

## Immunogenic Properties of Outer Membrane Proteins of Salmonella in Chicken

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**Abstract:** The aim of this study was to investigate the *Salmonella* infection in chicken in certain localities, as well as to evaluate the antigenicity of the outer membrane proteins (OMP) by Western blot and ELISA as a preliminary phase for vaccine production. Fecal samples collected from chicken suffering from diarrhea revealed the isolation of *Salmonella* in 17. 5% of the cases, while in the contact apparently healthy chicken, the incidence was 3. 4%. Four *Salmonella* serovares were elucidated namely, *S. Enteritidis* (7. 3 and 1. 7%), *S. pullorum* (5. 1 and 0. 8%), *S. typhimerium* (2. 9 and 0.8%) and *S. gallinarum* (2. 2and 0%) from diarrheic and apparently healthy chicken, respectively. SDS-electrophoretic analysis of the outer membrane proteins (OMP) revealed common antigen protein bands, especially between *S. typhimurium* and *S. enteritidis*. All serovars showed intense protein bands in the range from 20 to 45 kDa. In the Western blot analysis, serum antibodies from chicken infected with *S. enteritidis* reacted with protein bands at the range of 17- 31 kDa. Two protein bands were characteristic for *S.pullorum* and *S. gallinarum* (14. 4 and 24KkDa). Only one protein band (24 kDa) from the blotted OMP of *S. typhimurium*, reacted with serum from infected chicken. ELISA results detected the presence of serovar specific antibodies. It could be concluded that *Salmonella* OMP were major immunogenic antigens that could be used in ELISA or Western blot to detect and monitor *Salmonella* infection in chicken.

**Key words:** Outer membrane proteins • *Salmonella* • Enterobacteriaceae-chicken

### INTRODUCTION

*Salmonella* infections in chicken continue to be a major problem worldwide. Substantial economic losses were manifested through mortality and poor growth of infected chicken as well as the hazard of causing food poisoning to humans. Many outbreaks of *Salmonella* infections has been reported worldwide which encountered the most frequently isolated serovars as *S. typhimurium*, *S. enteritidis*, *S. gallinarum*, *S. pullorum*, *S. newport*, *S. cerro*, *S. montevideo*, *S. agona* and *S. dublin* which was considered the major host-adapted *Salmonella* for chicken [1,2]. The infection was always aggravated by poor hygienic conditions and inadequate nutrition. Chicks were most susceptible to infection due to their immature immune responses, undeveloped microflora in their gastrointestinal echo-system and the permanent exposure to the source of infection from the environment. Shedding *Salmonella* without clinical signs may be associated with chronic *Salmonella* infection (carrier), convalescence after acute infection

or a recent starting colonization [3]. Unlike the intermittent shedding of *Salmonella* in the feces, antibody levels do not fluctuate greatly on daily basis. Sero-conversion was used in many countries as a tool for *Salmonella* surveillance and control in cattle, pig and poultry industry at slaughter to identify infected flocks as a regulatory procedures for food safety and security program. Application of ELISA can provide information about the infection status of the flock, moreover the repeated testing of chicken differentiates those recently infected (increasing antibody titer) and those convalescent ones (decreasing titers) from *Salmonella* carriers, which would have relatively constant titers. However, further investigations has to be conducted on the outer membrane proteins (OMP) and their immunogenic potentials as a first step for potent vaccine production.

The objective of this study was to investigate the *Salmonella* infection in chicken in certain localities, as well as to evaluate the antigenicity of the outer membrane proteins (OMP) by Western blot and ELISA as a preliminary phase for vaccine production.

## MATERIALS AND METHODS

**Specimens:** A total number of 253 faecal samples were collected from 137 diarrheic chicken and 116 contact apparently healthy chicken. At the same time 253 blood samples were collected in order to correlate the incidence of *Salmonella* to the sero-conversion of each individual bird.

Samples were collected during a period of one year from chicken at governmental and private farms in Giza, Kafr El-Sheikh and Dakahlea governorates.

Specimens were transferred to the laboratory in an ice box with minimum delay, where fecal samples were examined bacteriologically for *Salmonella* isolation and identification. Blood samples were allowed to clot, then centrifuged at 700xg for 15min. Sera were collected and stored in frozen aliquots until used in the serological tests.

***Salmonella* Isolation and Identification:** Fecal samples were cultured into tetrathionate broth, incubated at 37°C and 42°C, respectively for 24hr. Loopful from these broth cultures were then streaked onto MacConkey and S. S agar plates, incubated at 37°C for 24-48 hrs and *Salmonella* suspected colonies were identified morphologically, then biochemically and serologically according to the Kauffman-White Scheme by slide agglutination test using polyvalent and monovalent (O) and (H) antisera.

