

## SERO-PREVALENCE OF SALMONELLA INFECTION IN ANIMALS IN NORTH INDIA

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### ABSTRACT

On screening of 647 serum samples from 72 pet dog, 42 mares, 333 cows (aborted 19, healthy breeding cows 194, cows with pyrexia 120) and 200 buffalo (healthy breeding buffaloes 80, slaughtered for meat 120) a total of 51.7% female and 25% male pet dogs, 48.3% cows with pyrexia of unknown origin (PUO), 47.4% cows those aborted within last 15 days of serum collection, 6.2% healthy breeding cows, 26.2% breeding mares, 10.7% breeding buffaloes and 8.3% buffaloes slaughtered for meat were positive for anti-*Salmonella* cytotoxin-I immunoglobulins in indirect enzyme linked immuno-sorbent assay (ELISA). The prevalence of anti-*Salmonella* IgGs was significantly high ( $\chi^2 < 0.001$ ) in dogs, mares and cows either aborted or sick with PUO than in other animals. Significantly high prevalence ( $\chi^2 < 0.001$ ) of anti-*Salmonella* antibodies in cows either with PUO or abortion indicated the important role of salmonellosis in health and production of cattle in Northern India.

**Key words:** *Salmonella*, ELISA, seroprevalence, horses, cattle, dogs, buffaloes, abortion

Salmonellosis, a zoonotic infection affecting wide range of animals and humans, is world wide in distribution (Wray, 1995). The problem is complex because of very high number of *Salmonella* serovars, latent infections and faecal shedding by the infected and silent carriers (Popoff and LeMinor, 1997). Except a few host-adapted serovars, most of the *Salmonella* cause more or less similar type of disease (mostly indistinguishable from other septicemia) in different species of animals and in human beings. Seroprevalence and microbiological investigations of any infectious disease helps in knowing the status of the disease and furnish information for its control strategies. This paper describes the prevalence of *Salmonella* antibodies, an indicator of salmonellosis in animals in Northern India.

### MATERIALS AND METHODS

In this study, *Salmonella* cytotoxin I antigen common to most of the *S. enterica* subspecies *enterica* serovars (Singh and Sharma, 2000) was used in indirect ELISA for detecting *Salmonella* antibodies in 647

serum samples from mares (42), dogs (72), cows (333) and buffaloes (200). Serum samples with known history (Table I), collected within one preceding year were procured from serum bank at Centre for Animal Disease Research and Diagnosis (CADRAD), Indian Veterinary Research Institute, Izatnagar and stored at  $-20^\circ\text{C}$ .

Titres of anti-*Salmonella* immunoglobulins G (IgGs) in serum samples of different animals were determined with single dilution (1:200) indirect horse-radish-peroxidase (HRPO) enzyme linked immunosorbant assay (I-ELISA). *Salmonella* cytotoxin I (SCT-I) specific antibody (raised in guinea pig) coated ELISA plates (Singh and Sharma, 1999, 2000) were used. The SCT-I produced from *S. Weltevreden* strain (Singh and Sharma, 1999) was used as the antigen on the coated ELISA plates for capturing anti-SCT-I antibodies in test serum sample. SCT-I binding IgGs in serum samples of cattle, mares, buffalo and dog serum samples were detected using HRPO conjugated anti-cattle, anti-horse, anti-buffalo and anti-dog IgG serum (National Institute of Immunology, New Delhi), respectively. Ortho-phenylenediamine (OPD) phosphate (Sigma, USA) was used as substrate for color development. Negative and positive controls using reference serum available at National *Salmonella*

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**Table 1**  
**Prevalence of anti-*Salmonella* SCT-I antibodies in different animals in North India**

