SALMONELLOSIS IN SWINE: A REVIEW OF SIGNIFICANT AREAS AFFECTING THE CARRIER STATE.

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THE ORGANISM

Introduction

*Salmonella* species (spp.) are facultatively anaerobic, non-spore forming, Gram-negative, facultative intracellular bacteria which belong to the family *Enterobacteriaceae*. The majority of *Salmonella* are motile, however, nonmotile mutants may occur and one serotype, *S. pullorum* is always nonmotile (LeMinor 1984). Infection of animals with various species of *Salmonella* may or may not result in serious disease. It does, however, serve as a reservoir for potential transmission to humans. The interplay of *Salmonella* spp. with its host is varied and may include host specificity, inapparent infections, recovered carriers (subclinical carriers), enteritis, septicemia, abortion and combinations of disease syndromes. *Salmonella* spp. are zoonotic agents and are readily transferred between animals, from animals to humans and between humans by direct or indirect means.

Classification

There is a variety of naming schemes associated with the genus *Salmonella* none of which are completely accepted by scientists in the field. DNA-DNA hybridization between salmonellas has indicated that there is not enough genetic variation to warrant species differentiation within the *Salmonella* genus (Hook 1990). Another study has divided the genus into three species, *S. typhi*, *S. choleraesuis*, and *S. enteritidis* (LeMinor 1984). In this schema *S. typhi* and *S. choleraesuis* each consist of a single serotype, while all other *Salmonella* are grouped under *S. enteritidis*.

Currently, it is recognized that the genus *Salmonella* is divided into two species, *S. enterica* and *S. bongori*. *Salmonella enterica* is further subdivided into six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *indica* and *houtenae* (Leminor and Popoff 1987; Reeves et al. 1989). Most *Salmonella* belong to *S. enterica* subsp. *enterica*. Members of this subspecies are given a name which is usually based on the geographic location where the serovar was first isolated.

At the present time, there are approximately 2300 serotypes of *Salmonella* which differ in antigenic structure, host adaptation and biochemical reactions. The most widely used, and probably the most useful method of differentiating between *Salmonella* spp., is the Kauffman-White scheme (Hook 1990). Serotypes are differentiated by exhaustive cross-absorption and cross-reaction with antisera from the existing serotypes. The antigens responsible for differentiation are the somatic O antigens, as well as the flagellar H antigens and the Vi antigen (Falkow and Mekalanos 1990). Using this system *Salmonella* are named using a genus species format such as *S. typhimurium*. However, the species nomenclature clearly does not define a species but rather a serotype of *Salmonella*.

PATHOGENESIS

Infectious dose

While ingestion of large numbers of *Salmonella* spp. may be required to initiate disease, disease is facilitated often by factors such as peristaltic impairment, interference with intestinal flora and elevation of gastric pH (Clarke and Gyles 1993). The LD$_{50}$ for *S. enteritidis* in germ free mice has been shown to be only 3-5 organisms. However, the comparable value in conventional mice is 10$^6$ CFU (Collins and Carter 1978). These data have implicated normal intestinal flora as one protective factor from development of clinical salmonellosis. They may also serve to explain the greater susceptibility of the very young whose intestinal flora is not fully developed.

Most studies suggest *Salmonella* spp. gain access to the host by an oral route, pass through
the stomach (during which time populations are greatly reduced), then colonize the intestine (Hale 1988). Intraluminal replication varies between serotypes. In swine, *S. typhimurium* replicates to much higher numbers intraluminally than *S. choleraesuis* which is inherently more invasive (Gianella et al. 1973; Reed et al. 1986).

**Adherence**

Attachment of *Salmonella* spp. to epithelial cells has been shown to be influenced by a nonfimbrial, mannose-resistant adhesin which can mediate attachment of *Salmonella* spp. to mammalian tissue culture cells in vitro (Tavendale et al. 1983). Interestingly, many of the serotypes which are less invasive in humans have the mannose-resistant adhesin, whereas the more invasive ones, such as *S. choleraesuis* and *S. typhi* lack the adhesin (Clarke and Gyles 1993). This indicates that other virulence factors mediate disease outcome. Most serotypes of *Salmonella* possess mannose-sensitive hemagglutinating pili (type 1) that bind to mannose derivatives on eukaryotic cells. However, the type 1 pili do not appear to play a significant role in adherence of the bacterium to the ileal mucosa (Finlay and Falkow 1988).

**Invasion**

Much of the knowledge regarding the penetration of the intestine by *Salmonella* spp. is based on work by Takeuchi (1967). The bacteria enter enterocytes through the microvilli or via the tight junctions in between enterocytes then migrate via membrane-bound vesicles to the basal region of the cell. The *Salmonella* pass through the enterocytes to the lamina propria where they stimulate an inflammatory response and are phagocytized by neutrophils and macrophages (Takeuchi and Sprinz 1967).

More recent studies have looked at the invasion process on a molecular basis and have divided this process into two stages. In the first stage, the bacteria adhere to an unidentified receptor on the epithelial cell surface and cause activation of a cascade of events on the cell surface which are mediated by the epidermal growth factor receptor (Galan et al. 1992b; Portnoy and Smith 1992). This series of events indicates that *Salmonella* invasion is dependant on both virulence factors of the pathogen and on the interaction of the host cell with the pathogen. In the second stage, invasion by *Salmonella* spp. induces a tyrosine phosphorylation of the epidermal growth factor receptor. It appears this phosphorylation induces increased intracellular calcium concentrations, microvilli depolarization, formation of extracellular blebs and internalization of the organism (Galan et al. 1992a; Portnoy and Smith 1992). It has been shown that invasion is regulated by several global regulatory systems which are induced by environmental factors such as low oxygen concentration, temperature, and osmolality (Galan and Curtiss 1990; Lee and Falkow 1990; Jones et al. 1992). Other factors influencing invasion include chemotaxis, motility and flagellar orientation (Jones et al. 1992). However, plasmids, which have been shown to be critical for virulence, have no effect on invasion (Gulig and Curtiss 1987).

