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Anti-*Salmonella* Antibodies: An Immunoepidemiological Study

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Abstract This research looks at the seroprevalence of anti-Salmonella antibodies in humans and chickens from Jamaica, West Indies. These antibodies were assessed by enzyme-linked immunosorbent assay (ELISA) and showed that 11.3% (6 out 53) human samples and 95.3% (102 out of 107) IgY samples had the presence of anti-*Salmonella* antibodies. These results suggest the presence of Salmonellosis as a contaminant in humans and endemic state in birds, which not necessarily means active disease.

Keywords: *immunoepidemiology, seroprevalence, anti-Salmonella antibody, humans, chickens, enzyme-linked immunosorbent assay (ELISA), Jamaica.*

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1. Introduction

The environment is exposed to a wide range of foodborne maladies such as Salmonellosis, which is accountable for thousands of deaths globally and billions of lost revenues to the poultry industry. *Salmonella*e are gram-negative, non-spore-forming facultatively anaerobic bacilli [1], and this genus is a member of the Enterobacteriaceae family [2].

S. enteric serovar Typhimurium infects laying hens and may cause human infection when cracked eggs are consumed. In contrast, S. enteric serovar Enteritidis (SE) contaminates the contents of intact eggs and is the major egg-associated human pathogen. It is postulated that *Salmonella spp* colonizes ovaries and oviducts of chickens and subsequently contaminates eggs as they formed [3].

Three key mediations intended at preventing the contamination and growth of *Salmonella spp*. in eggs have incorporated farm-based plans to prevent *Salmonella spp*. from being introduced into egg-laying flocks, early and sustained refrigeration of shell eggs, and education of consumers and food workers about the risk of consuming raw or undercooked eggs. Since 1996, the incidence of *Salmonella spp* infection in humans has significantly decreased, although many cases and outbreaks due to *Salmonella* contaminated eggs continue to occur [4].

Control of *Salmonella* is challenging because there are numerous possible sources of *Salmonella* contamination in an integrated poultry operation, including chicks, feed, rodents, wild birds, insects, transportation, farm environment, and processing plant environments [5]. It is tough for farmers to recognize chickens that have got a recent Salmonellosis and that are at risk for producing contaminated eggs [6]. The seroprevalence of anti-*Salmonella* antibodies has been carried out in different populations [7,8,9,10]. We aimed to investigate the seroprevalence of anti-Salmonella antibodies in the sera of healthy humans and in the eggs of healthy layer chickens (brown Leghorn).

2. Materials and Methods

Human serum samples were donated by the National Public Health Laboratory in Jamaica in 2005 as a part of the "Bacterial Antigen" project and kept at -20°C until used. These serum samples came from patients across the island.

Chickens were purchased at various farms across the island in 2009, and their eggs were collected, and the IgY samples were isolated and kept at -20^{o}C until used.

2.1. Chicken IgY Isolation

The IgY fraction was separated from the egg yolks of a diversity of laying hens. The chloroform-polyethylene glycol (PEG) procedure isolated the chicken IgYs [11]. In brief, the eggs were washed with warm water, and the egg yolk was separated, and a 1:3 solution made in phosphate- buffered saline (PBS) pH 7.4. An identical volume of chloroform was added to the mix, which was then centrifuged at (1,000 RPM) for 30 min at (RT). The supernatant was obtained and mixed with PEG 6000

(12%, w/v), stirred, and incubated (RT for 30 min) and again centrifuged. The precipitate containing IgY was dissolved in PBS at pH 7.4. A volume equivalent to 1/6 the volume of egg yolk was dialyzed against 1L PBS at pH 7.4 for 24 h at 4ºC. The IgY was stored at –20ºC until further analysis.

2.2. ELISA for Anti-*Salmonella* Antibodies

An enzyme-linked immunosorbent assay detected anti-Salmonella antibodies in humans and several avian species [4]. The methodology of this test was as follows: ninety-six well polystyrene microplates (U-shaped bottom, Sigma-Aldrich Co, St. Louis USA) were incubated at 4°C and overnight with 1 µg/well of the LPS from S. Typhimurium. The microplates were washed and blocked for 1 hour at 18°C [4]. The microplates were then rewashed (4 times). After that, a 50 µl aliquot of human serum or an IgY sample was added. After incubating for one hour at 18ºC, the microplates were washed as previously described and 50 µl peroxidase-labeled anti-IgY-protein A conjugate (prepared by mixing conjugates from Sigma-Aldrich Co.) in dilutions 1:5000 with PBS-Tween-20 was added [5]. After a further incubation and washing step, 50µl tetramethylbenzidine (TMB) was added. The microplates were further incubated for 15 minutes in the dark, and then 50µl 3M HCl was added to stop the reaction. The microplates were read at 450 nm. Mean optical density value (XOD) equal to or higher than 0.35 was taken as the cut-off point.