Animal	Sex	History	No. of animals tested	Positive for <i>Salmonella</i> antibodies
Dog	Male	Apparently healthy pet	12	3 (25.0%)
	Female	Apparently healthy pet	60	31 (51.7%)
Cattle	Female	(a) Apparently healthy for breeding	194	12 (6.2%)
		(b) Aborted	19	9 (47.4%)
		(c) clinically sick with pyrexia of unknown origin	120	58 (48.3%)
Buffaloes	Male	Apparently healthy for breeding	5	0 (0.0%)
	Female	Apparently healthy for breeding	75	8 (10.7%)
	Male	Slaughtered for meat	60	2 (3.3%)
	Female	Slaughtered for meat	60	8 (13.3%)
Horse	Mare	Apparently healthy breeding stock	42	11 (26.2%)
Total			647	137 (21.2%)

Centre, IVRI, Izatnagar were kept in each plate and colour intensity of reaction was read at 492 nm (Singh and Sharma, 2000). All tests were performed in triplicate using 1:200 diluted serum samples (in phosphate buffer, 0.15M, pH 7.2, 0.1% bovine serum albumin, Sigma, USA). To quantify anti-*Salmonella* IgGs, formula used was as under:

ELISA OD value = [(Average test OD - Average negative control OD) / Average negative control OD].

OD values with a mean = 1.9 (with no individual value less than 1.8) were taken as positive at which test has been reported 97.6% specific and 100% sensitive in goats (Chandra, 2002) and 95.7% specific and 100% sensitive in dogs (Verma, 2004) and horses (Babu, 2003).

## RESULTS AND DISCUSSION

None of the known *Salmonella* negative serum samples of dog, mare, cattle and buffalo yielded mean OD values exceeding 0.25 ( $\pm 0.051$ ), 0.39 ( $\pm 0.030$ ), 0.30 ( $\pm 0.048$ ) and 0.52 ( $\pm 0.032$ ), respectively, however all positive controls yielded OD value =  $2.13 \pm 0.054$ , irrespective of species of animal to which the serum belonged. Using the above criteria for positive test a total of 51.7% healthy female and 25% male pet dogs, 48.3% cows with pyrexia of unknown origin (PUO), 47.4% cows which aborted within previous 15 days of serum collection, 6.2% healthy breeding cows, 26.2% breeding mares, 10.7% breeding buffaloes and 8.3%

buffaloes slaughtered for meat were detected positive for *Salmonella* antibodies (Table 1).

Statistical analysis of the results (Table 2) using  $\chi^2$  test revealed that significantly more numbers of female buffaloes either on farm ( $\chi^2$ , 0.05) or slaughtered for meat ( $\chi^2$ , 0.06) were positive for anti-*Salmonella* IgGs in their serum than the male buffaloes, however difference in male and female populations of dog was statistically insignificant ( $\chi^2 > 0.2$ ). At the same time there was no significant difference between prevalence of anti-*Salmonella* IgGs among buffaloes kept on farm and slaughtered for meat ( $\chi^2 > 0.7$ ). Comparison of results of detection of anti-*Salmonella* IgGs in serum samples of females of different species the difference was highly significant ( $\chi^2 < 0.001$ ), and more number of bitches and mares were positive for *Salmonella* antibodies than cows and buffaloes ( $\chi^2 < 0.001$ ). Similar higher prevalence of anti-*Salmonella* IgGs was evident in male dogs ( $\chi^2$ , 0.006) than in male buffaloes. On comparison of prevalence of anti-*Salmonella* antibodies in sick and healthy cows significantly ( $\chi^2 < 0.001$ ) more number of cows sick with PUO or those aborted had positive titres than healthy stock indicating the important role of salmonellosis in health and production of cattle in Northern India.

