ZOONOTIC DISEASES, HUMAN HEALTH AND FARM ANIMAL WELFARE

Salmonella

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INTRODUCTION

Salmonella is a major worldwide problem for both animal and public health. It is a Gram-negative bacterium that was first described as the cause of 'hog cholera' in pigs in 1885 by American veterinarian, Theobald Smith, who named the species *Salmonella choleraesuis* in honour of his mentor Daniel Salmon. Over the years, the scientific classification of the genus *Salmonella* has gone through many changes. At present we classify *Salmonella* into just two species: *Salmonella bongori* that is primarily associated with reptiles[1] and *Salmonella enterica*. It is the second of these species that is associated with disease in both humans and animals and is divided into around 2,500 subspecies or serovars which are generally treated as individual species for ease[2]. Most of the 2,500 *Salmonella* varieties can infect a wide range of species and are capable of causing diarrhoea in humans. All of these may have a zoonotic source, in that it can be acquired from animals either directly or more usually from infected food products of animal origin such as meat, milk and eggs.

Throughout the world the most important foodborne *Salmonella* serovars are *Salmonella* Typhimurium and *Salmonella* Enteritidis, both in terms of number of cases and the severity of infection caused. As described earlier *Salmonella* is a worldwide pathogen. The importance of foodborne infection in the worldwide burden of diarrhoeal disease is not known, though is likely to form a substantial component of the 2.5 million deaths due to diarrhoea. *Salmonella enterica* is a robust and adaptable organism. Being able to infect a wide range of hosts and survive well in the environment allows *Salmonella* to thrive in livestock production and be transmitted onto humans through food[3].

Salmonella infection (salmonellosis)

The majority of *Salmonella enterica* are capable of causing gastroenteritis in humans. Generally disease is more severe and more likely to lead to medical treatment and be reported following infection with more aggressive or virulent varieties such as *S.* Typhimurium, *S*. Enteritidis or *Salmonella* Virchow. Typically human salmonellosis leads to painful gastroenteritis accompanied by fever, diarrhoea and vomiting. In some cases the infection becomes invasive and life threatening. Indeed in the UK alone there are around 80-100 deaths caused by *Salmonella* infection. As with many infections the young and the old are most vulnerable.

Salmonella typically attacks the small intestine (ileum). It has evolved a range of systems to ensure its survival through the stomach and is capable of growing both with and without oxygen - allowing easy survival in the gut. During infection *Salmonella* bacteria adhere to the gut wall. They then invade through cells making up the gut wall[4]. This causes damage and inflammation caused by both the bacterium and the host immune system responding to infection. Occasionally infection is poorly controlled and becomes spread through the body that can lead to death[5]. Such invasive *Salmonella* infection is a major cause of death amongst the HIV positive population in sub-Saharan Africa[6].

Sources of human salmonellosis

The majority of human *Salmonella* infections come from contaminated food and in particular meat, eggs and unpasteurized milk. Other foods may also be a source when contaminated with the faeces of livestock or wildlife. This may include fruit and vegetables including sprouted seeds and salad vegetables such as a major US outbreak associated with salad onions used in Mexican foods[7]. The droppings of wild birds are thought to have contaminated both chocolate in the EU[8] and peanut butter in the USA[9] leading to substantive outbreaks in recent years. Nevertheless most infection comes from animal products. Poultry meat and eggs and pork and pork products are amongst of the main sources.

Salmonella infection in chickens

The link between *Salmonella* and poultry is well understood by the public, largely through the *Salmonella* in eggs scare in 1988. At that point *Salmonella* was endemic in UK egg production and cases of *S.* Enteritidis Phage Type 4, the form of *Salmonella* associated with UK egg production were in the region of 3,000-4,0000 per annum. Changes in legislation and production practice have greatly improved the situation, though the link between *Salmonella* and the chicken remains an important source of foodborne infection.

