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A Study on Prevalence and Virulence Characterisation of *Salmonella enterica* subsp. *enterica* Isolated from Poultry and Related Environment in India

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In the present study 24 isolates of *Salmonella enterica* with a prevalence of 8.21% were recovered from a total of 292 samples collected from poultry and related environment. Twenty four isolates were found to belong to five different serovar while two isolates were rough. Among them, the serotype *S*. Haifa (58.33%) was found highly abundant followed by *S*. Heidelberg (20.83%), *S*. Virchow, *S*. Agona and *S*. Kentucky (4.16% each). Interestingly, serotype *Salmonella* Haifa is isolated and reported for the first time in India in present investigation. Out of 24 *Salmonella* isolates subjected to rabbit ligated ileal loop (RLIL) assay only 12 were found to be enterotoxigenic with dilatation index ranged from 0.40 to 0.43. These 12 isolates belonged to 4 serotypes viz *S*. Virchow (1), *S*. Heidelberg (2), *S*. Kentucky (1) and *S*. Haifa (7) while 1 isolate belonged to rough strain. All *Salmonella* isolates produced beneath the colony haemolysis (BCH) on blood agar made from washed erythrocytes of goat. None of the isolates were found positive for the production of enzymes viz. phenylalanine deaminase, lecithinase, phospholipase A, DNase, caseinase and gelatinase..

Key words: *Salmonella enterica*, prevalence, enterotoxin, rabbit ligated ileal loop assay.

Salmonellosis is an important infectious disease affecting chicken globally (Rajagopal and Ming, 2013). Poultry has been recognized as one of the main sources for disseminating *Salmonella* infection to man and various other animals as it serve as a reservoir for a large number of *Salmonella* serotypes which are potentially pathogenic to birds and man (Vandeplas *et al.*, 2010). In India, Salmonellosis is considered to be endemic with 6.7 to 97.6% prevalence reported from chicken carcasses (Ramya *et al*., 2013; Kaushik *et al.*, 2014). Salmonellosis in poultry has been recognized as the primary risk factor associated with the recent increase in public health problem (Aarestrup *et al*., 2007). More than 2,541 serovar of *Salmonella* have been described by National Salmonella Reference Laboratory, Galway, Ireland till now which have different distribution in different animal species and geographical region. Some serovars of *Salmonella* show host-specificity and are true poultry pathogens viz. *Salmonella enterica* serovar Gallinarum and *S*. Pullorum. *Salmonella* infections with other serovars like *S*. Enteritidis, *S*. Typhimurium, *S*. Hadar and *S*. Heidelberg seldom cause disease in poultry, but are of major concern to public health (Breytenbach, 2004).

The pathogenesis of *Salmonella* has been linked to secretion of various enzymes like enterotoxin, Haemolysin, DNase, Caseinase, Gelitinase which have significant role in onset of disease. *Salmonella* enterotoxin interacts with ileal mucosa and disrupts normal intestinal

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function, which results in an acute inflammatory cell influx, secretion and enteritis (Wood *et al.*, 1998) while other enzymes contribute to a variable extent in establishment and spread of infection in susceptible host.

Hence, the study was conducted to study the prevalence of *Salmoenlla* in various poultry farms and determine their pathogenic potential.

MATERIALS AND METHODS

Sampling:

Two hundred and ninety two different samples namely cloacal swabs of culled, sick and unproductive birds, caecal swabs, meat including different organs collected from dressed broiler birds, feed, litter, water used for drinking by birds and egg and its contents etc were collected from five different poultry farms. The detail of the sample collection has been presented in Table 1.

Isolation and Purification

Samples were inoculated in buffered peptone water (1:10) for pre-enrichment at 37°C for 16 h. One ml of pre-enriched broth was transferred into tetrathionate broth (TTB) for selective enrichment of salmonellae and incubated at 42°C for 48 h. The TTB-enriched culture was then inoculated on MacConkey2 s agar and incubated at 37°C for 24 h. Pale, non-lactose fermenter colonies were picked and plated on Brilliant Green Agar. Suspected colonies of *Salmonella* were further purified by subculture and transferred onto nutrient agar slants. These isolates were then subjected to standard morphological and biochemical tests, as described by Buchanan and Gibbon (14), to ascertain their identity as *Salmonella*.

Serological Typing of *Salmonella* Isolates;

All the isolates belonging to genus *Salmonella* were sent to National Salmonella center, IVRI, Izatnagar (UP) for serological typing.