**Preparation of the *Salmonella* Outer Membrane Protein (OMP):** The four *Salmonella* isolates were cultured on tryptic soy agar in Roux flasks and incubated at 37°C for 24hr. Bacterial cells were harvested into 10mM HEPES buffer pH 7.4 and washed three times with normal saline. The bacterial cells were sonicated for 15 min. then centrifuged at 1700xg for 20min. The sediment was discarded and the supernatant was centrifuged at 60,000xg and the pellet was dissolved in 2% sarkosyl in 10mM HEPES buffer and was left overnight at 4°C. The preparation was then centrifuged at 60,000xg for 1 hr. and the pellet was dissolved in 10mM Tris HCl pH 7.2 and finally dialysed in 0.15M NaCl, 50mM PBS pH 7.4 for 24 hr. at 4°C. [4,5]. The protein content of these OMP preparations were then estimated according to Lowry *et al.* [6].

## Sodium Dodecyl sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) of *Salmonella* OMP:

Equal volumes of the different *Salmonella* OMP preparations and sampling buffer were vortexed for 15 seconds and heated in thermomixer for 5 min then loaded in the gel along with a molecular protein marker range from 16.5-175kDa [7].

**Western Blot Analysis:** Nitrocellulose membrane of 0.2µm porosity was used to transfer the electrophoresed proteins by the Western blot technique. The blotted-OMP-protein strips for each *Salmonella* serovar were incubated with 1:100 diluted sera from chickens that were positive as shown by isolation of the corresponding serovar. The strips were then washed three times with PBST and incubated with rabbit anti-bovine IgG conjugated with the horse radish peroxidase for 1 hr at room temperature in dilution 1:2000 in PBS pH 7.4. The conjugate was then washed and the color was developed by adding the substrate (30mg 4-chloro-1-naphtholdissolved, 10ml cold methanol, 30u. l hydrogen peroxide in 50 ml PBS pH7.4).

**ELISA Procedure:** The ELISA was performed in polystyrene 96 well microtiter plates (Dyatech). The plates were coated separately with 5 µg/ml of each *Salmonella* eluted-OMP preparations in carbonate bicarbonate buffer pH 9.6 [3].

## RESULTS

Episodes of diarrhea in chicken were reported in some governmental and private farms in Giza, Kafr El-Sheikh and Dakahlea and Sharkia Governorates. Fecal samples collected as well as those collected from apparently healthy contact showed higher incidence of *Salmonella*; 17.5 and 3.4%, respectively. Four different *Salmonella* serovars were elucidated namely, *S. enteritidis* (7.3 and 1.7%), *S. pullorum* (5.1 and 0.8%), *S. typhimurium* (2.9 and 0.8%) and *S. gallinarum* (2.2 and 0%) from diarrheic and apparently healthy chicken, respectively (Table 1 and Figure 1).

The OMP electrophoretic analysis of the four *Salmonella* serovars was displayed in Figure 1 (a) The four serovars shared many protein bands as intense protein bands in the range from 20-45kDa was a general character of the four OMP preparations (common *Salmonella* antigen). In the Western blot analysis, serum antibodies from chicken infected with *S. typhimurium* reacted with protein bands at 17 - 31 kDa.



Fig. 1: Immunoblotting of salmonella. lane M. Marker, lane 1.S. Enteritidis, lane, 2.S. Pullorum. lane, 3.S. typhimurium, lane, 4 S. gallinarum (OMP)

Table 1: Frequency of different Salmonella serovars in the faeces of diarrheic and apparently healthy chicken

| SALMONELLA ISOLATES   | Incidence in diarrheic chicken | Incidence in apparently healthy chicken |
|-----------------------|--------------------------------|---|
| <i>S. enteritidis</i> | 10/137<br>7.3%                 | 2/116<br>1.7%                           |
| <i>S. pullorum</i>    | 7/137<br>5.1%                  | 1/116<br>0.8%                           |
| <i>S. typhimurium</i> | 4/137<br>2.9%                  | 1/116<br>0.8%                           |
| <i>S. gallinarum</i>  | 3/137<br>2.2%                  | 0/116<br>0%                             |
| Total                 | 24/137<br>17.5%                | 4/116<br>3.4%                           |

Table 2: Serological detection of Salmonella antibodies in the serum of diarrheic and apparently healthy chicken

| ELISA Designation                      | No. of ELISA positive in diarrheic chicken | No. of ELISA positive in apparently healthy chicken |
|--|--|---|
| <i>S. enteritidis</i> ELISA<br>OD>0.32 | 15/137<br>10.9%                            | 5/116<br>4.3%                                       |
| <i>S. pullorum</i> ELISA<br>OD>0.3     | 10/137<br>7.3%                             | 4/116<br>2.6%                                       |
| <i>S. typhimurium</i> ELISA<br>OD>0.3  | 7/137<br>5.1%                              | 2/116<br>1.7%                                       |
| <i>S. gallinarum</i> ELISA<br>OD>0.27  | 4/137<br>2.9%                              | 1/116<br>0.9%                                       |
| Total positive samples                 | 36/137 (26.3%)*                            | 12/116 (10.3%)**                                    |
| Total positive cases                   | 33/137 (24.1%)                             | 11/116 (9.5%)                                       |

