Environmental Microbiology (2001) **3**(7), 421–430

Minireview

The chicken, the egg and *Salmonella enteritidis*

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Summary

***Salmonella enterica* serovar Enteritidis is the cause of the food-borne salmonellosis pandemic in humans, in part because it has the unique ability to contaminate eggs without causing discernible illness in the birds infected. The infection route to humans involves colonization, survival and multiplication of the pathogen in the hen house environment, the bird and, finally, the egg. This review highlights the stages of transmission and discusses evidence that altered bacterial growth patterns and specific cell surface characteristics contribute to the adaptation of *S. enteritidis* to these diverse environments.**

Salmonella enteritidis, a human pathogen that contaminates hen eggs

Human illness caused by infection with *Salmonella enterica* serovar Enteritidis (*S. enteritidis*) increased worldwide beginning as early as the mid-1970s and, by 1990, this serovar displaced *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) as the primary cause of salmonellosis in the world (Baumler *et al*., 2000; Anonymous, 2001). Epidemiological investigations in Hungary, the United Kingdom, the United States and Germany confirmed that the food most associated with increased illness in people was the egg (Anonymous, 1988; 2001; St Louis *et al*., 1988; PHLS, 1989; Ullmann and Scholtze, 1989). An example of the extent to which illness increased through much of western Europe is shown in Fig. 1, which depicts the progression of the pandemic in England and Wales (Anonymous, 2001). Germany, Austria and Poland experienced 100–200 cases of *S. enteritidis* salmonellosis per 100 000 people, whereas England peaked at 40 cases and France at 10 cases per 100 000 people (Gomez *et al*., 1997). During 1997, *S. enteritidis* accounted for 85% of all cases of human salmonellosis in Europe, but incidence has declined from this peak (Fisher, 2001). Further investigations established that phage type (PT) 4 *S. enteritidis* predominated during the pandemic (Fig. 1), but other phage types were often detected. During this same period, salmonellosis from *S. typhimurium* and other serotypes remained relatively stable throughout Europe and the US, as reflected in the data obtained from England and Wales (Fig. 1). In contrast to the experience of Europe, the US and Canada have experienced only a four- to sixfold increase in human illness (currently at four cases per 100 000 people). There is concern about the potential for *S. enteritidis* in the US to increase to European levels, because of the recent emergence of PT4 *S. enteritidis* in California and other western states (Ward *et al*., 1987; Anonymous, 1999).

Salmonella enteritidis is the only human pathogen that contaminates eggs routinely, even though the on-farm environment of the chicken is a rich source of a number of *Salmonella* serotypes (Soerjadi-Liem and Cumming, 1984; Caldwell *et al*., 1995; Byrd *et al*., 1999). Identification of eggs as virtually the sole source of *S. enteritidis* outbreaks suggested that intensive sampling of birds and diversion of suspect eggs from the market could be used to achieve control; instead, farmers, public health officials and retailers have been confounded by the intractable nature of the problem. The *S. enteritidis* pandemic involves interactions of the pathogen with multiple environments, which include the hen house, the bird, the egg, as well as the human host (Fig. 2). Identification of bacterial characteristics unique to *S. enteritidis* should help to explain how it differs from other *Salmonella* serotypes in these environments. *Salmonella pullorum* and *Salmonella gallinarum* are other closely related serovar group D1 avian-adapted *Salmonella* that are historically associated with egg contamination (Snoeyenbos, 1991; Li *et al*., 1993; Shivaprasad, 2000). Other *Salmonella* can internally contaminate eggs in experiments, and *Salmonella heidelberg* is monitored closely because of its association with poultry products (Anonymous, 1986; Keller *et al*., 1997; Williams *et al*., 1998; Leach *et al*., 1999). However, *S. enteritidis* is currently the only *Salmonella* serotype that causes frequent human illness associated with egg contamination, which determines its unique threat to food safety.

