MECHANISM OF HOST-AGENT INTERACTIONS IN SUBCLINICAL SALMONELLA INFECTION IN PIG HERDS

FEDORKA-CRAY, P.J.

Salmonella spp. are ubiquitous in nature and have been recovered from nearly all vertebrates (Taylor and McCoy, 1969) and there is little dispute that Salmonella is an intestinal pathogen (D’Aoust, 1991; Hsu, 1989). Typically, the organism is thought to pass through the stomach after oral exposure, enter into the intestine, replicate intraluminally, pass through the glycocalyx, undergo endocytosis through the cell cytoplasm, then exocytosis through the basement membrane (Hale and Formal, 1988). Malabsorption and/or the release of prostaglandins may play a role in the manifestation of diarrhea (Gianella et al., 1973). Other factors that may influence fluid loss include the presence of an enterotoxin or cytotoxin/hemolysin (Prasada et al., 1990; Libby et al., 1990; Reitmeyer, 1986).

In addition to the economic impact of salmonellosis on the human population, it is also a major economic disease of swine resulting in millions of dollars in lost income to the pork industry (Schwartz, 1990). Although there have been over 50 serotypes of Salmonella isolated from swine carcasses at slaughter, little is known about their pathogenicity. Disease manifests itself in postweaning pigs of all ages and is most often attributed to S. choleraesuis var. kunzendorf and S. typhimurium. Infection in swine typically results in diarrhea and septicemia, with reduced feed efficiency and decreased weight gain (Wilcock and Schwartz, 1992; Williams, 1980). Salmonella choleraesuis is the primary serotype isolated from swine and is associated with septicemia (Wilcock and Schwartz, 1992; Roof et al., 1992; Fraser, 1991). Salmonella typhimurium (or S. typhimurium var. copenhagen) is associated with enterocolitis (Wilcock and Schwartz, 1992; Roof et al., 1992; Fraser, 1991). Both S. choleraesuis and S. typhimurium persistently infect swine (Wilcock and Olander, 1978; Wood et al., 1989; Wood and Rose, 1992; Fedorka-Cray et al., 1994; Gray et al., 1995; Gray et al., 1996a,b) and both are known to infect humans. Pneumonia (Turk et al., 1992) and rectal strictures (Wilcock and Olander, 1977) have also been reported, but their occurrence varies according to the serotype of Salmonella involved. Salmonella agona has not been recognized as a significant pathogen of swine despite its importance in the human population (Williams, 1980). Meningitis, encephalitis, or caseous lymphadenitis may be prevalent in some cases.

Ferris and Miller (1996) reported the top ten Salmonella serotypes recovered from swine in 1996. Many other serotypes of Salmonella are associated with food borne disease in humans and include S. anatum, S. enteritidis, S. heidelberg, S. mbandaka, S. newport, and S. reading (Levine et al., 1991). The top ten serotypes recovered from humans are reported by the Centers for Disease Control (CDC, 1994). Comparison of the swine and human serotypes indicate that three serotypes, S. typhimurium, S. heidelberg, and S. agona appear on both lists. This may be influenced by the fact that a majority of the isolates reported by Ferris and Miller (1996) were

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USDA-ARS-National Animal Disease Center, Enteric Diseases and Food Safety Research Unit, 2300 Dayton Road, Ames, IA 50010
submitted as clinical cases, indicative of a primary or secondary associated infection. Serotypes from non-clinical isolates recovered from swine may or may not correlate with the CDC serotypes.

The USDA: Animal Plant Health Inspection Service (APHIS): Veterinary Service (VS) conducted a study of the health and management of grower/finisher swine as part of the National Animal Health Monitoring System's (NAHMS) Swine '95 Survey (Bush, 1995). A stratified random sample of producers with grower/finisher pigs from the major swine producing states representing 91% of the United States swine inventory was selected for the study. A total of 152 farms participated in the large study. Samples were collected either from July through September or from November through January the following year. From each farm, a maximum of 50 fresh fecal samples were collected from the floor of pens containing late finisher pigs with a maximum number of 10 pens per farm sampled.

A total of 6,655 samples were cultured for Salmonella. The sample and herd prevalence rates were 6.2% (415/6,655 positive) and 38.2% (58/152 positive), respectively. The number of serotypes recovered from the positive farms ranged from one to six (one serotype was recovered from 34 farms). The ten most common serotypes recovered were S. derby (33.5%), S. agona (13.0%), S. typhimurium (copenhagen) (10.1%), S. brandenburg (8.0%), S. mbandaka (7.7%), S. typhimurium (3.6%), S. heidelberg (3.6%), S. anatum (1.9%), S. enteritidis PT13A (1.7%) and S. wortington (1.7%). Among the positive farms (n=58), 15.8% were positive for S. derby while 6.6% of the farms were positive for S. agona.

