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Specific adhesion and invasion of *Salmonella* Enteritidis in the vagina of laying hens

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Abstract

Salmonella Enteritidis is the predominant serovar associated with egg-borne salmonellosis in humans. The colonization of *S.* Enteritidis in the vagina may play a role in the production of *S.* Enteritidis-contaminated eggs. In the first experiment, the in vitro adhesion of *S.* Enteritidis in vaginal and follicular explants was compared with that of *S.* Typhimurium by bacteriological isolation methods. The mean number of *S.* Enteritidis associated with vaginal explants was significantly ($P < 0.05$) higher than *S.* Typhimurium associated with vaginal explants and both serovars associated with follicular explants. In the second experiment, the in vitro adhesion and invasion of *S.* Enteritidis strains in the vaginal epithelium was compared with that of several strains of *S.* Agona, *S.* Infantis, *S.* Hadar, *S.* Heidelberg, *S.* Montevideo and *S.* Typhimurium, by immunohistochemical methods. The mean number of *Salmonella* in the vaginal epithelium depended on their lipopolysaccharide (LPS) type, with the rank order as follows: LPS type O9 (*S.* Enteritidis) > LPS type O4 (*S.* Agona, *S.* Typhimurium and *S.* Heidelberg) > LPS type O7 (*S.* Montevideo and *S.* Infantis) and LPS type O8 (*S.* Hadar). This rank order of *Salmonella* invasiveness is in accordance with the frequency of *Salmonella* outbreaks involving contaminated eggs. These findings suggest that *S.* Enteritidis has a higher ability to colonize the vaginal epithelium than other serovars, and the *Salmonella* LPS type may play an essential role in tropism of the reproductive tract.

Keywords: *Salmonella* Enteritidis; Egg contamination; Vagina; Adhesion; Invasion; Lipopolysaccharide

1. Introduction

Outbreaks of human salmonellosis caused by *Salmonella* Enteritidis have dramatically increased throughout the world since the mid-to-late 1980s and have become an important problem for the poultry industry and public health (Rodrigue et al., 1990; Fris and Van den Bos, 1995; Hogue et al., 1997). Epidemiologic analyses have suggested contaminated eggs or egg products as the major source of infection (Van de Giessen et al., 1992; Altekruse et al., 1993; Hedberg et al., 1993; Henzler et al.,

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1994). However, it has not been confirmed why *S.* Enteritidis has been the predominant serovar associated with egg-borne salmonellosis in humans. As *S.* Enteritidis is the serovar most frequently isolated from eggs, it may have unique characteristics or ability to contaminate eggs. Understanding the mechanism of egg contamination with *S.* Enteritidis is essential to eradicate human salmonellosis by contaminated eggs.

S. Enteritidis is found in all egg components laid by infected hens: yolk, albumen, eggshell membrane and eggshell. Two possible routes of egg contamination by *S.* Enteritidis are considered: (1) direct contamination of yolk, albumen, eggshell membranes or eggshells before oviposition originating from infected reproductive organs and (2) penetration through the eggshell from the colonized cloaca or feces after or during oviposition. Different segments of the reproductive organs may differ in their susceptibility to *S.* Enteritidis colonization and invasion. Thiagarajan et al. (1994, 1996) describe that *S.* Enteritidis can invade and multiply in granulosa cells of the preovulatory follicle. De Buck et al. (2003, 2004a) emphasize that the isthmus is the most frequently and heavily contaminated segment of the oviduct. Our previous experiments demonstrated that hens inoculated intravaginally with *S.* Enteritidis produced higher number of contaminated eggs than hens inoculated intravaginally with other *Salmonella* serovars and that the colonization of *S.* Enteritidis in the vagina led to a frequent incidence of egg contamination (Miyamoto et al., 1997; Okamura et al., 2001), which is consistent with the results of several studies that focused on the role of the ascending infection in egg contamination (Keller et al., 1995; Reiber et al., 1995).

The aim of this study is to confirm whether *S.* Enteritidis has higher capability to colonize the vagina than other serovars, which may cause the frequent incidence of contaminated eggs. We compared the in vitro adhesion of *S.* Enteritidis in vaginal and preovulatory follicular explants with that of *S.* Typhimurium, and then compared the in vitro adhesion and invasion of *S.* Enteritidis in the vaginal epithelium with that of several strains of *S.* Agona, *S.* Infantis, *S.* Hadar, *S.* Heidelberg, *S.* Montevideo and Typhimurium.

2. Materials and methods

2.1. Experimental birds

Approximately 300-day-old Boris Brown (Hy-Line Brown) laying hens were obtained from the single flock of a local commercial layer farm (NICHIWA, Hyogo, Japan). They were free of any apparent diseases throughout the growing and laying periods. Experimental hens were housed in individual cages in an isolated facility under a 12-h light/12-h dark photoperiod. They were provided with non-medicated food and water supplied ad libitum. No *Salmonella* was detected from cloacal swabs and feces of all birds during the acclimation period, over 10 days before use. The birds were managed according to The Standards Relating to the Care and Management of Experimental Animals (Japan).

2.2. Preparation of vaginal and follicular explants

Hens were euthanized by an intravenous injection of pentobarbital sodium solution. The vagina and ovary were collected aseptically and washed gently with Dulbecco's Modified Eagle's Medium (DMEM, Sigma–Aldrich Japan K.K., Tokyo, Japan). The vaginal segments placed on sterilized Petri dishes filled with DMEM were opened longitudinally and 8 mm diameter pieces of vaginal explants were cut aseptically using Biopsy Punch (Kai industries, Gifu, Japan). The ovarian epithelia were removed and 4–6 mm diameter preovulatory follicles were collected aseptically. The vaginal and follicular explants were placed onto sterilized 24-well flat bottom tissue culture plates including 900 μl of DMEM.

2.3. Bacteria

The *Salmonella* strains used in this study and their original isolation sources are shown in Table 1. All strains were supplied by Dr. T. Tsukamoto of the Osaka Prefectural Institute of Public Health and were originally isolated from samples associated with food poisoning. Bacteria for inoculation were cultured in Trypto-Soya Broth (Nissui, Tokyo, Japan) for 20 h at 37 °C. The number of bacterial inocula was adjusted to approximately 1×10^4 CFU/ml with DMEM.