and was 17% in one and 6% in the other; it also varied between slaughter days. *Salmonella* was isolated from 47% of the pigs; the highest prevalence was in rectal contents (25.6%) whereas the lowest prevalence was on the carcasses (1.4%). In other samples the *Salmonella* prevalence was: tonsils (19.6%), livers, tongues and mesenteric lymph nodes (9.3% each). She commented that her results do not seem to differ from those of previous surveys during the last 3 decades in The Netherlands *i.e.* 21-34% (Edel and Kampelmacher, 1970, Oosterom *et al.*, 1985).

The Slaughter Process

The slaughter process in a well run pig abattoir is capable of reducing the level of surface contamination of carcasses because of the scalding and singeing stages. Davies *et al.*, (1999b) found that although 88.2% of the carcasses were contaminated with *Salmonella* after bleeding, the proportion was reduced to 5.7% after scalding and after singeing none was contaminated. Snijders (1976) recommended scalding water temperatures above 60°C to prevent *Salmonella* contamination, which was confirmed by Sorqvist and Danielsson-Tham (1990). Jones *et al.*, (1984) found that water enters the stick wound during scalding and may spread haematogenously to other parts of the carcass and suggested that steam scalding should be used. Likewise, Rheault and Quessy (1999a) cultured 276 stick wounds of which 0.9% were contaminated with *Salmonella*, 40.6% coliforms and 27.7% *E. coli*. However, any *Salmonella* that survive these stages can be spread between carcasses by the dehairing

equipment (Gill and Bryant, 1993). Most of the contamination, however, results from escape of intestinal contents during evisceration (Saide-Albornoz *et al.*, 1995). The studies of Davies *et al.* (1999b) indicated that there was little increase in carcass contamination after evisceration although further increases caused by trimming and meat inspection have been described (Mousing *et al.*, 1997). However Davies *et al.*, (1999b) commented that there were marked differences in the frequency of intestinal rupture during evisceration by different shifts and suggested that the training of abattoir staff and the monitoring of good evisceration techniques should receive a high priority. Likewise production systems in which pigs receive minimal roughage and little exercise should be discouraged because they increase the frequency of intestinal rupture during slaughter (Miller *et al.*, 1997). Hazard analysis critical control point (HACCP) style procedures have been widely adopted in abattoirs but the level of microbial monitoring to verify the critical control points and the correct application of procedures is often insufficient (Langer, 1995). Employment of HACCP principles in the abattoir, however, appear to have decreased the recovery of *Salmonella* from swine carcasses (FSIS report 1999). Rheault and Quessy, (1999b) examined 2841 samples from the environment of 4 abattoirs before and after operations. They found 4% of environment samples, including gloves and aprons, 21.3% of faeces from incoming pigs and 6.3% of the carcasses positive for *Salmonella* although the latter was lower in the abattoir working with HACCP. Likewise, Limpitakis *et al.*, (1999) found 178/1874 samples, which included floors, hands, knives, *etc.* positive for *Salmonella*. In addition 7.3% of carcasses were positive. Swanenburg (2000) isolated *Salmonella* from 29.4% of 477 environmental samples. When the eviscerator wore a plastic glove and sanitized his knife in water at 82°C contamination was reduced by 50%, using a disinfectant reduced it by 75%. Giovannacci *et al.*, (2001) used molecular genetic techniques to study the persistence and spread of *Salmonella* in 2 abattoirs. They found that most of the pig, pork and environmental samples, collected during the working day were contaminated with *Salmonella* as were the environmental samples

collected before work began. In contrast no *Salmonella* were isolated from the samples from the cutting room before the start of work, though during work *Salmonella* was isolated. In one abattoir, Swanenburg *et al.*, (2000) isolated *Salmonella* from 33% of the samples from the carcass splitter and the same serovar was isolated on 2 days running, suggesting that the machine was persistently contaminated. Carr *et al.*, (1998) and Saide-Albornoz *et al.*, (1995) concluded that chilling and trimming were important critical control points during slaughter and storage. They concluded that carcass contamination was associated with environmental contamination and from carrier pigs which then spread the *Salmonella* to the cutting room. However no *Salmonella* was continuously isolated from the abattoir environment. Berends *et al.*, (1997) showed that 70% of carcass contamination with *Salmonella* was derived from carrier pigs and the other 30% from cross-contamination. Van Netten *et al.*, (1995) used a lactic acid spray to reduce carcass contamination and while its use reduced *Salmonella* contamination, its use also caused deterioration in the organoleptic quality of the meat.

Mousing *et al.*, (1997) investigated the consequences of changing from traditional to an entirely visual system of meat inspection in 183,383 pigs and suggested that 0.7 /1000 *Salmonella* contaminated carcasses would be missed. However visual inspection would reduce cross-contamination and costs. Likewise, steam scalding, hot water (79-81°C) (Jensen and Christensen, 2001), use of bactericidal sprays on the carcass and removal of the head and pluck together have all been shown to reduce carcass contamination but their use is not permitted in many countries. Likewise it has been shown that the level of *Salmonella* contamination on carcasses can be reduced by up to 30% if the head is not split after slaughter (Olsen *et al.*, 2001).

Measures to avoid contamination of pork in the abattoir

Swanenburg (2000) suggested that to prevent *Salmonella* contamination of pork it was necessary to:

- 1/ Avoid direct and indirect contact between different herds along the whole pork production chain, especially between *Salmonella*-free and infected herds.
- 2/ Avoid exposure of pigs to contaminated environments, especially in the lairage.
- 3/ Avoid stress during transport and in the lairage to reduce *Salmonella* excretion and susceptibility.
- 4/ Avoid environmental contamination by infected pigs.
- 5/ Avoid carcass contamination by residual flora in the abattoir.

Berends *et al.*, (1996) investigated the factors affecting the microbiological quality of meat, they found:

- 1/ A correlation between the number of live animals with *Salmonella* in their faeces and carcass contamination.
- 2/ Live animals with *Salmonella* were 3-4 times more likely to result in a *Salmonella* positive carcass than a *Salmonella* free animal.
- 3/ For *Salmonella* contamination of the carcasses at the end of the line 15% could be attributed to cross contamination via slaughter equipment and handling by abattoir personnel, and 85% could be attributed to faulty evisceration technique.
- 4/ Risk factors were: dirty polishing machines (5-15% contamination after singeing), mistakes during evisceration (60-90%), and further dressing (5-35%).

However the numbers of *Salmonella* per cm² were usually less than 1-2 organisms and they suggested that although this number was unlikely to cause food poisoning, it was an important source of cross contamination to other meat and meat products.

Pork and pork product contamination

More recent reports indicate that a wide range of *Salmonella* may be present in fresh pork (Bozzano *et al.*, 1993; Fernandez-Escartin *et al.*, 1995). In Greece, 285 of pork pig carcasses were found to be contaminated with *Salmonella* (Epling *et al.*, 1993). Carcass contamination was 17.5% in Canada (Lammerding *et al.*, 1988), 21% in The Netherlands (Oosterom *et al.*, 1985), 6.5% in the USA (FSIS

Report, 1999) and 27% in Belgium (Korsak *et al.*, 1998). Pig meat products such as hot vacuum packaged pork (Van Laack *et al.*, 1993), especially those incorporating low grade material such as mechanically recovered meat (Banks and Board, 1983), are frequently contaminated with *Salmonella*. In the USA, 12.4% of fresh pork sausages were positive for *Salmonella* (Johnston *et al.*, 1982). More recently in the UK, a new phage type of *S. Typhimurium*, U 310, has been associated with pig products and human infection (Ward and Threlfall, 2001).

Human Infections

In many countries the relationship between human illness and *Salmonella* contamination of pig meat is unclear, with the possible exception of Denmark where approximately 10-20 % of human cases are

considered to be related to pork and pork products (Wegener *et al.*, 1994; Hald and Wegener, 1999a). In 1998, the case rate *i.e.* *Salmonella* cases/ 100,000 human population associated with pork for Denmark, The Netherlands and Germany were 7-11, 14-19 and 18-23/ 10⁵ respectively, although a word of caution is necessary because of the use of different reporting systems in each of the countries (Hald and Wegener, 1999a). In the UK, recent outbreaks of *S. Typhimurium* associated with pork products were reported by Maguire *et al.*, (1993) and Cornell and Neal (1998). Other *Salmonella* outbreaks associated with pork or pork products are: *S. Infantis* (Wegener and Baggesen, 1996), *S. Manhattan*, (Hald *et al.*, 1999b) and *S. Typhimurium* DT 104 (Baggesen *et al.*, 1999b). In the last outbreak there were 25 cases of whom 2 died.

