Induction of a homologous and heterologous invasion–inhibition effect after administration of *Salmonella* strains to newly hatched chicks

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A B S T R A C T

Administration of live *Salmonella* strains to day-old chicks provides protection against infection within hours by intestinal colonisation–inhibition. However, the extent to which the oral application of live *Salmonella* wild-type or vaccine strains may induce an early invasion–inhibition effect is unknown. Potentially protective pre-treatment strains of *Salmonella* Enteritidis and Infantis were examined for their ability (i) to colonise the caeca, to invade the liver, to induce an influx of granulocytes in caecal mucosa and, (ii) for their capacity to inhibit the systemic invasion of homologous and heterologous *Salmonella* challenge organisms. The highly invasive strain *Salmonella* Enteritidis induced a strong influx of heterophils in the caecal mucosa followed by a complete invasion–inhibition of both homologous and heterologous *Salmonella* challenge organisms administered 24 h later. Pre-treatment with a less invasive *Salmonella* Infantis resulted in a lower influx of granulocytes in the caecal tissue followed by a complete invasion–inhibition of the homologous serovar Infantis but only an incomplete invasion–inhibition of heterologous serovars. This invasion–inhibition effect has not been described previously in chickens and should be considered in the development of novel live *Salmonella* vaccines to prevent an early invasion of extra-intestinal organs by *Salmonella* challenge organisms in young chicks.

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1. Introduction

Poultry meat and eggs are considered to be the major source of *Salmonella* infection for humans [1]. As a component of comprehensive *Salmonella* control programmes in poultry immunisation with both live and inactivated *Salmonella* vaccines represents one of the most important methods to increase the resistance to infection of both very young and adult chickens [2]. Protective effects induced by vaccination of birds include the reduced intestinal colonisation and the diminished systemic invasion by *Salmonella* wild-type organisms. These are induced by effectors of the adaptive immune response but only some weeks after vaccination [2]. There is thus an “immunity gap” during the first days or weeks of life of the chickens. It has been shown that oral administration of live *Salmonella* strains to day-old chicks provides protection against infection with related *Salmonella* organisms within hours by an intestinal colonisation–inhibition effect which is most certainly the result of microbial physiological processes [3–8].

However, we do not know to what extent the application of live wild-type *Salmonella* or attenuated vaccine strains might also stimulate a rapidly induced early invasion–inhibition effect in poultry. Such an effect could be highly significant as it might prevent the early invasion of extra-intestinal organs which could be followed by a persistent systemic infection with the associated development of the carrier state. Therefore, early inhibition of invasion might be a practical prophylactic tool to avoid transovarian *Salmonella* transmission from persistently infected breeder birds to their progeny.

The aim of the study was to gain more insight in the potential extent and the efficacy of a possible extra-intestinal systemic invasion–inhibition effect after oral administration of different live *Salmonella* serovars to day-old chicks against subsequently inoculated homologous and heterologous *Salmonella* challenge strains. Additionally, in order to better evaluate the possible contribution of immune cells infiltrating the lamina propria of the gut to the expression of an invasion–inhibition effect between *Salmonella* organisms the influx of granulocytes after inoculation of *Salmonella* strains was examined.

2. Materials and methods

2.1. Chickens

Specific pathogen-free White Leghorn chickens were hatched at the facilities of the Friedrich-Loeffler-Institute, Jena, Germany, from...
eggs obtained from Charles River Deutschland GmbH (Extertal, Germany). Experimental and control groups were each kept in cages (all birds of the group in one cage) in separate rooms; commercial feed (in powder form without antibiotics or other additives) and drinking water were both available ad libitum. Cleaning and feeding regimes were organised which prevented cross-contamination effectively throughout the experiments. Animal experiments were performed in accordance with the German Animal Protection Act (registration number: 04-01/04).

