Population of Salmonella serovar typhimurium in the Cecum of Gnotobiotic Chickens with Escherichia coli and Intestinal Bacteria

Tsuneo FUKATA, Hirokazu TSUTSUI, Eiichiro BABA, and Akira ARAKAWA

Department of Veterinary Medicine, College of Agriculture, University of Osaka Prefecture, 4-804 Mozu-umemachi, Sakai, Osaka 591, Japan

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ABSTRACT. To test the interaction between various species of bacteria and Salmonella serovar typhimurium (S. typhimurium), the population of S. typhimurium was measured in the cecum of gnotobiotic chickens in the presence of Escherichia coli (E. coli) and one of the four intestinal bacteria; Lactobacillus acidophilus, Clostridium perfringens, Bifidobacterium thermophilum and Bacteroides vulgatus. Competitive exclusion of S. typhimurium by di-flora chicken was not demonstrated. But the population of S. typhimurium was temporarily suppressed in di-flora chickens with E. coli and L. acidophilus. In penta-flora chickens with E. coli and these four intestinal bacteria, the population of S. typhimurium was suppressed for only 2 days. In normalized chickens, the population of S. typhimurium was markedly suppressed.—KEY WORDS: Escherichia coli, gnotobiotic chicken, Salmonella serovar typhimurium, suppression.


Greater susceptibility of the newly hatched chicken to oral infection of food-poisoning salmonellae, when compared with adult chickens, is attributed to the virtual absence of intestinal microflora [13]. The resistance of newly hatched chickens can be conferred by oral administration of a suspension of feces or cecal contents obtained from adult chickens [13, 14]. Prevention of establishment of salmonellae in chickens harboring adult intestinal flora was named ‘competitive exclusion’ [11, 19, 22]. The influence of intestinal microflora on exclusion of salmonella colonization in chickens was examined in large-scale field trials [1, 19, 23]. However, these trials seemed to have limits and its practicability had been questioned [1].

Barrow and Tucker [4] indicated that salmonellae were excluded from ceca of conventional chickens inoculated with some kinds of Escherichia coli (E. coli) originating from sewage and an abattoir. In ceca of mono-flora chickens, Salmonella serovar typhimurium (S. typhimurium) was excluded by E. coli, but not by Lactobacillus acidophilus (L. acidophilus), Bacteroides vulgatus (B. vulgatus), Bifidobacterium thermophilum (B. thermophilum) or Clostridium perfringens (C. perfringens) [5].

In the present study, competitive exclusion of S. typhimurium was examined in di-flora chickens with E. coli and one of the four intestinal bacteria, and in penta-flora chickens with E. coli and four bacteria.

MATERIALS AND METHODS

Chickens: Methods for preparing germ-free chickens, White Leghorn, Hy-Line, were described previously [5, 6].

Microorganisms: S. typhimurium was supplied by the National Institute of Animal Health in Tsukuba, Japan. L. acidophilus, C. perfringens, B. vulgatus and B. thermophilum were supplied by Dr. Benno of the Institute of Physical and Chemical Research in Saitama, Japan. A standard strain of E. coli, O-150 antigen, was used.

Media and culture methods: Growth media were trypticase soy broth (Nissui Pharmaceutical Co., Japan) for S. typhimurium and E. coli and Gifu anaerobic media (GAM) broth (Nissui) for other bacteria. Bacteria inocula were prepared from broth cultured at 37°C for 20 hr. The selective media were mannitol lysine crystal violet brilliant green (MLCB) agar (Nissui) for S. typhimurium. The number of total bacteria was counted on GAM agar cultured anaerobically (Oxoid Ltd., England) at 37°C for 48 hr.

Experimental design: Experiment (I). Population of S. typhimurium in di-flora chicken. A suspension of E. coli and L. acidophilus, C. perfringens, B. vulgatus or B. thermophilum each consisting of 10⁸ colony forming units (CFU) was inoculated into 2-day-old germ-free chickens. Two days later, 10⁴ CFU/ml of S. typhimurium was orally inoculated. The number of S. typhimurium and total bacteria in
the cecal contents were counted at 1, 2, 4, 6 and 8 days after S. typhimurium inoculation. The sample was weighed and added to 99 volumes (w/v) of sterile buffer solution [12]. One ml of the mixture was withdrawn and diluted with 9 ml of sterile buffer solution. Likewise, each sample mixture was diluted at serial 10-fold steps. From each suspension, 0.1 ml was withdrawn and spread onto each agar plate. The colonies developed were counted. The number of bacteria was expressed as \( \log_{10} \) of CFU/g of cecal contents.

Experiment (II). Population of S. typhimurium in penta-flora chicken. A combined suspension of E. coli, L. acidophilus, C. perfringens, B. vulgatus and B. thermophilum was inoculated orally at each 10^6 CFU/bird into 2-day-old germ-free chickens. Two days later, 10^8 CFU/ml of S. typhimurium was inoculated. This experiment was conducted at the same schedule as experiment (I).