**Intracellular survival**

Once inside the cell, *Salmonella* spp. multiply in membrane-limited vacuoles with a generation time of about 50 minutes. *Salmonella* spp. can survive within many cells; however, the most significant cell is probably the macrophage. Mutations which destroy this capacity include loss of LPS and certain auxotrophic and regulatory events. Cell wall composition has been shown to influence intracellular survival of *S. choleraesuis* (Griffith 1982). In the intracellular compartment the bacteria are protected from nonspecific defenses such as complement as well as antibody and some antibiotics (Falkow and Mekalanos 1990).

*Salmonella* spp. synthesize over 30 proteins which are selectively induced during infection of macrophages. Two of these are heat shock proteins, GroEL and DnaK. Avirulent, macrophage sensitive mutants have been shown to produce heat shock proteins but fail to synthesize different subsets of proteins normally induced within the macrophage. This indicates that these proteins are important for survival (Buchmeier and Heffron 1990).
**Intestinal inflammation, fluid production**

*Salmonella* spp. are known to produce cholera-like and shiga-like enterotoxins and these toxins may induce diarrhea independent of mucosal damage (Kinsey et al. 1976; Clarke and Gyles 1987). The *Salmonella* enterotoxin gene has been shown to be coded on the chromosome (Chopra et al. 1987). It must be noted however, that the production of toxins *in vitro* is somewhat obscure and not easily studied in the case of *Salmonella*. Therefore, the overall importance of toxin production is not understood. In general, diarrhea observed with salmonellosis is believed to be primarily associated with the inflammatory response induced by *Salmonella* spp. (Gianella 1979). This response stimulates local prostaglandin synthesis resulting in activation of the adenylate cyclase system increasing the secretion of fluid and electrolytes into the lumen (Falkow and Mekalanos 1990). The systemic signs and lesions relating to the septic form of this disease are commonly attributed to endotoxemia as a result of bacterial dissemination.

Cell free extracts of *Salmonella* have been shown to be cytotoxic and inhibit protein synthesis in eukaryotic cells (Koo and Peterson 1982, Koo et al. 1984). These studies provide a molecular basis for the cellular damage caused by *Salmonella* cytotoxin during experimental salmonellosis (Koo et al. 1984).

**Extraintestinal infection**

The struggle between *Salmonella* spp. and the host is usually not localized in the intestine. This is especially true with *S. choleraesuis* infection (Cherubin et al. 1974; Wilcock 1979; Reed et al. 1986). Bacteria which may be intracellular or free in the mucosa and submucosa are transported by the lymphatics to the regional lymph nodes which contribute to the inflammatory response. From the lymph nodes, *Salmonella* spp. may travel via the efferent lymph vessels and drain into the circulatory system. They are filtered out of circulation via the reticuloendothelial system, usually by the spleen and liver. Release of endotoxin into the circulation may account for many of the systemic effects of disease including fever and vascular damage. Thrombosis of the small vessels may lead to ischemic necrosis of the extremities and tips of the ears, particularly with *S. choleraesuis* infection in swine. Failure to contain the infection will result in septicemia resulting in pneumonia, meningitis and septic arthritis (Wray and Sojka 1977).

**Upper respiratory infection**

It has suggested that infection of the upper respiratory tract may influence the outcome of infection. Aerosol experiments in chickens and mice have shown that infections with *Salmonella* spp. can be achieved more regularly via the lungs than by oral inoculation (Clemmer et al. 1960; Darlow et al. 1961).

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 (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, Fedorka-Cray et al. (1995) has demonstrated that the lung is a primary site of colonization following intranasal inoculation of esophagotomized pigs. Additionally, Gray et al. (1995c) has also demonstrated that the lung is colonized in swine that are naturally exposed to pigs infected with *S. choleraesuis*. These data illustrate that lung colonization is not an artifact of experimental inoculation.

Swine have a large number of alveolar macrophages in the lung (Winkler and Cheville 1987). Fedorka-Cray et al. (1995) hypothesized that swine alveolar macrophages may have an impaired ability to contain *Salmonella* spp. within the early hours after infection. However, once uptake has occurred, the alveolar macrophages may act as a vehicle for dissemination of
Salmonella spp. (Fedorka-Cray et al. 1995).

**Virulence factors**

Many potential virulence factors have been identified for *Salmonella* spp. but few have been tested critically for their contribution to virulence. It has been estimated that *Salmonella* spp. possess over 200 virulence factors, only a fraction of which have been characterized (Curtiss 1994). 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.

Several serovars have been shown to produce enterotoxins specifically cholera-like toxin (Prasad et al. 1990, 1992). Very little is known about this toxin as it relates to the pathogenesis of *Salmonella* spp. If it acts similarly to cholera toxin, the B subunit of the protein will bind to the Gm ganglioside on the membrane of intestinal epithelial cells after which the A subunit is internalized causing activation of cAMP and prostaglandin synthesis. These changes would result in fluid and electrolyte secretion (Falkow and Mekalanos 1990).

A common feature of *Salmonella* spp. induced enteritis is severe damage to intestinal epithelial cells likely 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* spp., *Shigella* and enteroinvasive *E. coli*. This cloned protein is a 26 kDa cell-associated hemolysin and its role in virulence is under study.