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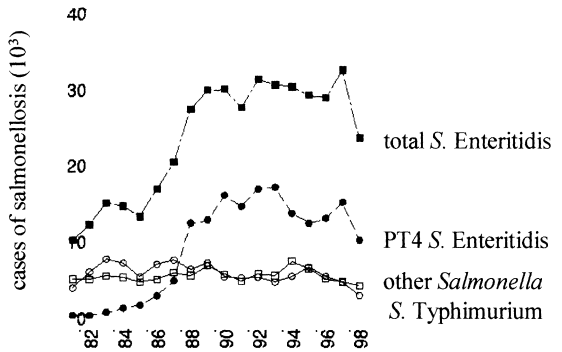

Fig. 1. *S. enteritidis* food-borne pandemic of human disease from 1981 to 1998. Example of the incidence of salmonellosis in humans in England and Wales, from data available at http://www.phls.co.uk. The corresponding incidence in other countries ranges from fivefold higher (Germany) to sixfold less (US and Canada).

The route to poultry infection – colonization of the hen house and its pests

Poultry are raised in hen houses that vary in construction, moisture and organic material; they are populated by different pests, such as rodents, insects and wild birds; and poultry are managed with different practices, including the use of moulting and biocontainment. Thus, the hen house is not a single environment, but a number of niches in which bacteria survive and multiply (Fig. 2). Egg contamination is prevented in part by stocking the hen house with culture-negative chicks from uninfected breeder and multiplier birds (USDA-APHIS, 1999). However, *S. enteritidis* can be cultured from insect and animal hosts living in and around hen houses, which means that exclusion requires stringent biocontainment practices (Davies and Wray, 1995a; Kinde *et al.*, 1996a; Olsen and Hammack, 2000). Reintroduction of the pathogen from pests into adequately cleaned houses stocked with culture-negative birds is a constant threat that is a major contributor to the egg contamination problem (Fig. 2). Better hen house design might aid control by excluding pests and improving air quality, because poor ventilation and high dust levels appear to aid dissemination of bacteria among chickens by colonization of mucosal surfaces (i.e. nares and conjunctiva) (Humphrey *et al.*, 1992; Holt *et al.*, 1998). Bacteriological screening of the hen house helps to identify flocks at risk (Davies and Wray, 1996a; Hogue *et al.*, 1997; Henzler *et al.*, 1998). However, it can be difficult to isolate *S. enteritidis* from hen houses, even when a flock is producing contaminated eggs (Henzler *et al.*, 1994; Caldwell *et al.*, 1995; Davies and Wray, 1996a; Riemann *et al.*, 1998; Fuzihara *et al.*, 2000). Inhibiting the growth and survival of *S. enteritidis* in the hen house is an emerging area of research that may aid control (Clay and Board, 1992; Guard-Petter, 1997; Himathongkham *et al.*, 1999; Mattick *et al.*, 2000).

A particular, but variable, risk factor for egg contamination is the presence of a resident infected mouse population (Fig. 2) (USDA, 1998). The house mouse *Mus musculus* is the dominant rodent species in hen houses, and it is a rich source of organ-invasive *S. enteritidis* (Henzler and Opitz, 1992; Davies and Wray, 1995b; 1996b; Guard-Petter *et al.*, 1997; Henzler *et al.*, 1998). Rodent control measures have decreased *S. enteritidis* in the hen house (Davies and Wray, 1996b; Henzler *et al.*, 1998; Anonymous, 2000a). It cannot be ruled out that *Mus musculus* may enrich both chicken and human pathogenic variants in the hen house.

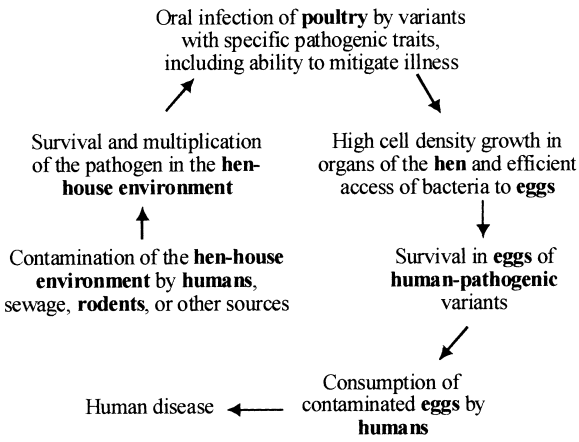

Fig. 2. The route to human infection by *S. enteritidis*. A diversity of environments, living and inanimate, are colonized or infected. Eggs are said to be contaminated rather than infected, as they are not fertile and do not contain living cells. For epidemiological purposes, the route is primarily linear, with humans as the end host; however, transmission of infection from humans to chickens suggests that the route can be cyclical.