CONTROL MEASURES

It is clear that biosecurity and hygiene precautions to control *Salmonella* should be taken throughout the pig meat production and distribution chain from nucleus breeders to hot dog stalls (Simonsen *et al.*, 1987).

During the last decade, the structure of the swine industry has changed markedly, with the introduction of large integrated systems and breeding pyramids akin to those of the poultry industry. Control measures are of increasing importance because of consumer concerns about food-borne zoonoses. Epidemiological studies in recent years suggest that *Salmonella* infection of sucking piglets is much lower than that of older animals because of lactogenic immunity and that the application of an Integrated Quality Control (IQC) Systems agreed upon by all staff, can reduce the prevalence of *Salmonella* on pig farms. Application of these systems require some knowledge of the *Salmonella* prevalence on an individual farm and this can be monitored as indicated earlier either by serology or culture. The main components of an IQC System are:

Biosecurity

As indicated previously there are many routes by which *Salmonella* can be introduced onto a farm and the organism is often disseminated widely on farms. Control measures include changes of clothing and boots for visitors, bird and rodent control, foot-baths containing active disinfectant outside houses, limiting access to the site by visitors and lorries, *etc.* Farm size, stocking densities and pig density within a region all have a negative effect on the *Salmonella* status of a farm, perhaps by predisposing to *Salmonella* spread within and between farms.

All-in All-out systems:

Effective cleaning and disinfection are important aspects of disease control. It is generally accepted that farms should operate an All-in All-out (AIAO) policy, with adequate cleaning and disinfection after

the pen is empty. Linton *et al.*, (1970) found that uninfected animals, which remained in disinfected pens usually stayed free of *Salmonella* but as the number of pigs per pen increased a higher prevalence of infection was found. Tielen *et al.*, (1997) found that *Salmonella* negative piglets placed in clean accommodation remained free despite serological evidence of *Salmonella* in the sows. In Denmark, removal of 10 week old pigs from breeding farms infected with *S. Typhimurium* to clean premises appeared to be effective in prevention of infection at market age (Dahl, *et al.*, 1997). Offsite weaning at 10-16 days prevented *Salmonella* infection in grow to finish pigs moved to a clean environment (Nietfeld *et al.*, 1998). Fedorka-Cray *et al.*, (1997b) weaned pigs at 14-21 days and removed them to clean accommodation where the piglets remained free of *Salmonella*. Improved disinfection of weaner and grower pens on several farms produced significant reduction in the incidence of positive batches (from 80 to 11% on one farm) (Davies and Wray, 1997a).

A word of caution should be introduced, however, as other investigators have shown no benefit in reducing the prevalence of *Salmonella* species using AIAO management of finishing pigs compared with conventional farrow-to-finish systems in North Carolina, USA and higher prevalence of *Salmonella* in multiple site AIAO systems. In 1995 AIAO was practised on 42.4 % of operations with finishing accommodation, comprising 51% of national hog production (Davies *et al.*, 1997). A comparison of continuous flow (CF) systems and AIAO systems in the USA found little difference between the 2 systems when tissues collected in the abattoir were examined for *Salmonella* (Proescholdt *et al.*, 1999).

In Sweden, depopulation is used to control *Salmonella* infection in pigs, but this is not always successful, and Wahlstrom *et al.*, (1997) described a farm on which *S. Derby* was endemic and they found that it was not possible to eliminate the infection without permanently decreasing the pig

population by 50%. Depopulation has also been used in Denmark to eradicate *S. Typhimurium* DT 104 from the national pig herd (Mogelmoose *et al.*, 1999) but it was not always successful because contamination persisted on some of the farms and the replacement pigs became infected. A reappraisal of this strategy has resulted in intervention plans being produced for *S. Typhimurium* DT 104 infected herds and herds being declared free on bacteriological and serological testing: so far this strategy has been successful in 15 herds (Mogelmoose *et al.* (2001). In Denmark, a task-force was established to clarify why chronic infected level three herds were unable to reduce their *Salmonella* problem. Intervention plans were formulated for 78 herds and 12 months after the implementation of the plan, 61 (78%) of herds were assigned to level one, 12 (15%) herds to level 2 and 5 herds remained at level 3. Of the 61 herds, 45 maintained their status six months in a row. Bagger and Nielsen, (2001) concluded that it is possible to reduce the prevalence of *Salmonella* in chronically infected herds by a combination of intensive advising, enforced implementation of intervention plans and economic pressure.

In the UK, intervention strategies to control *Salmonella* such as feed acidification and the use of fermented liquid feed were not successful: the most successful strategy in batch systems was dry

cleaning and disinfection with 2% formaldehyde. However in many cases the beneficial effects were undermined by the introduction of *Salmonella* infected pigs and the failure to disinfect other areas and McLaren *et al.*, (2001) concluded that the use of single interventions was unlikely to be successful. A chronically infected herd was depopulated, the pens were cleaned and disinfected and then left empty for six months. However *Salmonella* was detected in the soil of the sow paddock and in mice six months after depopulation and on re-population, *Salmonella* was detected in replacement breeding stock and though the level of infection remained low initially, within a year *Salmonella* was widespread (Davies *et al.*, 2001a).

Feeding Systems

Many batches of animal feed are contaminated with *Salmonella* (Wray and Wray, 2000). Studies in a number of countries have shown that the particle size and dietary constituents may affect *Salmonella* colonisation in the gut. Likewise, dry feed versus wet feed has also been shown to play a role in *Salmonella* colonisation. Naturally fermented feed is now being recommended for reduction of *Salmonella* infection in Denmark. Likewise the use of acidified feed may improve the response to treatment and reduce the spread of disease.

ANTIBIOTIC RESISTANCE OF *SALMONELLA* FROM PIGS

The use of antibacterial drugs for treatment and prophylaxis of various diseases in pigs may result in the development of antibacterial resistant *Salmonella*. In many cases *Salmonella* may not cause disease in pigs and the organism may be exposed to the selective pressure of an antibacterial drug when it is being used to treat a disease caused by another bacterial species. Tetracyclines account for 40% of the antibacterial sales in the UK and they are mostly used in pigs and poultry in medicated feedstuffs to treat various disorders, including respiratory disease. Tetracyclines are one of the oldest antibacterial drug and have been on the veterinary market for more than 50 years, and many *Salmonella* isolated from pigs are resistant to the drug. In the UK, 77% of the *Salmonella* isolated from pigs were resistant to tetracyclines in 1999, and resistance was also frequent to other antibacterial drugs (see Annex) and there does seem to be a relationship between antibiotic resistance in *Salmonella* from pigs and antibiotic use, especially tetracyclines.

A more serious problem is the high prevalence of multiple resistant (resistant to 4 or more antibacterial drugs) *Salmonella* isolated from pigs in the UK. This is predominantly associated with 3 different phage types of *S. Typhimurium*: DTs 104, 193 and 208. The widespread occurrence of DT 104 in other species of farm animals and man has caused public concern in the UK and other countries where it has occurred. An important aspect of the epidemiology of DT 104 is its spread within the animal populations, which has been caused by movement of animals through markets, purchase and transport. Since it is resistant to 5 or more antibacterial drugs, the use of such drugs on the farm may favour its establishment in the intestine. Further research is clearly needed to study the epidemiology of multiple resistant *Salmonella*, the genetic basis for their resistance and the factors which enable them to spread rapidly within the animal population.

Although the antibacterial drugs used for growth enhancement are unlikely to have any direct action

on *Salmonella*, which is a Gram-negative bacterium, because their mode of action is predominantly against Gram-positive bacteria. The use of some growth enhancers however, has been shown to promote *Salmonella* colonisation of the intestine and increased shedding in the faeces by inhibiting the protective microbiota. Some growth enhancers have been banned within the EU because they cause resistance in commensal bacteria, e.g. *Enterococci*, *Escherichia coli*, which may enter the food chain and spread to man, where they may either cause disease or transmit their resistance genes to other bacteria. It is likely that in the next few years the use of growth enhancers will be phased out. This may affect pig performance by increasing the prevalence of gastro-intestinal disease and increase the use of antibacterial drugs for therapy, which in turn may affect *Salmonella*. Antibacterial drug resistance in *Salmonella* is only a small part of the problem because many other bacterial species, e.g., *E. coli*, *Enterococci*, *Campylobacter*, which are normal components of the intestinal microbiota may show much higher levels of resistance than *Salmonella*. If these micro-organisms enter the food chain, they may either cause nosocomial infections in man or add to the reservoir of antibacterial drug resistance genes. The problem of antibacterial resistance is not a problem of *Salmonella* alone, and to tackle it one needs to consider the picture as a whole and address resistance in other bacterial species, such as *E. coli*, *Enterococci*, *Staphylococci* in addition to *Salmonella*.

