Macrophage recruitment and activation: a model for comparing resistance to *Salmonella* Enteritidis in different broiler breeds


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**Summary**

A model for comparing resistance to *Salmonella* Enteritidis was evaluated in different broiler breeds. The recruitment and phagocytic activity of peritoneal macrophages were assessed in three different broiler breeds (A, B and C) which are farmed world-wide. Assessment was performed after three days of intraperitoneal (i.p.) administration of 3% Sephadex G-200 (10 ml), initiated at twenty-one days of age, followed by contact with i.p. live *S. Enteritidis* (10 ml, 1.2 x 10^8 colony forming units/ml) for 45 min. Assessment included determination of the number of i.p. macrophages recruited, the number of i.p. phagocytised *S. Enteritidis* cells per macrophage, the levels of degranulated i.p. β-glucuronidase and β-galactosidase, and the count of surviving *S. Enteritidis* cells. Confirmation of the significance of the model was obtained by comparing resistance to field infection by *S. Enteritidis* in the three broiler breeds.

The recruitment of i.p. macrophages in response to challenge with Sephadex and *S. Enteritidis* was significantly higher (*P < 0.05*) in birds of breed A (mean cumulative i.p. macrophage count, in 10 fields of microscopic slide smear magnified at x 1,000, was equal to 61.7), compared to recruitment in birds of breed B (33.3) or breed C (41.2). The mean number of phagocyted *S. Enteritidis* cells per i.p. macrophage in birds of breed A (2.68) was significantly higher (*P < 0.05*) than in breed B (0.83) and insignificantly higher (*P > 0.05*) than in breed C (2.35). In addition, the highest level of recruitment and phagocytic activity of macrophages, in birds of breed A, was associated with a higher significant mean i.p. β-glucuronidase activity (10,425.5 units/ml) than in breed B (3,438.2 units/ml) or breed C (3,356.34 units/ml) (*P < 0.05*). Moreover, birds of breed A demonstrated a higher mean i.p. β-galactosidase activity (2.226 units/ml) than birds of breed B (0.852 units/ml) or breed C (1.852 units/ml) (*P > 0.05*).

The higher level of recruitment and activity of i.p. macrophages and the higher rate of degranulation of i.p. enzymes in breed A were associated with a greater number of surviving i.p. *S. Enteritidis* cells. In response to outbreaks of *S. Enteritidis* in the field, the average mortality was significantly higher in flocks of breed A (3.2%) than in flocks of breed B (1.2%) or breed C (0.96%) (*P < 0.05*). These data provide an indication of the significance of the model in reflecting the differences in resistance to *S. Enteritidis* of broiler breeds reared in a farm environment.

**Keywords**

Introduction

Salmonella Enteritidis infection in poultry is attracting increasing attention due to the recent acquisition of higher virulence and invasiveness by some strains (5, 14). The acquisition of a high molecular weight plasmid of around 50 kilobases (kb) is incriminated in this increased virulence, associated with S. Enteritidis invasiveness, leading to infection of the ovary in hens (7). This allows S. Enteritidis to disseminate more widely, due to transmission to offspring through infected ovaries and hatching eggs (29). In consequence, a dramatic rise in S. Enteritidis prevalence in humans has occurred world-wide, since the mid-1980s, due to consumption of poultry and poultry products contaminated with S. Enteritidis (10, 28, 29).

The rise in S. Enteritidis infections in humans, with poultry incriminated as the major reservoir of these organisms, has encouraged the development of different approaches to control S. Enteritidis infection in poultry. The approaches developed include immunoprophylaxis of chicken by lymphokines (18, 32), chemotherapy (15, 21), vaccination (3, 16) and competitive exclusion by microflora (5, 24). However, the development of an approach for controlling S. Enteritidis infection by breed resistance in chickens has received little attention (6), compared to similar research undertaken in mice of different genetic lines (29).