2.3. Statistical Analysis

Data were analyzed to compare the differences in proportions of anti-Salmonella antibodies in human and chicken samples. SPSS version 21 was used to analyze the results statistically.

3. Results and Discussion

The method of Polson (1990) was successfully applied in the preparations of purified IgY fractions from birds with the objective of immunodetection. This study aimed to know the statistical significance of anti-*Salmonella* antibodies in a cohort of humans and chickens was. As Table 1 shows, 11.3% (6 out 53) human samples and 95.3% representing 102 out of 107 IgY samples showed the presence of anti-*Salmonella* antibodies.

This report suggests that *Salmonella spp* are microorganisms to whom chickens are most exposed to and therefore, their immune system fight it by the production of humoral immune responses (antibody). Humans are less exposed to these ubiquitous bacteria than chickens, and they fulfill most effective preventive measures of hygiene to avoid contamination. It explains why only 11.3% of the samples were positive for anti-*Salmonella* antibodies. Besides, some humans are vaccinated against *S.* Typhi, and they develop specific antibodies that cross-react among *Salmonella spp*, but we do not know if any of the human samples came from individuals vaccinated against typhoid fever.

Table 2 shows the differences in proportions of the seroprevalence of anti-Salmonella antibodies in humans and chickens. There was a statistically significant difference ($p < 0.05$) between the samples. Also, a 95% confidence interval was calculated for difference in proportions of both human and chicken samples. For human was 0.0278-0.1982 and for the chicken was 0.9129-0.9931.

Table 1. Presence of anti-*Salmonella* antibodies in egg yolk and human serum by indirect ELISA

Species	Positive (%)
Human	11.3 (6/53)
Chicken	95.3 (102/107)

Table 2. Table showing the differences in proportions between human and chicken samples with 95% confidence intervals

	Humans	Chickens	Difference
Proportion of antibodies	0.113	0.953	0.84
Sample size	53	107	
95% confidence interval	0.0278 - 0.1982	0.9129 - 0.9931	0.6858 - 0.9942

[Z value 10.7, p value < 0.05].

We wanted to know the exposure of humans to *Salmonella spp* because, in Jamaica, the prevalence of Salmonellosis is unknown in man. However, the bacteria have been isolated from chicken farms and chicken products (e.g., *Salmonella* Montevideo and *Salmonella* Augustenborg) [12]. Seepersad-Singh and Adesiyun [13] studied the antimicrobial resistance of *Salmonella spp* and their prevalence in various animal species in Trinidad and Tobago. They reported the presence of S. Montevideo in one of two isolates retrieved from reptiles. Akter et al., 2007 [14] reported that Salmonellosis is a common problem in poultry farms of Bangladesh. The indiscriminate use of antibiotics to control this infectious disease results in drug resistance.

Nsutebu et al. (2002) reported a cross-sectional study in a group of 230 consecutive blood donors in Yaoundé, Cameroon. They found a baseline titer of up to 1:400 for anti-Salmonella antibodies [15]. Serum samples from 641 workers of poultry plant showed a level of anti-Salmonella antibodies in 60.7% of workers from a poultry plant and 9.8% of meat-packing plant workers [16]. This last study suggests that the labor place is a critical risk factor for Salmonellosis.

Purifying IgY from the yolk of the avian egg is of interest as a source of specific antibodies for oral administration to prevent infection (Larsson et al., 1993) [17]. Immunoglobulin Y antibodies have been used in the immunodiagnosis of several infectious diseases [18-20]. The use of avian eggs in antibody production results in a reduction in the use of laboratory animals used for this purpose. Besides, immunized chickens produce larger quantities of antibodies than do rodents in the laboratory (Schade et al., 1991) [21]. The hens are farmyard animals and are therefore less expensive than laboratory animals such as rabbits.

Antibodies developed in birds recognize more epitopes on mammalian proteins. It is indeed more advantageous to

use IgY in immunoassays (e.g., ELISAs), which detect mammalian and bird proteins. It is especially true when the antigen is a highly conserved protein such as a hormone (Gassmann et al., 1990; Rosol et al., 1993) [22,23]. Chicken IgY does not react with mammalian anti-IgG antibodies, including rheumatoid factors present in human serum. In immunological assays, the interference caused by rheumatoid factors can be problematic, particularly as the sensitivity of the assay increases (Boscato and Stuart, 1988) [24]. If the chicken IgY is used, interference by anti-IgG antibodies can be eliminated (Larsson and Holmadahl, 1990) [25].

Future study of the seroprevalence of anti-Salmonella antibodies in the Caribbean should look at antibodies developed against the O and H antigens of *Salmonella spp*. In addition to search for antibodies in people handling uncooked or undercooked meat and animal husbandry. However, our results suggest the presence of Salmonellosis as a contaminant in humans and endemic state in birds, which not necessarily means active disease.

The limitations of this study include:

1. The small sample in humans
2. We do not know if any of the human samples came from individuals vaccinated against typhoid fever.

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