Rabbit Ligated Ileal Loop Assay (RLIL)

The technique of Bergdoll (1988) was followed for RLIL. Test cultures of Salmonella strains were grown in glucose broth at 37ÚC for 24 hours. Adult rabbits approximately weighing about 1 to 1.5 kg were procured and kept off feed for 24 hours prior to test but water was provided ad libidum. The rabbits were anaesthetised by injecting xylazine 5mg/kg body weight and ketamine 25mg/kg body weight. After shaving the surgical site, abdomen was cut opened with midline incision of 5-7 cm length with aseptic precaution. The small intestine was extravasated and proximal end of small intestine was located. By leaving first 20-30 cm, twelve to thirteen segments were made by ligating the intestine tightly with silk thread. Bacterial cultures (about 1 ml) were injected and uninoculated glucose broth served as control. The intestine was replaced in the abdominal cavity and muscles and skin were sutured with silk thread with continuous suture. Rabbits were given water ad lib. Rabbit died after about 18 hours. Their abdomen was opened and inoculated gut loops were observed for distention and presence of haemorrhages. Volume of the fluid accumulated inside and length of each segment was measured.

Dilatation Index (DI)

The dilatation index (DI) was calculated by the method of Thapliyal and Singh (1978).

$$DI = \frac{\text{Volume of fluid accumulated (ml)}}{\text{Length of loop (cm)}}$$

The dilatation index of 0.4 or more was considered to be positive for the presence of LT toxin.

Enzyme production Assay

Lecithinase and phospholipase A production

All the strains were stabbed on egg yolk agar plates and incubated at 37°C for 48 h to examine 12 hourly for zone of opacity/clearance around lecithinase/phospholipase A producing colonies.

DNase Activity Test

Samples were spot inoculated on DNAase plates and incubated at 37°C for 48 h to see the clearance around colonies for detection of extracellular DNase activity. Plates were further exposed to ultraviolet rays (150 to 3900 °A) for half an hour and reincubated at 37°C for 24 hours. Colonies showing development of purple violet zone were considered positive for cell bound DNase activity.

Caseinase Activity Test

Milk agar plates were spot inoculated with test cultures and incubated at 37°C for 48 hours. Clearance zone around colony was indication of positive test.

Gelatin Liquefaction

Stab cultures made in gelatin agar tubes were incubated up to 7 days at 37°C to observe gelatin liquefaction. Before reading the results, the cultures were held at 4°C for half an hour.

Haemolysin Assay

All cultures were spot inoculated on blood agar plates containing washed erythrocytes of goat. After overnight incubation at 37°C, zone of clearance was measured around the colonies in case of haemolysin positive isolates (Albesa, 1989).

RESULTS

A total of 24 *Salmonella* isolates were obtained from 294 different samples of five poultry farms following the conventional method of isolation and identification. Thus, the prevalence recorded in the study was 8.21%. All 24 isolates were obtained from exotic breeds (White Leghorn and Rhode Island Red) of poultry and none isolate was obtained from native breeds (Aseel and Kadaknath). Further, all 24 isolates were only recovered from caecal swabs and none from clocal swab, feed, litter, meat, water and egg and its contents. The majority of the isolates belonged to *S.* Haifa (14, 58.33%) followed by *S*. Heidelberg (5, 20.83%), *S*. Virchow (1, 4.16%), *S.* Agona (1, 4.16%), *S.* Kentucky (1, 4.16%),) and two (8.33%) rough strains.

Twelve out of 24 *Salmonella* isolates were found to be enterotoxigenic. Range of dilatation index of enterotoxigenic *Salmonella* isolates was recorded from 0.40 to 0.43 which was considered very specific for positive reaction. The distribution of enterotoxigenic and nonenterotoxigenic *Salmonella* isolates according to different serotypes with their dilatation index is summarized in table 2.

All isolated serotypes of *Salmonella* produced beneath the colony haemolysis (BCH) on blood agar made from washed erythrocytes of goat. No clear zone haemolysis (CZH) was recorded by any of the *Salmonella* isolates. All *Salmonella* isolates did not appear to produce enzymes urease, phenylalanine deaminase, lecithinase, phospholipase A, DNase, caseinase and gelatinase.

DISCUSSION

Out of the five Poultry farms studied for occurrence of salmonellosis, CVAS poultry farm was found free from salmonellosis which can be accounted to good hygienic and scientific management of poultry farm in comparison to the farms located in other area. The results showed that none of the *Salmonella* isolates was found from native birds (Kadaknath and Aseel breed). But all 24 *Salmonella* isolates were obtained from exotic birds (White Leg Horn breed). Similar findings were reported by Kapoor *et al.* (1981) who reported that native breeds of poultry (Kadaknath and Aseel breed) were free from *Salmonella*, but 3.2 percent exotic birds were faecal excreters of *Salmonella* organism. *Salmonella* were more frequently isolated from the caeca than from any other organs, tissues or other parts of the gastrointestinal tract. Desmidt *et al.* (1997) also found caecum to be important carrier site for *Salmonella*e containing highest

Table 1. Samples collected from various Poultry Farms and its related environment.