ANTIMICROBIAL SUSCEPTIBILITY

Since the inception of penicillin, antibiotics have been used to treat bacterial infections in animals and humans for decades (Martel and Coudert 1993). Their use has expanded to not only include prevention of disease in animals but also growth promotion (Wray et al 1993). Misuse of antimicrobials may result in complications such as reinfection or long term carriage, drug induced toxicity, or a development of resistance (Hook 1990). The development of resistance has become a prominent issue in the application of antimicrobics (Cohen 1992; Perez-Trallero and Zigorraga 1995). Bacteria that survive the selective pressure that accompanies antibiotic usage can become resistant to that antibiotic. Susceptibility testing of bacterial isolates not only allows for discrimination between isolates, but allows for assessment of developing resistance. Evangelisti et al (1975) observed more resistant S. typhimurium in chickens after treatment with oxytetracycline in feed. Smith and Tucker (1978) did not observe any decrease in infection after treatment with neomycin in chickens infected with Salmonella. Additionally, they noted the development of neomycin resistant E. coli following treatment. Ling et al (1992) also observed the emergence of resistant bacteria after treating a previously sensitive strain of Salmonella typhi with cotrimoxazole. Antibiotic resistant organisms have been observed after use of medicated feed for the promotion of growth (DuPont and Steele 1987). This usually comes about through the long-term use of medicated feed containing low concentrations of antibiotics (Cherubin 1984).

It is possible to observe no change in clinical signs of infection after a presumably effective antibiotic has been administered. Evangelisti et al. (1975) saw no response to treatment with
oxytetracycline in swine inoculated with *Salmonella typhimurium*. Jacks et al. (1988) observed similar results following use of eflornithin in swine experimentally infected with *Salmonella typhimurium*. In both studies, no differences were observed from treated and untreated groups. Additionally, in a study involving pigs from a *Salmonella*-infected farm, no decrease in number of positive pigs following treatment with enrofloxacin was observed (Dahl et al 1996).

Carrier animals may also be fostered during antibiotic treatment. If the bacterium is not eliminated, it may persist as a latent infection within the host. Treatment can also cause persistent shedding of the bacteria (Hook 1990). Aserkoff and Bennett (1969) have found that some antibiotics actually prolong excretion of bacteria, especially *Salmonella*, in humans. Additionally, antimicrobial treatment may increase the opportunity for transmission of the bacterium to others.

CARRIER STATE

Because of our apparent inability to control salmonellosis in the meat animal reservoir, it has become the most important zoonosis in developed countries. In a study of food borne disease from 1977 to 1984, Bryan (1988) observed that pork was responsible for 11% of the *Salmonella* outbreaks attributed to meat. Factors involved in *Salmonella* food borne outbreaks are multifactorial. Swine, along with poultry, cattle and seafood, are important carriers of *Salmonella* (Bean and Griffin, 1990; Lammerding, 1988). Naive animals are infected from contaminated feed, chronic carriers which are introduced into the population, infected rodents, or from contaminated farm personnel (Heard, 1969; Williams and Newell, 1968; Wilcock and Schwartz, 1992; Duhamel et al., 1992). At the abattoir, the initial source of contamination is the carrier pig and transmission is thought to occur by pig-to-pig contact or from exposure to the contaminated physical environment (Newell and Williams 1971). These infected animals, in turn, contaminate the premises, equipment, and personnel leading to contamination of the final product (Williams and Newell, 1970; Newell and Williams, 1971; Morgan et al., 1987). The initial source, however, remains the persistently infected pig. Current efforts to identify and eradicate the carrier population has been impeded by a lack of information regarding the epidemiology and pathogenesis of salmonellosis in swine. Because *Salmonella* species are widely distributed and persist well in the environment, elimination and control has been difficult (Morse and Duncan, 1974).

Wood et al. (1991) demonstrated that *S. newport* can colonize and persist in swine up to 28 weeks following challenge. Reed et al. (1985) demonstrated that *S. heidelberg* can colonize pigs, however, his study was concluded after 5 days and no information is available regarding the carrier state. Similar studies lasting 5 days were also conducted for *S. typhimurium* and *S. choleraesuis* (Reed et al., 1986). Lavall et al. (1991) indicated that sows are carriers and a possible source of infection and Newell and Williams (1971) isolated *Salmonella* from one week old pigs. We demonstrated that pigs can become infected within 48 h after exposure to a population of pigs shedding low numbers of *S. typhimurium* (Fedorova-Cray et al., 1994). The carrier state has also been examined following exposure to *S. typhimurium*. (Wilcock and Olander, 1978; Wood et al., 1989; Wood and Rose, 1992; Fedorova-Cray and Stabel, 1993). Gray et al. (1995, 1996a, 1996b) conducted some of the most comprehensive studies published on the carrier state of *S. choleraesuis* in swine. However, a paucity of information still exists regarding persistence of other serotypes and regardless of serotype, the mechanism(s) that lead to persistence in the host are still undefined.
Inoculation Routes: Nhalue (1991) has shown that survival and multiplication of different Salmonella serotypes or strains in vivo may depend on different critical properties to overcome host defense in mice. The upper respiratory tract in mice is thought to play a major role in the carrier state (Tannock and Smith, 1971) and the avian respiratory tract was studied to assess effects following intratracheal challenge with an avirulent S. typhimurium (Toth et al., 1992). Wray et al. (1988) demonstrated that airborne transmission can be a primary mode of infection of S. typhimurium in mice and cattle. Hardman et al. (1991) have suggested that aerosols may play a role in transmission of Salmonella between cattle penned individually. In the absence of contact with the gut epithelium, we have demonstrated that 3 h following exposure of respiratory tissues to Salmonella, the bacteria can be recovered from the intestine (Fedorka-Cray et al., 1995). This clearly implicates the upper respiratory tract as a portal of entry. The significance of populations of Salmonella that bypass trafficking through the stomach has yet to be determined.

