PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY AND RISK ASSESSMENTS OF NONTYPHOIDAL SALMONELLA FROM HEALTHY AND DIARRHEIC DOGS

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Dogs are also important asymptomatic carriers of *Salmonella* and it's antimicrobial resistant with the possibility of transmission to humans. Therefore, study was conducted to estimate the prevalence, risk factors and antimicrobial resistance profiling. A total of 415 rectal swab samples were collected and examined for presence of non-typhoidal *Salmonella*. Non-typhoidal *Salmonella* were isolated from 26 (6.3%) of the rectal swab samples, with significantly higher occurrence in diarrheic (15.2%) than non-diarrheic (5.5%) dogs, female than male, households with raw meat as a main feed type (23.1%; 95% CI=5–53.8) than leftover fed (10.1%) and practiced mixed feeding system (17%). *Salmonella* isolates showed higher resistance to ampicillin (41.7%), while all isolates were fully susceptible to gentamicin.

Keywords: Antimicrobial resistance, Dog, Non-typhoidal *Salmonella*, Prevalence, Risk factors.

***S**almonella* causes subclinical disease to acute fatal septicaemia, chronic diarrhoea and death in numerous animal species and in human beings (McGavin *et al.,* 2001). Animals can shed *Salmonella* serotypes into the environment without any apparent clinical signs. *Salmonella* is widespread in the environment and commonly found in farm effluents, human sewage and in any material subjected to faecal contamination Due to considerable geographical and temporal variation in the ₽ prevalence of *Salmonella* species in animals and humans, understanding the role of animals in zoonotic transmission is important to monitor salmonellosis (Leonard, 2014). Non-typhoidal *Salmonella* is an important zoonosis worldwide. It is reported that globally an estimated 65–380 million illnesses and 43–88 thousand deaths of human beings were associated with non-typhoidal *S.* enterica from the year 1990 to 2012 (Kirk *et al*., 2010). One of the sources for human salmonellosis is faeces of pet dogs and there have been reports on transmission of *Salmonella* from dogs to humans (Sato *et al*., 2000). It was reported that dogs can harbour large bacterial load (102 –106 per 100g of faeces) in their intestine, which can be shed in their faeces for several months. Thus, this carriage could be of significant importance to public health as dogs have close contact with family members in households. The concern of antimicrobial resistance is particularly important in developing countries, because of inadequate adherence to prudent use of antimicrobials; unhygienic living conditions; and close contact and sharing of houses between animals and humans (Feasey *et al*., 2012). Moreover, there is lack of information on the risk factors for dog salmonellosis and there is limited information on the antimicrobial susceptibility profiles of clinical isolates. Therefore, the objectives of this study were to estimate the prevalence of non-typhoidal *Salmonella* isolates, to assess the risk factors associated with *Salmonella* occurrence, and to identify antimicrobial susceptibility profiles of the isolates from apparently healthy and diarrheic dogs in all the six districtsof Kumaon region of Uttarakhand, India.

Materials and Methods

The study was conducted in 415 pet dogs comprising of 382 apparently healthy and 33 suffering with diarrhoea of all the six districts of Kumaon region of Uttarakhand, India. All dogs included in the study didn't take any medication with antimicrobial activity for the past 4weeks prior to sampling.A cross-sectional study was conducted from January 2022 to December
2022 to estimate the prevalence of *Salmonella* from rectal swab sample of 5 dogs. Dogs were sampled through door-to-door visit from households. Experimental study was conducted in Department of Veterinary Public Health and Epidemiology, to identify the bacteria and examine antimicrobial susceptibility patterns of *Salmonella* isolates. The sample size was determined using the formula given by by assuming simple random sampling. As there was no previous study on dog salmonellosis, the sample size was determined by assuming 50% expected prevalence; 5% desired absolute precision at 95% confidence interval; and based on the assumption of large dog population existing in the region putting under following formula.

$$n = \frac{1.962 \times Pexp\,(1 - Pexp)}{d^2}$$

Where n=sample size.
Pexp=expected Prevalence.
d=desired absolute precision

Prior to sample collection, individual animal's history of medication with antimicrobial agents was noted. Five rectal swab sampleswere collected from each dog after proper restraining with the help of the owner. The samples were placed into sterile buffered peptone water and transported to Department of Veterinary Public Health and Epidemiology, in box containing ice packs. Samples were processed for bacterial culture within 12h of arrival. In addition, questionnaire and observational survey were used to gather data on feeding practices as cooked animal products and mixed (raw meat, cooked animal products and household leftover) and sampled animal attributes such as sex, breed, body condition, and age.