Control of antibacterial resistance in *Salmonella* can be achieved by:

- 1/ Preventing the introduction and spread of *Salmonella* onto farms and between farms. by improved biosecurity. The methods will equally apply to all *Salmonella* irrespective of their resistance status.
- 2/ The responsible and prudent use of antibacterial drugs, for which guidelines have been produced in many countries and by international organisations such as OIE and WHO.

- 3/ Development of alternatives to antibacterial drugs
e.g. vaccines, competitive exclusion.
- 4/ Improved husbandry and management to reduce the prevalence of disease on the farms.
- 5/ Since DTs 193 and 208 appear to be endemic on some farms, improved hygiene, cleaning and disinfection and perhaps depopulation may be necessary although in Denmark some pig herds that were infected with DT 104 and slaughtered became reinfected and the policy has been is no longer in operation.
- 6/ Increased surveillance of antibacterial resistance in *Salmonella* and other bacterial species so that when resistance to newer antibacterial drugs is detected control measures can be instituted.

GENERAL COMMENTS

Salmonella control presents a challenge to all involved in the pig industry. It is important that control must be based on a detailed knowledge of the epidemiology of infection and a specific control programme for each enterprise. Many strategies have been tried and tested *e.g.* vaccination, use of antibacterials, although the latter should be discouraged because of the risk of producing resistant microorganisms *etc.* None have been successful on its own, but improved hygiene and disease security, combined with vaccination, are methods that have been successfully used in other sectors of the livestock industry. Implementation on the farm of the HACCP principles, which are currently being studied in Europe (Bokma-Bakker and Mul, 2001) and in the US, may provide a model

for future production practices.

While HACCP in the abattoir will assist in reducing carcass contamination, on farm control and testing has a cost. Mark *et al.*, (1999) found that pork producers would be willing to conduct on-farm *Salmonella* testing if they could recover the cost of sample collection c. \$1.48-4.72 per pig depending on the method of testing. Consumers would also be willing to pay more, suggested figures were \$1-0.25/lb. though the amount varied as to socio-economic group, with women with children and senior citizens being the most willing (Miller and Unnevehr, 1999).

CONTROL SCHEMES IN DIFFERENT COUNTRIES

SWEDEN

Due to the low incidences of *Salmonella* infections in animals, the control and surveillance programme which was instituted in Sweden is generally held up to be the gold standard on which other countries base their monitoring systems.

General outline

The *Salmonella* control programme in swine was instituted more than 30 years ago and has the following requirements.

1/ Testing of all sanitary slaughtered animals as well as any suspect animal at normal slaughter for the presence of *Salmonella*.

2/ General surveillance by clinical checks made by practising and animal health veterinarians.

3/ Control of imported and domestically feed.

If *Salmonella* is found infected herds are put under restrictions. The restrictions are lifted when the herd is considered free from infection. Compensation is paid to herd owners for costs due to restrictions on infected farms. As swine, for all practical reasons, are free from *Salmonella*, and as infected herds are put under restrictions in is not considered necessary to create a system for testing all herds.

On farm control

Nucleus and multiplier herds

Depending on herd size 10-55 faeces samples are collected at each farm annually which enables a prevalence of 5% positive animals to be detected (with 95% confidence).

Herds at risk

Sampling is similar to the above.

Herds not covered by slaughter house base control programme

All sows are sampled twice a year in herds with 50 sows or less. In larger herds a sufficient number to detect a prevalence of 5% *Salmonella* with 95% confidence are sampled twice a year. Pooled faeces

are taken from each pen with growers and finishers. In fattening herds corresponding numbers of samples are taken. The herd is considered as *Salmonella* negative if the 2 successive samplings gave negative results.

Importation

The number of imported animals is very limited. Practically all imported animals have to stay in quarantine stations for varying periods. Therefore, the need for specific *Salmonella* importation regulations has been limited. However, if importation of animals increase specific regulations will be put in place.

Quarantine

Animals have to be isolated on arrival and tested for *Salmonella* with negative results before contact with other animals is allowed. The animals are sampled twice with at least 2 weeks between each sample.

Feed control

Feed producers delivering feed to swine herds have to conform with general rules and hygienic requirements as laid down in the feed legislation. However, there are no requirements for heating of such feed.

Movement control

Control is not considered necessary unless herds are put under restrictions.

Hygienic measures

There is no specific programme for hygienic measures as related to *Salmonella* in conventional herds. Rules only exist in herds found infected with *Salmonella*. Hygienic and clinical control is the responsibility of the practising and animal health veterinarians. If conditions might affect animal welfare, the authorities have to be notified.

Abattoirs

The sampling frame is all fattening pigs slaughtered in Sweden during 1 year and will detect a prevalence of *Salmonella* of 0.1% with 95% confidence limits. In 1994, a total of 7697 samples were examined. Sampling is performed on all working days and collected evenly throughout the day and the prevalence of *Salmonella* was 0.1%. In addition, approximately 6000 pigs are slaughtered under special conditions and *Salmonella* isolation is always performed.

Measures if *Salmonella* is isolated

Rules governing measures to be taken if *Salmonella* is found in herds are detailed in: LFSS 1982: 39. Any isolation of *Salmonella* in a herd has to be reported to the authorities and the isolate sent to the National Veterinary Institute for serotyping. The

herd is put under restrictions and investigated. Restrictions include access limited to the veterinarians and officials, prevention of the selling and buying of animals and certain other hygienic measures. Restrictions are not lifted until the herd is found free of infection. Stricter rules apply when *S. Typhimurium* is involved.

Animal owners may be compensated for the measures laid down and as a rule up to 70% of the cost may be reimbursed.

Follow up studies

In order to control the efficiency of the *Salmonella* control programme slaughter house surveys are performed.

DENMARK

The Danish Salmonella Surveillance and Control Programme for pigs operates at all stages of the production chain and has been applied nationally since 1995. An ELISA technique which can detect 90-95% of the *Salmonella* serogroups in pigs is used for monitoring and in any one year ~800,000 are analyzed and results are available monthly. Based on the results of the previous 3 months the herds are classified into 3 categories:

Level 1. Herd with no or very low *Salmonella* prevalence (95% of herds).

Level 2. Herds with a higher *Salmonella* prevalence (3.1% of herds).

Level 3. A higher proportion of reactors (1.3% of herds).

This year, August 1st, 2001, the programme will be amended at a number of stages.

1/ Animal feeds.

The control with feedstuffs continues unchanged. Compound feedstuffs are heat treated at 81°C to

eliminate *Salmonella*. Mandatory testing is done in all plants producing animal feed. The testing requires microbiological analysis of compound feedstuffs, as well as samples from critical control points during production. (In the year 2000 only 0.3% of samples were *Salmonella* positive).

2/ Breeding and multiplying herds.

The surveillance of these herds remains unchanged. Each month, all herds are tested for *Salmonella* antibodies in serum samples and based on the results a *Salmonella* index is calculated. If the index exceeds 5, faeces from pens are collected and cultured for *Salmonella*. If the index exceeds 15 a sales ban is imposed on breeding pigs, which is not lifted until the index is below 15.

If a sow herd sells weaners to a *Salmonella* level 2 or 3 finishing herd, pen samples must be examined for *Salmonella*.

3/ Slaughter pigs herds

The classification scheme for slaughter pigs will be adjusted on the following points:

- The sampling frame will be simplified into 60, 75, or 100 samples per year depending on the herd size.
- Herds with an annual kill of less than 200 pigs will no longer be sampled, which leaves 1.6% of slaughter pigs outside the scheme.
- The cut off for evaluating individual meat-juice samples will be reduced from an OD% 40 to OD% 0. Thereby doubling the number of positive samples.
- The previous 3 months serological samples will be weighed 0.2:0.2:0.6, and the weighed mean is called the serological *Salmonella* index.
- A herd will be assigned to one of three levels monthly. The limit between level 1 and level 2 will be set at index 40, and between level 2 and 3 will be index 70.