The biology of *Salmonella* infection in the chicken is such that the bird may carry *Salmonella* in its intestines or various sites trough the body with little or no ill effect to the animal[10]. Infected chicken may shed large numbers of *Salmonella* bacteria in their faeces for many weeks or even months[11]. In the gut *Salmonella* usually colonise the caeca of chickens, which are tube like structures that branch off the main chicken intestine. The caeca are regularly emptied by the bird leading to shedding in faeces. Colonisation levels may be high with as many as 100 million bacteria per gram of gut content found in experimental infection. This allows the spread of infection through a flock. This can lead to contamination of meat (muscle tissue) or more usually through cross contamination by intestinal content at slaughter. The industrial nature of both production and slaughter make spread of infection relatively easy.

S. Enteritidis is also able to colonise the reproductive tract of laying hens, again usually without disease[12]. This can lead to infection of the developing egg yolk in the ovaries or in the oviduct as the shell develops[13]. This occurs almost exclusively with *S.* Enteritidis or *S.* Pullorum, though very occasionally has been seen in other serovars. Furthermore faecal contamination after egg laying may also occur with many serovars, which appears to be a problem in intensive, cage-based systems[14].

Infection also induces strong immune responses which means that vaccination has been possible[15]. However protection is largely limited to closely related *Salmonella* serovars. For example there is good cross protection between the closely related *S.* Gallinarum and *S.* Enteritidis which has been exploited by vaccination[16], but protection between the more distantly related *S.* Typhimurium and *S.* Enteritidis is limited[17]. As such vaccines currently used are limited in their range of protection and there is a need for vaccines that can protect against many serovars.

Given its importance as a vehicle for human infection, the mechanisms that underlie egg infection are poorly defined. *S.* Enteritidis survives well and may multiply within eggs, though the mechanisms by which it infects the reproductive tract are poorly understood. A number of bacterial factors that allow attachment and invasion of the ovary and oviduct have been identified, mainly bacterial components that allow attachment to the host cell[18]. It is also clear that changes in the host have a significant role. Typically commercial egg laying breeds

commence egg laying at around 18-20 weeks of age. The rapid development of the reproductive tract and eggs within it place a considerable nutritional and physiological stress on the hen along with profound hormonal changes. The effects on the immune system around this period may be considered analogous in some ways to pregnancy in mammals with immunosuppression particularly of cellular immunity at around 16-20 weeks of age. This has been shown to lead to recrudescence or 'breakout' of infection in birds persistently infected with low numbers *S*. Pullorum and is associated with infection of the reproductive tract by this serovar[19]. More recently it has been shown that birds, even when vaccinated, are more susceptible to *S*. Enteritidis during the onset of lay[20].

Transmission of infection occurs relatively easily within flocks. As chickens are coprophagic (eat the faeces of other birds) and given the high levels of caecal colonisation and faecal shedding then once *Salmonella* enters a flock then it is readily spread in commercial production[21]. As such, prevention of *Salmonella* entering a flock through good biosecurity is essential. *Salmonella* may be introduced from rodents or wild birds or may be introduced on the clothing or vehicles of workers and others entering the farm[22]. Contaminated feed or water is another major source of infection[23]. The major 2010 *S*. Enteritidis outbreak in the USA that lead to nearly 2,000 human cases and recall of over 500 million eggs was related directly to contamination in the feed mill.

Once *Salmonella* enters a farm it may be difficult to remove requiring stringent disinfection and may be re-circulated through rodents or insects on a farm[24]. To a certain extent it has been suggested that *Salmonella* is easier to prevent on animals housed indoors than in free-range production as control of wildlife, insects and disinfection of the 'natural' environment is difficult. However some recent studies have suggested that the role of wildlife, and in particular wild birds, is lower than previously thought with a prevalence level of less than 0.2% in healthy wild birds[25]. As discussed above they may be important source of re-circulating *Salmonella* on a farm, but risk of introducing infection has probably been overstated. Additionally the greater the intensity of production the more likely is the spread of the infection and flocks with poor welfare the more susceptible the animal. It has also been suggested that aerosol transmission may occur for *S*. Enteritidis in commercial egg production, though is not as well understood as the faecal-oral route[26]. Given the difficulty in recognizing infected animals, then serological testing or bacteriological testing of faeces has been required to identify infection in flocks[27]. The development of improved testing and control has been successful in significantly reducing *Salmonella* in UK poultry.

