Indian Journal of Animal Sciences **85** (4): 367–369, April 2015/Short communication

Prevalence of emerging *Salmonella* serovars of zoonotic importance and their molecular characterization*

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Received: 25 March 2014; Accepted: 5 November 2014

Key words: Emerging serovars, Molecular characterization, Prevalence, *Salmonella*

In recent years the number of *Salmonella* serovars has increased. More than 2,579 serovars of *Salmonella* has been recorded across the world (WHO 2007). Among these, the most common non-typhoidal serovar isolated from human is *S.* Typhimurium followed by *S*. Enteritidis, whereas, the most common serovar of non-human origin is *S*. Typhimurium followed by *S*. Newport (CDC 2006). *S.* Typhimurium has diverse host range, which includes humans, cattle, pigs, sheep, horses, rodents and birds (Kingsley and Baumler 2000). Raw and inadequately cooked meat, eggs, milk and especially poultry are most commonly implicated vehicle for *Salmonella* infection (Rabsch *et al*. 2001). Factors such as increase in international travel and trade, inadvertent introduction of pathogens into new geographic areas, microbial adaptation and changes in food production system have resulted in emergence of *Salmonella* world-wide. Therefore, present study was undertaken to isolate and identify the *Salmonella* from man and animals and to know the type of pathogenic serovars existing in Pantnagar areas that are affecting the public health as well as health and production in the animals.

Samples used for isolation of *Salmonella* in the present study consisted of 682 samples comprising autopsied poultry tissues (60), poultry droppings (60), pig faeces (189), sheep faeces (11), cattle dung (105), buffalo dung (103) and human stool samples (154) from Pantnagar, Nagla, Jawahar nagar, Lalkuan and Rudrapur. The pre-enrichment medium for isolation of *Salmonella* was buffered peptone water. Selective enrichment media used were Tetrathionate broth, Rappaport-Vassiliadis broth and Selenite F broth. Selective plating media used was brilliant green agar (BGA), bismuth sulphite agar (BSA), Hektoen enteric agar (HEA) and xylose lysine deoxycholate (XLD) agar. These commercially available media were procured commercially. O antiserum poly A-I and Vi was procured for serological identification (slide agglutination test) of *Salmonella* spp. Oligonucleotide primers of *inv*A genes (for molecular confirmation of *Salmonella* as well as virulence status) used in present study was synthesized.

Salmonella organisms from faecal samples and samples from foods of animal origin were isolated as per WHO/CDC manual (2003) and USDA/FSIS guidebook (2002), respectively. *Salmonella* organisms were identified on the basis of cultural, morphological and biochemical reactions (Ewing 1986, Old 1996). Further, *Salmonella* organisms were identified by slide agglutination test using O antiserum poly A-I and Vi for their serological identification. Finally, *Salmonella* organisms were subjected to molecular identification as well as characterization using PCR technique by targeting *inv*A gene fragment following Chiu and Ou (1996).

The amplification was carried out in 25 µl reaction volume. The PCR thermal cycling conditions used for amplification of *inv*A gene include initial denaturation (94°C for 1 min) followed by 30 cycles of denaturation (94°C for 30 sec), primer annealing (60°C for 30 sec) and extension (72°C for 2 min). The final extension was applied at 72°C for 10 min. Agarose gel electrophoresis of amplified PCR products was carried out in 1.5% agarose gel in 0.5 × TBE buffer as per Sambrook and Russell (2001). *Salmonella* isolates identified on the basis of cultural, morphological and biochemical characters, slide agglutination test and molecular characterization were sent for serotyping to National *Salmonella* Center, Division of Bacteriology and Mycology, IVRI, Izatnagar, Bareilly.

Isolation of multiple serovars of *Salmonella* was approached to know the involvement of different types of *Salmonella* serovars in *Salmonella* infection. It was a significant finding that multiple serovars of *Salmonella* were recovered from poultry. As per the present study, it may be concluded that isolation of multiple *Salmonella* serovars can be approached to recover different types of *Salmonella* serovars from animal as well as human samples. Moreover, the involvement of multiple *Salmonella* serovars in the salmonellosis may pose strong virulence effect in the

*Part of Ph. D. thesis submitted by first author to G. B. Pant University of Agriculture and Technology, Pantnagar.

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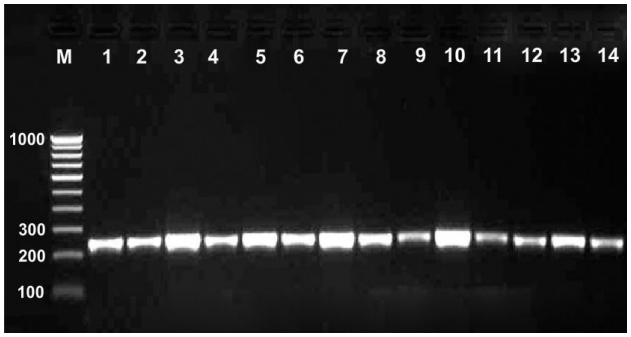

Fig. 1. Agarose gel electrophoresis showing amplified PCR products of *inv*A gene (244 bp). Lane M, 100 bp Marker; lane 1–14: *Salmonella* isolates.

pathogenesis of this disease.