2.2. Bacterial strains and culture

Salmonella (S.) enterica subspecies enterica serovars Enteritidis 147 (SE 147), Typhimurium 9098 (STM 9098) and Infantis 1326 (SINF 1326) were used in this study. The potential of these strains to colonise the gut and to invade internal organs of chickens has been characterised in detail [3,8–10]. To facilitate exact enumeration of the strains in caecal content and liver spontaneous nalidixic acid-resistant (N) mutants were produced [11] for challenge. These resistances have no perceptible impact on the in vivo results [3,10]. The bacteria used for pre-treatment or infection of the birds was cultivated in nutrient broth (SIFIN, Berlin, Germany). Doses were estimated by measuring extinction at 600 nm against a calibration graph determined for each strain used, and subsequently confirmed by plate counting on nutrient agar (SIFIN). All strains had been stored in the Microbank system (PRO-LAB Diagnostics, Ontario, Canada) at −20 °C.

2.3. Experimental design and bacteriology

The pre-treatment strains Salmonella Enteritidis 147 (SE 147) and Infantis 1326 (SINF 1326) were examined for their ability (i) to colonise the caeca, to invade the liver and, (ii) for their capacity to inhibit the intestinal colonisation and the systemic invasion of homologous and heterologous Salmonella challenge organisms administered 24 h after pre-treatment of day-old chicks. The viable count of the Salmonella wild-type strains inoculated orally into the crop at 1 day of life was 1–2 × 10⁷ CFU/bird in 0.1 ml. This dose ensured high caecal colonisation without morbidity [6]. SE 147 and SINF 1326 were enumerated in caecal content, in caeca after rinsing the mucosa with phosphate buffered saline and in liver at days 1 (4 h after application), 2, 3, 4, 5, 8 and 10 of life from 4 birds/group, respectively [10]. Homogenised organ samples were diluted and plated on deoxycholate-citrate agar (SIFIN, Germany) to detect the Salmonella organisms and incubated at 37 °C for 18–24 h. Liver samples were pre-enriched in buffered peptone water (SIFIN, Germany), incubated at 37 °C for 18–24 h and streaked onto deoxycholate-citrate agar. Additionally, caeca were taken from each animal from the infected groups and a control group and frozen in liquid nitrogen until use for immunohistochemistry.

The capacity of intestinal colonisation–inhibition and systemic invasion–inhibition was investigated using a standard protocol [3,7,8]. In one experiment 3 groups of 20 chickens were pre-treated on their first day of life with SE 147. The 3 pre-treated and 3 untreated groups (20 animals/group) were challenged orally at day 2 of life with either SE 147N, SINF 1326N or STM 9098N at a dose of 1–2 × 10⁷ CFU/bird. In another experiment 3 groups of 20 chickens were pre-treated on their first day of life with SINF 1326. The 3 pre-treated and 3 untreated groups (20 animals/group) were challenged orally at day 2 of life with either SE 147N, SINF 1326N or STM 9098N at a dose of 1–2 × 10⁵ CFU/bird. The challenge strains were enumerated in whole caeca with contents and liver from 4 birds/group at days 3, 4, 5, 8 and 10 of age using a standard method described previously [10]. Briefly, dilutions of homogenised organ samples were plated on deoxycholate–citrate agar with sodium nalidixate (50 μg/ml) to detect the challenge organisms and incubated at 37 °C for 18–24 h. Organ samples were pre-enriched in buffered peptone water, incubated at 37 °C for 18–24 h and streaked onto deoxycholate-citrate agar with sodium nalidixate.

2.4. Immunohistochemistry

To study the possible involvement of components of the innate immune response in the invasion–inhibition effect between Salmonella strains, the influx of granulocytes into the caecal mucosa was examined immunohistochemically. Additionally, the invasion of the pre-treatment strains SE 147 and SINF 1326 was analysed in order to show the proportion of bacteria having entered lower regions of the caecal mucosa. For that reason, frozen caeca of animals from groups only pre-treated with SE 147 or SINF 1326 were taken each at the same time post infection, after cutting sections of 7 μm thicknesses were prepared. The staining of granulocytes and Salmonella organisms was performed as described [9]. Briefly, sections were fixed in acetone and subsequently incubated with the monoclonal anti-granulocyte antibody IC6 (kindly provided by R. Ducatelle, University Gent, Belgium). After that, the bound primary antibodies were detected by the peroxidase anti-peroxidase method using the secondary goat anti-mouse immunoglobulin (Sigma, Deisenhoven, Germany) and peroxidase-anti-peroxidase complex (Sigma). The enzyme-linked antibody was visualized by reaction with 3.3′ diaminobenzidine (Merk, Darmstadt, Germany).

