

## EXPERIMENTAL TOXICITY STUDIES OF SALMONELLA SEROVARS ISOLATED FROM PIGS, IN MICE AND GERMINATING SEEDS

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### ABSTRACT

*Experimental studies on the effects produced by Salmonella serovars was conducted on mice and germinating seeds. For this study six isolates of Salmonella were isolated from 101 faecal samples of pigs collected randomly at different piggeries. The 6 isolates of Salmonella were typed as S.Derby (3) S.Typhimurium (2) S. Typhisuis (1) at National Salmonella & Escherichia centre, Kasauli, Himachal Pradesh. To study the effects of the isolates 0.1 ml of cell free culture supernatant of Salmonella serovars was inoculated intraperitoneally to mice and the culture suspension having concentration of approximately  $2.7 \times 10^9$  cfu/ml Salmonella serovars was applied on the germinating seeds. The Salmonella serovars which were toxic to mice were also found to be toxic to seeds causing inhibition of germination. The present investigation indicated that germinating seeds can be taken as a model to determine virulence of Salmonella serovar instead of experimental mice to avoid unnecessary suffering to the laboratory animals.*

**Key Words:** Salmonella, toxicity, mice, germinating seeds.

### INTRODUCTION

Salmonellosis is a wide spread disease that affects wide range of animals and human beings. The food animals like pigs once infected remain as chronic carriers. The carrier pigs cause severe economic loss to the pig rearing farmers due to piglet mortality and lower growth rate and remain as a lateral infection for human beings. (Singh et al.,1980).

In the present investigation Salmonella serovars were isolated from the faecal samples of pigs in three districts of Andhra Pradesh and the toxic effects of the serovars was studied and compared in mice and germinating seeds (Mahtab Z.Siddiqui et al., 2006).

### MATERIALS AND METHODS

A total of 101 faecal samples were collected aseptically from the pigs of different age

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groups of Chittoor, Vishakhapatnam and Guntur districts of Andhra Pradesh state. Isolation was done by using 1% buffered peptone water as transport medium to the laboratory. Rappaport - Vassiliadis and Selenite F broth were used as primary enrichment media. Subsequently brilliant green agar, MacConkey agar, Xylose Lysine Deoxycholate agar, Salmonella Shigella agar and Rappaport Vassiliadis modified semi solid media were used for isolation. The morphological, cultural and biochemical characteristics of bacterial cultures were performed as per Edwards Ewing (1972). Cultures identified as Salmonella were sent to National Salmonella and Escherichia center, Central Research Institute, Kasauli, Himachal Pradesh for typing.

**Bacterial Strain:** A total of six isolates of Salmonella were typed. Among them three were S. Derby two were S. Typhimurium and one was S. Typhisuis.

**Laboratory mice :** Twenty four mice weighing about 25-30g maintained in experimental animal house, Department of Microbiology, College of Veterinary Science, Tirupati, were employed for bio-assay to assess virulence of Salmonella isolates.

**Mice Inoculation :** Culture supernatant Salmonella serovars was prepared by centrifugation of 18 h old brain heart infusion broth ( BHI ) culture of Salmonella.

Six mice for each serovars were inoculated with 0.1 ml of CFCS of Salmonella isolates intraperitoneally (I/P). The control group was administered with 0.1 ml of phosphate buffer saline (PBS).

The inoculated mice were observed for a period of 7 days for pathogenicity and mortality. Mice that succumbed during the period were necropsied. Remaining mice were sacrificed on the seventh day and necropsied. Gross lesions in the

liver, spleen, trachea, heart and intestines were observed and tissue samples were preserved in 10% formal saline for further histopathological studies.

**Germinating seeds :** Certified seeds of ground nut ( Tirupati 1), cowpea and jowar (NTJ 2) were obtained by the courtesy of department of Plant Pathology, Acharya N.G.Ranga Agricultural University ( ANGRAU) to study the effect of the organisms on germination of the seeds.

Bacterial cultures were grown in trypticase soy broth for 24 h at 37°C. The broth cultures were pelleted by centrifugation at 5000 x g and washed twice with PBS and resuspended in the same buffer. The concentration of each of the culture suspension was adjusted to the 9th tube (approx.  $2.7 \times 10^9$  cfu/ml) of McFarland standard tubes for turbidity tests.

Seeds were checked for the presence of Salmonella or E. coli prior to their use. The seeds were soaked in sufficient quantity of each of cell suspension in recommended dose. The seeds were incubated in pre-sterilized glass Petri dish moist chambers with absorbent cotton overnight at room temperature. The seeds were spread approximately 10 numbers on sterile Petri dish and incubated at room temperature.

The seeds were observed 48 h of post incubation for germination and non-germination. The appropriate controls were maintained with PBS.

## RESULTS AND DISCUSSION

The overall incidence of Salmonella in fecal samples of pigs was 5.94%. Pathogenicity studies carried out with serovars viz., S. Derby, S. Typhimurium and S. Typhisuis in mice revealed

variations in virulence, mortality and clinical observations.

The effects of I/P inoculation in mice are dullness, depression and anorexia. The mortality in mice started from 18 h post inoculation and continued for 7 days. During acute phase of illness mice developed hind limb paralysis, coma and death.

Among the serovars tested, the serovar S. Typhimurium had exhibited maximum virulence with mortality of two mice within 24 h, three mice within 48 h and the remaining one mouse within a week, whereas S. Derby and S. Typhisuis revealed moderate mortality in mice from 24 h to one week. (Table 1)

The mean gut weight and body weight ratio (GW : BW) of 0.12 to 0.14 were considered as severely cytotoxic and between 0.08 to less than 0.11 as moderately cytotoxic serovars. (Table 2).

