Veterinary Microbiology 192 (2016) 135–144

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

In vitro invasive capacity of *Salmonella* strains into sections of the layer hen oviduct

Andrea R. McWhorter, Kapil K. Chousalkar*

School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy Campus, 5173, Australia

ARTICLE INFO

Article history:
Received 4 March 2016
Received in revised form 26 June 2016
Accepted 7 July 2016

Keywords:
Salmonella
Invasion
Oviduct
Egg

ABSTRACT

Raw or undercooked eggs and egg products are frequently identified as the source of *Salmonella* following outbreaks of foodborne gastrointestinal disease. Some *Salmonella* serovars, such as *Salmonella* Enteriditis, have a high tropism for the oviduct of laying hens. Oviduct colonization with *S.* Enteriditis can result in both internal and external contamination of an egg. While oviduct invasion is not limited to *S.* Enteriditis, the invasive capacities of other serovars is not widely known. In this study, the *in vitro* invasive ability of eighteen *Salmonella* isolates of representative serovars into different segments of the oviduct was assessed. All *Salmonella* isolates tested were invasive and the highest bacterial invasion was observed in segments of the isthmus and vagina. *S.* Bredeney consistently exhibited the lowest invasion into all sections of the oviduct. Interestingly, the *S.* Typhimurium definitive types included in this study did not exhibit significantly greater invasion capacity than other serovars. In this study, the genomic capacity of the selected isolates of representative *Salmonella* serovars to colonize the layer hen oviduct was also investigated. Previous studies have identified several genes upregulated during oviduct colonization by *S.* Enteriditis. Single gene comparison of 107 genes from eleven *Salmonella* isolates was conducted to determine whether these oviduct colonization genes were present within each bacterial genome. The degree of homology with corresponding sequences in *S.* Enteriditis P125109 was also determined for each gene. Genes encoding the O-antigen as well as phage and virulence plasmid genes were among the most highly variable and may serve specific roles in oviduct invasion.

1. Introduction

Members of the bacterial species *Salmonella enterica* have established a unique niche within commercial poultry environments. While many *S. enterica* serovars are considered commensal organisms for chickens, the contamination of poultry meat, eggs or egg products within the food supply chain represents a major global public health concern. In particular, the consumption of raw or undercooked egg-related products as well as poor handling practices during processing are frequently linked with outbreaks of human salmonellosis (Threlfall et al., 2014).

Salmonella enterica is highly diverse, with over 2500 serovars characterized to date (Guibourdenche et al., 2010). Of these, strains of *Salmonella* Enteriditis exhibit a particularly high tropism for the layer hen oviduct and have been shown to colonize this organ following both experimental and natural infection (De Buck et al., 2004c; Gantois et al., 2009). Bacterial colonization of the oviduct can lead to contamination of the yolk, albumin, egg shell membranes or egg shell prior to oviposition (Gantois et al., 2009; Gast et al., 2013). Infection of the layer hen reproductive tract can arise through one of two mechanisms: (i) bacterial migration through the cloaca to the vagina and further up the oviduct (Gantois et al., 2009) or (ii) systemic infection resulting in oviduct colonization. This latter mechanism has been documented for *S.* Enteriditis, *S.* Heidelberg, *S.* Hadar, *S.* Virchow and *S.* Typhimurium (Gast et al., 2013; Gast et al., 2011; Okamura et al., 2001).

Bacterial invasion and colonization are complex processes that involve unique interactions between host and bacteria. Bacteria have to overcome several host barriers including thick mucus and mucin layers within the oviduct as well as the secretion of host immune factors such as β-defensins (Ebers et al., 2009; Michailidis et al., 2012), Toll-like receptors (Michailidis et al., 2010) and surface secretory antibody (Withanage et al., 1999). In addition, certain strains of *S.* Enteriditis and *S.* Typhimurium exhibit higher tropism for the oviduct than other serovars (Gantois et al., 2008b; Keller et al., 1997; Mizumoto et al., 2005). Interestingly, *S.* Enteriditis

* Corresponding author at: School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy, 5173, SA, Australia.
E-mail address: kapil.chousalkar@adelaide.edu.au (K.K. Chousalkar).

http://dx.doi.org/10.1016/j.vetmic.2016.07.009
0378-1135/

appears to increase its tropism for the oviduct upon serial passage through the host (Gast et al., 2003) but this property has not been described for other *Salmonella enterica* serovars.

In Australia, *S.* Enteritidis infection in poultry is a notifiable animal disease but it is not endemic within the commercial egg industry. While many *Salmonella* serovars are commonly identified from Australian flocks, it is definitive types of *S.* Typhimurium that represent the greatest public health risk (Threlfall et al., 2014). Increasing rates of salmonellosis associated with the consumption of egg products suggests either increased egg surface bacterial contamination or contamination of egg internal contents. To date, limited investigation of bacterial colonization of the layer hen oviduct by serovars other than *S.* Enteriditis has been performed (Gantois et al., 2008b; Keller et al., 1997; Mizumoto et al., 2005). Factors which may affect oviduct colonization include type I fimbrae (Buck et al., 2004; Li et al., 2003) and LPS O-antigen group (Mizumoto et al., 2005). In this study, we selected eighteen *Salmonella* isolates of different serovars commonly isolated from egg farms. The ability of each isolate to invade the layer hen reproductive tract was assessed using explants of the infundibulum, isthmus, magnum, shell gland and vagina.

