Enterotoxigenicity of *Salmonella* Gallinarum strains isolated from poultry

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**SUMMARY**

Twenty *Salmonella* Gallinarum strains isolated from poultry were tested for Enterotoxigenicity activity using rabbit and chicken ligated intestine loop technique and the rabbit vascular permeability factor activity test. One ml of cell-free filtrate (CFF) of each isolate was injected into chicken ligated loops and rabbit ligated loops, one ml of whole cell culture(WCC) of each isolate was injected into chicken ligated loops and 0.1 ml of crude enterotoxin was injected internally in rabbit skin.

Enterotoxigenic activity measured as mean dilatation index (DI) produced by *Salmonella* Gallinarum enterotoxins in rabbit loop test was significantly lower than that in the chicken intestine loop (p < 0.05) and did not differ significantly than the mean DI of WCC in the chicken intestine segment (p > 0.05). The filtrates from *Salmonella* Gallinarum strains gave permeability factor in 41.6 % of the cases. These data seem to support the concept of specificity of chicken intestinal loop method as suitable for detection of enterotoxigenicity of *Salmonella* Gallinarum.

**KEY-WORDS** : *Salmonella* Gallinarum - enterotoxin - chicken loop test - vascular permeability factor.

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**RÉSUMÉ**

Entérotoxigénicité de souches de *Salmonella* Gallinarum isolées de volailles. Par T. KOKOSHAROV.

L’entérotoxigénicité de 20 souches de *Salmonella* Gallinarum isolées de volaille, a été testée par le test de l’anse intestinale de poulet et de lapin ligaturée et par le test de perméabilité vasculaire chez le lapin.

Un ml de filtrat acellulaire de chaque souche a été injecté dans les anses intestinales ligaturées de poulet et de lapin et un ml de culture bactérienne de chaque souche a été injecté dans l’anse intestinale ligaturée de poulet. 0,1 ml d’entérotoxine brute a été injecté dans la peau du lapin.

L’activité entérotoxique mesurée par l’index de dilatation (DI) due aux entérotoxines de *Salmonella* Gallinarum sur l’intestin de lapin est significativement plus faible que sur l’intestin de poulet (p < 0.05). Elle ne diffère pas significativement de l’activité de la culture bactérienne sur l’intestin de poulet (p > 0.05).

Les filtrats de *Salmonella* Gallinarum contiennent un facteur de perméabilité dans 41.6 % des cas.

Ces résultats montrent la plus grande sensibilité de l’intestin de poulet pour la mise en évidence des entérotoxines de *Salmonella* Gallinarum.

**MOTS-CLÉS** : *Salmonella* Gallinarum - test de l’intestin de poulet ligaturé - facteur vasculaire de perméabilité.
Introduction

The pathogenesis of fluid secretion in salmonellosis is not clearly understood. The demonstration that endotoxin of *Vibro Cholerae* and *Escherichia coli* play a vital role in diarrheal disease provoked interest in the possible role of enterotoxin in salmonellosis [2]. It is clear that *Salmonella* colonize and invade the intestinal mucosa and cause mucosal inflammation, fluid secretion and tissue damage [4, 9, 13] and to effect an increase in vascular permeability when injected into rabbit skin [11, 5]. *Salmonella* Typhimurium enterotoxin has been induced the lipid peroxidation of the enterocyte membrane thereby leading to a lose of cell viability [8].

An understanding of the mechanism of action of bacterial toxins has led not only to a better comprehension of the microbial pathogenesis [10], but also to the development of new therapeutics. A heat-labile enterotoxin of *Escherichia coli* is an effective oral adjuvant when co-administered with soluble antigen of *Salmonella* Dublin in mice [3]. The mechanisms underlying the symptom of diarrhoea are based on microbial pathogenic factors and complex host responses [7]. One of these factor is the enterotoxin [9, 1].

*Salmonella* Gallinarum was identified as causative agents of fowl typhoid more than 100 years ago [14]. SMITH and HELL'S [16] made experiment in animals and strains of *Salmonella* Gallinarum of poultry origin have been also tested. The two *Salmonella* Gallinarum and one *Salmonella* Pullorum tested strains have yielded negative results for enterotoxin production. Later SINGH et al. [15] demonstrated that *Salmonella* Gallinarum cytotoxin indiced lesions and symptoms quite similar to acute fowl typhoid.

Because the enterotoxin production of *Salmonella* Gallinarum strains is not elucidated it was decided to study the biological activity of the viable *Salmonella* Gallinarum and their enterotoxins in vivo by the rabbit and chicken intestinal loop assays and the rabbit vascular permeability factor activity test.

