Research Note

Reduction of Campylobacter jejuni in a Simulated Chicken Digestive Tract by Lactobacilli Cultures

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ABSTRACT

Studies were conducted to investigate the impact of a selected lactobacilli mixed culture on Campylobacter jejuni in simulated chicken digestive tract models. Veronal buffer solutions corresponding to the pH of successive segments of the chicken digestive tract were prepared. The lactobacilli mixtures were prepared by mixing four fresh lactobacilli cultures, including Lactobacillus acidophilus, Lactobacillus fermentum, Lactobacillus crispatus, and Lactobacillus brevis. The C. jejuni and lactobacilli mixture were mixed with sterile poultry feed, and the previously prepared veronal buffer solutions were then added separately. The mixture was incubated at 41.1°C for various lengths of time with periodic agitation. The feed passage time for five segments of the digestive tract were adopted: crop (pH 4.5), 30 min; proventriculus (pH 4.4), 15 min; gizzard (pH 2.6), 90 min; small intestine (pH 6.2), 90 min; and large intestine (pH 6.3), 15 min. The Campylobacter and lactobacilli were enumerated. An antagonistic effect on C. jejuni by the tested lactobacilli spp. was found in individual sections and the complete simulated digestive tract models. In the simulated complete chicken digestion system, no C. jejuni were found during the final incubation period when a lactobacilli mixture was present. The results of this in vitro study indicate the potential value of future in vivo studies.

Pathogenic microorganisms represent a potential that can determine whether a meat or poultry business remains in operation. The U.S. Centers for Disease Control and Prevention (CDC) (5) reported that a high percentage of foodborne illnesses are associated with meat, poultry, and egg products. They estimated that foodborne pathogens are responsible for 76 million illnesses and 5,000 deaths in the United States each year (5). The major area of concern in the processing of poultry is the removal and elimination of as much microbial contamination as possible and the prevention of cross-contamination of any potential pathogens that may be present. Reducing or eliminating the pathogenic bacteria in the gastrointestinal tract of chicken can decrease the risk of cross-contamination during evisceration.

Campylobacter spp. have been isolated from 14 to 91% of cecal and rectal samples and carcasses of healthy chickens (4, 13, 27, 30, 31). Since C. jejuni was first recognized as the cause of an outbreak in 1979, it has been implicated in 53 foodborne disease outbreaks in the United States between 1979 and 1987, affecting 1,547 individuals, with two deaths (2). The CDC (5) reported that C. jejuni is the leading cause of bacterial diarrhea in the United States. There are probably numbers of cases in excess of estimated cases of salmonellosis. In 1999, the CDC estimated that C. jejuni caused approximately 1,963,141 illnesses in the United States or about 14.2% of all foodborne illnesses (5).

In 1973, Nurmi and Rantala (24) introduced a technique called competitive exclusion (CE) to increase the resistance of young chicks to Salmonella infection by orally inoculating them with the intestinal content of Salmonella-free adult birds. During the past two decades, there have been many studies on the efficacy of CE for the control of pathogenic bacteria such as Escherichia coli and Salmonella (7, 10, 11, 20, 23, 33); however, few studies have reported on the inhibition of Campylobacter spp. by CE concept (16, 17, 36).

Lactic acid bacteria have been used as the component of defined cultures of the CE technique. They are present naturally in the chicken gut and are also introduced to suppress the growth of harmful bacteria (1). During the last two decades, lactic acid bacteria have been tried for use as an oral antimicrobial agent in chicken’s production. However, the impact of C. jejuni by lactobacilli cultures in the chicken’s digestive tract was not clear. A simulated model was used in the human gastrointestinal to evaluate the survival of microorganisms in several studies (3, 14, 18, 19, 22, 25). Therefore, the objective of this research was to investigate the impact of lactobacilli cultures on the growth and survival of C. jejuni by a simulated chicken digestive tract model.

MATERIALS AND METHODS

Cultures. C. jejuni (ATCC 29428) culture was obtained from the American Type Culture Collection (ATCC, Rockville, Md.) and cultured on modified Campylobacter agar slants (Difco Laboratories, Detroit, Mich.) at 42°C in a microaerophilic environment (BBL, Cockeysville, Md.) (14). Four strains of lactobacilli,
TABLE 1. Survival of C. jejuni in individual sections of simulated chicken digestive tract as affected by the presence of lactobacilli spp.a