New Financial Penalties

The level of penalty will correspond to the *Salmonella* index level: level 1 - no penalty, level 2- 2% of the value of the carcass, and level 3-4% of the carcass value.

Level 2 and 3 herds

The veterinary authorities require that faecal samples are taken in order to identify the *Salmonella* serotype. Herds assigned to level 3 have to be slaughtered under special hygienic precautions. This is done at specially designated slaughterhouses at the end of the day to prevent cross-contamination with other carcasses. Carcasses from level 3 herds also have to be heat-treated or subject to other special treatment.

New surveillance method for *Salmonella* in fresh pork

Since 1993 fresh pork has been surveyed for *Salmonella* species at the slaughterhouses every month. A new method of *Salmonella* testing on carcasses was introduced by January 1st 2001; 5 carcasses per slaughter day are swabbed at 3 defined areas (the sternum, the hind leg near the tail and the jowl) at 100cm² for each sample. The swabbing areas were originally defined by USDA, USA. This method is more sensitive than the one previously used, and the number of positive samples recorded

is expected to increase. Preliminary results for 2001 show a prevalence of 1.4-1.8%. This should be regarded as an effect of the improved test sensitivity and not increased *Salmonella* prevalence as such.

DT 104 Herds

Herds infected with multiresistant *S. Typhimurium* DT 104 have to follow additional restrictions. The herd is given a Zoonotic Restrictions Order. This includes a requirement for a herd intervention plan, restriction on livestock trade, and a requirement for special slurry handling. The herd intervention plan is made to ensure that *Salmonella* reducing measures are implemented in the herd for at least 12 months, and the restriction on livestock trade is to prevent the spread of DT 104 infection to other herds.

Hot water decontamination

Finishing pigs infected with multiresistant *Salmonella* Typhimurium DT 104 may either be slaughtered under special hygienic conditions as with level 3 herds with subsequent heat-treatment or may be decontaminated with hot water. Decontamination is applied to carcasses after removal of organs. The carcass is showered with 80°C hot water for 14-16 seconds, which produces a significant reduction in the bacterial count on the surface. Five carcasses from each batch are tested to ensure that the process is effective. If *Salmonella* sp. is not detected, the whole batch may be used for fresh consumption.

The Danish *Salmonella* Control programme has been applied nationally since 1995 and the following table shows the prevalence of *Salmonella* especially Typhimurium before and after the commencement of the programme.

Prevalence of *Salmonella* before and after the commencement of the programme (Christensen *et al*, 1999).

| | 1993/94 | 1998 |
|---|--------------------------|-------|
| <i>Salmonella</i> herd prevalence | 22.2% | 11.4% |
| Overall prevalence | 6.2% | 3.2 |
| <i>S. Typhimurium</i> herd prevalence | 13.4% | 7.1% |
| Proportion <i>S. Typhimurium</i> isolates | 64.4% | 60.8% |
| Herd types in 1998 with <i>Salmonella</i> | breeding/ multiplying | 11.7% |
| | Farrow/ grower | 16.7% |
| | Slaughter | 11.4% |

THE NETHERLANDS

Current legislation and “voluntary rules” in the pig industry which affect the Salmonella-status of pork.

Currently, legislation involving the pig industry originates from two sources: the government which produces mandatory laws for all parties involved in the pig industry and the Product Boards. Product Boards are organisations of a certain kind of industry with regulatory powers. These regulations are mandatory for all companies which under the aegis of that specific Product Board. For example, All farmers and slaughterhouses are under the supervision of the Product Board for Livestock, Meat and Eggs. There is a Product Board Animal Feed for supervision of all feed mills. Legislation or rules made by the Product Boards could be described as “voluntary” because they were set up by the industry itself for purposes of self regulation but once set they are mandatory for that sector. In addition Product Boards facilitate in creating quality control system rules which are not mandatory but truly voluntary rules, for example the IQC schemes. Participants work according to signed contracts and inspection schemes in an industry wide implemented quality scheme.

In the summary given below voluntary and mandatory rules for the pig producing sector are

described which could affect the *Salmonella*-status of pig herds.

Feed

About 75% of all weaners, growers, fatteners and finishers (finishers for short) receive heat treated pelleted compound feed which is produced for about 98-99% under GMP guidelines. All IQC-contracted enterprises receive 100% GMP-feed. These GMP guidelines include rules on monitoring and intervention of *Salmonella* contamination of feed and feed ingredients and are set up and carried out on behalf of the Product Board for Animal Feed (PBAF). *Salmonella* is found in less than 1% of the pig feed and *S. Typhimurium* is only a small minority of the isolated strains. By October 1st this year all pig feed mills must have a certified HACCP system (PBAF rule).

The remainder 25% of the finishing pigs receive a complete fermented liquid feed based on by-products from the human food industry of which several risk factor analysis have demonstrated that it has a profound inhibiting effect of *Salmonella*-infections. GMP guidelines for these kind of feeds are being developed.

Tylosin has been banned as a growth promoter in finishing feed.

Pig herds

Government legislation prescribes a hygiene barrier facility including a changing room in which clean boots and overalls are available for visitors and where all people entering the herd should wash their hands with water and soap. Disinfection trays or boot brushes with a disinfectant are required by law at the entrance of all herds. The farmer is required to see that visitors comply to these rules:

- 1/ Farm yards should be closed (required by law), preventing entrance of unauthorised vehicles. This can be done by a clearly visible rope, chain of gate.
- 2/ Herds receiving pigs are required by law to have a washing place for vehicles delivering pigs and are required to supply water to enable lorry drivers to clean their vehicles after delivery of the pigs.

3/ The farmer is required to see that lorry drivers comply to these rules. A log book registering all cleaning and disinfection activities should be kept with the vehicle.

4/ Herds are required by law to have a clinical inspection of all pigs by a veterinarian at least once a month (swine vesicular disease [SVD]-control scheme).

5/ Herds are required by law to have a cooled storage facility to store dead pigs until they are picked up by the lorry from the rendering plant.

6/ Finisher herds can have a maximum of three suppliers of pigs (required by law). Identification and Registration (I and R) of all herds (1 and more pigs) and an automated system issuing transport permits upon request by farmers (RVL - Regeling Varkens Leveringen - Regulation Pig Deliveries) allows control on the compliance with this rule.

Integrated Quality Control (IQC) requires:

- 1/ Bird proofing of pig houses.
- 2/ Fly and rodent control.
- 3/ Keeping doors locked to prevent unauthorised entry of visitors.
- 4/ A bell at the main entrance to announce visitors.
- 5/ Visitor registration (law).
- 6/ Limited antibiotic use on veterinary prescription only with longer withdrawal times before slaughter than prescribed by law.
- 7/ Stress reduction by feed withdrawal before delivery of market age pigs to the slaughterhouse. By using no tranquilizers before and during transport. And no electric goads are to be used.

About 85% of all finishing herds participate in a IQC programme.

Practically all pigs are kept on partially slatted floors in total confinement, in an AIAO management system with high pressure cleaning of facilities between batches of pigs. Minimum stocking density for finishers is 0.7 m² per finisher from 25 kg upwards (law).

Transport

Lorries can carry only pigs from one herd at the time to another herd or slaughterhouse (law). After each delivery, lorries have to be cleaned and disinfected. At slaughterhouses the result is checked by an employee of the National Livestock and Meat Inspection Agency (RVV). Data on cleaning and disinfection are registered in a log book kept with the vehicle.

The transport sector has its own IQC-system, including guidelines on animal handling, driving habits and stocking density during transport. Stress reduction is realised by a nearly 100% loading lifts on the lorries. Animal welfare is measured by a required inspection on transport loss and carcass damage. In addition, training of drivers and workers is required.

Slaughterhouses

GMP-guidelines (created and controlled by the Product Boards for Livestock, Meat and Eggs [PVE]) include monitoring for proper singeing, full stomachs during slaughter, faecal carcass contamination, carcass contamination with bile, and prescribes a number of carcass samples to be investigated to verify hygienic handling by measuring the quantity of Enterobacteriaceae as a result of faecal contamination.