One of the principal components of comparing breed-resistance to infection is evaluation of the innate immunity of the host to the pathogen (19). The immune cell with a significant role in innate immunity is the macrophage (30). The Sephadex model for induction of intraperitoneal (i.p.) macrophage recruitment and activation in poultry has been used successfully (12, 25). In addition, the degranulation of lysosomal and/or cytoplasmic enzymes by the macrophages during phagocytosis, including β-glucuronidase and β-galactosidase, has been employed previously to assess the phagocytic-activity of these cells (2, 22, 23, 27, 34).

Developing countries import breeder chickens from the developed world, which differ in genetic make-up, resulting in differences in immune potentials and resistance to diseases in the broiler offspring (4). To the knowledge of the authors, this is the first attempt to use peritoneal macrophage recruitment and activity as a model to compare the resistance to S. Enteritidis in three different internationally-used broiler breeds. Confirmation of the significance of the model was assessed using data from field outbreaks of S. Enteritidis in the three broiler breeds.

Materials and methods

Experimental design and sampling

Three different twenty-one-day-old broiler breeds (A, B and C), free of Salmonella infection, as confirmed by cloacal swab cultures at one, fourteen and twenty days of age, were included in the experimental design. Birds of each breed were divided into six groups (treatments) with six replicates (birds) in each treatment (Table I). Each bird in the first three groups of each breed was administered a 10-ml volume of Sephadex G200 (3% weight/volume in sterile distilled water). Birds in groups 4-6 of each breed were deprived of the Sephadex treatments. The Sephadex was administered intraperitoneally, in the right side of the abdomen. At twenty-four days of age, different groups in the same breed received different i.p. challenges of 10-ml volume in the right side of the abdomen. The challenges were as follows:

- group 1 received live log phase-S. Enteritidis (1.2 × 10⁸ colony forming units (cfu)/ml of tryptose phosphate broth (TPB)); the poultry S. Enteritidis strain had acquired a ~50 kb plasmid associated with high virulence and invasiveness, as reported previously (5)
- group 2 received sterile TPB

Table I

**Experimental design and distribution of broilers of different breeds within treatments**

<table>
<thead>
<tr>
<th>Breed</th>
<th>Isolation room number</th>
<th>Number of broilers</th>
<th>Treatment</th>
<th>Nature of challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>6</td>
<td>SE in TPB</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6</td>
<td>+</td>
<td>TPB</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6</td>
<td>+</td>
<td>Saline</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6</td>
<td>-</td>
<td>SE in TPB</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6</td>
<td>-</td>
<td>TPB</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>6</td>
<td>SE in TPB</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>+</td>
<td>TPB</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>+</td>
<td>Saline</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>-</td>
<td>SE in TPB</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>-</td>
<td>TPB</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>6</td>
<td>SE in TPB</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>6</td>
<td>+</td>
<td>TPB</td>
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<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>+</td>
<td>Saline</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>-</td>
<td>SE in TPB</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>-</td>
<td>TPB</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>-</td>
<td>Saline</td>
</tr>
</tbody>
</table>

a) intraperitoneal administration of 10 ml of 3% Sephadex G-200 to each twenty-one-day-old bird, in the right side of the abdomen
b) intraperitoneal administration of 10 ml challenge in the right side of the abdomen at twenty-four days of age
c) SE live count = 1.2 × 10⁸ cfu per ml of TPB

SE : Salmonella Enteritidis
TPB : tryptose phosphate broth
An aseptic technique was used to cut the skin overlying the breed at 45 min following the i.p. challenges. After 45 min, the birds were cervically dislocated, allowing sampling for different analyses.

Sampling was performed from differently treated birds in each breed at 45 min following the i.p. challenges. After 45 min, the birds were cervically dislocated, allowing sampling for different analyses.

An aseptic technique was used to cut the skin overlying the right abdominal cavity and i.p. fluid was collected in sterile vials and stored at -20°C for enzyme assays (β-glucuronidase and β-galactosidase). Another portion of the i.p. fluid was transferred to an assigned well in a microtitre plate for a S. Enteritidis count. One drop (~50 µl) of i.p. fluid was dispensed and smeared on a microscope slide, dried at room temperature for approximately 5 min, fixed with acetone for 10 min and frozen at -20°C for 48 h before being subjected to an immunofluorescence technique to count the recruited macrophages and phagocytised S. Enteritidis cells per macrophage cell.