Control of *Salmonella* in the UK

Both legislation and industry practice have lead to considerable reductions of *Salmonella* in UK poultry, especially in the egg sector. Recent European legislation has formalized controls throughout Europe. Under these regulations baseline surveys of *Salmonella* prevalence in breeding flocks, layer and broiler chicken flocks, turkeys and pigs were made by the European Food Safety Authority (EFSA). Each member state was required to develop and implement a series of National Control Plans (NCP) for *Salmonella* and set out targets for reduction in *Salmonella.* In the UK these NCP were rolled out initially in breeding flocks in 2007/08, followed a year later by laying flocks and finally broiler production in 2009.Although it is perhaps too early to judge the success of the NCP in the UK, anecdotal evidence does suggest an ongoing decline in *Salmonella* levels in the UK flock.

Even prior to the introduction of NCP for egg layers, the 'Lion Mark Scheme' adopted by most UK egg producers in 1998 had greatly reduced the prevalence of *S*. Enteritidis in the UK flock with positive flock levels falling from around 200 in 1997 to less than 10 by 2003. The scheme

introduced many of the measures included in the subsequent NCP and also introduced the use of vaccination for hens in both free range and standard production schemes. Initially killed, injected vaccines were introduced, these largely being superseded by live attenuated vaccines delivered via drinking water[28]. The success of this scheme is clear, both in the reduction of positive flocks and human cases in the UK.

Although vaccination has been successful in layers, it has not been adopted in the broiler industry for a number of reasons, chiefly cost[29]. Although the breeding stock of broilers are usually vaccinated against *Salmonella*, the narrow profit margin on an individual animal means the cost of both the vaccine and its delivery are simply too expensive. Coupled to this is that the young age of the broiler chicken at slaughter, typically 39-42 days of age, means it is difficult to get good levels of protective immunity to *Salmonella* and the requirements for withdrawal periods of live *Salmonella* vaccines to prevent entry into the food chain make vaccination an unrealistic prospect[30]. Indeed such practicalities are likely to limit the use of vaccines for the control of *Campylobacter* too. Other approaches including pre-and probiotics usually via feed and the acidification of drinking water have all been utilized in the control of *Salmonella,* though the success of these approaches has at best been moderate[31].

Salmonella in worldwide egg production

Production systems and in particular housing appear to have an impact on infection and faecal shedding, though it should be noted no system is free from *Salmonella*. Large flock sizes, particularly with birds of mixed ages, clearly increase the levels of *Salmonella*[32]. The effect of the housing system is less clear. Some studies show that caged birds have much higher levels of *Salmonella*[33]. Indeed the paper by Namata and others[34], suggests that the likelihood of infection is ten times higher in caged birds over free-range hens. Others have shown that caged systems have lower or equivalent levels of *Salmonella* to free-range or floor housed hens[35]. Recent experimental evidence also shows that alternative systems and free range production have increased risks of transmitting infection from bird-to-bird[36]. There also seems to be an increased risk of contamination of eggs after laying in highly mechanized intensive systems due to difficulties of cleaning cages and equipment leading to contamination[37]. Nevertheless the consensus of opinion is that production systems with higher welfare do not increase the risk of *Salmonella* infection and on balance are likely to have a lower risk of infection[38]. Indeed as chickens are naturally social foraging animals, holding birds singly in cages is simply a convenience of production that leads to poor animal health and welfare which is likely to impact negatively on both egg productivity and quality.