Some slaughterhouses have a USA export certificate which includes monitoring and, if necessary, corrective action on the *Salmonella* contamination of pig carcasses. Hygienic handling without decontamination in the Netherlands result in low levels of *Salmonellae*, similar to that found in the USA where decontamination is permitted. Many slaughterhouses are implementing/ have implemented HACCP rules, including *Salmonella* as a hazard.

None of these rules were specifically set up to reduce *Salmonella* contamination of the end product with the exception of GMP guidelines on Enterobacteriaceae monitoring by assessment of Enterobacteriaceae levels that relate to the contamination with *Salmonellae* and intervention for

feed before transport and hygiene in the slaughterhouses. However, all of them might contribute to the reduction of *Salmonella* in one way or another. A specific *Salmonella* control scheme to reduce the *Salmonella* contamination of pork has not been set up yet. However, the sector has agreed that such a scheme should be set up and the Product Boards for Livestock, Meat and Eggs is in consultation with the sector on the contents such a scheme. This autumn a pilot study will be started to see if the intervention measures described by Swanenburg (2000) can be implemented in a practical situation. Specifically the logistic slaughtering will be tested. Results will be implemented in the control scheme.

GERMANY

After a preparatory period of 1-2 years it was proposed to establish the infra structure of abattoirs (27) fattening farms (2400) laboratories and government institutions and to work out the logistics it is proposed to monitor *Salmonella* using the meat-juice ELISA. The first step was to encourage slaughter houses to participate in a voluntary programme, but it has been concluded that the programme can only continue if it is obligatory for all and supported by the government.

The testing protocol will examine: all animals in herds < 5; 45 in herds <100, 50 in herds of 100-200 animals and 60 in herd >200. Depending on the results no action will be taken if the seoprevalence is <20%, if 20-40 veterinary consultation and if >40% control measures under veterinary supervision will be established. As yet the programme has not been implemented in legislation though some companies are undertaking voluntary testing.

FRANCE

In France, companies have their own *Salmonella* control schemes. A preliminary and experimental plan has also been set up nationwide under the aegis of the Ministry of Agriculture since 1999.

UNITED KINGDOM

While individual companies may have their own *Salmonella* control schemes, there is no nationally organised programme. The British Pig Executive is planning to introduce a national *Salmonella* Monitoring Programme as part of its Zoonoses Action Plan in 2002.

Under the 1989 Zoonoses Order, the isolation of *Salmonella* from a number of different animal species including pigs is reportable to The Ministry. Under the Order, The Ministry has powers to carry out further investigations and prevent the movement of animals and equipment. Advice will also be offered to the farmer to help control infection on the farm. To assist the farmer, codes of practice for the prevention and control of *Salmonella* on pig farms (and incidentally, other types of farms) are available (MAFF Publications, Admail 6000, London SW1A 2XX).

The Animal By-Products Order, 1999 requires official quarterly testing of products that may be incorporated in animal feeding stuffs. Likewise, imported animal protein is also subject to legislation and testing. Voluntary codes of practice have also been produced to give guidance to the industry.

SALMONELLA RESEARCH REQUIREMENTS IN PIGS IN THE UK

The British pig industry is going through the most protracted crisis in living memory. A period of nearly two years in which pig prices have been below the cost of production has resulted in a substantial decline in British pig production. This has had an impact on the remainder of the supply chain with feed suppliers, abattoirs, processing companies and others all affected. Among the challenges facing the industry are:

- 1/ Global over supply of pig meat.
- 2/ An open and increasingly competitive EU market, which is likely to be exacerbated with the enlargement of the EU, especially the membership of Poland and Hungary, and access of cheaper US and Canadian pork to the European market.
- 3/ Erosion of technical efficiency in relation to our main competitors.
- 4/ The apparent lack of a perceived interest by individual sectors within the British pig meat supply chain.

To meet these challenges a strategic plan has been produced by the British Pig Executive, the plan is based on three main elements:

- 1/ Reduce costs to a competitive European level.
- 2/ Defend and add value to the market by consumer and product segmentation.
- 3/ Develop market segmentation in key European markets and move beyond UK dependence.

Food safety, welfare, disease control and the environment, will all impinge on the 3 above elements and the fragmented nature of the British pig industry already means that it is lagging behind its main competitors in addressing some of these issues. Equally the relationships between the various issues are complex e.g improved welfare may be at the expense of the environment, possibly

productivity and food safety considerations. In the UK there appear to be a number of farm assurance schemes, each with a different set of standards and it is important that for the UK as a whole there should be a common standard if the UK is to compete with its competitors.

Another factor that now needs to be taken into account is the aftermath of the current Foot and mouth Disease (FMD) epidemic. While some units that have been slaughtered out may be repopulated and possibly enlarged, others will be left empty or converted to other uses. Some consider that important factors in the current FMD, and earlier Swine Fever, outbreaks were the size of modern day production units and transport of animals. Davies (2001) considered that for these acute, infectious viral diseases there were 3 possible solutions:

- 1/ Vaccination.
- 2/ Increase and enforce sanitary blocks, and the use of all-in, all-out systems for fattening pigs.
- 3/ Much advocated in the press is the dismantling of very large livestock units and gradual dispersal of the dense animal populations that have developed during the last 2-3 decades.

The last option may be desirable on sanitary grounds and may well happen in Western Europe after Central European countries join the Union. Indeed, in the long term, economic pig production in large vertically integrated operations in Central Europe, financed by overseas capital and with the potential to increase cereal production, may make it difficult for the UK producers to compete.

Future trends in pig production systems

Effects < 5years

- 1/ Minimal investment in intensive housing. Limited further increase in outdoor breeding herds, and in marginal land/ climate areas some move of outdoor breeding herds at least partially indoors.

- 2/ Likely increase of herd size, but some farmers will leave the industry.
- 3/ Move to greater use of artificial insemination/ embryo transfer, leading to reduced purchase of boars.
- 4/ Banning of remaining antibiotic digestive enhancers (growth promoters).
- 5/ Welfare pressures by consumers/ retailers on production systems, *e.g.* slatted floors, farrowing crates, leading to the use of straw.
- 6/ Environmental considerations. The EU Rural Environment Protection Scheme (REPS) programme places a strong emphasis on controlling nutrient use, especially phosphates and nitrates, in agriculture and so requires regular analysis of soil surface, (streams/ run off) and ground water (deep wells).

Effects 5-15 years

- 1/ Further division of the industry into 2 sectors:
 - i/ based on intensive housing within EU/ UK welfare regulations producing low cost product efficiently. Likely to be larger enterprises, some of which will be corporate owned. There will also be a move towards a more vertically integrated approach. These operations are likely to have a pyramidal-type structure, with genetically improved herds at the top, and possibly with A1 studs linked in.
 - ii/ Higher cost production systems with extra limitations on use of slatted flooring *etc.* These will be mainly relatively small producers and privately owned enterprises *e.g.* organic, rare-breed types, selling into niche markets and within their locality.
- 2/ Continued production of breeding pigs, but mainly for an export market.

Effects > 15 years

- 1/ Increasing controls, EU based, on stocking density in geographical regions, production systems and individual unit size.
- 2/ A withdrawal from the global marketing of pigs

because of significant problems of residues, disease control and environmental contamination.

- 3/ Possibly a large reduction in pig production in the UK.

Salmonella Control

Consumers are getting more individualistic and idiosyncratic in their food requirements, and demands such as food safety and health are high on the political agenda. In the UK, the Food Standards Agency (FSA) has just published its strategic plan for the next five 5 years (see <http://www.foodstandards.gov.uk>). The plan stresses the importance of putting the consumer interest first in developing food policy and regulation, through a farm to fork approach to food safety.

FSA has set a target of reducing foodborne illnesses by 20%, and it plans to improve existing surveillance systems to monitor how well it is meeting its targets. It considers that the safety of food is the primary responsibility of those who produce and process it and for the enforcement agencies to oversee them. FSA plans to work with industry to promote good practice and to implement HACCP procedures.

Salmonella control is more advanced in Scandinavian countries, with programmes in Sweden and Finland directed towards elimination. The Northern European countries appear to be following the Danish suppression programme, which is based on serological testing on farm and at the abattoir to identify heavily infected farms. Measures are then taken to reduce the number of *Salmonella* infected pigs entering the abattoir (details of different schemes will be found in Annex). In the short term, it is likely that semi-compulsory or compulsory programmes will be introduced in the UK perhaps under the auspices of the Food Standards Agency or the European Union (see earlier). Introduction will be easier if the industry becomes vertically integrated, with *Salmonella* control of food supplies, pig farms and of slaughter and meat processing plants.