The LPS of *Salmonella* spp. is a major determinant of host specificity and virulence. 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* spp. in the intestinal tract (Nalue and Lindberg 1990). The LPS component of *Salmonella* spp. also contributes to vascular damage and thrombosis. Endotoxin properties result in fever, disseminated intravascular coagulation, circulatory collapse and endotoxic shock associated with salmonellosis (Takeuchi and Sprinz 1967; Clarke 1985).

Motility provided by flagella appears to be important for invasion for some, but not all, serotypes 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 nonsiderophore mechanism for scavenging iron (Clarke and Gyles 1993).

Finally, heat shock proteins have been shown to be produced by *S. typhimurium* inside murine macrophages. Mutants which 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).
DISEASE IN SWINE

Associated serotypes
Clinical swine salmonellosis can be separated into two syndromes. *Salmonella typhimurium* is associated with enterocolitis, while *S. choleraesuis* is usually associated with septicemia. In the United States clinical swine salmonellosis is almost solely due to infection with *S. typhimurium* or *S. choleraesuis*. Clinical disease has also been associated with *S. typhiuis*. 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; Glock 1994). 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. In contrast, other countries see clinical disease from many serotypes and *S. choleraesuis* may or may not be one of them (Nielsen 1995).

The vast majority of *S. choleraesuis* outbreaks in swine are due to the H2S producing variant *kurzendorf* (Wilcock and Schwartz 1992). However, the non-H2S producing *S. choleraesuis* has been as high as number 2 in the top 10 most common *Salmonella* isolates from swine in a given year (Ferris and Thomas 1993).

Populations affected
Intensely 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 serotype is rare in adult animals; however, infection is not. *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 regardless of age. Disease from *S. choleraesuis* in the adult is not a common occurrence. However, if a susceptible population is exposed, the animals will be affected significantly (Wilcock and Schwartz 1992). It is not known how common subclinical infection is in the adult. Normally only moribund, suspect cases are cultured for *S. choleraesuis*. In suckling pigs disease is distinctly uncommon but infection is not (Gooch and Haddock 1969; Wilcock et al. 1976). The occurrence of salmonellosis in suckling pigs is rare, presumably because of lactogenic immunity, while neonatal swine are susceptible to oral challenge with salmonellae and develop disease similar to that observed in weaned pigs (Wilcock and Olander 1978).

Septicemia
The septic form of porcine salmonellosis is usually caused by *S. choleraesuis*. Affected pigs are inappetent, lethargic and febrile with temperatures up to 107°F. Respiratory signs may consist of a shallow moist cough and diaphragmatic breathing. Clinical signs first appear after 24-36 hours of infection (Reed et al. 1986). Often, producers will find the first evidence of disease as dead pigs with cyanotic extremities and abdomens. In most outbreaks, mortality is high and morbidity is variable but generally less than 10% (Reed et al. 1986; Wilcock and Schwartz 1992). Diarrhea is normally not a feature of *S. choleraesuis* infection until at least the fourth or fifth day of infection. It may last from five to seven days after onset if chronic reinfection is not occurring.

Gross lesions include colitis, infarction of gastric mucosa, swollen mesenteric lymph nodes, splenomegaly, hepatomegaly and lung congestion. Random white foci of necrosis are often observed on the liver (Reed et al. 1986; Wilcock and Schwartz 1992).

The microscopic lesion which is most often associated with *S. choleraesuis* in swine is the paratyphoid nodule. This lesion can be viewed in the liver as clusters of histiocytes amid foci of acute coagulative hepatocellular necrosis and corresponds to the white foci seen grossly (Lawson and Dow 1966). Other lesions may include fibrinous thrombi in venules of gastric mucosa, cyanotic skin and glomerular capillaries. Swelling of histiocytes and epithelial cells typical of gram negative sepsis, as well as hyperplasia of reticular cells of the spleen and lymph nodes are often observed (Wilcock et al. 1976).
Enterocolitis

Salmonella spp. enterocolitis in pigs is typically associated with S. typhimurium infection and occasionally with S. choleraesuis infection. In contrast to the septicemic disease, the initial sign of infection is often a watery yellow diarrhea. Infected pigs are inappetent, febrile and lethargic. Mortality is usually very low, however, morbidity can be very high within a few days after infection (Wilcock and Schwartz 1992).

The major gross lesion at necropsy is focal or diffuse necrotic colitis and typhilitis. Mesenteric lymph nodes are greatly enlarged. Intestinal lesions develop as red, rough mucosal surfaces that may also have gray-yellow debris. Colon and cecal 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).

Histopathologic 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 1986).

EPIDEMIOLOGY

General introduction

Members of the genus Salmonella are extremely ubiquitous in nature, recovered from nearly all vertebrates as well as insects and are often referred to as universal pathogens (Taylor and McCoy 1969; Falkow and Mekalanos 1990). Taken as a whole, it is useful to group Salmonella spp. into three groups on the basis of host-adapted preference. The first group are Salmonella serotypes highly adapted to humans. The prototype of this group is the typhoidal bacillus, S. typhi. The second group are Salmonella serotypes highly adapted to specific hosts other than humans. Some examples of this group are S. pullorum or S. gallinarum which are adapted to avian hosts or S. typhisuis and S. choleraesuis which are adapted to swine. In addition, S. abortusovis is a serotype highly adapted to sheep and is a major cause of abortion in ewes. There are also serotypes such as S. dublin which is viewed primarily as a pathogen of cattle but is often found in other hosts (LeMinor 1984). However, some serotypes in this second group can cause severe disease in humans which may result in high mortality. This has been observed following infection by S. choleraesuis (Cherubin 1980). The third group of salmonellae would be those with a broad host range. Most Salmonella spp. belong to this category and S. typhimurium is the best known serotype of this group. It is the serotype most frequently associated with gastroenteritis worldwide (Falkow and Mekalanos 1990).

