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THE VALIDITY OF ENZYME LINKED IMMUNOSORBANT ASSAY IN DETECTION OF SALMONELLA IN DRESSED POULTRY

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INTRODUCTION

Salmonellae which are endemic in many poultry flocks, may causing minimal poultry health problems. However during the slaughter of such poultry by assembly-line procedures encourages cross contamiantion particulary in the slaughter operation with continous water immersion chilling often takes place . This fact explains why the poultry industry has received most of the blame for contamination of the food chain with salmonellae.

Traditional methods for the isolation of salmonellae from poultry which involve non-selective pre-enrichment followed by secondary enrichment in selective broth media and subculture onto selective differential agars, have been used by many authors, (Wassef et al., 1980; El-Mossalami et al., 1986 and Nouman et al., 1986). Such methods are both labour, intensive and time consuming while a sample can provisionally be declared salmonella positive within 3 days, a final answer on a negative sample can take up to 5 days. Therefore a wide interest in a more rapid methods for the detection of salonella and a number

of procedures of varying complexity have been put forward. These include; the use of immuno fluorscent techique (Safwat et al., 1981) Enrichment serology, radiometry, enzyme immunoassays and the used of gene probes (Emswiler et al., 1984; Rigby 1984; and Todd et al., 1986).

The trend in this study is to verify the suitablity of the enzyme immunoassay as rapid test for the detection of salmonellae in dressed broilers.

MATERIALS AND METHODS

I . Survey of market dressed broilers for salmonellae contamination by ordinary cultural method. For that purpose, 166 freshly dressed broilers were collected from Cairo poultry plants. At the laboratory, the skin of such dressed broilers was sampled and examined for salmonella contamination according to Van Schothorst et al. (1976).

II . Efficiency of the Enzyme Linked Immunosorbant Assay (ELISA) in screening dressed broilers for salmonella as natural contaminants in comparison with the cultural method.

Out of the above mentioned samples, 66 dressed broilers were subjected to screening for salmonella using the ELISA parallel to the culture method.

III. Efficiency of ELISA in screening dressed broilers for salmonella as experimental contaminants.

Twenty broilers were collected alive, slaughtered, hand dressed, every carcass was palced in sterile polyethylen bag containing 100 ml-peptone broth to which one ml. 24 hours old Salmonella typhimurium broth culture was added. The carcase being shaked. Then every carcass was examined by both methods.

ELISA TECHNIQUE: (Emswiler 1984)

A. **Antigen preparation:** From the chopped skin, 25 gms were added to 250 ml lactose broth, incubated 24 hours at 37°C. Then one ml was transferred to tetrathionate broth and incubated 24 hours at 37°C. One ml was inoculated H-broth tubes (10 ml.) incubated 6 hours at 37°C. Tubes were then centrifuged at 1000 rpm for 20 minutes and the sadiment were resuspended in 2 ml. sterile phosphate buffer saline (pH 7.4). The harvest was heated for one hour in boiling water bath.

   The determined optimum dilution for the antigen was $^1/_{50}$; for the antiserum was $^1/_{20}$ and for the conjugate it was $^1/_{2000}$.

B. **Plate configuration: (Figur 1):** The column 1A-H was blank (no antisera); The column 2 A & B was positive control, C & D was negative control. Tested samples started from column 3.

C. **Procedure:** Starting form column 3, 50µl of the prepared samples were put in duplicate wells, plate was covered, incubated at 37°C for 3 hours, plate washed twice, dried by tapping on a towel. Fifty µL. per well of diluted antiserum was transferred to the antigen coated palte, covered, incubated at 37°C for 60 minutes, rewashed 3 times and dried. Fifty µL. of diluted conjugate in tris ELISA buffer was added to each well, covered, incubated at 37°C for 50 minutes, wash 3 times, dry, 100 µL. per well of ABTS + H_2 O_2 substrate in citric acid buffer, shake on a shaker 20 minutes. Add 100µL. stopping solution per well, read on a reader.