IMMUNITY

Traditionally, neutrophils have been thought to play an important role in inflammatory and immune responses mainly by phagocytosis and killing of bacteria (Conlan, 1997). Neutrophils are regulated locally and systemically by various mediators such as cytokines and hormones. Salmonellae appear to be able to evade and resist killing by phagocytes (Buchmeier and Hefferson, 1991; Roof and Kramer, 1989). The characterization of neutrophil functions in healthy and Salmonella spp. infected swine has received limited attention. Smith et al. (1981a) were the first to demonstrate neutrophil bactericidal activity in pigs. They observed enhanced in vitro killing of S. aureus by porcine neutrophils at 48 h post S. typhimurium inoculation. It was also demonstrated that porcine neutrophil adherence to nylon fiber columns increased 6 to 48 h after oral inoculation with S. choleraesuis (Smith et al., 1981b).

INTERVENTION STRATEGIES

Competitive Exclusion: Use of competitive exclusion may provide a safe and practical means for control of Salmonella in swine. Competitive exclusion implies the prevention of entry into a given compartment because that space is already occupied, the competing entity is better suited to establish and maintain itself in that environment or the competing entity is producing a product hostile (toxic) to its competition (Bailey, 1987). In the early 1970's, Nurmi and Rantala (1973) demonstrated that the susceptibility of broiler chicks to Salmonellae colonization was probably due to the delayed establishment of intestinal microflora in chickens reared according to modern mass production methods. They also showed that Salmonellae infections could be prevented by feeding the chicks anaerobic cultures of normal intestinal adult fowl flora ("competitive-exclusion" or CE; Nurmi and Rantala, 1973; Rantala and Nurmi, 1973). Since that time the efficacy of the CE concept has been demonstrated in many laboratories (Bailey, 1987; Barnes et al. 1980; Bailey et al., 1991), although little work has been done in other species.

Vaccines: Adhesins, mannose resistant and sensitive, may play a role in attachment of the organism to the gut surface and virulence (Lockman and Curtiss, 1992; Falkow and Mekalanos, 1990). Flagella also allow for association with the gut epithelium and may play a role in survival within macrophages (Weinstein et al., 1984). LPS protects Salmonella from complement killing and
uptake by phagocytes (Groisman, 1990;Jiminez-Lucho and Leive, 1990). The lipid A portion of
the LPS can activate macrophages and cause severe vascular changes (Nilofer and Kuni, 1990).
Enterotoxin, cytotoxin and/or hemolysin may also play a role (Libby et al., 1990). Outer membrane
proteins have also been shown to induce a protective immune response in mice (Foulaki et al, 1989;
Udhayakumar and Muthukkaruppan, 1987). Siderophores (Visca et al, 1991; Jiwa, 1987) and porins
(Matsui and Arai, 1990) may also play a role in virulence. One other virulence determinate that is
required for invasion is a virulence plasmid (Helmut et al, 1985). Virulence plasmids for S.
typhimurium and S. choleraesuis are known to be different (Kawahara et al, 1988; Gulig and
Curtiss, 1987). Disease appears to be attenuated following loss of this plasmid.

Protection against disease may be mediated through successful vaccination regimes. Typically,
vaccines are composed of those determinants thought to be involved in disease. Failure to
incorporate protective antigen(s) into a vaccine may result in inadequate or no protection.
Historically, killed vaccines do not provide for immune elimination of Salmonella from peripheral
organs as has been reported for live vaccines (Eisenstein and Sultzer, 1983; Collins, 1974).
Salmonella appears to require the presence of more than one virulence determinant to cause disease
(Groisman et al, 1990). This makes production of an efficacious vaccine challenging. Control of
Salmonella on the farm is currently undertaken by changes in management protocols or by use
of live attenuated vaccines (Hassan and Curtiss, 1990; Curtiss et al, 1991; Collins et al, 1991; Roof
et al., 1992; Kramer et al., 1992). Although these vaccines are efficacious in reducing morbidity
and mortality associated with salmonellosis, carrier animals are still evident within the population.

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