Salmonella in pork products

Wilcock and Schwartz (1992) consider the epidemiology of Salmonella in swine as two relatively separate problems: 1) The contamination of pork carcasses and retail products with Salmonella spp. and 2) salmonellosis as a disease of pigs. They also point out that failure of prevalence surveys to distinguish the two conditions has led to considerable confusion about the etiology and epidemiology of clinical salmonellosis in swine. It should be noted that infection of swine and swine products by a wide variety of serotypes is common, but clinical disease caused by serotypes other than S. typhimurium or S. choleraesuis is distinctly uncommon.

Due to the potential threat of foodborne illness in humans resulting from consumption of contaminated pork, it is appropriate that we briefly consider this subject. The results of these
studies will not be discussed in depth here. It is accepted that the infected pig leaving the farm is most often considered the original source of abattoir infections. Also of importance is that *S. choleraesuis* is rarely associated with contamination of carcasses and pork products. As mentioned earlier, *S. choleraesuis* is a host adapted serotype that rarely infects humans; however, in cases where it has infected humans, it presents a disease of grave consequence that is difficult to diagnose and treat (Cherubin 1980).

In contrast, the top 10 serotypes isolated from swine in 1994 (Ferris and Thomas 1994) include at least 3 (*S. typhimurium, S. heidelberg, S. agona*) of the top 10 serotypes commonly associated with human disease (Bean and Griffin 1992). This indicates that serotypes which commonly cause disease in humans may be closely associated with pork and pork products.

**Distribution and prevalence**

Salmonellosis as a disease in swine occurs worldwide but varies markedly in estimated prevalence and mortality as there seems to be variation in prevalence between serotypes. Some of this variation can likely be explained by the virulence of the specific strains endemic to an area or from the genetic variation of breeding stock. Investigators have added to the confusion in this area. Often reviews report epidemiologic data from slaughterhouse or federal surveillance studies which are unsupported by clinical or pathologic criteria for salmonellosis (Wilcock and Schwartz 1990). There is also marked variation in the prevalence of *Salmonella* spp. responsible for disease production in data reported from diagnostic laboratories. There could be many explanations for this amount of variation. Of particular concern are variations in bacteriologic culture methods utilized to isolate the organism. *Salmonella typhimurium* is much less difficult to isolate than *S. choleraesuis* because it grows readily in all of the standard selective media used, whereas host adapted strains often require more specialized media (Ewing 1986).

Overall, regardless of animal species, the number one *Salmonella* isolate is *S. enteritidis*. Ferris and Frerichs (1990) found that *S. choleraesuis* has been, and is currently, the second most frequently isolated *Salmonella* spp. from all animal sources in the United States since 1979. The isolation rate was greater than 99% from swine when compared to all other animals.

In the late eighties and early nineties, the reported isolations of salmonellosis due to *S. choleraesuis* were increasing. One laboratory reported 256 isolations in 1981 with gradual increases to 788 in 1989. In 1989 *S. choleraesuis* was isolated from >95% of swine salmonellosis cases while *S. typhimurium* represented 4% of cases (Schwartz 1990). Causes for the increase are unknown. Recent reports indicate the trend may be declining (Ferris and Thomas 1994).

**Cost**

Owen (1990) estimated that the cost of salmonellosis as a disease to Iowa swine producers ranks second to swine dysentery caused by *Serpulina hyodysenteriae*. The National Animal Health Monitoring Survey estimated that swine salmonellosis is responsible for 28 million dollars in annual production losses in Iowa and 100 million in losses nationwide (Schwartz 1990). There are no estimates of costs associated with subclinical infections of *Salmonella* in swine.

**Source of infection**

As previously noted, *Salmonella* is ubiquitous in nature. However, if one recognizes that *S. choleraesuis* is the most frequent porcine isolate, but is rarely isolated from swine feeds or non-porcine salmonella reservoirs, the conclusion must be drawn that the infected shedding pig is the source of new infections (Wilcock and Schwartz 1992). It has been shown that experimentally challenged pigs can shed up to 10^6/g of *S. choleraesuis* (Smith and Jones 1967) and 10^7/g of *S. typhimurium* (Gutzmann et al. 1976) in the feces. The challenge inocula used in these studies were as high as 10^11 CFU which seems to be an inoculum unlikely found in the environment. The minimum infective dose for either *S. choleraesuis* or *S. typhimurium* has not been established. Often investigators infect swine with doses of 10^8-10^11 CFU. Fedorka-Cray et al. (1994)
demonstrated that pigs infected with $10^4$ CFU of *S. typhimurium* will develop a short term carrier state. Gray et al. (1995b) demonstrated that experimental infection of pigs with $10^3$ CFU of *S. choleraesuis* will be cleared with no apparent shedding or clinical signs. In contrast, a dose of $10^6$ CFU results in persistent infection for at least 9 weeks.

Natural transmission studies with *S. typhimurium* in swine have indicated that subclinical carriers develop when naive swine are exposed to a population of swine shedding $<10^3$ CFU (Fedorka-Cray et al. 1994). Gray et al. (1995c) demonstrated that natural exposure of *Salmonella*-free swine to a population shedding $10^3$ CFU *S. choleraesuis* g of feces will result in a severe clinical outbreak with some of the population carrying the organism for at least 12 weeks. In comparison to experimental models, the naturally exposed swine would have needed to ingest between 250 g ($10^6$ CFU dose) and 25000 g ($10^8$ CFU dose) of feces each to manifest the severe clinical signs observed in this experiment. This amount of coprophagia is unlikely. These data suggest that during natural transmission, the infectious dose of *S. choleraesuis* is much lower than experimental models have previously described. Swine may also be exposed to a large dose of *S. choleraesuis* by a mechanism other than fecal-oral transmission.