2.5. Image analysis

The quantification of the number of granulocytes as well as the Salmonella-positively stained area in caecum was accomplished by means of image analysis (SIS, Münster, Germany). Granulocytes were counted within an interactively drawn region of interest (ROI) including the lamina propria as well as epithelial cells of caecum. The absolute number of granulocytes was calculated to 1 mm² mucosa. At least three ROIs per animal were analysed.

The invasion of bacteria was studied by determining the percentage of Salmonella-positively stained area including lamina propria and epithelial cells of caecum from animals only pre-treated with SE 147 or SINF 1326 at day 1 of life. For each animal, at least five independent regions were examined.

2.6. Statistical analysis

For immunohistochemistry the student t test for comparison of two independent samples was used to evaluate differences between the groups (both pre-treated groups versus the control group). P values of <0.05 were considered significant. Viable bacterial counts were converted into logarithmic form. For statistical purposes a viable count of log₁₀ < 1.47 (the limit for direct plate detection) from a sample detected positive only after enrichment was rated as log₁₀ = 1.0. A sample which yielded no Salmonella growth after enrichment was rated as log₁₀ = 0. Data were evaluated by variance analysis. The factors considered were group and time. P values <0.05 were regarded as statistically significant (software: statgraphics plus, Inc. Rockville, MD, U.S.A.).

3. Results

3.1. Course of infection after administration of pre-treatment strains SE 147 and SINF 1326

The oral administration of pre-treatment strains SE 147 and SINF 1326 at a dose of 1–2 × 10⁷ CFU/bird resulted in a high level of caecal colonisation (Table 1) but did not induce morbidity or clinical
signs. There was no significant difference in the ability of either strain to colonise the caeca. The pre-treatment strains SE 147 and SINF 1326 reached very similar numbers in both caecal content and caeca mucosa (after rinsing the mucosa). Despite its capacity to colonise the gut, SINF 1326 showed a significantly (P < 0.05) reduced invasion of the liver at days 1, 2, 3, 4, 7 and 9 after administration in comparison with SE 147. The differences ranged from 2.1 to 2.8 log10 units indicating the decreased invasiveness and lower virulence of SINF 1326.

### 3.2. Quantification of the pre-treatment strains SE 147 and SINF 1326 in caecal wall

The potential of the pre-treatment strains SE 147 and SINF 1326 to invade the caecal mucosa was analysed by immunohistochemistry and image analysis. Compared to pure bacteriology, microscopy enables to identify the location of Salmonella either on the epithelial lining or in lower regions of the gut mucosa. Both Salmonella serovars were capable of invading the caecal mucosa albeit to different degrees (Fig. 1). SE 147 was detected in epithelium and lamina propria basement regions of the caeca in significantly (P < 0.05) larger numbers than SINF 1326 from 4 h to 9 days after infection. The strain SE 147 was, however, detected also in lower zones of the lamina propria. SINF 1326 entered only solely epithelial cells and sub-epithelial regions.

### 3.3. Colonisation–inhibition and invasion–inhibition of homologous and heterologous Salmonella serovars by SE 147

The challenge strains SE 147N, STM 9098N and SINF1326N administered to the un-treated control groups at day 2 of life colonised the caeca very rapidly, reaching high numbers of more than 10^8 CFU/g already by 24 h after administration and bacterial counts of ca. 10^9 CFU/g during the first week of life. The most profound level of caecal colisation–inhibition was produced by SE 147 against the homologous variant SE 147N and remained in force at least until 10 days of age (Table 2). The intestinal colonisation of STM 9098N and SINF 1326N was, in contrast to untreated controls considerably reduced by SE 147 but only for a short period of about 48 h. Despite the significantly (P < 0.05) reduced number of challenge organisms in the groups pre-treated with SE 147 until 8 days of age, the counts of STM 9098N or SINF 1326N in caeca increased steadily and reached the same level as in untreated controls by day 10 of life.

In un-treated groups all challenge strains invaded the liver in countable but different numbers. SE 147N and STM 9098N reached nearly identical values of ca. 5 × 10^2 CFU/g up to day 5 of age and SE 147N even more than 10^3 CFU/g at days 8 and 10 of age. Invasion of SINF 1326N was, however, significantly (P < 0.05) lower than of SE 147N and STM 9098N. Pre-treatment of day-old chicks with SE 147 completely prevented the invasion of both the homologous challenge strain SE 147N and the heterologous strains STM 9098N and SINF 1326N. Even after pre-enrichment cultures of the caeca did not reveal any Salmonella challenge organisms.