Detection of *Salmonella* antibodies in large number of healthy and sick animals is not surprising because salmonellosis is hyper-endemic in India in human beings (John, 1996) however, little is known about its prevalence in livestock and pet animals. Of

**Table 2**  
**Chi-test statistics for anti-*Salmonella* IgG positive animals**

Groups of animals compared	n	Positive for <i>Salmonella</i> antibodies	Expected to be positive for <i>Salmonella</i> antibodies	Chi-test values
<b>Healthy female animals</b>				
Bitch	60	31	9.7	
Cow	194	12	31.5	
Buffalo	135	16	21.9	
Mare	42	11	6.8	0.000
<b>Healthy male animals</b>				
Dog	12	3	0.8	
Buffalo	65	2	4.2	0.006
<b>Total cattle tested</b>				
Sick	139	67	33.0	
Healthy	194	12	46.0	0.000
<b>All buffaloes according to use</b>				
Slaughtered for meat	120	10	10.8	
Kept for breeding and milk production	80	8	7.2	0.700
<b>Buffaloes slaughtered according to sex</b>				
Male	60	2	5.0	
Female	60	8	5.0	0.058
<b>All buffaloes according to sex</b>				
Male	65	2	5.9	
Female	135	16	12.2	0.053
<b>Dogs in respect of sex</b>				
Male	12	3	5.7	
Female	60	31	28.3	0.220

2500 *Salmonella* serovars reported, more than 720 serovars are prevalent in India and have been isolated from wild, pet and domestic animals and humans (Saxena *et al.*, 1989, Verma and Gupta, 1995, Verma *et al.*, 2001, Singh, 2007). Presence of *Salmonella* infected animals in community might be an important factor in maintaining the disease in endemic form in India because most of the serovars prevalent in animals have zoonotic potential and have also been commonly isolated from human cases of salmonellosis (Saxena *et al.*, 1989, John, 1996, Singh, 2007).

High prevalence of *Salmonella* antibodies in healthy pet and domestic animals could be attributed to poor hygienic standards of animal husbandry and close contact between human beings and animals. This hypothesis is further substantiated by earlier observations in dogs, reported to carry similar *Salmonella* serovars as detected in human beings (Sood and Basu, 1988, Saxena *et al.*, 1989, Verma and Gupta, 1995, John, 1996, Singh, 2007). Another important factor responsible for

high prevalence of *Salmonella* antibodies in dogs might be their diet because *Salmonella* is a food-borne pathogen and >12 % of meat and meat products in India have been reported positive for *Salmonella* (Sharma *et al.*, 1995, Thapliyal, 1995). Similar high prevalence of *Salmonella* antibodies have also been reported earlier in pet dogs using microagglutination tests with a battery of O and H antigens of different *Salmonella* serovars (Verma *et al.*, 2008).

High prevalence of *Salmonella* antibodies in cows with PUO and those aborted indicated an important role of *Salmonella* in inducing abortion and non-specific illness in cattle. Among buffaloes, healthy slaughtered males had lower prevalence of *Salmonella* antibodies than healthy females. It might be due to the fact that most of the healthy males are slaughtered at young age, while females are slaughtered at older age when these become unproductive. The other probable cause might be better colonization of *Salmonella* in female buffaloes causing repeat breeding, low

productivity, metritis and mastitis problems. Similar higher prevalence of *Salmonella* antibodies has been reported earlier in female than in male in goats (Chandra *et al.*, 2006, 2007) and horses (Singh and Sharma, 2000).

*Salmonella* has often been reported to be associated with abortion in mares either as primary cause or as secondary opportunist when mares are under stress from other viral and bacterial infections or physical stress due to work or environment (Kilborne, 1983). High prevalence of *Salmonella* antibodies in breeding mares (with no history of vaccination in last three years) in India are in concurrence to bacteriological findings reported earlier (Singh and Sharma, 2000, Singh, 2007, Singh *et al.*, 2007).

The study concludes that anti-*Salmonella* IgGs were more commonly present in serum samples of mares and dogs than in cattle and buffaloes. Significantly, female animals had high sero-positive *Salmonella* infection than male animals. Similarly, sick and aborted cows were significantly more sero-positive for *Salmonella* infection than the healthy stock indicating that salmonellosis might be an important infection in animals in Northern India.

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