Control measures will have to be applied throughout

the production chain, and also include the feed mills for which Codes of Practice have been produced (MAFF). Control measures on the farm, and also in the abattoir, would include the use of ELISA serology to identify current levels of infection and in the longer term an indication of the success of intervention strategies. Feed is likely to be either liquid, possibly fermented, or meal because many recent studies have shown a positive effect in *Salmonella* reduction. Other aspects of *Salmonella* control on pig farms will be found in the Code of Practice for the Prevention and Control of *Salmonella* on Pig Farms produced by MAFF/SERAD. In the abattoir HACCP systems will need to be validated by bacterial culture on a regular basis, and changes made to inspection and abattoir procedures.

Immediate Research Requirements

Control and on-farm monitoring

1/ To meet the FSA strategic plan will require a proactive approach by the pig industry and an increased investment in research and development into on farm-reduction of *Salmonella*. The first step will be:

To determine *Salmonella* levels on individual farms. This can be estimated by the use of the meat-juice ELISA and blood testing of breeding animals. However, isolation of *Salmonella* by bacteriological culture will be necessary to confirm the farm's *Salmonella* status. Likewise it will be necessary to have more targeted epidemiological studies and this will necessitate the use of molecular genetic techniques to study epidemiology. The techniques are now available to provide greater precision and accuracy in determining the spread of *Salmonella* on farms and between farms. Further research is also clearly needed to study the epidemiology of multiple resistant *Salmonella*, the genetic basis for their resistance and the factors which enable them to spread rapidly within the animal population.

Therefore there will need to be an agreed plan for

the pig industry as a whole, which will need validation and adjusting as research findings become available. This will also necessitate epidemiological studies on the different types of production systems *e.g.* single, multisite, all-in all-out, outdoor systems so that meaningful HACCP systems can be developed and possible strategies for monitoring *Salmonella* intervention strategies.

ELISAs are being used in a number of countries for monitoring pig herds, but the capital cost of developing a comprehensive fully automated system, as in Denmark, is huge and it will be necessary to develop ELISA tests that can be used for pooled blood samples, and so reduce costs. A number of ELISA kits are now on the market and these will need validation so that the results are comparable, and to agree an OD cut-off point nationally, but preferably internationally (in Denmark, previously 40%, now 20%; Davies RH and van der Wolf, P [personal communication] consider it should be 10%).

A rapid "pig-side" test is also desirable to determine possible *Salmonella* status of purchased animals, animals before slaughter *etc.*

2/ Antibiotic resistance has become a political issue in recent years, and a number of antibiotic growth enhancers (AGEs) have now been banned in the EU. It is claimed that the use of AGEs results in a potential improvement of 3-5% production efficacy. Likewise in countries where AGEs have been banned therapeutic use of antibacterial drugs has increased in the short term. It will be necessary to develop strategies to reduce antibacterial use, these are likely to be:

i/ The development of improved killed and live attenuated vaccines for *Salmonella* and other porcine pathogens that can be administered orally to prevent infection and disease. It is also desirable to develop a polyvalent vaccine effective against a range of different *Salmonella* serovars (while a *S. Typhimurium* vaccine would be expected to give some cross protection against

S. Derby, it is by no means certain that it would protect against *S. Choleraesuis*).

If vaccines were to be widely used, strategies would need to be developed so that their use does not invalidate monitoring by ELISA. This would necessitate vaccines that have a unique immunological marker that enables the serological response of vaccinated animals to be differentiated from that of natural infection.

There are a number of *Salmonella* vaccines currently on the market, and in the short term their use under field conditions needs evaluation.

ii/ Competitive exclusion (CE) has been widely used in the poultry industry to prevent *Salmonella* infection in day-old-birds. While CE preparations have been developed in the USA, their use under experimental conditions has been equivocal and further research is necessary into use of different CE preparations and bacterial preparations *e.g. lactobacilli*, yeasts, *etc.*

iii/ Feed has been identified as a major influence on the prevalence of *Salmonella* in pigs. Thus, the use of either coarse rations, including barley or fermented liquid feed have been shown to reduce the relative risk of *Salmonella* carriage, however, their use has a negative effect on performance.

Similarly the type of feed affects the likelihood of intestinal rupture during evisceration and hence carcass contamination. While studies are taking place into the use of liquid feed, further work is necessary to determine possible mechanisms, including bacterial ecology of the intestine and studies on the physiology of digestion.

iv/ Organic acids, such as formic, lactic and propionic acids, have been incorporated into feed or given in the drinking water to reduce *Salmonella* colonisation. While some have reported successful experiments, others have found no advantage.

Welfare

In the EU animal welfare is high on the political agenda. Consumer pressure, through the retailer, could radically influence systems of intensive pig production, particularly the confinement of sows, the use of totally slatted floors and the use of farrowing crates for lactating sows. Some aspects of welfare impinge on *Salmonella* control and research is necessary into the effect of straw as compared with the use of slats, especially as slats reduce the faecal-oral contact and *Salmonella* contamination in the pen. Slats, however, may increase stress and offer more harborage for mice. Conversely straw may improve the health and reduce *Salmonella* contamination, although push-through drainage systems associated with many straw based systems may increase the risk.

Processing

The EU veterinary committee recommended in 1996 that visual post-mortem inspection of pig meat should be accepted for pigs coming from herds in which ante-mortem inspection is conducted within a HACCP based quality system. As pointed out previously there are many areas during the slaughter and processing where *Salmonella* contamination may occur, while HACCP procedures are already in place in abattoirs there is need for their validation by bacteriological methods. Such studies would include pre-slaughter intervention on farms to prevent cross-infection during transport or in the lairage, and other areas of the abattoir. Changes to traditional methods of slaughter *e.g.* removal of lungs and trachea intact with the head to prevent tonsil exposure. Likewise there is a need to evaluate carcass contamination and the best methods to use, whether it should be done before or after chilling, and the number of samples to take, whether surface swabs are used or excision.

The Environment

The impact of pork production on the environment is another area where regulatory changes will have an influence on pig farming. The EU Rural Environmental Protection Scheme places a strong emphasis on controlling nutrient use, especially phosphates and nitrates in agriculture and so requires regular analysis of soil, surface and ground water.

This in turn affects the spreading of slurries on land not only for their effect on mineral build up (*e.g.* the use of zinc oxide is said to increase zinc levels in soil) but also spreading of pathogens. One problem

highlighted in recent years, was the disinfection of anthrax contaminated pig slurry - the only solution was to treat it, at great expense, as industrial waste.

Suggested time scale for research and development requirements into *Salmonella* infections in pigs

Research is a continuum and it is better to consider it as short, intermediate and long-term, and the following provides a summary of points raised previously.

Short-term

- 1/ Production and validation of an agreed sampling protocol to be applied to the industry.
- 2/ Evaluation of ELISA kits and development of:
 - i/ an assay that can be used for pooled serum/meat juice samples
 - ii/ a pig-side test.
- 3/ Welfare: straw v. slats.
4. Evaluation of HACCP procedures in the pork production chain, which would include evaluation of cleansing and disinfection regimens.
- 5/ Levels of *Salmonella* and other pathogens contamination in slurries and abattoir effluent, and methods for disinfection.
- 6/ The development and testing of new pig diets *e.g.* Fermented Liquid Feeds and the use of compounds such as organic acids, compound feeds with increased grit size and barley levels and other factors to enhance gut health.
- 7/ Abattoir best practices relating to the equipment and procedures.
- 8/ Pre-slaughter interventions to prevent *Salmonella* contamination in the abattoir, *e.g.* high levels of acidified feed followed by CE.

Medium-term

- 1/ Development of live attenuated vaccines that can be administered orally and which possess a marker to allow differentiation from naturally occurring *Salmonella* and which do not interfere with the interpretation of serological tests.
- 2/ The mechanisms of competitive exclusion (CE) in

Salmonella prevention and the development of CE products.