The lack of systematic disease surveillance in either animal or human populations in developing nations means we cannot clearly assess the impact of *Salmonella*, though in countries such as Thailand with rapidly expanding industries for both domestic consumption and export have recognized the problem[39]. More is known about *Salmonella* in developed nations. The EFSA baseline survey gave a clear snapshot of *Salmonella* in European poultry and more recent data suggests levels in egg production at least are falling across Europe. Figures for 2008 suggest that less than 1% of UK laying flocks are infected with *Salmonella* and around 2% in France - both countries having high management standards including vaccination[40]. Levels in Spain, from where imported eggs have been implicated in a number of UK outbreaks, are still considerably higher at nearly 14%, though this is a considerable drop from the initial baseline survey[41].

Perhaps it is the levels of *Salmonella* in US poultry and subsequent transmission to humans that are most alarming. The Centers for Disease Control in the US estimates it has over 1.2 million cases of human salmonellosis a year compared to around 50,000 cases each year in the UK

estimated by the Health Protection Agency. Surveillance of *Salmonella* by the US Department of Agriculture is considerably less rigorous than that employed by Animal Health Veterinary Laboratories Agency in the UK. Vaccination is used by around 50% of US egg producers compared to the over 99% in the UK. Management practice is considerably less welfare-friendly than the UK due to a practice known as molting. Moulting is the forced moulting (or molting in American English) of feathers through withdrawal of feed and water, or provision of low energy feeds for up to 14 days. This causes a flock to moult its feathers and replace with new. It also causes regression of the reproductive tract stopping the production of eggs. When feed is re-introduced, the hen's reproductive system is rejuvenated, leading to laying of larger higher quality eggs at a high rate. The practice extends the length of time hens produce high quality saleable eggs and is commonplace in US egg production, though is rightly prohibited in the EU and many developed poultry industries, though the practice was employed prior to industrial-scale production in many countries. Whilst chickens naturally moult in the early autumn, forced moulting has profound effects on chicken health and welfare and particularly on the immune system which results in increased susceptibility to both intestinal and egg infection with *Salmonella*[42], and may lead to the infection increased levels of disease[43]. The practice is driven by economics, but leads to an increased public health risk as well as a period high physical and psychological stress for the animals[44].

Salmonella in broiler chicken production

Levels in broilers range from 3% in UK carcasses, 7.6% in France, 14.9% in Spain, 17.6% in Germany up to 85% in Hungary[45]. In general terms the further south or east in Europe, the higher the level of *Salmonella*. To some extent this is a reflection of the application of effective controls. *Salmonella* is at low levels in Fennoscandanavian countries with either no *Salmonell*a detected or low levels (0.2% of broiler carcasses in Sweden). These countries were the first to adopt rigorous, even draconian, controls for *Salmonella* in poultry production. In contrast, controls in the USA are less stringent than in much of Europe with higher stocking densities and lower welfare standards. United States Department of Agriculture (USDA) figures suggest as much as 23% of US poultry meat is infected with *Salmonella* compared to less than 2% in the UK. Perhaps of most concern is that the use of growth-promoting antibiotics, banned in the EU in 1999 due to concerns that they play a major role in the development of antimicrobial resistance, is still commonplace with the US Food and Drug Administration only proposing a voluntary code of practice in 2010. In many countries there is no information as to *Salmonella* levels in broilers, though in those countries such as Thailand seeking to export fresh poultry meat are making steps to understand and control *Salmonella*. The high levels of *Salmonella* in some nations are of concern and it may be that the development of industries in such nations where poor management and lower welfare standards lead to continued high levels of *Salmonella*.