### 3.4. Colonisation–inhibition and invasion–inhibition of homologous and heterologous Salmonella serovars by SINF 1326

In this experiment, the challenge strains SE 147N, STM 9098N and SINF1326N administered to the un-treated control groups at 2 days of life colonised the caeca and invaded the liver to the same extent (Table 3) as in the previous experiment (Table 2). SINF 1326 inhibited the caecal colonisation of SINF1326N very effectively to 10^2 CFU/g from day 3 until day 50 of age. The heterologous challenge organisms SE 147N and STM 9098N were, in contrast to untreated controls but only for a short period. From day 5 onwards, SINF 1326 did not produce any colonisation–inhibition effect against these heterologous serovars.

Pre-treatment of day-old chicks with SINF 1326 prevented completely the invasion of the homologous strain SINF 1326N into the liver. The invasion of the heterologous challenge strains STM 9098N and SE 147N was inhibited completely by SINF 1326 until day 5 or day 4 of life, respectively, but afterwards these heterologous challenge organisms were detected in counts of less than 50 CFU/g liver.

### 3.5. Quantification of granulocytes in caeca

To characterise the potential of the pre-treatment strains SE 147 and SINF 1326 to trigger an influx of cells of the innate immune response into the gut mucosa, the occurrence of granulocytes in the caeca was examined by microscopy (Fig. 2). In animals pre-treated with SE 147 at day 1 of life, a strong increase of granulocytes was

### Table 1

Number of *Salmonella Enteritidis* 147 (A) and *Salmonella Infantis* (B) 1326 (log_{10} CFU/g) in liver, caeca and caecal contents of chickens after oral administration with 2 × 10^7 CFU/bird at 1 day of age.

<table>
<thead>
<tr>
<th>Time after administration</th>
<th><em>Salmonella Enteritidis</em> 147 (A) (log_{10} CFU/g)</th>
<th><em>Salmonella Infantis</em> 1326 (B) (log_{10} CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver Caeca Caecal contents</td>
<td>Liver Caeca Caecal contents</td>
</tr>
<tr>
<td>4 h</td>
<td>0.4 7.1 8.2</td>
<td>0.7 7.4 8.4</td>
</tr>
<tr>
<td>1 day</td>
<td>3.0 8.5 9.3</td>
<td>0.8 8.0 9.8</td>
</tr>
<tr>
<td>2 days</td>
<td>3.6 8.3 8.9</td>
<td>1.1 7.7 9.4</td>
</tr>
<tr>
<td>3 days</td>
<td>3.6 8.0 9.4</td>
<td>1.0 7.6 9.3</td>
</tr>
<tr>
<td>4 days</td>
<td>3.8 7.9 9.2</td>
<td>0.8 7.5 9.0</td>
</tr>
<tr>
<td>7 days</td>
<td>3.1 7.7 9.0</td>
<td>0.4 7.3 8.9</td>
</tr>
<tr>
<td>9 days</td>
<td>2.8 7.6 8.9</td>
<td></td>
</tr>
</tbody>
</table>

A, B: Significantly lower than group A (*Salmonella Enteritidis* 147) or B (*Salmonella Infantis* 1326). Standard error: liver: 0.152 caeca: 0.131 caecal contents: 0.071
at 2 days of age with or without pre-treatment with S.

Number of newly hatched chickens, there is an immunity gap response after administration of live Salmonella during the first few days of their life. It has been shown that oral administration of live Salmonella strains to day-old chicks provides protection against infection within hours by intestinal colonisation–inhibition [3,4,7,8]. Our results on colonisation–inhibition between Salmonella organisms in newly hatched chickens in this study confirm (i) that the most profound level of intestinal colonisation–inhibition occurs between homologous strains and (ii) that inhibition between strains of heterologous Salmonella serovars is effective only for about 48 h as has been found previously [3,5–8]. Later the differences in caecal colonisation by the challenge strains between pre-treated and un-treated birds amounted in most cases to less than 1.5 log10 units which are not relevant in view of the high total caecal colonisation. The reason for the only initially occurring inhibition effect is still unclear. The finding that colonisation–inhibition is most effective only between isogenic strains, already considerably lower between strains of the same Salmonella serovar and detectable merely for a very short time between heterologous serovars [8,12] is strongly indicative that this effect is not host-related but rather a non-host-related microbiological effect as suggested earlier [3] because a host-related mechanism should be effective against all serovars.