**CARRIER STATE AND SHEDDING**

*Species other than swine*

Although *Salmonella* spp. may survive for long periods in the environment, it is widely believed that the carrier animal is the major source of infections for both animals and humans (Wray and Sojka 1977).

The carrier state is defined as the absence of evidence of disease in animals that are able to transmit infection to susceptible individuals (Thrusfield 1986). Carrier animals develop as a result of the interaction of several factors including the serotype of *Salmonella*, age of the animal, and number of bacteria ingested. Young cattle often shed *Salmonella* only during convalescence, whereas adults are more likely to become chronic shredders. In addition a low dose which is insufficient to cause disease may result in a carrier state (Wray and Sojka 1977).

In cattle various types of carrier states have been identified (Wray and Sojka 1977). The active carrier state may follow recovery from clinical disease and cattle may excrete *Salmonella* spp. for months or years in the milk and/or the feces. The active carrier state often persists in the presence of high serum antibody titers to *Salmonella* O and H antigens.

Passive carriers are described as cattle which ingest *Salmonella* spp. and pass the organisms through the intestine into the feces with little or no invasion of the mesenteric lymph nodes. These animals cease shedding *Salmonella* spp. shortly after they have been removed from the contaminated environment (Wray and Sojka 1977). Latent carriers are cattle which have deep tissue infection with *Salmonella* spp. but do not excrete the organism in their feces (Wray and Sojka 1977). Excretion may be reactivated by unknown mechanisms.

Treatment of adult animals with antibiotics during the course of disease is ineffective in eliminating the carrier state in cattle infected with *S. dublin* (Wray and Sojka 1977). It is widely accepted that the antibiotic treatment of humans following infection with *S. typhi* (typhoid) is contraindicated as it prolongs the carrier state (Askerkoff and Bennett 1969).

Certain stress factors have been shown to promote activation or reactivation of clinical signs and shedding in *Salmonella* carrier cattle. These factors include, but are not limited to, transportation of animals, overcrowding, corticosteroids, parturition and concurrent infection. Tannock and Smith (1971b) described the carriage of *S. typhimurium* in sheep for up to 6 weeks after intranasal inoculation. When the same inoculum was given orally by use of a gelatin
capsule a prolonged carrier state was not observed. Tannock and Smith (1971a) also compared the effect of route of inoculation on the carrier state in mice. They concluded the upper respiratory tract provides a focus of infection. When the inoculation route is intranasal, a carrier state results for at least 6 weeks. As observed in sheep, the gastric route of inoculation did not result in development of a carrier state.

*Carrier state in swine*

After experimental challenge of swine with *S. typhimurium*, *Salmonella* could be isolated from the feces daily for the first 10 days and frequently over 4-5 months. Four to seven months post challenge carrier animals were necropsied and greater than 90% of the pigs were positive for *S. typhimurium* in the mesenteric lymph node, tonsil, cecum or feces (Wilcock and Olander 1978; Wood et al. 1989). In an unrelated experiment it has been shown that a sub-clinical, undetectable infection can progress to a high level of shedding after the occurrence of stressful events such as farrowing or transport to slaughter (Wilcock and Schwartz 1992).

In contrast to this minimal understanding of the carrier state of *S. typhimurium* in swine, the duration of shedding and the locations of organisms for *S. choleraesuis* in carrier swine has not been studied until recently. Gray et al. (1995a) has recently described the carrier state of *S. choleraesuis* in swine. These data demonstrate that a subclinical carrier state exists for at least 12 weeks after experimental challenge. The tissues in which *S. choleraesuis* can most commonly be found in carrier swine are the ileocolic junction, ileocolic lymph node, cecal contents, tonsil, lung and colon, regardless of route of inoculation. Swine can also shed *S. choleraesuis* in the feces sporadically throughout the 12 week period (Gray et al. 1995a). A dose dependent effect on persistence of *S. choleraesuis* in swine has also been observed (Gray et al. 1995b). Lower challenge doses such as $10^3$ CFU may be cleared by pigs. In contrast, a moderate dose ($10^6$ CFU) may result in persistent infection for at least 9 weeks. High challenge doses ($10^9$ CFU) have been shown to result in long term carriers which may be related to an observed lymphocyte immunosuppression.

Recent experiments have indicated that *S. choleraesuis* which have been shed from infected swine can survive for at least 3 months in a wet fecal slurry and at least 6 months in dry, desiccated feces (Gray et al. 1995d). This indicates the importance of decontamination of the environment when reduction of *Salmonella* spp. is a goal.

The influence of antibiotics on the frequency and duration of shedding of *Salmonella* in swine is not well understood. However, it is known that antibiotics do not affect the magnitude or intensity of shedding of *S. typhimurium* in swine (DeGeeter et al. 1976; Jacks et al. 1988). Conversely, it has been shown that antibiotics may reduce the magnitude and duration of shedding of *S. choleraesuis* (Jacks et al. 1981).

**IMMUNITY AND VACCINATION**

*Introduction*

There is continual debate over the importance of the humoral versus the cell-mediated immune response following *Salmonella* infections. This controversy stems from the pathogen's ability to reside successfully in both an intracellular and extracellular environment. Taken as a whole, these data suggest that both humoral and the cell-mediated response are important in resistance to *Salmonella* spp. infection. At the present time, there is a lack of information regarding the immune response in swine following *Salmonella* spp. infection.