Salmonella in pigs

Like the chicken the major public health concern is that *Salmonella* may colonise the pig without ill effect on the animal leading to contamination of pork and pork products at or prior to slaughter[46]. However unlike the chicken, weaner pigs of 6-12 weeks of age may develop diarrhoeal disease in much the same way as humans. It is also pigs of this age that are most susceptible to colonization. Many of the factors associated with infection in poultry hold true in pig production. Whilst in 2010 Animal Health Veterinary Laboratories Agency (AHVLA) found 172 pig herds with *Salmonella* compared to 474 poultry flocks, it should be remembered that the UK slaughters in the region of 10 million pigs per annum, but over 600 million chickens[47]. Indeed some estimates suggest that over 20% of UK pigs have *Salmonella* within their lymph nodes at slaughter. It is thought around 20% of human salmonellosis in the EU is due to

consumption of pork or pork products[48]. As such *Salmonella* in pork is a major public health problem, largely unrecognized by the general public.

S. Typhimurium is by far the most prevalent serovar in pigs making up around 65-70% of all *Salmonella* isolated from pigs in the UK[49]. *Salmonella* Derby also seems to be particularly associated with pigs in the EU[50].

Salmonella infection in the pig

S. Typhimurium is capable of causing diarrhoea or colonizing the lower gastrointestinal tract without causing disease. However unlike the chicken *S*. Typhimurium may colonise and persist in the tonsils and lymph nodes of pigs[51]. This does not happen in birds as they lack structure organ structure of tonsils and lymph nodes found in mammals. The mechanisms by which diarrhoeal disease is caused are broadly those found in human salmonellosis, something that has been exploited by use of piglets as experimental models for salmonellosis[52]. The factors that influence colonization of the gut or lymphoid tissue are poorly understood.

As with poultry, faecal shedding may occur within pig herds leading to transmission. This may occur in the farrowing shed, around weaning, during growing or finishing. Frequently there is a level of maternally-derived immunity in neonatal piglets which may inhibit infection[53]. As such, as described above, much infection occurs when pigs are 6-12 weeks of age, though infection also occurs frequently in younger piglets. Mixing of weaner pigs from separate pens, sheds or farms is considered to be a major factor is the spread of infection. As with chickens, colonization of the gastrointestinal tract during growth may lead to contamination of meat at slaughter[54]. There is increasing evidence that stress on colonized pigs, particularly long transport to slaughter may increase faecal shedding or lead a spread of infection from colonized lymph nodes again leading to shedding at time of slaughter[55].

Antimicrobial resistance and the emergence of monophasic *Salmonella*

Resistance to multiple antibiotic drugs (multi-drug resistance) appears to be a particular problem in pigs. Surveillance of the UK pig herd from 2003-2008 indicated two dominant types of *S.* Typhimurium - DT193 and U288, with 93% of isolates resistant to at least one antimicrobial drug and 67% of isolates resistant to between four and nine antimicrobials[56]. The frequent use of therapeutic and prophylactic antibiotic treatment in pigs is considered to have driven these high levels of resistance[57]. A particular concern throughout Europe and the USA is the emergence of a multi-antimicrobial resistant 'monophasic' *S*. Typhimurium strain[58] that differs in its surface to usual forms of *Salmonella*. The reasons for the increasing prevalence of the monophasic form are unclear, but it may provide and advantage in avoiding being detected by the pig's immune system.

Control and surveillance of *Salmonella* in pig production

Many of the principles for control in poultry also hold true for pigs[59]. Control has, perhaps not surprisingly, concentrated on weaner pigs, though measures to control *Salmonella* in older pigs are being explored. Interventions include feed manipulation, competitive exclusion via probiotic products and treatment of feed to eliminate *Salmonella*[60]. Other steps such as segregation of litters until after weaning may successfully reduce *Salmonella* levels, but are labour, and therefore cost, intensive. Generally it is considered that alternate systems of pig production may make *Salmonella* control more difficult, especially outdoor systems where disinfection and segregation are more difficult[61]. Indeed in the Netherlands highest *Salmonella* levels were found in organically reared pigs.

Vaccination is not as yet widely used in the control of *Salmonella* in pigs. Although there are vaccines available commercially, they do not really offer the protection needed. The requirement for an effective vaccine is clear to both veterinarians and the pig industry[62]. However the lack of basic research in salmonellosis of pigs makes achieving the development of effective vaccines difficult.

