

Seroprevalence study on salmonellosis in apparently healthy dogs by enzyme-linked immunosorbent assay

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ABSTRACT

Sera samples (575) were collected from 17 breeds of apparently healthy dogs from different parts of the country and tested for the presence of antibodies against cytotoxin-I antigen ELISA from *S. Weltevreden* (BM1613) to know the seroprevalence of salmonellosis. The overall seroprevalence was 58.08%. Place-wise the highest seroprevalence was in sera samples collected from Dehradun (92.00%), while breed-wise maximum sera samples of Labrador (81.58%) showed the presence of anti- cytotoxin-I antibodies of salmonella. The difference of seroprevalence of salmonellosis between 2 sexes was nonsignificant and the age group of 7–9 years and above showed the highest seroprevalence (63.37%). Our results showed a high seroprevalence of salmonellosis in dogs, which indicates a serious threat to human health.

Key words: Dog, ELISA, *Salmonella*, *Salmonellosis*

Salmonellosis of pet animals particularly dog plays an important role in epidemiology of the disease in man. Children are especially the most vulnerable due to their closeness to dogs. Sato *et al.* (2000) reported occurrence of *Salmonella* Virchow infection in infant transmitted by household dogs. It has been seen that 7.7% of healthy dogs carry *Salmonella* representing 20 different serotypes (Shimi *et al.* 1976). However, most of them suffer from sub-clinical or latent infection (Kozak *et al.* 2003) and act as carrier, so it is not easy to isolate *Salmonella* from them due to inconsistent excretion of pathogen. Therefore, immunological methods become a necessity for diagnosis of salmonellosis, which includes precipitation, agglutination, immunofluorescence tests and enzyme-linked immunosorbent assay (ELISA). Only little is known about prevalence of *Salmonella* infection or *Salmonella* antibodies (an indication of past or subclinical infections and/or carrier stage) in dog while it is the most important companion animal for old and weak persons who are prone for *Salmonella* infection, therefore insight into

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status of salmonellosis in dogs may be of significance from public health point of view (Wray and Wray 1999). The present paper deals with study on seroprevalence of salmonellosis in dogs by cytotoxin-I antigen based ELISA.

MATERIALS AND METHODS

Collection of serum samples: About 3.0 ml of blood from 575 dogs, which came for routine vaccination purpose, was collected from different sources (Table 1) in aseptic conditions using labelled sterile disposable syringes during October 2004 to May 2005. Serum was separated and stored at –20°C in small aliquots till use.

Antigen: Cytotoxin-I antigen was prepared from *S. Weltevreden* (BM1613 strain) as per Singh and Sharma (2000).

Enzyme linked immunosorbent assay (ELISA): ELISA was performed using *Salmonella* cytotoxin-I specific dog antibodies and cytotoxin-I antigen (Singh and Sharma 2000).

Briefly, each of the 96 wells of flat bottom polystyrene ELISA plates was coated with 100µl of anticytotoxin-I (dog) and incubated in humid chamber at 37°C for 6 h. The plates were washed thrice with PBST (phosphate buffer saline with 0.05% Tween–20). Thereafter remaining site in each well was blocked with 300 µl freshly prepared 5% (w/v) skim milk powder. After overnight incubation at 37°C, plates were washed with PBST thrice. Then to the wells of ELISA plates, 100 µl antigen prepared at the concentration of 0.1 mg protein/ml in 1 M Tris was added into the wells and incubated

for 2 h at 37°C as above. Wells were emptied and washed as earlier and to each well, a 100 µl of diluted (1: 200 in PBS with 0.1% BSA) test serum was poured in triplicate and incubated for 2 h at 37°C. Wells were emptied and washed as earlier and to each well, a 100µl of suitably diluted (1: 5000 in PBS with 0.1% BSA) anti dog IgG HRPO conjugate was added and incubated for 2 h at 37°C, plates were washed thrice with PBST as above and 100µl of freshly prepared substrate solution containing Orthophenyline diamine (OPD) and H₂O₂ made in citrate phosphate buffer (pH 4.6, 0.1 M) and plates were incubated for 20 min at 37°C in dark. The reaction was stopped by adding 100µl of 1 M H₂SO₄ in each well. Optical density (OD) of each well was read at 492 nm with ELISA reader. ELISA values were calculated as per Coker-Vann *et al.* (1984):

$$= (\text{Test OD} - \text{Negative control OD}) / \text{Negative control OD}$$

An ELISA value of 1.3 and above was considered as positive.

Statistical analysis: The chi-square analysis was applied (Snedecor and Cochran 1967) on results to know the significant difference between source, breed, sex and age on seroprevalence of salmonellosis in dogs.

RESULTS AND DISCUSSION

Dogs suffered from salmonellosis after ingestion of infected water, or consumption of raw and untreated food (Green 1998). The infected humans can also transmit the disease to companion animals (Becker *et al.* 1992). The factors playing an important role in the occurrence of salmonellosis in dogs include their coprophagia nature and the ability of the *Salmonella* to survive in various environments (Saladiova *et al.* 2001).