The macroscopic observations revealed congestion of liver, lungs, intestines and heart together with enlargement of spleen. The intestines were distended with watery fluid. Further it was observed that the whole body of the mice was oedematous and swollen as compared to PBS inoculated control.

The Salmonella serovars were re-isolated from blood, heart, liver and faecal samples of the experimentally infected mice by streaking on MacConkey Agar (MCA) and Brilliant Green Agar (BGA) plates.

Histopathological observations of the liver revealed intense degeneration of the parenchymatous cells. The hepatocytes revealed notable degenerative changes with passive hyperaemia and haemorrhagic foci as persistent feature. Increased survival of animals led to focal

areas of necrosis and mononuclear cell infiltration. Sinusoidal haemorrhages, karyomegali and binucleated cells were seen in highly potent cytotoxin producing strains. The intestinal changes included degeneration and desquamation of epithelial cells of villi and serofibrinous exudation in the mucosa with passive congestion. Sections of the spleen revealed sinusoidal congestion, paucity of lymphoidal cells, rarefied germinal centres, focal areas of necrosis, depletion of cells.

In the present investigation among the three serovars tested, S. Typhimurium was found to be the most pathogenic serovar to mice producing high mortality. S. Derby and S. Typhisuis were moderately pathogenic to mice. Characteristic lesions viz., congestion of liver, lungs, intestines, enlargement of spleen and heart were observed. The clinical signs supported by necropsy finding and as evidenced by histopathological observations and re-isolation of the bacteria in vitro confirming their association with pathogenicity. These findings were in agreement with Singh et al (1997).

The observation on pathogenicity in mice revealed that the invasiveness of Salmonella is considered to be an important attribute for virulence which is in accordance with D'Aoust (1989); Hsu (2005).

Among the various serovars of Salmonella employed in the present study, S. Derby inhibited 100% germination followed by S. Typhimurium and S. Typhisuis. In appropriate controls the germinating seeds exhibited 100% germination indicating the inhibition in germination was due to Salmonella (Table 3).

The Salmonella serovars were re-isolated from the washings of the infected grains by inoculating in MCA and BGA agar plates.

**Table 1:**  
**Results of pathogenicity study in mice**

Sl. No.	<i>Salmonella</i> serovars	Mortality		
		24 h	48 h	After 48 h up to 7 days
1.	S.Derby	1	1	2
2.	S.Typhimurium	2	3	1
3.	S.Typhisuis	0	1	1
4.	Control	0	0	0

**Table 2:**  
**Mouse bio-assay of Salmonella serovars in mice**

<i>Salmonella</i> serovar	Pre inoculation body weight (g) (Mean ± SD)	Gut weight after death (g) (Mean ± SD)	GW/BW ratio
S.Derby	32 ± 2.83	3.95 ± 0.21	0.122*
S.Typhimurium	34 ± 1.41	3.85 ± 0.92	0.132*
S.Typhisuis	34 ± 0.71	3.7 ± 0.14	0.117*
Control	32 ± 0.71	2.3 ± 0.14	0.078*

\*Mean GW:BW ratio : 0.12 to 0.14 - severely cytotoxic

\* Mean GW:BW ratio : 0.08 to 0.11 - moderately cytotoxic

**Table 3:**  
**Germination inhibition percentages of different seeds by Salmonella serovars**

S. No.	Salmonella serovars	No of seeds germinated out of 10 seeds			% Inhibition		
		Groundnut	Cowpea	Jowar	Groundnut	Cowpea	Jowar
1	S.Derby	0	0	0	100	100	100
2	S.Typhimurium	1	0	2	90	100	80
3	S.Typhisuis	1	2	1	90	80	90
4	Control	10	10	10	0	0	0

The internal structures like cotyledons were affected in the groundnut seed whereas in cowpea and jowar the seed coats were affected which in turn had shown deleterious effects in the process of germination of seeds.

Instead of experimental animals germinating seeds were used because of ethical and humanitarian grounds (Mahatab Z.Siddiqui et al., 2006). A modest attempt was made to study the pathogenic effects of the isolates separately in mice and on germinating seed and to determine pathogenicity of isolated organism, seed model can be taken up as an alternative preliminary experiment instead of experimental animals to avoid pain to the animals and to reduce the cost of the experiments.

#### REFERENCES

- D'Aoust, J. Y. (1989) Food borne Bacterial Pathogens, Marcel Dekker Inc. New York.
- Edwards, P.R. and Ewing, H.W.(1972). . . . Identification of Enterobacteria, 3rd edition.

Bergees publishing company Minneapolis, Minnesota.

Hsu, R.B., Lin, F.Y., Chen, R. J., Hsueh, P. R. and Wang, S.S. (2005), . . . . Antimicrobial

drug resistance in Salmonella infected aortic aneurisms. Animals of thoracic Surgery 80: 530-536

Mahtab Z.Siddiqui., Singh, B.R., Agarwal, R.K. and Srivastava, S.K. (2006)... An invitro seed model for determination of Salmonella serovars toxicity. Indian J.Comp. Microbiol. Immunol. Infect. Dis. 27: 42- 43.

Singh, S.P., Sethi, M.S. and Sharma, V.D.(1980)... Indian J Anim. Sci. 50:186-188.

Singh, Y., Sharma, V.D., Sharma, S.N. and Singh, B.R.(1997)... Experimental toxicity of

Salmonella Weltevreden cytotoxin in mice. Indian.J.of Comp. Microbiol. Infect. Dis.18:136-141.