Total 14 isolates of *Salmonella* were recovered from 682 samples and molecularly identified using PCR technique (Fig. 1). These isolates belonged to 5 different *Salmonella* serovars viz., *S.* Ughelli (5), *S.* Essen (3), *S.* Elisabethville (2), *S.* Lagos (2) and *S.* Drogana (2) which is of great zoonotic importance. The present findings indicated that a large number of *Salmonella* isolates were recovered from different kinds of samples. *Salmonella* Drogana was recovered from human source which is also of zoonotic importance.

In the present study, it is worth mentioning that *Salmonella* serovars, viz. *S.* Elisabethville, *S.* Essen, *S.* Lagos, *S.* Ughelli and *S.* Drogana were possibly recovered for the first time from different sources from Pantnagar and its vicinity. The emergence of these serovars might be due to introduction of organisms through the contaminated poultry or animal feeds or introduction of infected animal or poultry in these areas. However, Sharma and Thapliyal (1995) reported the prevalence of other serovars i.e., *S.* Typhimurium (1), *S.* Paratyphi B (2), *S.* Emek (2), *S.* Newport (4) and *S.* Saintpaul (56) in poultry. There was a peculiar finding that the serovar *S.* Drogana was recovered from human stool sample. As per reports available so far, this serovar appears to be the first isolate from human source in India. However, in India this serovar has been reported from non-human source. Singh *et al*. (2007) reported *S.* Drogana in equines from designated equine farms in India. Kidanemariama *et al*. (2010) also reported *S.* Drogana from non-human source i.e. poultry in a retrospective study on incidence of *Salmonella* in animals in South Africa during 1996 to 2006.

Table 1. Prevalence of emerging *Salmonella* serovars in different samples

S. No.	Sample		Serovar identified	No. of serovar	Prevalence of serovar (%)
1	Autopsied poultry tissues	(60)	*S.* Lagos	1	1.67
2	Poultry droppings	(60)	*S.* Ughelli	2	3.33
			S. Essen	1	1.67
			S. Elisabethville	2	1.9
3	Pig faeces	(189)	*S.* Ughelli	2	1.06
4	Sheep faeces	(11)	*S.* Essen	1	9.09
			S. Drogana	1	9.09
5	Cattle dung	(105)	*S.* Ughelli	1	0.95
			S. Essen	1	0.95
6	Buffalo dung	(103)	*S.* Lagos	1	0.97
7	Human stool samples	(154)	*S.* Drogana	1	0.65
	Total	(682)		14	

Figures in parenthesis are number of samples.

The highest prevalence of *S.* Ughelli, *S.* Essen, *S.* Elisabethville, *S.* Lagos and *S.* Drogana was found in poultry droppings, sheep faeces, poultry droppings, autopsied poultry tissues and sheep faeces (Table 1). Such a high prevalence of *Salmonella* in experimental sheep indicated towards the poor hygienic as well as managemental practices prevailing at the experimental house. However, Kane (1973) recorded comparatively lower prevalence (4.5%) of *Salmonella* in sheep at 2 slaughterhouses in the southern part of the North Island which might be due to good hygienic as well as managemental practices adopted at slaughterhouses. The lowest prevalence of *Salmonella* serovar was found as 0.65% in human stool samples which might be attributed to and good immune status of human beings and good personal hygiene adopted by them. However, Murugkar *et al.* (2005) reported higher prevalence rate (20.5%) of *Salmonella* in human beings which might be due to poor immune status of human beings, poor personal hygiene adopted by them and hyper-endemic zone of salmonellosis selected in the studies. Moreover, the prevalence also varies from time to time and from one geographical area to another due to emergence of organisms. In the present study, the most prevalent serovar was *S.* Ughelli (35.71%) followed by *S.* Essen (21.43%). The prevalence of *S.* Elisabethville, *S.* Lagos and *S.* Drogana was found as 14.28% each.

In the present study, emerging *Salmonella* serovars, viz. *S.* Elisabethville, *S.* Essen, *S.* Lagos, *S.* Ughelli and *S.* Drogana were first time recovered from Pantnagar and its vicinity, which might be attributed to introduction of these serovars from one geographical area to another within the country or even from a foreign land through animal and poultry feeds, foods of animal origin for human consumption or any imported products of animal origin. All these emerging serovars are of great zoonotic importance and may pose serious threat to the public health as well as health and production in the animals. Therefore, a comprehensive strategy should be made and followed regarding regular surveillance, effective prevention and treatment of disease.

SUMMARY

Samples (682) comprising autopsied poultry tissues, poultry droppings, pig faeces, sheep faeces, cattle dung, buffalo dung and human stool samples were collected and processed for isolation of *Salmonella* spp. Total 14 *Salmonella* isolates were recovered belonging to *S.* Ughelli

(5), *S*. Essen (3), *S*. Elisabethville (2), *S*. Lagos (2) and *S*. Drogana (2). These serovars emerged for the first time in Pantnagar and nearby areas. The serovar *S*. Drogana was recovered from human stool sample appears to be the first isolate from human source in India. Multiple *Salmonella* serovars were recovered from poultry droppings. The highest prevalence of *S*. Ughelli, *S*. Essen, *S*. Elisabethville, *S*. Lagos and *S*. Drogana was found in poultry droppings, sheep faeces, poultry droppings, autopsied poultry tissues and sheep faeces. Molecular characterization of *Salmonella* showed the presence of *inv*A gene in all the serovars isolated in the present study which suggested their molecular confirmation as well as virulence character.

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