As with poultry, EFSA baseline surveys have been used to develop targets for *Salmonella* reduction via NCPs. Prevalence rates vary across Europe from minimal levels in Finland, to over 30% in Spain[63]. However surveillance is better developed in some countries, such as the UK, Netherlands and Denmark and as such these figures are probably more reflective of the true levels. There is relatively little epidemiological information in developing countries with only a few small studies conducted in Africa where prevalence is quite low but pig production is on a small scale. In contrast pig production is extremely important in South East Asia where studies in Vietnam have suggested a high prevalence of *Salmonella*. The worldwide prevalence is summarized in the table below.

Worldwide prevalence of *Salmonella* in pigs at slaughter[64]

Country	*Salmonella* prevalence	Comments	Reference
Finland	0.1%		EFSA, 2010
Netherlands	9%		EFSA, 2010
Germany	12%		EFSA, 2010
France	19%		EFSA, 2010
United Kingdom	22%		EFSA, 2010
Spain	31%		EFSA, 2010
Ethiopia	18%	Limited commercial pig production as pork not eaten by Ethiopian Orthodox Christians and Muslims	Davies, 2011
Kenya	12%		Davies, 2011
USA	10%	Estimated Figure	Davies, 2011
Mexico	50%	Estimated Figure	Davies, 2011
Vietnam	67-93%		Boyen, 2008 Davies,2011

The UK pig industry has taken steps towards the identification and control of *Salmonella* through two schemes led by the British Pig Executive (BPEX) in conjunction with DEFRA. The first scheme, the Zoonosis Action Plan or ZAP scheme, was introduced in 2002 to help producers identify and therefore act to control *Salmonella* in their herds. However the test used did not give an indication of current infection within herds and was limited in testing a small number of *Salmonella* types and as such uptake to this voluntary scheme was poor. Changes in legislation and the requirement of a NCP for pigs have lead to the scheme being updated in 2008 and renamed the Zoonosis National Control Plan (ZNCP) with more frequent and stringent testing. The ZNCP also introduced the 'Platinum Pig Award' given to producers with an annual prevalence of less than 10%.

CONCLUDING REMARKS

As pig and poultry production increase worldwide, so does the potential risk of foodborne salmonellosis. Whilst it is clear that well-managed production systems can limit *Salmonella*, complete elimination is unlikely. The UK has some of the higher welfare standards for animal production, though to a significant number of consumers even these standards are unacceptable. In a global economy the trade in meat and animals has focused on cost, rather than maintaining standards of welfare, hygiene or disease control. The UK egg industry has been successful in reducing *S.* Enteritidis in eggs though vaccination and improvements in biosecurity. It is compliant with EU legislation on welfare and cage size, but competes with industries that are not and produce eggs with a greater risk of *Salmonella* infection. Levels of *Salmonella* in US egg production have remained higher than those of the UK, a reflection both on lower vaccination rates and poorer management and welfare in farms. The scale of US intensive production is bigger UK production in every way with one exception: the space afforded to the UK laying hen is bigger than the space given to its American counterpart. As we in the UK dismantle our 'battery cages' they are sold on to developing egg industries where welfare standards are lower and the risk of both animal and zoonotic infection is greater.

Microbial pathogens constantly evolve and find new niches. The epidemic of monophasic *Salmonella* in pigs is such an example. Changes in production or attempts to control disease can have unexpected effects. Indeed the emergence of *S.* Entertidis in chickens may have been a consequence of creating a niche following the virtual eradication of *S*. Pullorum and *S*. Gallinarum coupled with intensification of the industry. The prevalence of zoonotic pathogens such as *Salmonella* may wax and wane over time and as we develop and apply new interventions in control or the pathogen evolves. However they remain a threat to human and animal health. An integrated approach to animal production that provides safe and sufficient food to meet our growing global requirements must consider animal health and welfare as part of control of zoonotic infections.

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