

Competitive exclusion against *Salmonella gallinarum* of *Salmonella enteritidis* infected chickens

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To evaluate the degree of competitive exclusion against *Salmonella gallinarum* (*S. gallinarum*) of *Salmonella enteritidis* (*S. enteritidis*) infected chickens, fifty-six, 4-week old Hyline layer suspected of *S. enteritidis* infection were challenged with *S. gallinarum*. All chickens were tested for *S. enteritidis* isolation using cloacal swabs and serum plate agglutination test using *S. enteritidis* Ag. before challenge and classified into four groups (SE isolated, SE nonisolated, SE seropositive and SE seronegative). None of the SE isolated and the SE seropositive groups died after challenge and the average weight gains were 245.5g and 254.6g, respectively. But in the SE nonisolated and the SE seronegative groups, mortality was 18.2% and 20.6% and the average weight gains were 150.1g and 111.2g. The incidence of reisolation of *S. gallinarum* of the SE isolated and the SE seropositive groups were 41.7% and 47.6% from liver, 33.3% and 47.6% from spleen and 8.3% and 14.3% from cecum, respectively, and the SE nonisolated and the SE seronegative group were 63.6% and 64.7% from liver, 84.1% and 88.2% from spleen and 47.7% and 52.9% from cecum. The serological response of the SE isolated and the SE seropositive groups hardly changed from 75.0 and 81.8% before challenge to 75.0 and 85.7% after. But, the other two groups were found to be significantly higher after challenge and increased from 0 and 18.2% to 100%. Consequently, *S. enteritidis* preinfected chickens were found to be significant different in terms of mortality, weight gain, reisolation of *S. gallinarum* and serological response compared to noninfected chickens. Moreover, our study shows that *S. enteritidis* infected chickens appear strong competitive exclusion against the colonization of *S. gallinarum*.

Key words: *Salmonella gallinarum*, *Salmonella enteritidis*, competitive exclusion

Introduction

Fowl typhoid (FT) is a septicemic disease of domesticated birds and the course may be acute or chronic with high mortality depending on the virulence of *Salmonella gallinarum*. The gross lesion has been described as a swelling and redness of liver, spleen and kidney. These lesions are frequently seen in young birds and bronze and swollen livers are commonly observed in the subacute and chronic stages [13, 15].

Outbreaks of FT in Korea have been reported since 1992 and economic losses coupled by FT in brown layers are very serious [9]. Recently FT has broken out country-wide because of the failure of epidemic prevention and poor hygiene.

S. enteritidis colonization of poultry remains an issue of increasing food safety and public health concern. Its pathogenesis has very different consequences for hatched poultry than for more mature birds [13, 15]. *S. enteritidis* infection can sometimes lead to illness and death at high frequencies, especially the phage type 4 [13]. The incidence of *S. enteritidis* in poultry flocks is being reported continuously in Korea [8], but the rate is very much lower than that of FT, so *S. enteritidis* infection in Korea hasn't been considered so seriously in poultry.

S. gallinarum and *S. enteritidis* possess the same O antigens 1, 9, 12, but *S. enteritidis* possesses flagella antigens, whereas *S. gallinarum* does not [13]. It is known that the same O antigen structure has cross immune response, so various types of salmonella vaccine have been tested to immunize chickens and protect against the shedding of these organisms [1, 4, 7].

The objectives of the present study were to evaluate the degree of competitive exclusion by cross immune response against *S. gallinarum* of *S. enteritidis* infected chickens, although not live *S. enteritidis* vaccination.

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Materials and Methods

Bacterial strain

S. gallinarum challenge strain, 98252 was originally isolated from cases of fowl typhoid, the strain was stored in tryptic soy broth (TSB; Biolife, Milano, Italy) containing 30% glycerol at -70°C . Broth cultures were made by subculturing from the frozen stock culture with a platinum loop to 10 ml volumes of TSB and incubating for 24 hrs at 37°C . The density of the incubated broth was adjusted using a spectrophotometer (Shimadzu, Japan) and counted on plate count agar (Biolife, Milano, Italy) before challenge.

Chickens

Fifty-six, 4-week old Hyline layer suspected of *S. enteritidis* infection were obtained commercially. All chickens were tested for *S. enteritidis* isolation using cloacal swabs and serum plate agglutination test using *S. enteritidis* Ag. and housed in a isolation room during the test period. They had no any evident signs of Newcastle disease, Infectious bronchitis, Colibacillosis and so on.

Experiment designs

All chickens regardless of *S. enteritidis* infection were orally challenged with 3.1×10^7 cfu of *S. gallinarum* and mortality and weight was evaluated. *S. gallinarum* was reisolated from liver, spleen and cecum and serological response by the serum plate agglutination test using *S. gallinarum* antigen was performed after 14-days.