*Humoral immunity*

Antibodies against *Salmonella* are common in sera following exposure to the pathogen. Passive transfer of antibodies against *Salmonella* spp. to offspring is observed (Royal 1968).
Antibody can provide protection through opsonization of the pathogen, neutralization of toxins and initiation antibody dependent cell-mediated cytoxicity. Many antigens from *Salmonella* have been shown to induce antibodies including LPS (Jimenez-Lucho and Leive 1990), proteins (Foulaki et al. 1989; Saxen et al. 1986; Udhayakumar and Muthukkaruppan 1987) and ribosomal fractions (Eisenstein 1975).

The importance of humoral immunity in protection from *Salmonella* infection is discussed by Eisenstein and Sultzer (1983). The experiments conducted in mice show increased host survival following challenge, increased duration of survival and passive protection against homologous strain challenge. Evidence suggests that specific antibodies in the colostrum of cows vaccinated with *Salmonella* may interact with organisms in the lumen of the gut of calves and influence the outcome of infection (Royal 1968).

Natural and antibody-dependent antibacterial mechanisms may be important in defense against *Salmonella* spp., particularly in the gastrointestinal tract. Secretory IgA is also found in the intestine of animals that have recovered from disease or that have been vaccinated orally (Clarke and Gyles 1994; Stabel 1993). Intestinal antibodies constitute the first line of specific immune defense to organisms which enter the gastrointestinal system.

The amount of specific antibody in serum has not been shown to correlate with protection against experimental challenge of calves vaccinated with either a live attenuated mutant or heat-killed preparations of *Salmonella* spp. (Habasha 1981; Lindberg and Robertsson 1983). In addition, not all animals which are infected or immunized with *Salmonella* develop titers to the organism even though they show increased survival from subsequent challenge (Clarke and Gyles 1994). Recently, antibody titers to nontyphoidal *Salmonella* O-antigen have been suggested to confer protection against challenge (Robbins et al. 1992).

**Cell-mediated immunity**

*Salmonella* is a facultative intracellular pathogen which can evade antibody in the intracellular environment. This suggests that a strong cellular response is needed for pathogen elimination (Sell 1987). Infection with *Salmonella* has been shown to effectively induce cell-mediated immunity (Hanna et al. 1979a; Hanna et al. 1979b; Hassan and Curtiss 1990; Jones et al. 1991). Transfer of sensitized T cells confers protection, whereas transfer of macrophages and B cells are not protective in the absence of T cells. Delayed-type hypersensitivity develops in animals with natural and experimental salmonellosis (Robertsson et al. 1982a,b; Lindberg and Robertsson 1983).

Generally, the cell-mediated immune response correlates well with protection in calves and mice immunized with live attenuated vaccines and subsequently challenged with large numbers of *Salmonella* spp. (Habasha 1981; Lindberg and Robertsson 1983; Eisenstein and Sultzer 1983). Cross-protection has been demonstrated for *S. typhimurium* and *S. dublin* and may be attributed to shared O-antigenic components and porin antigens (Habasha 1981; Lindberg and Robertsson 1983).

Interestingly, investigators have demonstrated that live vaccines were superior to killed bacteria in providing increased host survival which was facilitated by elimination of the organism from the spleen and liver (Collins 1974; Eisenstein and Sultzer 1983). Using a mouse model of typhoid fever the immunity induced by an araA mutant of *S. typhimurium* was attributed to natural killer cell activity that was observed in the early stages of disease. It was suggested that these cells may also contribute to host defense in the later stages of disease (Schafer and Eisenstein 1992).

**Mucosal immunity**

Mucosal surfaces are the major sites in the body in which antigens are encountered. Throughout life, they are continuously bombarded by antigens, whether they are ingested food
particles, microbes such as *Salmonella*, toxins, parasites, or allergens. Most infectious diseases develop on mucosal surfaces and in many the organism is limited to these sites. To combat this constant threat, vertebrates have developed a complex mucosal immune system that undertakes the task of limiting infections without interfering with the function of the fragile mucosal tissue (Klein 1989).

The major humoral immune factor at these sites is locally produced secretory IgA antibody (Mestecky 1987). It has been estimated that 65 to 90% of immunoglobulin-producing cells produce IgA and 75% of the total immunoglobulin produced in humans is IgA (Michalek et al. 1995).

An antigen that has come in contact with the mucosal surface must cross the epithelium to reach the lymphoid tissue in the lamina propria. On some mucosal surfaces, this crossing is affected by specialized cells overlying the lymphoid follicles (Michalek et al. 1995). In the intestine, and probably also in the respiratory system, crossing the epithelium is regulated by M cells. On the side opposite the luminal surface, lymphocytes attach themselves closely to the M cells. The M cells bind antigen on the luminal side, endocytose it and then exocytose the antigen to the lymphocytes on the opposite side. The M cells differentially bind antigens excluding commensal organisms and food antigens. Some invasive pathogens such as *Salmonella* spp. can use this transport mechanism to their advantage which results in access to macrophages and lymphocytes in which they can survive (Klein 1989).

The M cells transport material in both directions. Lymphocytes, for example, have been seen moving through M cells by diapedesis and then entering the lumen of the gut. It has been hypothesized that most of the lymphocytes found on the luminal surface of the gut mucosa may have reached their destination by this manner (Klein 1989). This may be the mechanism by which swine challenged with *S. typhimurium* by mechanisms completely excluding oral gastric exposure are observed to have *S. typhimurium* positive intestinal tissues after only a few hours (Fedorka-Cray et al. 1995). It should be noted that the M cells take up antigens, but are unable to carry out the crucial presentation step which is required to initiate an immune response because they lack class II Mhc molecules and are unable to process the antigen for presentation (Klein 1989). Therefore, the M cells must release the antigen to the mucosal associated lymphoid tissue (MALT) where presentation can take place.

