Some observations on the caecal microflora of the chickens during experimental acute fowl typhoid

T. KOKOSHAROV

Laboratory of Bacteriology, Regional Veterinary Station, 6300 Haskovo, Bulgaria

Contact address : Tel. 359 38 200 32, fax : 359 38 20 55 ; E-mail : kokosharov@yahoo.com

Introduction

The main mechanism regulating the microbial ecology in the gut of chickens and the importance that changes in the intestinal microflora play in birds are still poorly understood [17]. There has been an upsurge in interest in the role that the normal intestinal flora, both aerobic and anaerobic plays in protecting against Salmonella infection [3, 4, 9]. Paired caeca are situated at the junction of the small and large intestine and they normally contain a stable population of bacteria of very many different types [1, 6]. The flow rate is low in these regions which would allow for greater microbial multiplication in the lumen [18]. It is impossible to make detail microbial analysis of this heterogenous composite because the estimation of the most representative bacteria will do satisfactory notion for gastrointestinal microflora [20].

The effect of Salmonella Gallinarum infection on caecal microflora of the chickens have not been investigated. The purpose of this study was to provide quantitative and qualitative information in the microflora of the caecum in normal chickens and to evaluate the changes in the microflora of the caecum following peroral inoculation with Salmonella Gallinarum.

Materials and methods

BIRDS

The experiments were performed with fifty 5-month-old female Salmonella-free New Hampshire chickens. The experimental group was formed 14 days before to the study. The chickens received antibiotic-free food and water ad libitum.
BACTERIUM

A strain of field *Salmonella* Gallinarum isolated from a dead hen was stored on Dorset egg medium at 4 °C. The strain was recovered by inoculating a small portion of the stock into blood agar and incubated the agar over night at 37 °C. The bacterial suspension concentration 1.5x10⁹ colony forming bacterial (cfu) was made with sterile 0.85 % saline solution. The viable cell concentration of the challenge inoculum was confirmed counts on brilliant green agar plates.

EXPERIMENTAL PROCEDURES

A group of 50 chickens were inoculated with 1.5 ml of bacterial suspension into the crop. The control group was inoculated perorally with 1.5 ml of 0.85 % saline solution. At 1, 4, 7, 14 and 21 days post inoculation seven infected chickens were euthanatized and their caecal contents and from dead infected birds and from the control (noninfected) chickens, which were also analysed.

ISOLATION MEDIA

The aerobe media were:

1/ Brilliant green phenolrot agar—for *E. coli* and *Salmonella*.
2/ 5 % sheep blood agar with supplement 7.5 % sodium chloride - for *Staphylococcus* spp [5].
3/ Media Barnes containing sodium azide and 2, 3, 5-triphenyl- tetrazolium chloride (TTC) - for faecal streptococci (Enterooccus spp.).

The anaerobe media were:

1/ Media Wilson-Blair - for H2S-producing Clostridia.
2/ Bifidobacterium selective agar composed of BL agar base with 5 % sheep blood, 2 g lactose, 10 ml 1 % sodium azide (per 100 ml of media).
3/ Agar hydrolyzed milk with supplement drops of 5 % hinablau solution - for *Lactobacillus* spp.

QUANTITATIVE AND QUALITATIVE EXAMINATION OF MICROFLORA

Serial 10 - fold dilution of each specimen were performed and 100 µl aliquots of each dilution were then spread onto selective culture media. All determinations were carried out in duplicate and using different dilutions to ensure accurate values. Number of colonies on each plate were expressed logarithmically as log10 cfu/g caecal contents. The enumerations of H2S-producing Clostridia were made from Wilson-Blair media incubated at 37 °C for 24 h. We counted the black colonies. The other bacteria were enumerated after aerobically and anaerobically incubation at 37 °C for 48-72 h. The predominant organisms grown on the media were indentified to family or genus according to Bergey’s Manual of Systemic Bacteriology [21].

STATISTICAL ANALYSIS

All results were statistically analysed using the Student-Fisher test. Values were reported as mean standard error (SE).

Results

BACTERIOLOGICAL EXAMINATION OF AEROBIC MICROFLORA

The viable numbers of aerobic organisms per gram in luminal contents of the caeca at different time after inoculation with *Salmonella* Gallinarum of group of 5-month age chickens are shown in Table I.