Serum samples (575) were collected from apparently healthy dogs (except those of Allahabad) of 17 breeds from different parts of India and subjected for determination of antibodies against cytotoxin-I antigen by ELISA. Cytotoxin-I antigen is the common antigen found in all pathogenic *Salmonella* and *S. Weltevreden* (BM1613 strain) was reported to be the best source of the cytotoxin- I (Singh and Sharma 1999), which was also used by Singh and Sharma (2000) to

detect about 2 400 pathogenic serovars of *Salmonella*. It has been shown to be a suitable antigen for studying seroprevalence in other animals too (Chandra *et al.* 2007, Singh *et al.* 2010). A total of 334 serum samples were found positive for anti-*Salmonella* cytotoxin- I (anti-SCT-I) antibodies with ELISA. The overall seroprevalence of salmonellosis in dogs was quite high (58.08%) with ELISA (Table 1), which might be due to the ubiquitous nature of this organism.

There was significant difference in prevalence of antibodies against *Salmonella* in dog sera samples of different places of the country ($P < 0.05$). It was highest in Dehradun (92.00%) and the lowest in Gajraula (36.36%), although sera samples of Allahabad had showed the maximum seroprevalence but due to its smaller sample size that too belonged to diseased dogs (Table 1), had no significance. This variation in place-wise prevalence is very much expected and has been observed in other animals too, because in different parts of the country different serovars of *Salmonella* predominates (www.up.gov.up.nic.in/ivri/nsc). Being primarily an intestinal bacteria, salmonellae are widespread in the environment but reported to be more prevalent in areas of intensive animal husbandry practices such as kennels and dog training centers. However, the results of ELISA do not support it, though the prevalence of agglutinins against 'O' and 'H' antigens of *Salmonella* in dogs of NTCD, Tekanpur, was reported to be highest in earlier report of Verma *et al.* (2008).

Breed-wise distribution of anti-SCT-I antibodies (Table 2) revealed that these were most prevalent in Labrador (81.58%), which is a large sport breed, prefer to roam outside,

Table 2. Breed-wise seroprevalence of salmonellosis in dogs

Place	Sera samples tested	Sera samples positive to ELISA (% positive)
Non descript	160	85 (53.13)
German Shepherd	113	69 (61.06)
Pomeranian	90	41 (45.56)
Indian Spitz	48	26 (54.17)
Labrador	76	62 (81.58)
Dobermann	48	28 (58.33)
Others*	40	23 (57.50)
Total	575	334 (58.09)

*Others include Great Dane, Boxer, Bhutia, Lhasa Apso, Dach shund, Pug, Dalmatian, Lion Badger, Bull dog, Cocker Spaniel, Golden Retriever breeds.

Table 3. Sex-wise seroprevalence of salmonellosis in dogs

Sex	Sera samples tested	Sera samples positive to ELISA (% positive)
Male	304	172 (56.58)
Female	271	162 (59.78)
Total	575	334 (58.09)

Table 1. Place-wise seroprevalence of salmonellosis in dogs

Place	Sera samples tested	Sera samples positive to ELISA (% positive)
Bareilly	351	156 (44.44)
NTCD, Tekanpur	70	59 (84.28)
MVC, Chennai	68	61 (89.70)
Dehradoon	25	23 (92.00)
Sambhal	22	10 (45.45)
Pantnagar	20	13 (65.00)
Gajraula	11	04 (36.36)
Allahabad	08	08 (100)
Total	575	334 (58.08)

Table 4. Age-wise seroprevalence of salmonellosis in dogs

Age in years	Sera samples tested	Sera samples positive to ELISA (% positive)
<1	56	32 (57.14)
1-<3	206	112 (54.37)
3-<5	128	78 (60.94)
5-<7	84	48 (57.14)
7 & above	101	64 (63.37)
Total	575	334 (58.09)

thereby more chances of exposure to *Salmonella* infection. The effect of breed on *Salmonella* infection was also noticed earlier in poultry and other animals which have resulted into development of *Salmonella* resistant strains. The ELISA results showed that sex (Table 3) had no influence on the prevalence of anti-SCT-I antibodies in dogs as reported earlier by Verma *et al.* (2008) also.

Age-wise prevalence (Table 4) of anti-SCT-I antibodies revealed that these increased with age indicating that either susceptibility of dogs to *Salmonella* increases with age as in human beings and other animals (Wray and Wray 2000) or repeated sub-clinical infection with the prevalent serovar boost to keep the antibody titer high enough to be detected positive. Our results are in agreement with studies on human where the prevalence of antibody titres against 'O' and 'H' antigen employing widal test were reported to increase with age in the endemic regions of typhoid (Cherian *et al.* 1990, Deborah *et al.* 2001).

Our results indicated that strict monitoring and surveillance of salmonellosis is need of the hour to protect the dog from *Salmonella* infection, which in turn will protect the health of dog owners, children and public in general by preventing the contamination of environment through shedding of salmonellae by dogs.

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