In contrast to intestinal colonisation–inhibition pre-treatment of day-old chickens with SE 147 prevented completely the invasion into the liver of not only the homologous strain SE 147N but also the strains of the heterologous serovars STM and SINF. All challenge strains invaded the liver of untreated birds even with a dose of only 105 CFU/bird administered at day 2 of age with live counts in the range of 106 CFU/g for SE 147N to 50 CFU/g for SINF 1326N. In case of the invasion–inhibition between the variants of SE 147 it might be suspected that the caecal colonisation of SE 147N was too low to enable the strain to reach the lower zones of the gut or extra-intestinal organs. However, as the caecal colonisation of STM 9098N and SINF 1326N in pre-treated groups was nearly as high as in un-treated groups the invasion–inhibition must clearly be due to a different effect at least in these groups. Furthermore, because of the differences in colonisation–inhibition of the homologous and heterologous challenge strains by the pre-treatment strain SE 147 on the one hand coupled with the identical complete invasion–inhibition of these challenge strains on the other, there

<table>
<thead>
<tr>
<th>Day of life</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SE 147</td>
<td>SE 147N</td>
<td>SE 147N</td>
<td>SE 147N</td>
<td>SE 147N</td>
<td>SE 147N</td>
</tr>
<tr>
<td>2</td>
<td>SE 147N</td>
<td>SE 147N</td>
<td>SE 147N</td>
<td>SE 147N</td>
<td>SE 147N</td>
<td>SE 147N</td>
</tr>
<tr>
<td>4</td>
<td>0.4±0.6</td>
<td>0.5±0.6</td>
<td>1.2±0.6</td>
<td>0.4±0.6</td>
<td>0.5±0.6</td>
<td>1.2±0.6</td>
</tr>
<tr>
<td>5</td>
<td>0.4±0.6</td>
<td>0.5±0.6</td>
<td>1.2±0.6</td>
<td>0.4±0.6</td>
<td>0.5±0.6</td>
<td>1.2±0.6</td>
</tr>
<tr>
<td>8</td>
<td>0.4±0.6</td>
<td>0.5±0.6</td>
<td>1.2±0.6</td>
<td>0.4±0.6</td>
<td>0.5±0.6</td>
<td>1.2±0.6</td>
</tr>
<tr>
<td>10</td>
<td>0.4±0.6</td>
<td>0.5±0.6</td>
<td>1.2±0.6</td>
<td>0.4±0.6</td>
<td>0.5±0.6</td>
<td>1.2±0.6</td>
</tr>
</tbody>
</table>

1,2,3,4,5,6 Significantly lower than groups 1, 2, 3, 4, 5, and 6. Standard error: liver: 0.180 caeca: 0.531

4. Discussion

Little is known about the time of onset of a specific immune response after administration of live Salmonella organisms to very young chickens. As a result of the immunological immaturity of newly hatched chickens, there is an immunity gap already observed (P<0.05) 4 h after administration and continued until day 9. The highest value was seen 24 h after exposure of the chicks with SE 147. Chicks pre-treated with SINF 1326 at day 1 of life showed a reduced number of granulocytes per square millimeter caecal wall compared to birds inoculated with SE 147 during the whole experiment, the differences between both groups were significant (P<0.05) at days 2, 3, 4 and 8 of age. Whereas granulocytes were predominantly found in lamina propria and sub-mucosa during the first days after inoculation with SE 147 and later on generally beneath the epithelial lining and in the epithelium, after administration of SINF 1326 granulocytes were detected only in the epithelium and in the gut lumen from day 2 of life onwards (Fig. 3).

Table 3

Number of S. Enteritidis 147N, S. Typhimurium 9098N and S. Infantis 1326N (log10 CFU/g) in liver and whole caeca of chickens after oral administration of 2 × 105 CFU/bird at 2 days of age with or without pre-treatment with S. Enteritidis 147 at 1 day of age.