The mean SD values for *Salmonella* Gallinarum was log10 6.05 ± 0.98 ; log10 6.22 ± 1.15 ; 7.32 ± 0.86 on 1, 4 and 7 day P.I. and declined on 14 and 21 day. At the dead infected birds their amount was log10 7.55 ± 1.18/g caecal contents. The number of *E.coli* and *Staphylococcus* spp. from caecal samples were log10 7.15 ± 0.15 and log10 3.27 ± 0.37 cfu/g in the control birds, respectively, and gradually increased at 7 day P.I. and at the dead chickens (log10 8.10 ± 0.70 ; log10 8.43 ± 0.82 and log10 4.16 ± 0.25 ; log10 4.16 ± 0.35 cfu per gram of caecal contents, respectively). The difference was significant (P < 0.001).

The mean SE values for enterococci varied from log10 4.53 ± 0.24 cfu/g at the control chickens to log10 6.30 ± 0.59 in the 1st day P.I., log10 4.84 ± 0.47 at the 4th day, log10 5.44 ± 0.75 cfu/g at the 7th day, no change in recovery period and at the dead chickens.

BACTERIOLOGICAL EXAMINATION OF ANAEROBIC MICROFLORA

As demonstrated in Table I by the 7 days P.I. and the dead chickens there was a significant drop in the Lactobacilli and Bifidobacteria counts and reached log10 4.52 ± 0.25 ; log10 4.54 ± 0.30 and log10 4.56 ± 0.27 cfu/g, respectively. The difference was significantly. The count of H2S-producing Clostridia obtained from the cecal contents at 7 days P.I. and at the dead birds were significantly higher (log10 4.26 ± 0.84 ; log10 4.26 ± 0.84 ; log10 3.89 ± 0.10 cfu/g, respectively) than those from the caeca of control (noninfected) chickens (log10 2.86 ± 0.63 cfu/g).

All these changes were established when the infected birds showed of sings of systemic toxicity, including loss of appetite, a drooping attitude, anaemia and shrunken combs (data not shown).

The number of aerobes and anaerobes were returned to normal levels by 14 days P.I.

Discussion

Although that some authors [13] have been determined remarkable resistance of indigenous poultry microbial population by the introduction of new species, in our experiments we determined the capability of *Salmonella* Gallinarum to disturb ecological balance in caecum. This serovar is a poultry natural host-adapted causing outbreaks with heavy mortality [23].
In our previous study [10] at experimental induced acute fowl typhoid we established that the first 3-4 days P.I. the contagious chickens didn’t show change in their clinical parameters, the next 4-5 days they showed clinical symptoms of systemic toxicity and after 12-14 day they clinically normalized their health condition. On the basis of this observations all changes in the caecal microflora were found out in the clinical period of the infectious process and it is evidence that there was a strong interaction between infection with Salmonella Galinarum and observed changes. During the first 4 days P.I. and in the recovery period (14-21 days) the values of aerobic and anaerobic bacteria from the caecal contents didn’t distinguish from these of control chickens.

Four days were required for the development of a modification of the caecal flora and that systematically at the same time an increase in the potential pathogen is observed. E. coli, Staphylococcus spp., faecal streptococci and H2S-producing Clostridia rose to very large numbers in the caecum. The proteolytic properties of these Clostridia found in the intestine is a major factor to its detrimental effects [22]. It was also around this time that the Lactobacillus spp. and Bifidobacteria were established in low concentrations. The mechanism for this changes in bacteria has not been defined. Lactobacilli and Bifidobacteria are predominant in the caecal contents in the healthy chickens and may be their presence is considered clinical for maintaining the ecological balance of the caecal microflora. Our results are in accordance with this hypothesis. A more plausible explanation of our finding is that with the establishment of the Lactobacilli and Bifidobacteria in recovery period, the level of E. coli, Staphylococcus spp., Clostridia declined. Thus population of bacteria within the microflora of the caecum, appear to undergo significant changes fluctuation in number before a dynamic equilibrium is established between the species (14-21 days). It would seem probably that the development of these changes would provide competition for food sources, a decrease the pH and the production of volatile acids [2, 7, 8, 11, 12, 15]. The high number of Salmonella Galinarum in the caecal contents of the infected birds on days 1, 4, 7 and at dead chickens clearly indicated Salmonella Galinarum survival, replication and amplification of the challenge dosage. It is known that Lactobacilli and Bifidobacteria [15,16] protect against potentially harmful bacteria such as Salmonella. Therefore, an increase in the number of these strains will improve the status of microbial ecology in the chicken’s gut making it less sensitive to colonization by pathogens. A practical example of this hypothesis can be seen from studies on the therapeutic possibilities of supplementing diets with these bacterial species. The use of native gut microflora [19] and competitive exclusion culture [14], which have been contained these bacterial species, partially protect against Salmonella Galinarum and it was recommended in geographic areas where poultry production is adversely affected by fowl typhoid newly hatched chicks to be threatened with such bacterial cultures.