<table>
<thead>
<tr>
<th>Digestive section (simulated)</th>
<th>C. jejuni in controls</th>
<th>C. jejuni</th>
<th>Lactobacilli</th>
<th>C. jejuni</th>
<th>Lactobacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop (pH 4.5)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Initial</td>
<td>5.77 ± 0.11</td>
<td>5.42 ± 0.06</td>
<td>6.18 ± 0.08</td>
<td>5.64 ± 0.24</td>
<td>4.75 ± 0.18</td>
</tr>
<tr>
<td>After 30 min</td>
<td>5.44 ± 0.22</td>
<td>4.18 ± 0.18</td>
<td>6.13 ± 0.12</td>
<td>4.70 ± 0.06</td>
<td>4.75 ± 0.17</td>
</tr>
<tr>
<td>Different</td>
<td>0.33 ± 0.16 A</td>
<td>1.24 ± 0.06 A</td>
<td>0.05 ± 0.10</td>
<td>0.94 ± 0.12 A</td>
<td>0.00 ± 0.06</td>
</tr>
<tr>
<td>Proventriculus (pH 4.4)</td>
<td></td>
<td></td>
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<tr>
<td>Initial</td>
<td>5.72 ± 0.12</td>
<td>5.33 ± 0.09</td>
<td>6.15 ± 0.17</td>
<td>5.56 ± 0.18</td>
<td>4.75 ± 0.08</td>
</tr>
<tr>
<td>After 15 min</td>
<td>5.51 ± 0.19</td>
<td>4.47 ± 0.11</td>
<td>6.10 ± 0.05</td>
<td>4.87 ± 0.13</td>
<td>4.71 ± 0.09</td>
</tr>
<tr>
<td>Different</td>
<td>0.21 ± 0.16 A</td>
<td>0.86 ± 0.07 A</td>
<td>0.05 ± 0.03</td>
<td>0.69 ± 0.10 A</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Gizzard (pH 2.6)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>6.54 ± 0.28</td>
<td>4.91 ± 0.17</td>
<td>6.06 ± 0.07</td>
<td>4.99 ± 0.09</td>
<td>4.60 ± 0.32</td>
</tr>
<tr>
<td>After 90 min</td>
<td>3.29 ± 0.06</td>
<td>ND</td>
<td>6.03 ± 0.18</td>
<td>0.48 ± 0.17</td>
<td>4.47 ± 0.12</td>
</tr>
<tr>
<td>Different</td>
<td>2.05 ± 0.15 B</td>
<td>&lt;4.91 ± 0.12 A</td>
<td>0.02 ± 0.06</td>
<td>4.51 ± 0.09 A</td>
<td>0.15 ± 0.10</td>
</tr>
<tr>
<td>Small intestine (pH 6.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>5.74 ± 0.22</td>
<td>5.45 ± 0.21</td>
<td>6.21 ± 0.11</td>
<td>5.47 ± 0.13</td>
<td>4.72 ± 0.08</td>
</tr>
<tr>
<td>After 90 min</td>
<td>4.94 ± 0.10</td>
<td>3.44 ± 0.08</td>
<td>6.12 ± 0.16</td>
<td>3.60 ± 0.07</td>
<td>4.69 ± 0.11</td>
</tr>
<tr>
<td>Different</td>
<td>0.80 ± 0.13 B</td>
<td>2.01 ± 0.11 A</td>
<td>0.09 ± 0.05</td>
<td>1.87 ± 0.19 A</td>
<td>0.03 ± 0.08</td>
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<tr>
<td>Large intestine (pH 6.3)</td>
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<td></td>
</tr>
<tr>
<td>Initial</td>
<td>5.81 ± 0.13</td>
<td>5.46 ± 0.34</td>
<td>6.14 ± 0.02</td>
<td>5.57 ± 0.12</td>
<td>4.66 ± 0.16</td>
</tr>
<tr>
<td>After 15 min</td>
<td>5.69 ± 0.24</td>
<td>4.62 ± 0.19</td>
<td>6.17 ± 0.14</td>
<td>5.13 ± 0.18</td>
<td>4.66 ± 0.21</td>
</tr>
<tr>
<td>Different</td>
<td>0.12 ± 0.15 A</td>
<td>0.84 ± 0.16 A</td>
<td>−0.03 ± 0.10</td>
<td>0.44 ± 0.22 A</td>
<td>0.00 ± 0.09</td>
</tr>
</tbody>
</table>

a Each value represents the mean of four replications. ND, not detected.

b Mean ± SD log CFU of C. jejuni per ml of solution within a row not followed by common letter are different (P < 0.05).

Lactobacillus acidophilus (ATCC 9338), Lactobacillus fermentum (ATCC 4536), Lactobacillus crispatus (ATCC 33820), and Lactobacillus brevis (ATCC 4006), were also obtained from ATCC and cultured on Rogosa agar slants (Difco) at 30°C. Cultures were transferred periodically to maintain viability. The basis of microscopic examination (motility, Gram stain, shape), catalase, and oxidase tests were conducted after transfer to confirm the purity of cultures.

Fresh C. jejuni culture suspensions were prepared by transferring one loop of culture into 10 ml of brucella broth (Difco) with 5% lysed horse blood (BBL) and 1% Blaser's selective agent (Difco). The inoculum of C. jejuni was incubated at 42°C for 48 h before use. Fresh lactobacilli culture suspensions were prepared by transferring one loop of culture into 10 ml of Rogosa broth (Difco) and incubated at 35°C for 48 h.