Isolation and Identification of *Salmonella*

To isolate *Salmonella spp.*, liver and spleen samples were inoculated onto TSB and Salmonella-Shigella agar (SS agar; Biolife, Milano, Italy) and cecum/cloacal swabs samples were inoculated into tetrathionate brilliant green novobiocin broth, as described by Woo *et al.* [16], incubated for 48 hrs at 41°C and finally streaked on SS agar. To identify *Salmonella spp.* colonies on SS agar, C_8 -esterase-spot-test reagent (Biolife, Milano, Italy) was used and colonies produced a strong blue fluorescence under a wavelength of 366 nm was selected. Final identification of *S. gallinarum* and *S. enteritidis* was done from H_2S produc-

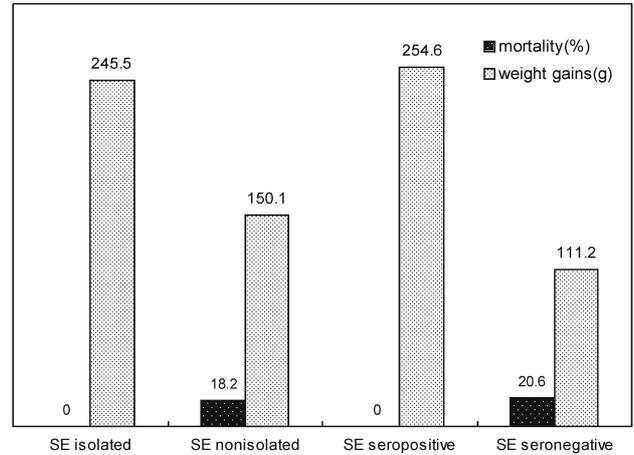


Fig. 1. Mortality and weight gains of *S. enteritidis* preinfected and noninfected groups during the 14-day test period after challenge with *S. gallinarum*

tion on SS agar and agglutination test using *Salmonella* O antiserum Group D₁ (Difco Laboratories, Detroit, MI) and *Salmonella* H antiserum single factor m (Difco Laboratories, Detroit, MI)

Results

Group classification by *S. enteritidis* identification

The results of the examination of *S. enteritidis* in chickens before challenge with *S. gallinarum* are shown in Table 1. According to results of *S. enteritidis* isolation or serological response, all chickens were classified into four groups, namely, SE isolated, SE nonisolated, SE seropositive and SE seronegative group, respectively.

Mortality and weight gain

Fig. 1 shows the comparison of mortality and weight gains of the *S. enteritidis* preinfected and noninfected groups after challenge with *S. gallinarum*. None of the SE isolated and the SE seropositive group died after challenge and the average weight gains were 245.5 g and 254.6 g, respectively. But in the SE nonisolated and the SE seronegative group, mortality were 18.2% and 20.6% and average weight gains were 150.1 g and 111.2 g, respectively.

Table 1. Classification of experimental groups by organism isolation from cloacal swabs and serological response to *S. enteritidis*(SE) antigen at 4-week old.

Groups	No. of reactors /No. of total chicks (%)	No. of positive/No. of reactors (%)	
		SE isolation	SPA*
SE isolated	12/56(21.4)	12/12(100)	11/12(91.7)
SE nonisolated	44/56(78.6)	0/44(0)	10/44(23.3)
SE seropositive	21/56(37.5)	11/21(52.4)	21/21(100)
SE seronegative	35/56(62.5)	1/35(2.9)	0/35(0)

*serum plate agglutination test

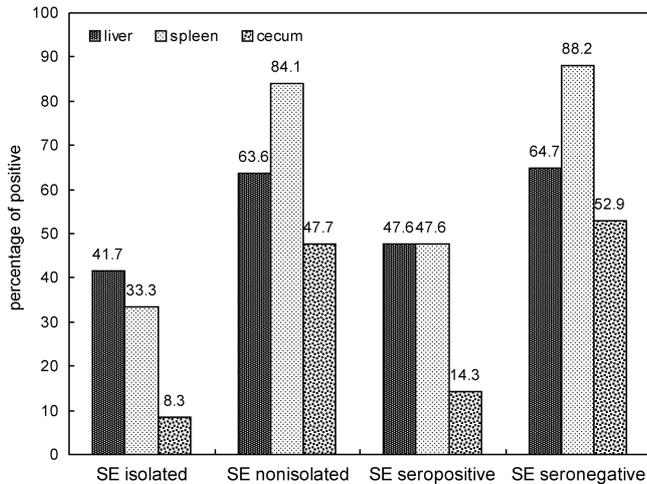


Fig. 2. Reisolation of *S. gallinarum* from organs of *S. enteritidis* preinfected and noninfected groups after 14-days challenge with *S. gallinarum*

Consequently, the *S. enteritidis* preinfected groups showed significant differences in mortality and weight gain compared to the noninfected groups.

Reisolation of *S. gallinarum*

Fig. 2 shows the incidence of the reisolation of *S. gallinarum* from organs. The SE isolated and the SE seropositive group showed reisolation rates of 41.7% and 47.6% from liver, 33.3% and 47.6% from spleen and 8.3% and 14.3% from cecum. But the SE nonisolated and the SE seronegative groups showed significantly higher reisolation rates from liver (63.6% and 64.7%), spleen (84.1% and 88.2%) and cecum (47.7% and 52.9%) than the *S. enteritidis* preinfected groups.