Protection of birds by other bacteria

It has been proposed that the absence of *S. pullorum* (and, by analogy, the nearly indistinguishable serotype *S.* gallinarum) from poultry stock in some countries with stringent control programmes may have encouraged the emergence of *S. enteritidis* (Baumler *et al.*, 2000; Rabsch *et al.*, 2000). Thus, competition between bacteria could affect the ability of *S. enteritidis* to survive and multiply within hen houses (Fig. 2). Investigation of interactions between *Salmonella* serotypes confirmed that competitive exclusion between avian-adapted serotypes and *S. enteritidis* is possible and that, sometimes, cross-protection can be demonstrated, as observed between *S. enteritidis* and other *Salmonella* serotypes (Schlecht, 1981; Hassan and Curtiss, 1994; 1997; Tabaraie *et al.*, 1994; Hormaeche *et al.*, 1996; Rabsch *et al.*, 2000; Nicholson and Baumler, 2001). Further investigation may provide additional evidence that there is some special aspect to interactions between *S. enteritidis* and the

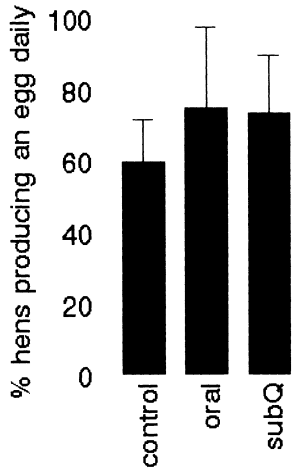

Fig. 3. Influence of *S. enteritidis* infection of hens on egg production. Eggs were collected for 21 days from uninfected control hens (mean of three different flocks), hens infected orally with 10^9 cfu *S. enteritidis* and hens infected subcutaneously (subQ) with 10^8 cfu *S. enteritidis*. Bars indicate daily standard deviations in daily production per hen. Incremental changes of 3% are considered to be substantial for flocks in production.

avian-adapted *Salmonella*. *S. enteritidis* infection can be decreased in poultry by competitive exclusion, which is currently practised most often by inoculating hatchling chicks with beneficial bacterial cultures (Nuotio *et al*., 1992; Methner *et al*., 1997).

Another management practice that alters the commensal gut flora of poultry is the practice of moulting, in which a temporary cessation in egg production is induced in older birds by withholding feed and water (Deitch *et al*., 1987). This practice increases productivity and extends the useful life of the flock. A consequence of moulting is increased shedding of *Salmonella* in faeces (Holt *et al*., 1994; Holt *et al*., 1998), and risk analysis suggests that it doubles the incidence of egg contamination (USDA, 1998). Moulting is not practised in Europe, as it is a stress for the bird (Kogut *et al*., 1999). The incidence of egg-associated salmonellosis in Europe is much higher than it is in the United States and, thus, there is no compelling reason to believe that cessation of moulting would substantially reduce the incidence of infection in humans. More research is needed to ascertain whether different management practices between continents result in different subpopulations of *S. enteritidis* that vary in their ability to contaminate eggs or to cause human illness. A further complicating factor in flocks is that other infectious agents of chickens may exacerbate problems (Phillips and Opitz, 1995; Qin *et al*., 1995; 1996).

Egg contamination by *S. enteritidis* is 'silent', unaccompanied by hen illness

There are typically no clinical signs in birds infected with *S. enteritidis* to suggest to farmers that the eggs they are producing might pose a public health threat (Fig. 2) (Hogue *et al*., 1997). In contrast, poultry infected with the other avian-adapted *Salmonella* that contaminate eggs, *S. pullorum* and *S. gallinarum*, experience increased mortality, drastic weight loss and sharply decreased egg production (Snoeyenbos, 1991; Shivaprasad, 2000). Silent infection in the bird is one way in which *S. enteritidis* resembles other carcass-contaminating *Salmonella* because, in general, *Salmonella* is not pathogenic in chickens except for the avian-adapted serotypes. Egg-contaminating *Salmonella* generate a unique type of lesion, the ovarian granuloma or 'Pullorum lesion', which is pathognomonic for evidence of infection associated with internal egg contamination (Snoeyenbos, 1991). It is an infrequent lesion after infection with *S. enteritidis*, but it has been reported. Remarkably, infection of hens by *S. enteritidis* has been seen to increase daily egg production in some experiments (Fig. 3, J. Guard-Petter, unpublished data), raising the possibility that poultry flocks might produce more eggs during active infection. In this case, evaluation of production records might give an indication of recent infection within flocks, although breeds of hens may differ in their response (Kinde *et al*., 2000). Increased egg production after infection has so far only been observed under experimental conditions, and further investigation is required to see if it occurs in production flocks.