D. Calculation:

1. Negative control mean (NC) = $\frac{\text{well 2C + well 2D}}{2}$

2. Positive control mean (PC) = $\frac{\text{well 2A + well 2B}}{2}$

3. S/P ratio = $\frac{\text{Sample mean}}{\text{PC - NC}}$

The S/P ratio greater than 0.5 is considered positive

RESULTS

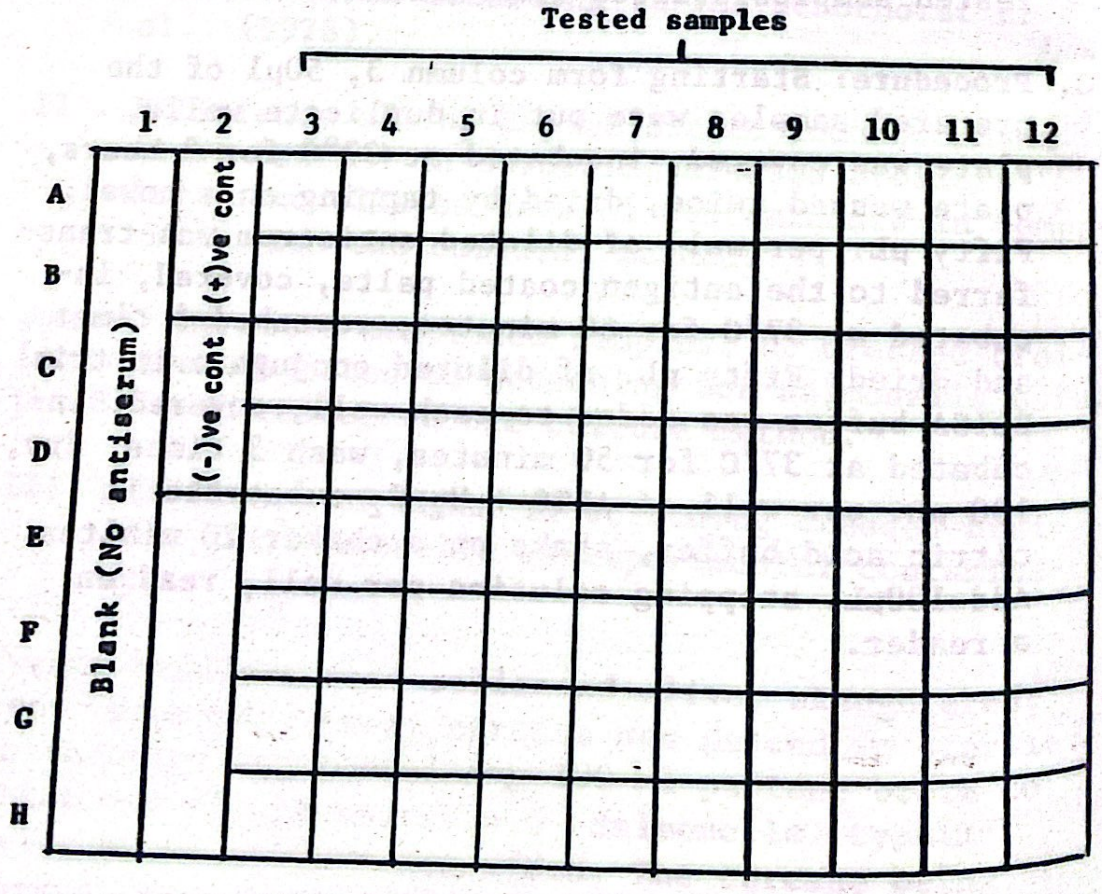

Table (1): The result of the survey for isolation of salmonella by using culture procedure only

Type	Somatic (10)	Flagellar (H)		Positive samples	
		Phase I	Phase II	No.	%
S.typhimurium	Group (b) 1,4,5,12	i	1,2	38	22.9
S.stanley	Group (B) 1,4 (5), 12, 27	d	1,2	4	2.4
Total				42	25.3

Table (2): Comparison between the ELISA and the cultural method in the detection of salmonella contaminated dressed broilers.

Contaminants	Natural		Experimental	
Tested samples	66		20	
Criteria	No.	%	No.	%
Total positive culture	19	28.8	20	100
Total positve ELISA	25	37.9	19	95
Positive both methods	18	27.3	19	95
Negative both methods	42	63.6	0	0
ELISA+ve Culture-ve	7	10.6	0	0
ELISA-ve Culture+ve	1	1.5	1	5

DISCUSSION

From the results achieved, it is evident that 25.3% of the examined broilers were positive for salmonella species when the culture method was used. S. typhimurium and S. stanly could be serotyped (Table 1). Such serotypes are frequent cause of human gastroenteritis.

In the comparison between the cultural method and the ELISA test for salmonella detection in dressed broilers, Table 2 reveals the following; Out of 66 samples examined for the detection of salmonella as natural contaminants, 19 samples were positive by the sultural method and 25 were positive by the ELISA Test. While 18 samples were positive by both methods 7 samples were positive ELISA negative culture but only one sample was negative ELISA positive culture. Such results thraw light on the efficiency of ELISA Test in the detection of salmonella as natural contaminants on dressed broilers. Similar conclusion was reported by Todd (1986). However, upon examination of 20 experimentally contaminated dressed broilers, all were recovered by the cultural method and only one sample failed detection by the ELISA test. This phenomenon could be explained by the fact that the salmonella antigen was of low concentration or due to an error in the application of ELISA technique in that particular sample (Emswriler et al., 1984).

From the results acheived one can safely conclude that, the ELISA test could be recommended as a twenty four hours test for the detection of salmonella contamination in dressed poultry. However, the cultural method is better applicable for the routine work when there is no need for a rapid answer, this is because the ELISA test despite being more rapid, time saving but more expensive.

SUMMARY

1. One hundred sixty six market freshly dressed broiler samples were analysed by the cultural method described for salmonella contamination;25.3% of the samples were found positive for salmonellae. The serotypes identified were S. typhimurium (22.9%) and S. stanley (2.4%).

2. In acomparative analysis one the naturally existing salmonella in dressed poultry, the ELISA test recovered a higher rate of salmonella contamination (37.9%) than cultural method (28.8%).

3. On artificially contaminated samples all samples were recovered by the cultural mehtod, while, 95% of samples were detected by the ELISA test. Only one sample was positive culture negative ELISA and the explaination for that phenomenon has been mentioned.

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