The collaboration of B lymphocytes with T helper lymphocytes (Th) may occur in the MALT or the draining lymph nodes. The B cells that encounter antigen and receive the necessary stimulation from Th cells leave the MALT without secreting antibody. As they mature and begin secreting antibody, they home back to the various MALTs where they settle back into the lamina propria (Bergmann 1986, Ogra and Karzon 1969). If the B cells encounter the same antigen again they begin to produce antibody resulting in a mucosal antibody response. What determines the B cells specificity to the MALT sites remains unclear. However, the homing of the B cells for the various MALTs is one characteristic of the mucosal immune system; another characteristic is the dominance of IgA-producing B-cells over any other B lymphocytes although IgM, IgG and IgE antibodies are also produced but in lesser amounts (Klein 1989).

The cells found in the MALT include lymphocytes, natural killer cells, macrophages, mast cells and eosinophils. At least some of the lymphocytes, the intraepithelial lymphocytes, seem to be unique in the mucosae. Among this population about 15% are T lymphocytes. There is a dispute regarding the remaining 85% of intraepithelial lymphocytes. The lamina propria contains true lymphocytes, both T and B cells, providing the necessary components for the initiation of an immune response in this area (Klein 1989).

Mucosal antibody responses to *Salmonella* antigens have been shown in swine after challenge (Gray et al. 1995 a,c) and vaccination (Stabel et al. 1993). However, there have not
been any studies providing a correlation of mucosal antibody response and increased resistance to salmonellosis in swine despite the critical importance of mucosal immunity.

**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-associate lymphoid tissue. These events induce the production of secretory IgA on mucosal surfaces (Clarke and Gyles 1994).

Recently the development of specific nonreverting 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* spp. smooth LPS (Levine et al. 1989). In several large trials utilizing human subjects this mutant has appeared to be very efficacious. Because of this success, this mutation has been employed for many *Salmonella* serotypes including *S. typhimurium* (Nnalu and Lindberg 1990). However, the *galE* mutation was not successful when utilized in *S. choleraesuis*. The O antigen of *Salmonella* serogroups are the main component of host specificity and facilitate survival in the gastrointestinal tract and entry onto deeper tissues (Nnalu and Lindberg 1990). The *galE* mutation in *S. choleraesuis* does not reduce virulence in swine. This is due to the fact that galactose is missing from the oligosaccharide repeating unit of the O antigen side chain of *S. choleraesuis* (Nnalu and Stocker 1986).

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 have been shown to be safe (Hook 1990; Robertson et al. 1983; Smith et al. 1984).

Mutations in global regulatory pathways have also been a popular means of attenuation. Several studies have utilized strains with deletions (*Δ*) 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 *Δcya Δcrp* 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. 1990; Stabel et al. 1991). A large study evaluating the safety and efficacy of a battery of *S. choleraesuis cya, crp* isogenic mutants in mice indicates that several of these strains are protective and safe (Kelly et al. 1992).

Recently 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. 1991). The nonspecific mutation was obtained by repeated passage through porcine neutrophils. The plasmidless variant lacks the ability to invade Vero cell monolayers and porcine neutrophils as well as having increased resistance to killing by H$_2$O$_2$ and phagocytic killing by porcine neutrophils (Roof et al. 1992).
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 spp.. Traditional culture methods are slow, cumbersome, expensive and require considerable manpower to complete. 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 can be achieved. In addition, the isolation of the organism serves as an invaluable source of epidemiologic data which cannot be overlooked.

It is always advisable to employ enrichment culture in the examination of various kinds of specimens for Salmonella spp. The first step in the culture process should include an assessment of the competing flora and the physical state of the Salmonella in the sample. For example, for a pelleted feed sample which has been heated and dried in the processing step there will likely be relatively few competing flora and the Salmonella may be in a desiccated state. This warrants use of a non-selective nutrient broth (Ewing 1986). In contrast, fecal samples contain large numbers of competing flora and the Salmonella may be in any stage of growth indicating the use of selective media for enrichment (Ewing 1986).

The media suggested for enrichment of Salmonella spp. in fecal specimens from carriers or suspected carriers are tetrathionate broth and selenite F broth (Ewing 1986). Many modifications have been made to these media and the application should be considered when choosing a medium for a specific purpose. Another useful medium for the enrichment of Salmonella spp. is Rappaport medium (Vassiliadis 1983), which utilizes malachite green and magnesium chloride as selective agents. However, this medium is easily overloaded when used as an initial enrichment and care must be exercised when developing an inoculation plan (1986).

Smith (1952) found it absolutely necessary to utilize media other than tetrathionate broth and selenite F broth for the isolation of S. choleraesuis, both of which have been reported to be toxic for S. choleraesuis. The same caution is warranted when any host adapted serotype is suspected as many of the of the host adapted serotypes do not grow well in the highly selective Salmonella media, including S. typhi (LeMinor 1984). It has been suggested that this may explain the infrequent isolation of S. choleraesuis in swine associated epidemiologic surveys (Ewing 1986). 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 serotypes as well as broad host range Salmonella spp. (Ewing 1986).

Many plating media have been devised for the isolation and differentiation of Salmonella spp. Plating media for use of isolation and differentiation of Salmonella spp. and other members of the genus Enterobacteriaceae may be divided into categories according to their selectivity.