Table I. — Changes in counts (mean ± SE) of aerobic and anaerobic bacteria in caeca of chicken following perorally Salmonella Gallinarum inoculation.

<table>
<thead>
<tr>
<th>Day P.I.</th>
<th>E.coli</th>
<th>Staphylococcus</th>
<th>Salmonella</th>
<th>Entero-coccus</th>
<th>H2S producing Clostridia</th>
<th>Lactobacillus</th>
<th>Bifidobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.15 ± 0.15</td>
<td>3.27 ± 0.24</td>
<td>0</td>
<td>4.53 ± 0.24</td>
<td>2.86 ± 0.63</td>
<td>6.05 ± 0.76</td>
<td>6.50 ± 0.38</td>
</tr>
<tr>
<td>1st day</td>
<td>7.30 ± 0.23</td>
<td>N.D.</td>
<td>6.05 ± 0.98</td>
<td>6.30 ± 0.59*</td>
<td>2.65 ± 0.81</td>
<td>6.13 ± 1.32</td>
<td>6.57 ± 0.09</td>
</tr>
<tr>
<td>4th day</td>
<td>7.03 ± 0.56</td>
<td>4.58 ± 0.13*</td>
<td>6.22 ± 1.15</td>
<td>4.84 ± 0.47</td>
<td>3.36 ± 1.12</td>
<td>6.34 ± 1.16</td>
<td>6.88 ± 0.45</td>
</tr>
<tr>
<td>7th day</td>
<td>8.10 ± 0.70*</td>
<td>4.16 ± 0.25*</td>
<td>7.32 ± 0.86</td>
<td>5.45 ± 0.75*</td>
<td>4.26 ± 0.84*</td>
<td>4.54 ± 0.25*</td>
<td>4.54 ± 0.30*</td>
</tr>
<tr>
<td>14th day</td>
<td>7.15 ± 0.16</td>
<td>N.D.</td>
<td>3.15 ± 0.66</td>
<td>6.46 ± 0.28</td>
<td>N.D.</td>
<td>6.67 ± 0.23</td>
<td>7.68 ± 0.60</td>
</tr>
<tr>
<td>21st day</td>
<td>7.45 ± 0.75</td>
<td>N.D.</td>
<td>2.46 ± 0.92</td>
<td>4.59 ± 0.29</td>
<td>2.83 ± 0.59</td>
<td>6.94 ± 0.22</td>
<td>7.04 ± 0.32</td>
</tr>
<tr>
<td>Dead</td>
<td>8.43 ± 0.82*</td>
<td>4.16 ± 0.35*</td>
<td>2.46 ± 0.92</td>
<td>4.28 ± 0.41</td>
<td>3.89 ± 0.10*</td>
<td>4.46 ± 0.26*</td>
<td>4.36 ± 0.27*</td>
</tr>
</tbody>
</table>

a - log10 mean cfu/g. Values followed by an asterisk are significantly different from that of control chickens
b - each group contained 7 chickens
N.D. - not determined

SOME OBSERVATIONS ON THE CAECAL MICROFLORA OF THE CHICKENS DURING EXPERIMENTAL ACUTE FOWL TYPHOID 533

The bacterial changes in caecal contents after the *Salmonella Gallinarum* administration gave a lot of information about some pathological mechanisms of acute fowl typhoid. The disturbance of microbial ecology in the clinical period of the disease may be ensure the severity of the pathological changes that followed.

References