Materials and methods

ANIMALS

Experiments were performed in New Zealand albino young rabbits, weighting 2 kg and two-month-old broiler chickens. During a seven-day-pre-test observation period bacteriological screening showed that the rabbit and chickens were negative for *Salmonella* Gallinarum organisms.

PREPARATION OF GRUDE ENTEROTOXIN

The 12 *Salmonella* Gallinarum strains isolated from death hens with diarrhoea were propagated in tryptone soy broth (TSB, Oxoid) and were incubated at 37°C for 24 hours. The cultures were then centrifuged in sterile centrifuged tubes at 3000 rpm for 30 min at room temperature. The resulting supernatant fluids were than filtered through sterile 0.46 mm membrane filters (Milipore Medical, Bedford, USA). Cell-free filtrates (CFFs) were stored at 4°C until their use in the enterotoxin assay [17]. The duplicate CFF of each strain was heated in a water bath at 56°C for 10 min to obtain heated cell-free filtrates (HCFFs).

PREPARATION OF WHOLE-CELL CULTURES

Whole-cell culture (WCC) of each strain *Salmonella* Gallinarum was prepared by growing the organism in 10 ml brain hearth infusion (BHI) broth for 23 hours. A viable count was carried out and the broth was standartized to contain 1 x 10⁸ cfu/ml (1).

ENTEROTOXIN ASSAY

The enterotoxigenicity of *Salmonella* Gallinarum strains was assayed using the rabbit ligated ileal loop technique [12] and chicken ileal loop method, a modification of this technique. Following 48 hours of fasting each rabbit and chicken was anaesthetised and jejunum and ileum were exteriorised through a midline incision. Ileo-jejunal loop measuring 6-10 cm were constricted. In experiment 1, rabbits were used each carrying seven loops, 1 ml of the crude enterotoxin (CFF) of each of the *Salmonella* Gallinarum isolate was injected into the loop, the last loop was inoculated with sterile BHI (control). In experiment 2, the procedure were similar to experiment 1, except that heat-cell-filtrates (HCFF) was injected instead CFF. In experiment 3, two-month-old chickens were used each carrying seven loops. One ml of standardised WCC of each of of the *Salmonella* Gallinarum isolate was injected into the loop, the last loop was control. The procedure in experiment 4 was similar to experiment 3, expect that 1 ml of crude enterotoxin(CFF) was injected instead of live bacteria. The viscers were returned into the abdomen cavity after inoculation and the incision closed. About 16-18 hours post-inoculation animals were sacrificed and the loops were again exteriorised. Enterotoxigenic response of the heat-treated, unheated cell-free filtrates and live *Salmonella* Gallinarum isolates was expressed as the mean dilata- tion index (DI) which is calculated as the ratio of volume (ml) of fluid in the loop to the final measured lenght (cm) of the loop. A volume of the least 1.0 ml of fluid accumulation per cm of intestinal loop was conside red a significant positive enterotoxic response.

STATISTICAL ANALYSIS

The student t-test was used to test the significance of the difference in the mean dilatation indices.
RABBIT SKIN TEST

Vascular permeability factor activity of enterotoxin of Salmonella Gallinarum isolated was examined by the method of SANDERFUR and PETERSON [11]. Rabbits were shaved and 0.1 ml injection of the crude enterotoxin samples were given intradermally with 26-gauge intradermal bevel needles. After 16 to 24 hours the zones of firm induratio(oedema) were palpated and estimated.

All the tests were performed at least twice in two different animals of the same species.

Results

DILATATION INDICES PRODUCED BY ENTEROTOXINS AND LIVE SALMONELLA GALLINARUM STRAINS

The mean dilatation indices (DIs) produces by cell-free filtrates (CFFs) into rabbit and chicken ileo-jejunal loop, whole-cell cultures (WCCs) into chicken intestinal loops are presented in Table I. Fluid accumulation Salmonella Gallinarum enterotoxin in chicken intestinal loop is illustrated in Fig. 1. The CFF of the 12 strains Salmonella Gallinarum gave positive results in 50.0 % of the cases in rabbit loop test and in 75.0 % in chicken loop test. The WCC of the same strains produces fluid secretion in 66.7 % in chicken intestinal loops. The DIs produced by Salmonella Gallinarum enterotoxins in rabbit intestinal loop ranged from 0.70 to 1.50 and between 0.70 and 2.80 in chickens intestinal segments. The DIs produced by live Salmonella Gallinarum enterotoxins in rabbit loop test was significantly in chickens intestinal loop range between 0.60 and 1.50. The mean DI produced by Salmonella Gallinarum enterotoxin in rabbit loop test was significantly lower than chicken intestinal loop (1.03 +/- 0.27 ; 1.40 +/- 0.67, respectively, p < 0.05) and did not differ significantly than the mean DI of DCC in the chicken intestinal segment (p > 0.05). None of the heated CFFs of all the strains Salmonella Gallinarum indiced any secretory response (data not shown). Spontaneous dilatation of ligated, nontreated intestinal segments was also common in chickens.