**Recruited macrophages and phagocytised Salmonella Enteritidis cells**

The number of recruited macrophages and the number of S. Enteritidis-phagocytised cells within macrophages in the peritoneal cavity fluid, in response to a 3-day challenge with S. Enteritidis, followed by contact with different i.p. challenges in different treatments for 45 min, were obtained using a modification of a previously described procedure (17, 26). Briefly, the acetone fixed smear of i.p. fluid cells was rinsed with distilled water, dried and flooded for 5 min with phosphate-buffered saline (pH 7.2). The smear was dried again and 100 µl (1:100 dilution) of S. Enteritidis-specific-hyperimmune chicken serum was added over the slide for 10 min and frozen at -20°C for 48 h before being subjected to an immunofluorescence technique to count the recruited macrophages and phagocytised S. Enteritidis cells per macrophage cell.

**Enzyme assays**

Two enzymes were assessed in the i.p. fluid (namely, β-glucuronidase and β-galactosidase), reflecting the macrophage phagocytic activities (22, 23). The level of the two enzymes in the i.p. fluid is directly proportional to phagocytic activity of the macrophages (33). The procedures followed in the assessment of β-glucuronidase and β-galactosidase have been described previously (13, 31).

**β-glucuronidase assay**

A standard curve was established using different levels of β-glucuronidase ranging between 0 and 12,500 units/ml. Each enzyme level was run in triplicate against p-nitrophenyl-β-D-glucopyranosiduronic acid substrate. The incubation time of the enzyme and the substrate was 15 h at 37°C. The reaction was stopped with 0.1 N NaOH and the optical density was measured at a wavelength of 410 nm. The i.p. fluid samples were each run in 100 µl volume added to 0.9 ml of the substrate. The rest of the procedure is as described above for the preparation of the standard curve.

**β-galactosidase assay**

A standard curve was established by using different levels of β-galactosidase ranging between 0 and 15 units/ml. Each enzyme level was run in triplicate against o-nitrophenyl-β-D-galactopyranoside. The mixture was incubated for 1 h at 37°C, the reaction was stopped with 0.4 M glycine-NaOH buffer (pH 10.8) and the optical density was measured at a wavelength of 430 nm. The i.p. fluid samples were each run in 0.5 ml volume added to 3.5 ml of the substrate. The rest of the procedure is as described above in the preparation of the standard curve.

**Survival of intraperitoneal Salmonella Enteritidis**

Serial dilutions of the i.p. fluid sample collected in the microtitre plate, using a 1 in 10 dilution factor and up to a dilution of 1 in 10^6, were performed, using sterile saline diluent. From each dilution, 25 µl was taken, plated on brilliant green agar plates and incubated at 37°C for 48 h. Plates containing between ten and thirty colonies were counted and the S. Enteritidis count in the i.p. fluid sample was calculated using the following formula:

\[
S. \text{Enteritidis (cfu/ml)} = \frac{\text{colony count} \times 1,000 \times \text{dilution factor}}{25}
\]

**Field outbreaks of salmonellosis**

Outbreaks of salmonellosis due to S. Enteritidis in six flocks per breed were examined. The flocks, each of approximately 10,000 birds, were managed similarly in terms of nutrition and preventive health programmes. The salmonellosis outbreaks occurred between ten and seventeen days of age. The cumulative mortalities during the S. Enteritidis outbreak in each flock were recorded and the average mortality in each breed during the outbreak was calculated. Confirmation of S. Enteritidis infection was obtained by clinical signs (somnolence, profuse diarrhoea, pasting of the vent area, drooping wings, shivering and huddling near heat sources) and by culture of the caeca. Culture of seven caeca was performed from randomly chosen dead birds from each flock, in tetrathionate broth at 37°C for 24 h. Subcultures were grown from the tetrathionate broth cultures onto brilliant...
green agar (BGA) plates. The BGA plates were incubated at 37°C for 24 h. Suspected colonies were confirmed as S. Enteritidis by biochemical characterisation on triple sugar iron slants, by absence of urease production and by serological characterisation of the somatic and flagellar antigens.