- 3/ Eradication/ reduction of *Salmonella* from infected premises. An effective step by step approach investigations and control.
- 4/ Abattoir procedures to limit carcase contamination and the possible use of irradiation for the final product.
- 5/ Epidemiology of infections within each type of production system. Research on the effect of different systems should progress through the short-term into the medium-term because of the evolution in the pig industry and continuous development of new systems.
- 6/ The patho-physiology of the intestine and the effects of different diets.
- 7/ Evaluation of biosecurity methods to prevent *Salmonella* infection of pig herds *e.g.* Minimal Disease Herds.
- 8/ Epidemiology and ecology of antibiotic resistant bacteria, including *Salmonella*, and factors affecting the development of resistance.

Long-term

- 1/ Breeding of genetically resistance pigs as a consequence of molecular genetic techniques and the use of embryo transfer.
- 2/ Nationally, totally integrated system of *Salmonella* control throughout the industry, with *Salmonella* control achieved by sound management, including nutritional and environmental factors.
- 3/ More standardised building and site design to reduce environmental contamination, facilitate waste clearance and increase biosecurity by uni-directional movement of pigs.

ACTIVE RESEARCH GROUPS IN THE UK

Epidemiology, surveillance and monitoring

Veterinary Laboratories Agencies - Dr. RH Davies and colleagues.

Scottish Agricultural College - Dr. WJ Smith.

University of Liverpool - Prof. KL Morgan and colleagues.

Vaccines and Immunology

Institute of Animal Health, Compton - Dr. PW Jones.

Cambridge University Veterinary School - Profs. I McConnell and DJ Maskell.

Veterinary Laboratory Agencies - Prof. MJ Woodward.

Public Health

Veterinary Laboratory Agencies - Drs. MJA Wooldridge, RH Davies and SJ Evans.

University of Bristol Veterinary School - Prof. TJ Humphrey and Dr. JEL Corry.

Public Health Laboratory Service - Prof. E Bolton.

Animal Feed

University of Plymouth - Dr. PH Brooks

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ANNEX

Table 1. *Salmonella* in pigs in the UK. Percentage of main serovars 1995-1999.

| Serovar | 1995 | 1996 | 1997 | 1998 | 1999 |
|-------------------------|------|------|------|------|------|
| Typhimurium | 64.4 | 58.9 | 62.8 | 57.5 | 67.9 |
| Derby | 11.9 | 14.8 | 11.7 | 15.1 | 11.5 |
| Goldcoast | 2.4 | 4.1 | 4.3 | 2.3 | 3.1 |
| Kedougou | 2.6 | 3.3 | 5.7 | 3 | 2.3 |
| Panama | 13 | 3.6 | 1.0 | 3 | 1.5 |
| Total no. of incidents. | 379 | 392 | 349 | 299 | 262 |

Table 2. *S. Typhimurium* in pigs in the UK – number of incident of different phage types 1995-1999.

| Phage type | 1995 | 1996 | 1997 | 1998 | 1999 |
|------------|------|------|------|------|------|
| 104 | 83 | 84 | 90 | 63 | 54 |
| 104B | 3 | 11 | 20 | 10 | 5 |
| 193 | 28 | 39 | 34 | 24 | 35 |
| 208 | 47 | 28 | 17 | 17 | 25 |
| U302 | 2 | 8 | 9 | 24 | 12 |
| Others | 81 | 61 | 49 | 34 | 47 |
| Total | 244 | 231 | 219 | 172 | 178 |

Table 3. Antibiotic resistance in *Salmonella* isolated from pigs. Percentage resistant to antibiotics in England and Wales 1995-1999.

| Year | No. of cultures | Sensitive to all | S ₂₅ | Su ₃₀₀ | T ₁₀ | N ₁₀ | Am ₁₀ | FR ₁₅ | Tm ₂₅ | C ₁₀ | Apr ₁₅ | NA ₃₀ |
|------|-----------------|------------------|-----------------|-------------------|-----------------|-----------------|------------------|------------------|------------------|-----------------|-------------------|------------------|
| 1999 | 878 | 15.1 | 32.6 | 54.0 | 77.7 | 3.8 | 32.5 | 0.5 | 30.5 | 20.7 | 2.5 | 6.0 |
| 1998 | 260 | 11.2 | 50.0 | 74.6 | 82.7 | 2.7 | 45.8 | 0.8 | 41.9 | 35.0 | 4.2 | 8.1 |
| 1997 | 361 | 15.8 | 53.2 | 71.5 | 78.9 | 3.3 | 44.3 | 1.9 | 32.7 | 36.0 | 2.8 | 8.0 |
| 1996 | 366 | 14.2 | 45.9 | 73.5 | 78.7 | 4.1 | 39.9 | 1.6 | 38.0 | 33.6 | 2.5 | 6.8 |
| 1995 | 499 | 9.6 | 51.1 | 79.8 | 83.2 | 6.0 | 44.1 | 1.0 | 40.9 | 33.5 | 3.2 | 1.6 |

S: streptomycin, Su: sulphonamide, T: tetracycline, N: neomycin, Am: ampicillin, FR: furazolidone, Tm: trimethoprim/ sulphamethoxazole, C: chloramphenicol, Apr: apramycin, NA: nalidixic acid.

Table 4. *Salmonella* in humans in England and Wales 1995-2000.

| Serovar | 1995 | 1996 | 1997 | 1998 | 1999 | 2000* |
|-----------------|-------|-------|-------|-------|-------|-------|
| Typhimurium | 6743 | 5542 | 4778 | 3039 | 2424 | 2651 |
| Enteritidis | 16044 | 18256 | 23008 | 16397 | 10775 | 8468 |
| Other serotypes | 6527 | 5185 | 4810 | 4292 | 4333 | 3725 |
| Total | 29314 | 28983 | 32596 | 0.728 | 17532 | 14844 |

* provisional

Table 5. The top 5 *Salmonella* serovars in the pigs' sector including animal food production in France.

| Serovar | 1996 | 1997 | 1998 | 1999 | 2000* |
|-----------------------------------|------|------|------|------|-------|
| Typhimurium | 342 | 335 | 312 | 379 | 121 |
| Derby | 165 | 205 | 188 | 162 | 131 |
| Brandenburg | 210 | 77 | 59 | 81 | 20 |
| Bredeney | 32 | 56 | 36 | 25 | 6 |
| Infantis | 25 | 44 | 33 | 92 | 16 |
| Total of isolates from pig sector | 943 | 919 | 786 | 949 | 384 |

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Table 6. Percentage of different *Salmonella* from humans in The Netherlands 1996-2000.

| Serovar | 1996 | 1997 | 1998 | 1999 | 2000 |
|--------------------------------------|-------------|-------------|-------------|-------------|-------------|
| Enteritidis | 44 | 46 | 43 | 41 | 47 |
| Typhimurium | 36 | 30 | 32 | 32 | 30 |
| Bovismorbificans | 2 | 1 | 2 | 2 | 1 |
| Brandenburg | 1 | 1 | 1 | 3 | 2 |
| Derby | 1 | 0 | 1 | 1 | 1 |
| Hadar | 2 | 2 | 2 | 2 | 1 |
| Infantis | 2 | 2 | 2 | 1 | 1 |
| Livingstone | 0 | 1 | 1 | 1 | 1 |
| London | 0 | 0 | 0 | 0 | 0 |
| Virchow | 1 | 2 | 1 | 1 | 1 |
| Panama | 1 | 1 | 1 | 1 | 0 |
| Others | 10 | 12 | 15 | 16 | 13 |
| Total no. of cultures received | 2889 | 2556 | 2266 | 2128 | 2059 |
| Incidence/10 ⁵ population | 28,81 | 25,49 | 22,59 | 21,22 | 20,53 |

Table 7. Percentage of different *Salmonella* from pigs in The Netherlands 1996-2000.

| Serovar | 1996 | 1997 | 1998 | 1999 | 2000 |
|------------------|-------------|-------------|-------------|-------------|-------------|
| Enteritidis | 0 | 0 | 0 | 0 | 0 |
| Typhimurium | 85 | 56 | 76 | 77 | 70 |
| Bovismorbificans | 0 | 0 | 1 | 1 | 0 |
| Brandenburg | 1 | 3 | 2 | 5 | 5 |
| Derby | 2 | 5 | 4 | 3 | 5 |
| Hadar | 0 | 0 | 0 | 0 | 0 |
| Infantis | 1 | 3 | 3 | 3 | 3 |
| Livingstone | 1 | 5 | 1 | 3 | 5 |
| London | 1 | 10 | 3 | 2 | 4 |
| Virchow | 0 | 0 | 1 | 0 | 0 |
| Panama | 4 | 7 | 2 | 1 | 1 |
| Others | 4 | 8 | 6 | 5 | 6 |
| Total | 544 | 1082 | 545 | 438 | 447 |