Preparation of the simulated chicken digestive tract model. Veronal buffer solutions at pH 4.5, 4.4, 2.6, 6.2, and 6.3 were prepared according to Gortner and Gortner (12). These pH values were chosen because they corresponded to the pH of successive segments of the chicken’s digestive tract (6). In a simulated complete chicken digestive tract, the pH values of each segment were adjusted either with 0.1 N HCl or with veronal buffer at pH 9.6.

Effects of lactobacilli spp. on C. jejuni in a simulated chicken digestive tract model. For the simulated individual segment of the digestive tract study, the lactobacilli mixture was prepared by mixing 0.25 ml each of four fresh lactobacilli suspensions. Fourteen milliliters of previously prepared veronal buffer solutions was mixed in the test tubes with 2 g of autoclaved sterile poultry feed. Two milliliters of lactobacilli (10⁶ or 10⁷ CFU/ml) mixture and C. jejuni (10⁵ CFU/ml) culture suspensions were added separately to the solution. Two milliliters of sterile peptone water was used instead of lactobacilli mixture suspensions as control. The pH values of the mixture solution were determined and adjusted for specific pH values of each digestive section according to experimental design. The tubes were incubated in a water bath at 41.4°C (chicken’s body temperature) for the following periods: pH 4.5 (crop), 30 min; pH 4.4 (proventriculus), 15 min; pH 2.6 (gizzard), 90 min; pH 6.2 (small intestine), 90 min; and pH 6.3 (large intestine), 15 min (6).

Before and after each incubation, the lactobacilli were enumerated using Rogosa agar. Plates were incubated in an anaerobic environment at 35°C for 48 h. The C. jejuni were enumerated by using a Campylobacter agar kit with Blaser’s supplement in a microaerophilic system (5% oxygen, 10% carbon dioxide, balance nitrogen) at 42°C for 48 h. The pour plating method was used for enumeration.

In the completed digestive system, 35 ml of veronal buffer solution (crop, pH 4.5) was mixed in the 250-ml flasks with 5 g of autoclaved sterile poultry feed. Five milliliters of lactobacilli mixture and C. jejuni culture suspensions were added separately into the solution. Two milliliters of sterile peptone water was used instead of lactobacilli mixture suspensions as control. The flasks were incubated in a water bath at 41.4°C. Peptone water was used for the controls. The pH values and incubation time of each segment of the digestive tract were adjusted and measured. Before and after incubation of each section, the lactobacilli C. jejuni were enumerated as described earlier.

Statistical analysis. Statistical analyses were performed to compare the effects of lactobacilli treatment on reduction of three types of pathogens in a simulated chicken digestive tract model.
A completely randomized design with four replications was used. Data were analyzed by analysis of variance according to the general linear model procedure of the Statistical Analysis System (28). When there were significant differences ($P < 0.05$), the least significant difference test was used to separate the means (35).

RESULTS AND DISCUSSION

An antagonistic effect on C. jejuni by the tested lactobacilli spp. was found in the individual simulated chicken digestive model. The reduction of C. jejuni by lactobacilli spp. was observed in the simulated gizzard and small intestine sections (Table 1). The mixture containing $10^4$ CFU/ml of lactobacilli performed as well as that containing $10^6$ CFU/ml in antagonistic effect on C. jejuni. The Campylobacter spp. were sensitive to low pH (below pH 5.0) (26). This explains why the greatest reduction was observed in the simulated gizzard (pH 2.6).

In the simulated complete chicken digestion system, no C. jejuni were found during the final incubation period when either $10^4$ or $10^6$ CFU/ml of lactobacilli mixture was present (Fig. 1). Regardless of the lactobacilli number, C. jejuni counts were reduced dramatically in the simulated gizzard section before leveling off in the simulated small and large intestinal sections. This indicated that the low pH environment in the gizzard section might help lactobacilli inhibit C. jejuni. In the final stage of the digestive tract system, the difference in C. jejuni between control and treatment groups was more than 1.5 log. Of all individual sections of the digestive tract that were simulated, the gizzard and small intestine sections were found to be critical for the C. jejuni growth but not other sections. However, in the simulated complete chicken digestive tract model, no C. jejuni were found during the final incubation period when the tested lactobacilli mixture was present. This observation indicated that a synergistic antagonistic effect on C. jejuni by tested lactobacilli mixture might exist during their passage through the digestive tract of the chicken.

C. jejuni have been reported to be the most common cause of gastroenteritis or enterocolitis in humans (29). C. jejuni are commonly found in a wide variety of domestic warm-blooded animals (9, 32), and poultry is most frequently infected by C. jejuni in the United States according to the estimate report of the CDC (5). No commercial vaccine is yet available to control Campylobacter infection in poultry, but there is the possibility of manipulating the intestinal microflora of chicks to increase resistance by CE (21, 29, 33, 36). The results of this study provided the information that there is a potential existence in reducing the incidence of C. jejuni in poultry by direct feeding of lactic acid bacteria. However, the intestinal tract is a complex environment and other factors (Eh, nutrients, toxic fermentation products, and attachment sites, etc.) may serve to protect or destroy microorganisms in vivo.

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