Source of Sample	Type of samples							
	Cloacal swabs	Caecal swabs	Feed	Litter	Meat	Water	Egg Contents	Total
CVAS PF	20	20	8	8	-	8	8	72
Sandhu PF	10	30	3	3	3	3	3	55
Bika PF	10	30	3	3	3	3	3	55
Satish PF	10	30	3	3	3	3	3	55
Rashid PF	10	30	3	3	3	3	3	55
Total	60	140	20	20	12	20	20	292

CVAS: College of Veterinary and Animal Science.
PF: Poultry Farm

number of *Salmonellae*. Thus study suggests caecal region was possibly the primary site for localization and penetration of *Salmonella*e as has been reported earlier (Fanelli *et al.*, 1971).

In India, several serovars have been reported from time to time in poultry. More than 53 different serovars have been reported from poultry alone in India and this number is ever increasing (Singh *et al.*, 2004). Here, in this study we obtained serotype *S*. Haifa for the first time in India as per the serotyping report of National Salmonella Center (Vet.) IVRI, Izatnagar (U.P.). Besides, serotype *S*. Heidelberg, *S*. Kentucky and *S*. Agona appear to have not been documented in the available literature from poultry. *S*. Haifa, *S*. Heidelberg, *S*. Kentucky, *S*. Virchow and *S*. Agona have been reported to cause non typhoidal salmonellosis in humans in several parts of world. All isolates have been reported earlier to be transmitted from poultry sources. However, in India very few reports of such infections have been reported which can be attributed to lack of proper diagnosis or non reporting. In our study, *S*. Haifa was the predominant serotype obtained which draws serious attention towards public health owing to the zoonotic threat it poses. There has been considerable variation in the occurrence of the most common *Salmonella* serovars in domestic fowl in different countries and at different times. Certain serovars have been known to become wide spread in a country or geographical area for a given period and then decrease in incidence to a point of little importance (Borland, 1975).

Table 2. Results of rabbit ligated ileal loop test

Strain No.	*Salmonella* serotype	Dilatation index	Enterotoxin production
1.	Virchow	0.40	+
2.	Heidelberg	0.06	-
3.	Heidelberg	0.16	-
4.	Heidelberg	0.41	+
5.	Heidelberg	0.43	+
6.	Heidelberg	0.046	-
7.	Agona	0.13	-
8.	Kentucky	0.42	+
9.	Rough strain	0.11	-
10.	Rough strain	0.41	+
11.	Haifa	0.02	-
12.	Haifa	0.04	-
13.	Haifa	0.40	+
14.	Haifa	0.41	+
15.	Haifa	0.17	-
16.	Haifa	0.42	+
17.	Haifa	0.12	-
18.	Haifa	0.40	+
19.	Haifa	0.01	-
20.	Haifa	0.27	-
21.	Haifa	0.41	+
22.	Haifa	0.07	-
23.	Haifa	0.40	+
24.	Haifa	0.41	+

RLIL assay is a simple and perfect test to evaluate the pathogenic potential of bacteria. In our study, we found 12 out of 24 isolates produced dilatation index corresponding to pathogenic trait. Results are in agreement to Borah *et al*. (2004), who observed diffuse haemorrhage involving all layers intestinal wall with dilation index ranging from 0.4 to 0.5. The results showed variability in enterotoxigenicity of *Salmonella* isolates among the serovars and even within the serotype. Thus, enterotoxigenicity of *Salmonella* isolates can be of epidemiological significance.

Our results of haemolysin study correlated well with those of Singh *et al.*, (2003). They reported that most of *Salmonella* serovars revealed beneath colony haemolysis (BCH) on washed erythrocytes. The study of haemolysin pattern has been related to differentiation of *Salmonella* isolates upto subserovar level. Haemolysin typing for *Salmonella* can be used as an efficient epidemiological tool (Agrawal, 2005). Inability of isolates to secrete DNase, lecithinase, phospholipase A, and caseinase is in contrast to the findings of Agrawal (2005). They have reported variable ability of *Salmonella* to secrete DNase, lecithinase, phospholipase A and caseinase. Non production of these enzymes can be attributed to the fact that all isolates might have been less pathogenic as they were isolated from apparently healthy birds.

In conclusion, the present study revealed the prevalence of *Salmonella* is comparatively more in exotic breeds compared to native ones. This study in unique as it reports for the first time *S*. Haifa in India from poultry source which has prime importance owing to its ability in cause serious gastroenteritis in humans. Serotypes like

S. Heidelberg, *S.* Kentucky, *S.* Virchow and *S.* Agona, although previously reported in India from humans but this study is the first to report it from poultry source. This can serve an important link of tracing non-typhoidal salmonellosis in human population in India for epidemiological control

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