Table 8. Number. of isolates of different *S. Typhimurium* phage types isolated from humans in The Netherlands 1996-2000.

| Phage type | 1996 | 1997 | 1998 | 1999 | 2000 |
|-------------------|-------------|-------------|-------------|-------------|-------------|
| DT104 (401 + 506) | 218 | 203 | 187 | 218 | 183 |
| 20* | 21 | 23 | 64 | 42 | 23 |
| 60 | 17 | 33 | 13 | 16 | 17 |
| 61 | 22 | 11 | 12 | 11 | 11 |
| 80 | 39 | 17 | 22 | 26 | 14 |
| 296 | 50 | 37 | 54 | 44 | 64 |
| 301 | 18 | 7 | 13 | 9 | 5 |
| 350 | 28 | 23 | 3 | 17 | 11 |
| 351 | 24 | 20 | 23 | 7 | 6 |
| 353 | 1 | 1 | 15 | 16 | 11 |
| 507 | 57 | 24 | 10 | 14 | 10 |
| 508 | 19 | 19 | 17 | 21 | 6 |
| 510 | 154 | 75 | 57 | 59 | 39 |
| 642 | 0 | 1 | 1 | 1 | 0 |
| Others | 334 | 292 | 195 | 179 | 205 |
| Total | 1002 | 786 | 686 | 680 | 605 |

* Dutch phage typing scheme

Table 9. Number of isolates of different *S. Typhimurium* phage types isolated from pigs in The Netherlands 1996-2000.

| Phage type | 1996 | 1997 | 1998 | 1999 | 2000 |
|-------------------|-------------|-------------|-------------|-------------|-------------|
| DT104 | 79 | 103 | 81 | 61 | 82 |
| 20* | 11 | 24 | 21 | 5 | 7 |
| 60 | 15 | 12 | 8 | 7 | 10 |
| 61 | 5 | 14 | 19 | 25 | 4 |
| 80 | 16 | 19 | 22 | 18 | 8 |
| 296 | 13 | 15 | 16 | 9 | 16 |
| 301 | 17 | 14 | 14 | 14 | 0 |
| 350 | 14 | 26 | 8 | 6 | 15 |
| 351 | 22 | 24 | 10 | 23 | 4 |
| 353 | 4 | 1 | 13 | 12 | 21 |
| 507 | 21 | 15 | 12 | 14 | 11 |
| 508 | 20 | 19 | 6 | 3 | 1 |
| 510 | 65 | 109 | 35 | 36 | 41 |
| 642 | 0 | 24 | 3 | 4 | 1 |
| Others | 153 | 207 | 52 | 100 | 94 |
| Total | 455 | 617 | 420 | 337 | 315 |

Table 10. Percentage of different *Salmonella* serovars from humans in Denmark 1997-2000.

| Serovar | 1997 | 1998 | 1999 | 2000 |
|------------------------|-------------|-------------|-------------|-------------|
| Enteritidis | 73.3 | 67.2 | 62 | 51.6 |
| Typhimurium | 16.8 | 17.5 | 17.9 | 18.7 |
| Agona | 0.5 | 0.8 | 1.8 | 3.1 |
| Infantis | 0.5 | 0.9 | 0.9 | 1 |
| Dublin | 0.3 | 0.3 | 0.6 | 0.6 |
| Senftenberg | <0.2 | - | 0.6 | 1.5 |
| Stanley | 0.4 | 0.5 | 0.6 | 0.9 |
| Panama | 0.2 | - | 0.3 | 0.5 |
| Derby | <0.2 | 0.6 | 0.3 | - |
| Others | 8 | 12.2 | 7.0 | 9.4 |
| Total no. of isolates. | 5015 | 3880 | 3268 | 2324 |

Table 11. Percentage of different *Salmonella* serovars from pigs in Denmark 1997-2000.

| Serovar | 1997 | | 1998 | | 1999 | | 2000 | |
|------------------------|------|------|------|------|-------|------|------|------|
| | Pig | Pork | Pig | Pork | Pig | Pork | Pig | Pork |
| Enteritidis | 0.8 | 0.2 | 0.9 | 0 | 0.6 | 0.3 | 0.4 | 0.5 |
| Typhimurium | 79.8 | 66.0 | 78.6 | 54.0 | 70.91 | 58.2 | 62.7 | 58.2 |
| Agona | 0.5 | 0.5 | 0.1 | 0 | 0.4 | 0.3 | 0.8 | 0.5 |
| Infantis | 4.6 | 9.3 | 3.7 | 17.2 | 5.2 | 8.5 | 5.2 | 5.1 |
| Dublin | - | - | 0.1 | 0.2 | 0.1 | 0.3 | 0.2 | 0 |
| Senftenberg | - | - | - | - | 0.1 | 0 | 0.2 | 0 |
| Stanley | 0.2 | - | 0.2 | 0 | 0.3 | 1.3 | 0.4 | 0 |
| Panama | - | - | - | - | 0.4 | 0.6 | 0.5 | 0 |
| Derby | - | - | 7.1 | 6.3 | 8.1 | 5.3 | - | - |
| Others | 14.1 | 24 | 9.3 | 22.3 | 13.8 | 25.2 | 23.1 | 14.4 |
| Total no. of isolates. | 1186 | 559 | 898 | 448 | 1121 | 318 | 1557 | 392 |

Table 12. Percentage of different *S. Typhimurium* phage types isolated from humans in Denmark 1997-2000.

| Phage type | 1997 | 1998 | 1999 | 2000 |
|-----------------------|------|------|------|------|
| DT104 | 7.1 | 13.1 | 25.2 | 10.5 |
| 12 | 58.8 | 43.3 | 20.1 | 19.3 |
| 288 | 3.4 | 3.8 | 9.2 | 0.7 |
| 17 | 1.0 | 2.1 | 4.8 | 1.2 |
| 170 | - | - | 4.8 | 3.6 |
| 66 | 3.5 | 4.9 | 4.4 | 5.3 |
| 120 | 1.6 | 1.7 | 4.1 | 7.6 |
| 193 | 2.3 | 3.4 | 3.0 | 3.6 |
| 110 | 1.8 | 0.6 | 2.3 | - |
| 135 | 2.4 | 3.6 | 1.8 | 3.3 |
| Others | 18.1 | 23.5 | 20.3 | 6.7 |
| Total no. of isolates | 621 | 474 | 437 | 419 |

Table 13. Percentage of different phage types of *S. Typhimurium* isolated from pigs in Denmark 1997-2000.

| Phage type | 1997 | | 1998 | | 1999 | | 2000 | |
|----------------------|------|------|------|------|------|------|------|------|
| | Pig | Pork | Pig | Pork | Pig | Pork | Pig | Pork |
| DT104 | 1.1 | 0.3 | 0.9 | 0.3 | 0.6 | 0.4 | 3.4 | 1.4 |
| 12 | 55.1 | 56.3 | 48.2 | 42.6 | 42.1 | 41.3 | 39.7 | 41.6 |
| 288 | 1.8 | 2.2 | 2.4 | 1.5 | 1.4 | 0.4 | 1.2 | 1.0 |
| 17 | 1.7 | 4.6 | 6.7 | 7.2 | 8.8 | 11.5 | 7.0 | 6.2 |
| 170 | - | - | - | - | 6.9 | 9.7 | 6.4 | 4.1 |
| 66 | 6.9 | 5.0 | 6.1 | 10.2 | 10 | 12.9 | 8.9 | 18.9 |
| 120 | 1.0 | 1.8 | 0.9 | 1.5 | 1.0 | 0.7 | 1.9 | 0.7 |
| 193 | 5.9 | 6.7 | 3.2 | 5.7 | 3.9 | 5.6 | 5.0 | 3.8 |
| 110 | 2.0 | 1.0 | 2.1 | 0.9 | 2.0 | 1.0 | - | - |
| 135 | 2.1 | 0.8 | 2.7 | 1.2 | 2.1 | 0 | 1.9 | 1.4 |
| Others | 19.4 | 21.3 | 26.7 | 28.9 | 21.2 | 16.5 | 8.8 | 16.9 |
| Total no of isolates | 1001 | 1024 | 749 | 333 | 843 | 288 | 931 | 291 |