Statistics
Statistical analysis was performed with the aid of commercial software, using the one way analysis of variance (ANOVA). Means were separated by the least significant difference (LSD) test (P < 0.05). The software plot function for regression was used to deduce the levels of ß-glucuronidase and ß-galactosidase from the established data in Table II.

Table II
Relationship between the enzyme level* and the optical density

<table>
<thead>
<tr>
<th>Enzyme level (unit/ml)</th>
<th>OD</th>
<th>β-glucuronidase</th>
<th>Enzyme level (unit/ml)</th>
<th>OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>12,000</td>
<td>2.328</td>
<td>15.0</td>
<td>1.929</td>
<td></td>
</tr>
<tr>
<td>12,000</td>
<td>1.965</td>
<td>12.0</td>
<td>1.779</td>
<td></td>
</tr>
<tr>
<td>6,250</td>
<td>1.242</td>
<td>7.5</td>
<td>1.328</td>
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<tr>
<td>6,000</td>
<td>0.895</td>
<td>6.0</td>
<td>1.129</td>
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</tr>
<tr>
<td>3,125</td>
<td>0.583</td>
<td>3.75</td>
<td>0.936</td>
<td></td>
</tr>
<tr>
<td>3,000</td>
<td>0.438</td>
<td>3.00</td>
<td>0.750</td>
<td></td>
</tr>
<tr>
<td>1,562</td>
<td>0.284</td>
<td>1.87</td>
<td>0.502</td>
<td></td>
</tr>
<tr>
<td>1,500</td>
<td>0.240</td>
<td>1.50</td>
<td>0.482</td>
<td></td>
</tr>
<tr>
<td>781</td>
<td>0.150</td>
<td>0.93</td>
<td>0.285</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td>0.142</td>
<td>0.75</td>
<td>0.285</td>
<td></td>
</tr>
<tr>
<td>300.0</td>
<td>0.104</td>
<td>0.48</td>
<td>0.193</td>
<td></td>
</tr>
<tr>
<td>250.0</td>
<td>0.098</td>
<td>0.375</td>
<td>0.184</td>
<td></td>
</tr>
<tr>
<td>195.3</td>
<td>0.072</td>
<td>0.167</td>
<td>0.120</td>
<td></td>
</tr>
<tr>
<td>187.9</td>
<td>0.070</td>
<td>0.030</td>
<td>0.040</td>
<td></td>
</tr>
<tr>
<td>97.6</td>
<td>0.065</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>93.7</td>
<td>0.063</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.052</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* each enzyme level was run in triplicate
OD: optical density

Results
The quantitative values of the parameters included in the model are presented in Tables III, IV, V, VI and VII. The mean number of recruited peritoneal macrophages following different challenges in the three different broiler breeds is presented in Table III. The macrophage recruitment effect in response to Sephadex and S. Enteritidis challenge was more apparent in broilers of breed A.

The phagocytic activity of peritoneal macrophages on S. Enteritidis cells, in broilers of the three breeds, is shown in Table IV. Again, broilers of breed A showed a higher number of phagocytised S. Enteritidis cells per macrophage, in comparison to those of breeds B and C (P < 0.05).
The average percentage mortality during the field outbreaks of S. Enteritidis in each of the three breeds is shown in Figure 1. The average percentage mortality was higher in breed A as detailed in the materials and methods.

80% of caeca of broilers showing typical signs of salmonellosis (3.2%), in comparison to breeds B (1.2%) and C (0.96%) (P < 0.05). Salmonella Enteritidis was isolated from more than female chickens has been observed previously (12). The 72-h sensitisation by Sephadex was sufficient to recruit peritoneal exudate cells containing more than 78% macrophages (12). In the present study, the macrophage recruitment effect by Sephadex was more apparent in broilers of breed A. Comparative differences in macrophage recruitment in response to i.p. challenges were observed in different genetic lines of White Leghorn chickens (26).