Contamination of the egg contents by *S. enteritidis*

Penetration of cracked shells by bacteria used to be a frequent cause of human illness before the introduction of grading schemes in the 1970s. However, egg contamination associated with *S. enteritidis* is believed to occur before deposition of the shell (Humphrey *et al*., 1991; Humphrey, 1994; Methner *et al*., 1995; Gast and Holt, 2000), by internal (vertical) transmission to the contents of the egg (yolk or albumen) via the reproductive tract (Fig. 2). It cannot be ruled out that *S. enteritidis* actively promotes access to the egg by penetration of a marginally faulty shell that excludes most other bacteria (Anonymous, 2000b). Once inside the egg, bacteria face an inhospitable environment. Egg white is rich in lysozyme and lacks available iron (Chart and Rowe, 1993), whereas yolk has antibodies (Desmidt *et al*., 1996; Terzolo *et al*., 1998; Holt *et al*., 2000). These factors help to limit the growth of bacteria in eggs, although on occasion eggs with very high numbers of bacteria are detected (Clay and Board, 1991; Humphrey and Whitehead, 1993; Humphrey, 1994; Baron *et al*., 1999; Gast and Holt, 2000). It is conceivable that some variants of *S. enteritidis* are more adapted for growth and survival in eggs (Keller *et al*., 1997), and that the egg is an environment that could

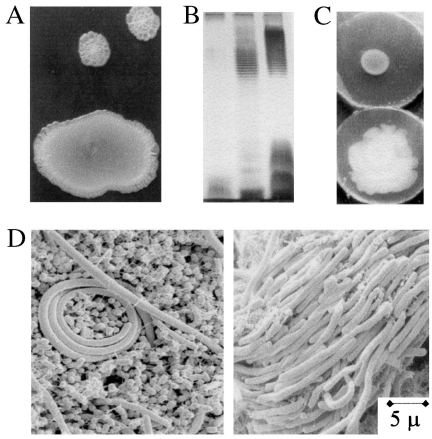

Fig. 4. Characterization of egg-contaminating *S. enteritidis*.
A. LPS O-chain and variation in glucosylation. Two colony morphologies arising from a heat-stressed culture (Guard-Petter *et al*., 1996); the two similar sized single-phenotype colonies at the top (10 mm diameter) make a cell surface organic matrix that forms a biofilm rich in glucosylated LPS, fimbriae and flagellae. The lower swarming colony (35 mm diameter) produces matrix at the edge only. The matrix-forming phenotype of *S. enteritidis* is commonly isolated from the spleens of mice.
B. LPS O-chain and variation in mass. Left to right: LPS from a rough variant lacking O-chain; low-molecular-mass (LMM) LPS O-chain typical of *S. typhimurium*; capsular-like high-molecular-mass (HMM) O-chain typical of *S. typhi* (SDS−12% polyacrylamide gel).
C. Swarming hyperflagellation and migration. Colonies of a swarming veterinary isolate (lower, 30 mm diameter) and a non-flagellated *flhD* mutant (8 mm diameter) on hard agar.
D. High cell density growth and phenotypic diversity. Left. Predominant cell morphology during growth from 10^{10} to 10^{11} cfu is a small (1.5 μm) flagellated coccoid form. Right. Predominant cell morphology after peak of high cell density growth, when massive cell lysis results in loss of the coccoid form and a precipitous drop in bacterial numbers, is an elongated rod lacking cell surface structures.

select for variants that are adapted to the human host (Fig. 2) (Rahman *et al*., 1997; Parker *et al*., 2001a).