Experiment (III). Exclusion of S. typhimurium in normalized chickens. Germ-free chickens were inoculated with one ml of a suspension of cecal contents of conventional chickens, diluted 2,000 times with buffer solution. Two days later, 10^6 CFU/ml of S. typhimurium was inoculated into each bird. Necropsy and bacteriological examination were conducted at the same schedule as experiment (I).

RESULTS

Experiment (I). Population of S. typhimurium inoculated into chickens with E. coli and L. acidophilus increased up to 10^7 CFU/g during the first 4 days after inoculation, but decreased to 10^5 CFU/g within 4 days (Fig. 1a).

The number of S. typhimurium inoculated into chickens with E. coli and C. perfringens maintained its level at 10^6–10^7 CFU/g throughout the experimental period (Fig. 1b).

Gnotobiotic chickens with E. coli and B. vulgatus showed an increase in S. typhimurium 2 days after inoculation, and maintained the level greater than 10^5 CFU/g (Fig. 1c).

The number of S. typhimurium in gnotobiotic chickens with E. coli and B. thermophilum increased gradually up to 10^7 CFU/g within 4 days following inoculation (Fig. 1d).

In the cecal contents of all di-flora chickens, the number of total organisms ranged from 10^9 to 10^10 CFU/g during experimental period (Fig. 1a-d).

Fig. 1. 1a-d. The number of bacteria in the cecal contents of gnotobiotic chickens with E. coli and test bacterium that inoculated with 10^6 CFU of S. typhimurium. ○: Average count of S. typhimurium and ●: Average count of E. coli and test bacterium. Bar: Standard deviation. Test bacterium: (a) L. acidophilus (n=4), (b) C. perfringens (n=4), (c) B. thermophilum (n=4-6), (d) B. vulgatus (n=4-8).

Fig. 2. The number of bacteria in the cecal contents of gnotobiotic chickens with E. coli, L. acidophilus, C. perfringens, B. thermophilum and B. vulgatus that inoculated with 10^6 CFU of S. typhimurium. ○: Average count of S. typhimurium and ●: Average count of bacteria (n=4). Bar: Standard deviation.

Experiment (II). The number of S. typhimurium and total organisms in penta-flora chickens are shown in Fig. 2. One day after inoculation of S. typhimurium, the number increased, but decreased from 2 to 4 days after inoculation and again increased gradually. The total number of organisms was 10^9-10^10 CFU/g.
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| Table 1. Exclusion of S. typhimurium in the cecal contents of the normalized chickens inoculated with 10^6 CFU of S. typhimurium |
|------------------|------------------|------------------|------------------|------------------|
|                  | Pre-inoculation | Days after S. typhimurium inoculation |
|                  |                  | 0    | 1    | 2    | 4    | 6    | 8    |
|                  | 0/4              |      |      |      |      |      |      |
|                  | 1/8               |      |      |      |      |      |      |

a) Number of positive birds/Number of birds inoculated.
b) Only one bird was positive for S. typhimurium; its number was 10^3 CFU/g.

Experiment (III). One day after S. typhimurium inoculation, 10^3 CFU/g of S. typhimurium was found in the cecal contents of one bird. The remaining birds showed negative for S. typhimurium. S. typhimurium was excluded (<10^3 CFU/g) from the cecal contents examined 2 to 8 days after inoculation (Table 1).

DISCUSSION

The authors reported the depressed colonization of S. typhimurium in mono-flora chickens with E. coli [5]. In this study, competitive exclusion of S. typhimurium by intestinal bacteria and E. coli was not demonstrated. In di-flora chickens with E. coli and B. thermophilum or L. acidophilus, S. typhimurium increased temporarily. In penta-flora chickens, the number of S. typhimurium decreased temporarily. These findings indicate that the inoculated bacteria have hardly any influence on the exclusion of S. typhimurium. In conventional chickens, Streptococcus faecalis did not exclude S. typhimurium tested by the cloacal swab method [20]. The mono-flora chickens with B. thermophilum and L. acidophilus were unable to exclude S. typhimurium in ceca [4]. Gnotobiotic chickens serially inoculated with L. acidophilus, or chickens given cecal contents or feces from normal chickens were able to slightly reduce salmonellae in the lower gut [19, 21].

Normalized chickens were rarely used for studying competitive exclusion of Salmonella. Lafont et al. [10] and Hudault et al. [7] showed that germ-free chickens were protected against S. typhimurium by inoculation with fecal flora from conventional chickens. They used the 10- or 16-day-old gnotobiotic chickens, however, chickens susceptible to salmonellosis are often younger than them. Therefore, in this study, 2-day-old chickens were used and S. typhimurium was inhibited by administration of cecal flora. The present findings and reports in the conventional chickens [2, 3, 8, 9, 11, 13, 14, 15, 16, 17, 18, 20] indicated that administration of intestinal flora to the young chickens shortly after hatching protects them from subsequent infection by salmonellae.

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REFERENCES