The challenge by S. Enteritidis following sensitisation using Sephadex for 72 h assisted in elevating the number of recruited macrophages in broilers of all three breeds (Table III). This is demonstrated by comparing the mean number of recruited macrophages in broiler treatments given Sephadex and S. Enteritidis in TPB versus groups given Sephadex and TPB alone in breeds A, B and C (81.7, 33.3 and 41.2 versus 63.3, 21.8 and 9.3, respectively) (P < 0.05). A similar pattern is presented in Table III when comparing the mean number of recruited macrophages in Sephadex-deprived broilers given S. Enteritidis in TPB versus those

The i.p. administration of Sephadex improved the macrophage recruitment in broilers of all three breeds, compared to broilers of the same breeds which were deprived of Sephadex (Table III). A similar effect of Sephadex on macrophage recruitment in twenty-five- to thirty-five-day-old

### Table V

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Breed</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sephadex + SE in TPB</td>
<td>10.425</td>
<td>3.438</td>
<td>2.432</td>
<td>3.356</td>
<td>1.186</td>
</tr>
<tr>
<td>Sephadex + TPB</td>
<td>18.276</td>
<td>4.168</td>
<td>4.938</td>
<td>3.252</td>
<td>7.82</td>
</tr>
<tr>
<td>Sephadex + saline</td>
<td>5.003</td>
<td>3.764</td>
<td>4.315</td>
<td>9.938</td>
<td>9.64</td>
</tr>
<tr>
<td>SE in TPB</td>
<td>5.507</td>
<td>2.306</td>
<td>5.086</td>
<td>1.886</td>
<td>6.25</td>
</tr>
<tr>
<td>TPB</td>
<td>2.793</td>
<td>2.265</td>
<td>2.064</td>
<td>3.251</td>
<td>3.14</td>
</tr>
<tr>
<td>Saline</td>
<td>924.8</td>
<td>1.441</td>
<td>3.047</td>
<td>4.901</td>
<td>1.148</td>
</tr>
<tr>
<td>SEM</td>
<td>1.843</td>
<td>2.704</td>
<td>1.185</td>
<td>5.54</td>
<td>5.18</td>
</tr>
</tbody>
</table>

a : mean values in a column followed by same superscript (a) are not significantly different by least significant difference (LSD) test (P > 0.05)
A-C : mean values in a row followed by different superscripts (A-C) are significantly different by LSD test (P < 0.05)
SE : Salmonella Enteritidis
SEM : standard error of the mean
TPB : tryptose phosphate broth

### Table VI

Mean peritoneal β-galactosidase levels (enzyme unit/ml) following different challenges in three breeds of broilers

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Breed</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sephadex + SE in TPB</td>
<td>2.225</td>
<td>0.852</td>
<td>1.832</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Sephadex + TPB</td>
<td>3.766</td>
<td>1.536</td>
<td>0.230</td>
<td>0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>Sephadex + saline</td>
<td>3.219</td>
<td>2.531</td>
<td>1.124</td>
<td>0.57</td>
<td>0.57</td>
</tr>
<tr>
<td>SE in TPB</td>
<td>7.610</td>
<td>1.776</td>
<td>0.406</td>
<td>1.33</td>
<td>1.33</td>
</tr>
<tr>
<td>TPB</td>
<td>2.246</td>
<td>0.562</td>
<td>0.412</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>Saline</td>
<td>0.062</td>
<td>0.794</td>
<td>0.453</td>
<td>0.22</td>
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</tr>
<tr>
<td>SEM</td>
<td>0.67</td>
<td>0.36</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
</tr>
</tbody>
</table>

a : mean values in a column followed by same superscript (a) are not significantly different by least significant difference (LSD) test (P > 0.05)
A-B : mean values in a row followed by different superscripts (A-B) are significantly different by LSD test (P < 0.05)
SE : Salmonella Enteritidis
SEM : standard error of the mean
TPB : tryptose phosphate broth