<table>
<thead>
<tr>
<th>Strain</th>
<th>DI of CFF</th>
<th>DI of WCC</th>
<th>PF in mm²</th>
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<tr>
<td>N</td>
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<td>1</td>
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<td>12</td>
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positive 6(50.0 %) 9(75.0 %) 8(66.7 %) 5(41.6 %)

X +/- SEM 1.03 +/- 0.27 1.40 +/- 0.67 a 1.07 +/- 0.38

DI - dilatation index
CFF - cell-free filtrates; WCC - whole-cell cultures; PF - permeability factor
a- (p < 0.05) significantly different from dilatation indices of cell-free filtrates in rabbits and whole-cell cultures in chickens

Table I. — Dilatation index produced by cell-free filtrates in rabbit an chicken intestines, whole-cell cultures in chickens intestines and alteration in vascular permeability in rabbit skin.
PERMEABILITY FACTOR OF THE CRUDE ENTEROTOXINS

The filtrates from the 12 *Salmonella Gallinarum* strains yielded permeability factor in 41.6% of the cases (Table I).

**Discussion**

This study for the first time shown that both cell-free filtrates (CFFs) and whole-cell cultures (WCCs) of the same *Salmonella Gallinarum* strains isolated from poultry with diarrhea induced fluid secretion in the rabbit and chickens intestinal loops.

There was demonstrable difference in the secretory responses elicited by the CFFs of the *Salmonella Gallinarum* isolated in rabbits and chicken intestinal segments. The dilution index (DI) of CFFs in chicken intestinal loops was significantly than DI of the same CFFs in rabbit model. The results indicate that the reaction of dilatation produced by a strain of *Salmonella Gallinarum* in ligated segment of a small intestine provides useful information on the ability of the strain to produce diarrhea. According CHAN and OBOEDBULEM [1] fluid secretion into the intestinal lumen is fundamental to the diarrhoeal syndrome. The information however may be misleading when applied to animals of a different species from the one in which the test has been performed. It is of the greatest value in its application to animals of the same species [16].

Before a strain can be enteropathogenic, which is interpreted here as its being able to produce enterotoxin, which we have now shown to be the essential property of strains that cause dilatation of ligated gut. The fact that the enteropathogenic *Salmonella Gallinarum* strains from poultry dilated ligated rabbit and chicken intestines in different degree is probably due to qualitative differences between the sensibility of the two animal species to *Salmonella Gallinarum* enterotoxin. It may be the consequence of testing strains in an animal species that is not their natural host. The chickens were a most suitable subject for ligated intestine experiment than rabbits. These experiments showed that it was necessary to study *Salmonella Gallinarum* virulence factor in the content of their interaction with the host cells of specific species.

For *Salmonella Gallinarum* the CFFs stimulated higher secretory responses that their corresponding WCC: the difference was significant (p < 0.05). This suggest that the factor(s) responsible for fluid secretion is liberated into the broth medium, hence in the filtrate. This difference in the expression of fluid secretory factor(s) by *Salmonella Gallinarum* raises questions about the actual mechanism of the secretory responses. Possible factors suggested to be responsible for fluid secretion include the ability of *Salmonella* to invade the intestinal mucosa and cause an inflammatory reaction [5], the ability of live bacterial cells with the fluid fraction to induce considerable higher secretory response than only its cell-free filtrate [9]. CHAN and OBOEDBULEM [1] support the concept of multiple mechanism for *Salmonella*-induced diarrhoea. Heat treatment appears to destroy the enterotoxic factor(s) in CFF as none of the heated CFFs of the *Salmonella Gallinarum* isolates induced measly fluid accumulation in the loops.

Not all *Salmonella Gallinarum* enterotoxins gave PF potency. The permeability of the small blood vessels of the intestinal villi caused by absorption of permeability factor of enterotoxin from the gut lumen, its increase leads to an accumulation of water, salts and plasma protein in the subepithelial spaces of the villi and the water and salts pass into the gut lumen [6].

These studies revealed that it seems little reason to doubt the importance of *Salmonella Gallinarum* enterotoxin as a cause of diarrhoea in the pathogenesis of fowl typhoid, the substance responsible for dilatation of ligated intestine.

**References**