The contribution of humans to the food-borne pandemic

An obvious risk factor for the emergence of pandemic food-borne salmonellosis in humans is the consumption of undercooked eggs (Fig. 2). Some progress has been made in protecting high-risk groups against egg-associated salmonellosis through education (Rao *et al*., 1987; Levine *et al*., 1991; Stein *et al*., 1993; Benamour *et al*., 1995; Green and Vinker, 1996; Roll *et al*., 1996; Shibusawa *et al*., 1997; Wright *et al*., 1997; Smith, 1998; Begue, 1999; Lee *et al*., 1999). However, the influence of humans on the current pandemic may be more complicated, as humans may transmit *S. enteritidis* directly to hens. PT4 S. *enteritidis* has predominated throughout much of the world since the early days of the pandemic, an example of which is shown in Fig. 1. In spite of its prevalence overseas and south of the border, PT4 *S. enteritidis* was not isolated in the United States until recently when chickens in California became infected from discharge of human sewage effluent upstream of the farm (Kinde *et al*., 1996a,b). Prior isolation of PT4 *S. enteritidis* had been seen in the United States in connection with infection in humans, especially international travellers and people living close to Mexico (Boyce *et al*., 1996). From the time of this initial isolation in chickens until today, the rapid spread and dissemination of PT4 *S. enteritidis* within the United States has been noted (Anonymous, 2000b). Wherever PT4 *S. enteritidis* is found, human illness appears to increase (Hogue *et al*., 1997), although recent reductions in illness from *S. enteritidis* have been realized (Anonymous, 2001; Olsen *et al*., 2001).

What are the special characteristics of *S. enteritidis*?

Salmonella enteritidis resembles *S. typhimurium* with regard to the known virulence mechanisms central to mammalian cell invasion and survival and growth in the host. Both pathogens have the highly conserved pathogenicity island-encoded type III secretion mechanisms and virulence effector proteins, and both harbour a large virulence plasmid, are motile and produce a galactose–rhamnose–mannose repeat unit of the lipopolysaccharide (LPS) O-chain backbone decorated with a dideoxyhexose that determines serotype (Galan and Curtiss, 1991; Reeves, 1993; Jones and Falkow, 1996; Ochman and Groisman, 1996; Blanc-Potard *et al*., 1999; Marcus *et al*., 2000). It has therefore not been obvious how *S. enteritidis* is especially able to follow the human infection route described and, above all, at a critical step, how it so successfully contaminates and grows in egg contents. *S. enteritidis* has been shown by a number of investigators to generate a striking degree of strain heterogeneity (Ward *et al*., 1987; Petter, 1993; Gast and Benson, 1995; Humphrey *et al*., 1995; Guard-Petter *et al*., 1999; Lu *et al*., 1999; Liebana *et al*., 2001; Parker *et al*. 2001b), suggesting that a complex network of characteristics may underlie its diverse behaviour. I describe here some emerging characteristics that correlate with infection and that may be central to the impact of this pathogen.

LPS microheterogeneity and assembly of a biofilm

Lipopolysaccharide (LPS) obtained from *S. enteritidis* differs greatly between strains in the degree of glucosylation that occurs in the O-chain region, ranging from a lack of glucosylation in stored isolates to an exceptionally high degree as recovered from variants freshly cultured from the spleens of naturally infected mice (Parker *et al*. 2001a). In contrast, *S. typhimurium* is well glucosylated on average, even when strains have been stored for years

or examined as low-passage isolates (Parker *et al*. 2001a). For *S. enteritidis*, the glucosylated LPS O-chain appears to contribute to an extracellular matrix or biofilm that is also rich in flagella and fimbriae (Guard-Petter *et al*., 1996; Humphrey *et al*., 1996; Allen-Vercoe *et al*., 1997). Biofilm correlates with enhanced oral invasiveness, but not egg contamination, in infected birds and, in tissue cell assay, with disruption of epithelial cells (Fig. 4A) (Guard-Petter *et al*., 1996; Solano *et al*., 2001). Biofilm-producing *S. enteritidis* may be a 'helper' phenotype that aids access of less orally invasive strains to the parenteral (post-mucosal) environment of the bird, as suggested by further infection studies in which a mixture of phenotypes gave good recovery of contaminated eggs (Fig. 4A) (Parker *et al*. 2001b). Because LPS O-chain glucosylation is a cell surface characteristic that is quickly lost by *S. enteritidis* in response to environmental conditions (Parker *et al*. 2001a), the mouse is probably an important secondary host because it persistently sheds orally invasive isolates into the hen house environment (Fig. 2) (USDA, 1998).