**Discussion**

The i.p. administration of Sephadex improved the macrophage recruitment in broilers of all three breeds, compared to broilers of the same breeds which were deprived of Sephadex (Table III). A similar effect of Sephadex on macrophage recruitment in twenty-five- to thirty-five-day-old

![Fig. 1](image)

Mean percentage mortality ± standard error in three broiler breeds A, B and C (n = 6 flocks per breed) observed during Salmonella Enteritidis outbreaks occurring between ten and seventeen days of age
given TPB alone in breeds A, B and C (12.0, 13.5 and 6.7 versus 1.3, 3.7 and 0.8, respectively) (P < 0.05). This result is consistent with a study in which the i.p. macrophage recruitment effect by live S. Enteritidis induced the accumulation of inflammatory phagocytes in baby chicks (18).

The in vivo sensitisation of broilers with i.p. Sephadex, followed by S. Enteritidis challenge in TPB, resulted in a significantly higher number of phagocytised S. Enteritidis cells per macrophage in breed A, compared to breeds B and C (P < 0.05). This is in agreement with a previous study which detected significant differences in an in vitro functional competence of i.p. macrophages from different genetic lines (26). These differences were observed following a single Sephadex injection which resulted in various rates of phagocytosis of sheep red blood cells by macrophages in chickens of different genetic lines.

A significantly higher number of phagocytised S. Enteritidis per i.p. macrophage was observed in broilers of breeds A and C in comparison to those of breed B (P < 0.05) (Table IV). Only broilers of breed C showed a significant improvement in macrophage phagocytosis of S. Enteritidis when sensitised with Sephadex and challenged with S. Enteritidis (2.35), as compared to broilers deprived of Sephadex sensitisation and challenged with S. Enteritidis only (0.63) (P < 0.05). These results indicate a breed difference in respect to activation of macrophage phagocytosis by Sephadex. A previous study demonstrated the impact of Sephadex treatment on macrophage activation (8).

When challenged with live S. Enteritidis, broilers of breed A were able to produce significantly higher levels of i.p. β-glucuronidase than those of breeds B or C (P < 0.05) (Table V), regardless of pre-sensitisation with Sephadex. This is probably due to the higher phagocytic activity of S. Enteritidis cells by peritoneal macrophages of breed A broilers (Table IV). A previous study revealed that release of the enzyme β-glucuronidase is directly proportional to cell uptake by phagocytes (33). The confirmation of this observation is given in Table V; in the challenges where live S. Enteritidis cells were absent, no significant difference (P > 0.05) in peritoneal-β-glucuronidase was detected among the three breeds.

Levels of i.p. β-galactosidase were always higher in all treatments of breed A, in comparison to those in breeds B and C, except in the groups receiving only the saline challenge, where the background β-galactosidase was the lowest in breed A (Table VI). This tendency for higher levels of β-galactosidase in breed A could also indicate a higher macrophage activity in the peritoneum, with a more significant rise in β-galactosidase when the challenge contains TPB, regardless of Sephadex pre-sensitisation (i.e. an apparent TPB effect on β-galactosidase level). This suggests that the smaller particles used in the challenge (present in TPB), in the absence of a large, complex S. Enteritidis cell-structure, may induce the release of different enzymes by the macrophages. This may indicate the presence of different genetic factors controlling each of the two enzymes (β-glucuronidase and β-galactosidase) in the macrophages.

More S. Enteritidis cells survived in broilers of breed A than in those of breeds B and C (Table VII). This survival could be due to a higher number of recruited macrophages and higher macrophage phagocytosis in broilers of breed A (Tables III and IV), which provided the correct intracellular environment for S. Enteritidis cells. Broilers of breed A treated with Sephadex, followed by challenge with S. Enteritidis in TPB showed a small reduction in S. Enteritidis count compared to those in breeds B and C. In addition, broilers deprived of pre-sensitisation with Sephadex and only challenged with S. Enteritidis in TPB, demonstrated an increase in S. Enteritidis count over the initial count used in the challenge. The survival and/or multiplication of Salmonella within macrophages has been studied in murine hosts and is thought to be due to a Salmonella gene encoding a cytosis required for virulence (20). Other observations suggest that the survival of Salmonella in murine macrophages is due to the role of the bacteria in inhibition of phagosome acidification (1) and phagosome-lysosome fusion inside the macrophage cell (9). Other research has demonstrated that macrophage toxicity does not require Salmonella-cell internalisation; the use of cytochalasin D, a drug that prevented bacterial uptake, did not prevent the induction of macrophage death by Salmonella (11).