<table>
<thead>
<tr>
<th>Day of life</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SINF 1326</td>
<td>SINF 1326N</td>
<td>SINF 1326</td>
<td>SINF 1326</td>
<td>SINF 1326</td>
<td>SINF 1326</td>
</tr>
</tbody>
</table>
seems to be no relation between both inhibitory effects. In comparison with SE 147, pre-treatment of day-old chicks with SINF 1326 completely prevented the invasion of the liver with the homologous strain SINF 1326N. The invasion of the heterologous challenge strains STM 9098N and SE 147N was also inhibited completely by SINF 1326 until day 5 or day 4 of life, but afterwards these heterologous challenge organisms were detected in counts of less than 50 CFU/g liver which was significantly (P < 0.05) lower than in untreated groups.

It was already shown that macrophages and other immune cells, especially heterophils are detectable in the lamina propria of the caeca by 24 h after administration of Salmonella strains [9,13–17]. Moreover, heterophils activated after prophylactic administration of Salmonella Enteritidis-immune lymphokines to the chicks greatly contributed to an increased host resistance to extra-intestinal Salmonella infection [15,18–20].

In the present study SE 147 and SINF 1326 were selected for pre-treatment of the chicks at day of hatch as their capabilities to colonise the gut and to invade extra-intestinal organs had been characterised previously [8–10,21]. Although both strains reached very similar numbers in the caeca, SINF 1326 showed a considerably diminished invasion of the epithelium and the lamina propria of the caeca as well as of the liver in comparison with SE 147. In birds pre-treated with the highly invasive strain SE 147 a strong increase of granulocytes was observed by day 1 after inoculation which was not only significantly (P < 0.05) but considerably higher than in birds pre-treated with the rather moderate invasive strain SINF 1326. Therefore, the more invasive strain SE 147 reveals itself as a strong- and the less invasive strain SINF 1326 as a weak inducer of influx and/or proliferation of granulocytes in the gut mucosa. It was shown that activated avian heterophils are able to phagocytise and kill Salmonella organisms very effectively and more efficiently than monocytes [20,22–24]. Furthermore, heterophil depletion studies indicate that these cells are decisive in the early protection against Salmonella Enteritidis [14,25]. Therefore, the influx of heterophils induced after pre-treatment of chicks with Salmonella strains administered at 1 day of age may be responsible for the invasion-inhibition of Salmonella challenge organisms inoculated subsequently in this study. Invasion of the intestinal mucosa by Salmonella spp. induces IL-8 production that initiates the recruitment and a continued migration of large numbers of heterophils from the blood to the lamina propria, the site of Salmonella invasion [15,19,23,25]. The continued migration of granulocytes was confirmed indirectly by our results as granulocytes were not only detected after initial invasion of the pre-treatment strains but at least until day 9 after pre-treatment.

The differences in invasion of the lamina propria between the pre-treatment strains SE 147 and SINF 1326 are most probably responsible for the different number of granulocytes infiltrating this site of invasion and, therefore, for the detected differences in invasion-inhibition against the respective heterologous serovars of these pre-treatment strains. Moreover, the lower number of infiltrating heterophils after pre-treatment with SINF 1326 most certainly resulted in the incomplete invasion-inhibition of the heterologous highly invasive challenge strains SE 147N and STM 9098N, whereas, the number of heterophils was sufficiently high to complete inhibit the invasion of the less invasive homologous strain SINF 1326N. It cannot be excluded that other immune cells are involved in invasion-inhibition, however, because of their immature characteristics T-lymphocytes and macrophages are not likely to play an important role during the first days after hatching [26,27].

Although we do not fully understand the immunological basis of the effect, this not previously described invasion-inhibition effect should be considered in the development of novel live Salmonella vaccines in the future as it could prevent early invasion of extra-intestinal organs which could be followed by a persistent infection with the potential for development of Salmonella carriers. Therefore, despite the necessary reduction in virulence, live Salmonella vaccines should still be able to invade at least to some extent, the lower zones of the intestinal lamina propria in order to stimulate an influx of heterophils and other immune cells into the gut to prevent the invasion of Salmonella challenge organisms in highly susceptible young chicks.

Acknowledgement

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