A further role in the pandemic is postulated for variants that produce glucosylated high-molecular-mass (HMM) LPS (referred to previously as HMW LPS) (Fig. 4B). This capsule-like LPS structure is similar to that produced by *S. typhi*, the host-adapted agent of typhoid fever in people (Hellerqvist *et al*., 1969; Rahman *et al*., 1997). *S. typhi* has a much longer average O-chain than does *S. typhimurium*, which can be accurately differentiated from what is referred to as 'long' or 'smooth' O-chain by chemical methods only (Parker *et al*. 2001a). As the chain length of LPS increases, so does the hydrophilicity of the outer cell membrane (Guard-Petter *et al*., 1999). Variants producing HMM LPS have been isolated from two sources, which are the egg and the spleen of the naturally infected mouse (Parker *et al*. 2001a). Current estimates are that nearly 40% of variants from eggs and 10% from the mouse spleen produce glucosylated HMM LPS. Whereas recovery of variants producing glucosylated HMM LPS from eggs suggests that some adaptation to humans may be occurring, recovery of the same variants from the naturally infected mouse suggests that it also aids propagation of the pandemic in chickens (Fig. 2). Another role for HMM LPS in the infection route from bird to human is that it may contribute to unusual pathology in the avian reproductive tract, as suggested in preliminary experiments that showed a striking ability of injected culture to induce regression of the avian reproductive tract. This effect was seen only with a wild-type strain, but not with its O-chain regulator of length mutant (Parker *et al*., 2000). Ovarian granulomas were not associated with HMM LPS, which suggests that *S.* enteritidis has pathogenic potential that *S. pullorum* does not. Experiments are in progress to determine if there are subtler changes that occur in the reproductive tract of birds after infection with *S. enteritidis* using the more natural oral route of infection.

Preliminary chemical characterization of the core region of LPS has revealed that there is a discrete difference between PT4 *S. enteritidis*, which is predominant in Europe, and PTs13a and PT8, which are endemic to the United States (R. Carlson and J. Guard-Petter, personal communication; Guard-Petter, 1999). How PT4 *S. enteritidis* differs from other phage types at the molecular level is an inherently important issue to address, because phage type does not have an identifiable role for virulence in the bird (Gast and Benson, 1995; Olsen *et al*., 1999; Gast and Holt, 2000); thus, the advantage that it imparts to the pathogen remains unexplained.

A frequently asked question is how much has genetic change in this pathogen contributed to strain heterogeneity. *S. enteritidis* strains from around the world are genetically similar, and stringent fingerprinting methods are required to differentiate lineages (Desai *et al*., 2001; Liebana *et al*., 2001a,b). However, single nucleotide polymorphisms (SNPs) have been linked to LPS O-chain microheterogeneity and to the source of the isolate (Parker *et al*., 2001a). *S. enteritidis* produces other types of mutants that are recoverable from the environment of hens (Allen-Vercoe *et al*., 1998; Humphrey *et al*., 1998; Guard-Petter *et al*., 1999). As undirected genetic mutation arises in *Salmonella* in response to environmental stressors (Kolter *et al*., 1993; Massey *et al*., 1999), it is reasonable that some of these genetic events enhance survival and fitness of the pathogen, whereas others are of no consequence or are even deleterious.

Motility and swarm cell differentiation

The avian-adapted egg-contaminating serotypes *S. pullorum* and *S. gallinarum* are *Salmonella* that lack flagella and associated motility. Mutation of *flhD* (part of the *flhDC* master operon regulator of flagellar biosynthesis) to eliminate flagellation in *S. enteritidis* unexpectedly enhanced oral invasiveness in poultry, whereas mutation of *fliC* (the flagellin structural gene) did not (C. T. Parker and J. Guard-Petter, submitted) (Fig. 4C). Flagellation has been shown otherwise to contribute to virulence in birds (Allen-Vercoe *et al*., 1999; Allen-Vercoe and Woodward, 1999). Alteration in gene expression from the flagellar master operon affects other cell functions, such as cell division and possibly virulence (Pruss *et al*., 1997; Claret and Hughes, 2000a,b). Because mutation of *fliC* did not enhance oral invasiveness, flagella and motility may not be important for achieving oral invasion in chickens. Instead, the ability of some *S. enteritidis* to alter cellular division greatly is considered the bacterial characteristic that is more likely to contribute to enhanced oral invasiveness. Some *Salmonella* classified as *S.*

pullorum might be *S. enteritidis* that has suppressed flagellation, which further supports the view that extreme variation in regulators of flagellar biosynthesis could be occurring in field isolates (Guard-Petter, 1997; Chaubal and Holt, 1999).