The significantly higher mortality of field flocks of breed A affected by S. Enteritidis, in comparison to that encountered in flocks of breeds B and C, is consistent with results obtained using the model. Broilers of breed A probably experienced greater S. Enteritidis cell survival due to the higher number of recruited macrophages and higher macrophage phagocytosis, which provided the correct intracellular environment for S. Enteritidis multiplication (20), and consequently, a higher mortality rate of the host. This verification affords significance to the model as a procedure that could be employed in the future in developing countries to assess differences in resistance of broiler breeds to a highly virulent and invasive strain of S. Enteritidis.

In conclusion, the model used for comparison of resistance to S. Enteritidis showed that broilers of breed A were able to recruit a greater number of i.p. macrophages with higher phagocytic activity, represented by increased S. Enteritidis internalisation in macrophages and higher degranulation of enzymes, but a lower ability to reduce the S. Enteritidis cell count. This is probably due to either the inability of the macrophages of breed A to cause efficient killing of S. Enteritidis cells, and/or the sensitivity of macrophages of this breed to cytotoxicity mechanisms.
acquired by the highly virulent S. Enteritidis strain used in the challenge. The results in field outbreaks of S. Enteritidis confirmed the results obtained by the model.

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Recrutement et activation des macrophages : modèle de comparaison de la résistance à *Salmonella* Enteritidis chez différentes races de poulets de chair


Résumé
Un modèle de comparaison de la résistance à *Salmonella* Enteritidis a été évalué chez différentes races de poulets de chair. Le recrutement et l’activité phagocytaire des macrophages péritonéaux ont été évalués chez trois races différentes de poulets de chair (A, B et C) élevées dans différents pays. L’évaluation a été faite trois jours après administration intrapéritonéale de Sephadex G-200 3 % (10 ml), inoculé à partir de l’âge de 21 jours, suivie d’un contact pendant 45 minutes avec des *S. Enteritidis* vivantes inoculées par voie péritonéale (10 ml, 1,2 × 10⁸ unités formant colonies/ml). L’évaluation comportait la détermination du nombre de macrophages recrutés et du nombre de *S. Enteritidis* phagocytables par ces macrophages, la détermination des niveaux de β-glucuronidase et de β-galactosidase dégranulées, ainsi que le comptage des *S. Enteritidis* survivantes dans le péritoine. Une confirmation de la signification du modèle a été obtenue en comparant la résistance à une infection naturelle due à *S. Enteritidis* chez les trois races de poulets de chair.

Le recrutement des macrophages dans le péritoine en réaction à l’inoculation de Sephadex et de *S. Enteritidis* était significativement supérieur (*P* < 0,05) chez les poulets de race A (le nombre moyen cumulé des macrophages, étalés sur 10 champs de lames microscopiques à un grossissement de 1 000, était égal à 81,7), à ceux obtenus chez les poulets de race B (33,3) ou de race C (41,2). Le nombre moyen de cellules *S. Enteritidis* phagocytables par ces macrophages chez les poulets de race A (2,68) était significativement plus élevé (*P* < 0,05) que celui de la race B (0,83) et à peine supérieur (*P* > 0,05) à celui de la race C (2,35). De plus, le niveau de recrutement et d’activité phagocytaire des macrophages, qui a été le plus élevé chez les poulets de race A, a été associé à une activité moyenne significative de β-glucuronidase dans le péritoine plus forte (10 425,5 unités/ml) que celle observée chez les poulets de race B (3 438,2 unités/ml) ou de race C (3 356,94 unités/ml) (*P* < 0,05). Par ailleurs, les poulets de race A ont présenté une activité moyenne plus élevée de β-galactosidase (2,225 unités/ml) que ceux de la race B (0,852 unités/ml) ou de la race C (1,852 unités/ml) (*P* > 0,05).
Le recrutement et l’activité plus importants des macrophages ainsi que le taux plus élevé de dégranulation des enzymes chez les poulets de race A ont été associés à un plus grand nombre de S. Enteritidis survivantes dans le péritoine. Après infection naturelle par S. Enteritidis, la mortalité moyenne a été nettement plus importante dans les élevages de poulets de race A (3,2 %) que dans ceux de race B (1,2 %) ou de race C (0,96 %) (P<0,05). Ces données permettent de mesurer l’intérêt du modèle pour apprécier les différences de résistance à S. Enteritidis chez les poulets de chair élevés à la ferme.