Bacterial genes important for oviduct colonization have been identified using gene knock-out (Bohez et al., 2008; Coward et al., 2012; Li et al., 2009), phenotype microarray (Raspoet et al., 2014), and *in vivo* expression technology screening methods (Gantois et al., 2008a). These studies have identified virulence genes within *Salmonella* pathogenicity island (SPI)-1 (Coward et al., 2012; Ebers et al., 2009; Li et al., 2009; Raspoet et al., 2014), SPI-2 (Bohez et al., 2008; Coward et al., 2012; Li et al., 2009; Raspoet et al., 2014), SPI-3 (Coward et al., 2012; Raspoet et al., 2014), and SPI-5 (Coward et al., 2012; Ebers et al., 2009) as well as those involved in DNA synthesis (Raspoet et al., 2014), stress responses (Gantois et al., 2008a; Raspoet et al., 2014), bacterial adhesion and motility (De Buck et al., 2004a; Raspoet et al., 2014), metabolism (Gantois et al., 2008a), and maintenance of cell membrane integrity (Gantois et al., 2008a). All of these genes play a role in bacterial colonization of the oviduct. Genes located on the virulence plasmid and phage related genes have also been found to be upregulated during oviduct colonization (Gantois et al., 2008a). These experiments, however, focused only on *S.* Enteriditis. Here, we amalgamate single gene homology data comparing multiple egg farm associated *Salmonella* isolates with *S.* Enteriditis. An additional aim was to identify whether isolates of other representative serovars also possess the genomic machinery required for oviduct colonization. Invasion results and sequence analyses were performed to identify *Salmonella* isolates with potential risk of evolving an increased tropism for the oviduct.

2. Methods

2.1. Salmonella *isolates*

Salmonella isolates were obtained from the *Salmonella* Reference Laboratory, Institute of Veterinary Medical Science (IMVS), and Adelaide, South Australia. Isolates used in these experiments were isolated from egg farms during routine surveys (Table 1). Long-term stocks were generated by preparing a bacterial suspension in brain heart infusion broth containing 20% glycerol and storing at −80 °C.

2.2. *Raising* Salmonella *negative pullets for oviduct invasion assay*

Fertile eggs were obtained from two breeds of commercial brown layer parent flocks. Eggs were fumigated with formaldehyde and incubated over a period of 21 days at 38 °C with relative humidity of 45–55% up to day 18 and then 55–65% up to hatching. Chicks (n = 26) were hatched at day 21 and housed in positive pressure rooms in animal house at the Roseworthy Campus of the University of Adelaide. This animal facility had been previously decontaminated with F10 veterinary disinfectant (Chemical Essentials, Australia) and then fumigated with formaldehyde. All animal pens, cages, trays, feeders, laboratory equipment, as well as the floor and walls of the rooms had previously been cleaned extensively with FoamCleanS (Chemtall, Australia) and SaniGuard (Chemtall, Australia). All equipment was moved into each of two rooms and a final fumigation with SaniGuard was performed before use.

All staff wore sterilised overalls, head-covers, shoe covers, masks and gloves while working within the facility. Feed was disinfected by either gamma irradiation or fumigation with formaldehyde. Water was disinfected by the addition of chloride tablets. *Salmonella* positive or negative status of the birds was monitored by traditional culture methods as well as PCR testing of fecal samples for the presence of the *invA* gene. Fecal, water and feed samples were tested for the presence of *Salmonella* every two weeks. The trial was terminated at week 30. All housing

Table 1
Isolate source *Salmonella* serovars.

Salmonella serovar used in this study	Isolate Source	Number of isolations from egg farms in 2014[a]
Bredeney	Feces	2
Typhimurium DT9	Egg Shell wash	41
Typhimurium DT44	Feces	2
Typhimurium DT135	Feces	45
Typhimurium DT170 = 108	Feces	12
Typhimurium DT193	Feces/Litter	3
Infantis	Egg shell wash	38
Lille	Feces	2
Montevideo	Feces	5
Oranienburg	Feces	1
Singapore	Feces	12
Virchow	Feces	21
Worthington	Feces	9
Cerro	Feces/Litter	2
Adelaide	Feces	3
Orion var +15, +34	Dust in layer shed	13
Zanzibar	Feces	34
Senftenberg	Feces	17

[a] Information was obtained from the *Salmonella* Reference Laboratory, Australian Salmonella Reference Centre, SA Pathology, Adelaide, Australia.