The OMP of *S. enteritidis* and *S. typhimurium* reacted relatively similar in the Western blot reaction. Two protein bands were demonstrated, 14.4 and 24 kDa. ELISA results detected the presence of *Salmonella* antibodies in the serum of diarrheic chicken in 24.1% of

the birds, while the rate of isolation of *Salmonella* from these diarrheic chickens was 17.5%. In apparently healthy chicken, ELISA detected the presence of *Salmonella* antibodies in their serum in 9.5% of the chicken, while the rate of isolation of *Salmonella* from these apparently healthy chicken was 3.4%. (Tables 1 and 2). There were some discrepancies in ELISA results with isolation results. ELISA recorded higher incidence of *Salmonella* infection with the four serovars, both in diarrheic and apparently healthy chicken (Table 2 and Figure1).

## DISCUSSION

*Salmonella* infection in chicken continue to be a major problem worldwide. They cause substantial economic loss both directly through mortality and poor growth after clinical disease and indirectly from chicken carriage leading to cases of human *Salmonella* infection which is a serious food-borne infection in man [3,8].

Fecal samples collected from chicken suffering from diarrhea showed higher incidence of *Salmonella* (17.5%) than contact apparently healthy chicken (3.4%). *Salmonella* serovars were elucidated from diarrheic and apparently healthy chicken. The antigenic structure of the 28 *Salmonella* isolates (Table 1). The frequencies of serovar isolation varied from one studied location to the other due to different managemental and hygienic regimens as well as geographical, environmental and individual differences [3, 5, 9, 10].

The OMP electrophoretic analysis of the *Salmonella* serovars (Figure1) showed that the serovars shared many protein bands which constitute the common genus antigen. The intense protein region which occupied the range from 20-45 kDa constituted the majority of the *Salmonella* specific OMP bands. The fainter bands which were revealed at the higher molecular weight region (higher than 67 kDa) and at the lower molecular weight region (lower than 20 kDa) were bands related or associated to the OMP, or residues of flagellar and pilus protein (lower than 20 kDa) or could be residues of *Salmonella* toxins (higher than 67 kDa) [5, 9, 11, 12]. OMP preparation from *S. enteritidis* and *S. typhimurium* showed greater similarities in their electrophoretic patterns, as both serovars share many factors in the somatic antigens, 0:1, 0:9 and 0:12. Many other virulence factors were expressed by *Salmonella* which pathologically influence the host cells and specific genes encoding these factors were regulated by many environmental conditions and regulatory pathways which

can be altered by many bacterial, frost and environmental factors [11, 12]. In the Western blot analysis, serum antibodies from chicken infected with *S. typhimurium* reacted with protein bands at molecular weight of 17-31 kDa. This range of protein which has been claimed, is the major outer membrane protein of *Salmonella typhimurium* [10,13]. The OMP of the two serovars *S. typhimurium* and *S. enteritidis* reacted relatively similar with the antisera collected from chicken that were infected with their respective serovars. Two protein bands were demonstrated, 14, 4 and 24 kDa. Only one protein band (24 kDa) from the blotted OMP of *S. typhimurium* reacted with serum from infected chicken. Figure 1 cross reactivity was noticed in Western blot analysis between *S. typhimurium*, *S. pullorum* and *S. enteritidis* due to the variable sharing of their somatic antigenic structure. Other protein band could be elicited and released in the serum of infected chicken, due to the severe inflammatory response and release of antibodies [10, 14].

OMP-ELISA results detected the presence of *Salmonella* antibodies in the serum of diarrheic chicken in 24.1% of the birds, while the rate of isolation of *Salmonella* from these diarrheic chicken was 17.5%. In apparently healthy chicken ELISA detected the presence of *Salmonella* antibodies in their serum in 9.5%, while the rate of isolation of *Salmonella* from these apparently healthy chicken was 3.4% (Table 1 and 2). This could be attributed to the intermittent shedding of *Salmonella* without clinical signs could be due to chronic *Salmonella* cases, convalescence after acute infection or a recent colonization [3, 4, 15]. Application of ELISA was a key tool to provide information about the infection status of the flock, moreover the repeated testing of chicken differentiates those recently infected chicken (increasing antibody titre) and those convalescent ones (decreasing titers) from *Salmonella* carriers, slight discrepancies were recorded in detecting *Salmonella* infected animals using OMP ELISA. Reactively positive with *S. typhimurium* ELISA and *S. enteritidis* ELISA. There were also two cases that gave positive results with *S. gallinarum* ELISA and *S. pullorum* ELISA.

*Salmonella* OMP can play an essential role in the induction of immune response in the birds and can be employed as an effective candidate vaccine which can confer solid and, active immunity and evade the hefty expenses of treatment of *Salmonella* infected calves which lately has acquired multiple potent antibiotic resistance [3, 15].

It was concluded that *Salmonella* OMP were major immunogenic antigens that could be used in ELISA or Western blot to detect and monitor *Salmonella* infection in chicken.

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