Mots-clés

Movilización y activación de macrófagos: modelo para comparar la resistencia a Salmonella Enteritidis de distintas razas de pollo de engorde


Resumen
Los autores evaluaron un modelo destinado a comparar la resistencia a Salmonella Enteritidis de distintas razas de pollo de engorde, determinando para ello los niveles de movilización y actividad fagocítica de macrófagos peritoneales en tres razas de pollo de engorde (A, B y C) presentes en explotaciones avícolas del mundo entero. El proceso se inició con la administración intraperitoneal de Sephadex G-200 al 3% (10 ml) a pollos de veintiún días de edad, seguida, tres días después de un contacto intraperitoneal con organismos vivos de S. Enteritidis (10 ml, 1,2 x 10^8 unidades que forman colonias/ml) durante 45 min. Los parámetros evaluados fueron los siguientes: número de macrófagos peritoneales movilizados; número de células de S. Enteritidis fagocitadas intraperitonealmente por los macrófagos; nivel intraperitoneal de β-glucuronidasa y β-galactosidasa desgranuladas; y recuento de células de S. Enteritidis supervivientes. Después se comprobó la adecuación del modelo, comparando sobre el terreno la resistencia de las tres razas de pollo a la infección por S. Enteritidis.

La movilización de macrófagos dentro del peritoneo en respuesta a la presencia de Sephadex y S. Enteritidis resultó significativamente mayor (P<0,05) en las aves de raza A, con una media acumulada de 81,7 macrófagos (en frotis de 10 campos observados microscópicamente a 1.000 aumentos), por sólo 33,3 en las aves de raza B y 41,2 en las de raza C. El promedio de organismos S. Enteritidis fagocitados por macrófagos dentro del peritoneo resultó significativamente mayor (P<0,05) en las aves de raza A (2,68) que en las de raza B (0,83), y no significativamente mayor (P>0,05) que en las de raza C (2,35). En las aves de raza A, además, el mayor nivel de movilización y actividad fagocítica de los macrófagos ha sido asociado al promedio significativamente más alto de actividad intraperitoneal de β-glucuronidasa: 10.425,5 unidades/ml, comparado con un valor de 3.438,2 unidades/ml en la raza B y de 3.356,94 unidades/ml en la raza C (P<0,05). Las aves de raza A exhibieron asimismo un promedio de actividad intraperitoneal de β-galactosidasa (2,225 unidades/ml) superior a las de raza B (0,852 unidades/ml) y a las de raza C (1,852 unidades/ml) (P>0,05).
El nivel superior de movilización y actividad de macrófagos intraperitoneales y la mayor tasa de desgranulación de enzimas intraperitoneales que exhibía la raza A venían asociadas a un mayor número de células de S. Enteritidis supervivientes en el peritoneo. Ante brotes de S. Enteritidis en condiciones naturales, la mortalidad media resultó significativamente mayor entre bandadas de raza A (3,2%) que entre bandadas de raza B (1,2%) o C (0,96%) (P < 0,05). Tales datos apuntan a la adecuación del modelo para reflejar las diferencias de resistencia a S. Enteritidis entre razas de pollos de engorde criadas en explotaciones avícolas.

Palabras clave

References