Differential media, with little selectivity for enterobacteriaceae, are used with some frequency. This group includes MacConkey agar on which the lactose negative Salmonella spp. appear as white colonies. All Salmonella spp. grow well on MacConkey agar (Ewing 1986). Moderately selective differential media includes Shigella-Salmonella agar and Hektoen Enteric (HE) agar. Hektoen Enteric agar is often considered a standard by which other Salmonella isolation media are measured (Dusch and Altwegg 1995). Again, all serotypes of Salmonella grow well on moderately selective differential media (Ewing 1986).

The final category is the highly selective media which include brilliant green (BG) agar. The BG agars are very popular and can be considered as a one purpose medium for the isolation of Salmonella spp. Salmonella appear as smooth pink colonies on BG agar. Only a few other genus of bacteria can also appear as pink colonies similar to Salmonella and include pseudomonads,
aeromonads, proteus and late lactose fermenting E. coli. Although these agars are very useful in the isolation of Salmonella spp. some serotypes such as S. typhi do not grow well on these agars (Ewing 1986).

A recent study compared HE agar, Rambach agar, 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 spp. 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).

In all cases, pooled fecal samples are preferred over rectal swabs for the detection of Salmonella-carrier pigs (McCall et al. 1966).

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. Assays utilized to detect microorganisms in food and feedstuffs are gaining widespread use in the industry and are called antigen-capture ELISA. 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 feces does 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 spp. in swine feces (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 of Salmonella per ml to detect the organism (Dziezek 1987). In order to achieve these numbers, a time consuming and expensive concentration protocol or a lengthy pre-enrichment must be employed. Some investigators have had success utilizing rapid enrichment protocols to detect Salmonella spp. in swine feces (Cherrington and Huis in't Veld 1993a,b).

The second use of ELISA is to detect animals which have been, or are currently, infected with Salmonella spp. This procedure is not new, and was first described by Carlsson et al. (1972) to detect antibodies specific for Salmonella LPS. Several studies have focused on this approach and have found that LPS antibody titer can be an important diagnostic tool for detection of Salmonella infected cattle (Smith et al. 1989; Spier et al. 1990, 1991).

The detection of antibodies to the O antigen of Salmonella has also been utilized successfully in swine (Nielsen et al. 1994). The mixed ELISA utilizes LPS produced by the method of Westphal (1965) from either S. typhimurium or S. choleraesuis. The majority of swine produce high titers to the O-antigen which are present whether or not shedding can be detected (Nielsen et al. 1994). The test can be utilized as a herd test but is not suited as an individual pig test. Unfortunately, experimentally and naturally infected swine have been shown to have a titer to LPS for at least 12 weeks after exposure to S. choleraesuis even after clearing the bacteria (Gray et al. 1995a,c). This may result in a number of ELISA positive pigs which 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, plasmidless S. choleraesuis vaccine do not initiate a humoral immune response to S. choleraesuis antigens (Gray et al. 1995e). This would suggest that swine vaccinated with this strain would appear as noninfected pigs on a diagnostic test.

Another ELISA has been utilized 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 higher magnitude of infectivity and a higher antibody response.
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 complimentary 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 spp. in food and clinical samples (Araj and Das Chugh 1987; Rahn et al. 1992; Cohen N.D. 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 per gram of sample without preenrichment (Ehrlich and Sirko 1994). Investigators have improved detection methods in PCR assays by combining it with immunomagnetic separation (Widjojoatmodjo et al 1991; Widjojoatmodjo et al. 1992) or by enrichment culture (Stone et al. 1994).

FUTURE DIRECTION

Control of infection caused by serotypes other than S. choleraesuis is reliant on detecting the carrier pig, contaminated feed, or environmental sources of infection. Pigs are most likely to develop disease during periods of stress or when exposed to large numbers of salmonellae. The commingling and transport of weanling pigs from different sources to finishing farms enhances activation of latent carriers and assures exposure of stressed pigs to salmonellae (Allred 1972).

Because S. choleraesuis is rarely, if ever, isolated from feed or feed ingredients the source of new infections would seem to be limited to carrier pigs and facilities previously contaminated with this serotype. It is not uncommon for outbreaks to occur in facilities with good sanitation suggesting that stress is a likely contributor to disease.

Management practices which allow filling of grower and finisher rooms with single source and age pigs is beneficial. In addition, careful attention to good management practices such as proper animal density, dry comfortable pens, temperatures and adequate ventilation is critical (Wilcock and Schwartz 1992).

The detection of carrier animals through culture is sporadic at best because of the unpredictable nature of fecal shedding. Even repeated negative cultures may not ensure that a herd or individual is not a potential source of infection. Use of Salmonella serology will determine if the animal has had previous exposure to salmonella but this has not been shown to have relevance to the carrier status or to the predictable probability of shedding of an individual animal (Nielsen et al. 1994; Kramer et al. 1994). However, serological testing of herds will provide valuable information regarding the ongoing prevalence of infection in the herd and allow a measurement regarding success of control strategies. Additionally, refusal to introduce animals which have a positive titer will eliminate the introduction of potential carriers, but may also eliminate a proportion of the population which is not infected (Wilcock and Schwartz 1992).

In the United States monitoring herds for Salmonella spp. is not commonly practiced. However, other countries have had success with a monitoring and reduction program (Nielsen 1995). Current interest suggests that monitoring herds for the presence of Salmonella spp. in the United States may become more common. In addition, there is a great deal of research being conducted regarding the analysis of hazards and the critical control points for reduction of
**Salmonella** spp. in both the pre and post-harvest setting of swine production.

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