Serological responses

Fig. 3 compares antibody produced in the *S. enteritidis* preinfected and noninfected groups after challenge with *S. gallinarum*. The SE isolated and the SE seropositive group showed very little difference before and after challenge from 75.0 and 81.8% to 75.0 and 85.7%. But the other two groups were significantly higher after challenge from 0 and 18.2% to 100%.

Discussion

Fowl typhoid (FT) is a septicemic disease of domestic birds and is caused by *S. gallinarum*, recognized in 1888 [13, 15]. By the early 1900s, many outbreaks both abroad and in the United States had been reported and FT is still a leading disease problem in many areas of the world except USA and other advanced countries. Indeed, FT has increased dramatically in parts of Latin America, the Middle East, Africa, and some European countries [3, 11, 12,

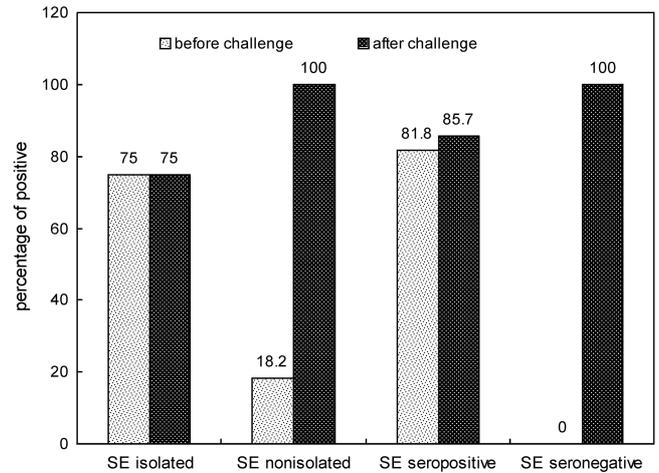


Fig. 3. Serological response on serum plate agglutination test of *S. enteritidis* preinfected and noninfected groups after 14-days challenge with *S. gallinarum*

14]. Outbreaks of FT in Korea were reported formally in 1992 and this has continued country-wide [9].

Unlike *S. gallinarum*, which has little human public health significance because it is extremely host specific [13], *S. enteritidis* contamination of eggs is a cause of human salmonellosis and continues to be a concern of food safety and public health [5, 6]. The incidence of human infection by *S. enteritidis* has steadily increased since the 1960s [2]. It was proposed that *S. enteritidis* became established in poultry flocks in the 1960s, which coincided with the eradication of the avian *Salmonella* pathogens *S. gallinarum* and *S. pullorum* from domestic fowl [2]. Because these three pathogens share a common immunodominant surface antigen (O9).

The O antigen of *S. enteritidis*, *S. pullorum* and *S. gallinarum* consists of the O12 antigen (a sugar backbone composed of O-polysaccharide repeating units) and the O9 antigen (a tyvelose sugar chain). Particularly, it is well known that O9-antigen induces the protective immunity and it was reported that vaccination with live *S. gallinarum* protects against colonization with *S. enteritidis* but not virulent *S. typhimurium*, which expresses serovar a different immunodominant determinant, the O4-antigen [4]. Furthermore, vaccination of mice with an *S. enteritidis* *aroA* mutant protected against subsequent challenge with a virulent *S. typhimurium* strain genetically engineered to express the O9, 12-antigen but not wild type *S. typhimurium* [7]. The present study shows that *S. enteritidis* preinfected poultry, although not live vaccine administered, were protected against colonization with *S. gallinarum*. This shows that the coexistence of *S. gallinarum* and *S. enteritidis* in poultry prompts competition as a result of the shared immunodominant O9-antigen, which generates cross-immunity.

Various killed vaccine preparation have been examined to control *Salmonella* infection in poultry, but none of these vaccines has been particularly effective in controlling *Salmonella* infection. This is because *S. gallinarum* is a cell-associated organism and humoral immunity offers only minimal prevention. Currently used vaccines against *S. gallinarum* in Korea that employ a killed bacterium are not widely used because of their cost and limited effectiveness. Moreover, it was reported that a combined vaccination program of live and killed bacterin provide better protection to laying hens than either vaccine administered alone [10].

The incidence of *S. enteritidis* infection in chicks has remained relatively constant with a low annual rate [8]. It is supposed that *S. gallinarum* infected poultry has increased immunity that protected birds against infection with *S. enteritidis*. Moreover, if *S. pullorum* and *S. gallinarum* were eliminated from commercial poultry in Korea, *S. enteritidis* infection could become endemic.

Although the main aim of this experiment was to assess the degree of competitive exclusion between *S. enteritidis* and *S. gallinarum*, we would propose that an effective live vaccine must be developed for both *S. enteritidis* and *S. gallinarum*.

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