Swarm cell differentiation is a developmental pathway characterized by cell elongation and hyperflagellation and, in enterobacteria, it is governed largely by upregulation of the flagellar master operon *flhDC* (Dufour *et al*., 1998; Fraser *et al*., 1999). Swarm cell differentiation results in enhanced virulence and migration of multicellular aggregates across surfaces (Allison *et al*., 1992; Darzins, 1994; Szymanski *et al*., 1995; Gardel and Mekalanos, 1996; Liaw *et al*., 2000; MacFarlane *et al*., 2001). Conditions for inducing swarm cell differentiation of *S. enteritidis* have been identified across normally inhibitory solid agar (Fig. 4A and C) (Guard-Petter, 1997). Spontaneous swarm cell migration was detected in a group of mouse spleen variants of *S. enteritidis* that were also producing HMM LPS (Guard-Petter *et al*., 1997). The structure of LPS is important for swarm cell migration in soft agar by *Salmonella* (Guard-Petter, 1997; Toguchi *et al*., 2000). As *S. enteritidis* produces a broad range of LPS structures (Parker *et al*., 2001a), it is possible that it migrates more aggressively within the environment of the hen house or the bird than do serotypes such as *S. typhimurium*, which produces a relatively homogeneous LPS structure. Interestingly, wild-type strains of *S. enteritidis* that can undergo spontaneous swarm cell differentiation have been highly virulent in chicks when injected, but not when given orally, which is an inverse result to that seen in chicks infected with the non-flagellated *flhD* mutant (C. T. Parker and J. Guard-Petter, submitted). These results also suggest that the ability of *S. enteritidis* to alter regulation between extremes of flagellation enhances its pathogenicity overall.

High cell density growth

Quorum sensing is a broadly conserved virulence mechanism among bacterial pathogens of plants and animals (Withers *et al*., 2001), and it has been surprising that no evidence of quorum sensing could be detected in pathogenic *Salmonella*. Recently, *S. enteritidis* has been shown to be capable of *luxR*-regulated high cell density growth (Guard-Petter, 1998). This type of bacterial cell communication occurs when localized concentrations of acyl-homoserine lactones (AHLs) exceed a threshold that effects the cell density-determined expression of specific genes, notably virulence genes, in the population (Fuqua and Greenberg, 1998; Hastings and Greenberg, 1999). The responsible *S. enteritidis* AHL has now been identified (P. Greenberg and J. Guard-Petter, personal communication). Variants of *S. enteritidis* that grow to high cell density are more efficient at contaminating eggs or causing illness in chicks (Guard-Petter, 1998). Cells undergo striking morphological change during high cell density growth, which exceeds 10^{10} bacterial cells ml^{-1} compared with a maximal cell density of 3×10^9 for strains not capable of doing so (Fig. 4D). Finding that *S. enteritidis* undergoes AHL-regulated high cell density growth suggests that epidemiological surveys may be indirectly detecting the emergence of quorum-sensing, virulence-enhanced *Salmonella* serotypes (Fig. 2). If this is so, methods for interrupting bacterial cell communication may result in new methods of control in the hen house environment (Holden *et al*., 1999).

Summary

It is emerging that *S. enteritidis* possesses particular characteristics that increase its fitness along the infection route; most especially, it has the ability to alter its cell surface dramatically, and its mode of growth may be especially significant. It is clear that this remarkable human pathogen has adapted to a wide diversity of environments, the hen house, the mouse, the chicken, the egg and the human, and that this adaptability has underpinned the pandemic of human food-borne illness. Although searches will continue for vaccine-based prevention against *S. enteritidis* hen infection and human disease, continued improvement and implementation of detection and environmental controls will also be effective in reducing egg contamination and therefore improving public health.

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