Isolation and identification of Salmonella

Isolation and identification of *Salmonella* from rectal swab samples were performed according to the procedure recommended by the international standard organization (ISO) for *Salmonella.* Rectal swab samples were transferred into a tube with 9ml of buffered peptone water, shaken for approximately 2min and incubated at 37±1°C for 18±2h. A portion of the culture (0.1ml) was transferred into a tube containing 10ml of selective enrichment liquid media (Rappaport-Vassiliadis) and incubated at 42°C for 24h. Similarly, 1ml of the culture was transferred to a tube containing 10ml of tetrathionate broth and incubated at 37°C for 24h. A loopful of inoculum from each of enrichment cultures was then inoculated on the surface of two different plates, xylose lysine deoxycholate (XLD) agar and brilliant green agar (BGA) thereafter, incubated at 37 °C for 24±3h. For confirmation, presumptive *Salmonella* colonies from both XLD and BGA agar were selected and streaked onto the surface of pre-dried nutrient agar plates and incubated at 37 °C for 24h. Colonies from nutrient agar were tested for catalase, oxidase, and Gram's reaction. Presumptive isolates were inoculated into the biochemical test tubes for identification: triple sugar iron (TSI) agar, Simmon's citrate agar, Sulphide Indole Motility (SIM) medium and incubated for 24 or 48h at 37 °C. Colonies producing an alkaline (red) slant with acid (yellow) butt with hydrogen sulphide production (blackening) on TSI, positive for citrate

utilization (blue color), and negative(yellow-brown ring) for tryptophan utilization (Indole test), and negative for urea utilization were considered as *Salmonella* (ISO 2002). In addition, all of the tested isolates were motile. Positive control isolate/strain was obtained from VTCC (VTCCBAA504), Hisar, India.

Antimicrobial susceptibility test of Salmonel -la isolates

The antimicrobial susceptibility of the isolates was determined using the disk diffusion method, following the Clinical and Laboratory Standards Institute guidelines (CLSI 2018). After sub-culturing the obtained isolates on tryptic soy agar, 3 to 4 pure colonies were selected and inoculated into tubes containing 5ml of tryptic soy broth (TSB). These tubes were then incubated at 37°C for 4-5 hours. To standardize the turbidity, the suspensions were adjusted to 0.5 McFarland turbidity standards using sterile saline solution.Using a sterile cotton swab, excess inoculum was removed by firmly pressing and rotating the swab on the inside wall of the tube above the fluid level. The swab was then spread across the entire surface of a Mueller-Hinton agar plate. After allowing the plates at room temperature for 5-10 minutes to remove excess moisture, antimicrobial discs were placed on the plate using sterile forceps. The plates were inverted and incubated overnight at 35°C.The diameters of the resulting zones of inhibition were measured to the nearest millimetres using a transparent plastic ruler. Interpretation of susceptibility, intermediate, or resistance categories was based on the CLSI guidelines. For the purpose of analysis, all readings classified as intermediate were considered as resistant unless indicated. The antimicrobial discs were amoxicillin + clavulanic acid (20/10µg), gentamicin (10µg), tetracycline (30µg), sulfamethoxazole and trimethoprim (23.75 and 1.25µg), and ampicillin (10µg).

Data analysis

Data collection involved inputting and encoding all gathered information into a Microsoft Excel spread sheet. For statistical analysis, STATA software version 11.0 (STATACORP, 2009) was utilized. Prior to analysis, dog ages were categorized into two groups: young (less than 2 years) and old (over 2 years). Additionally, body condition scores were assigned using a 5-point scale, ranging from emaciated to obese. Descriptive statistics, including frequency and percentage, were employed to depict community practices, knowledge, and awareness concerning the disease. To evaluate the correlation between risk factors and Salmonella prevalence, Chi-square, Fisher exact test, and logistic regression analyses were employed. Significance was determined by a P-value less than 0.5 in all instances. In all the cases, $P<0.5$ was considered as significant association.

Prevalence

The prevalence of Salmonella varied across different districts (Table 2). The highest prevalence was observed in Nainital (10%), followed by Almora (9.9%), Bageshwar (7.0%), Pithoragarh (3.4%), Champawat (1.6%), and Udham Singh Nagar (0%). Among the 209 households surveyed, Salmonella was detected in 12.4%, with varying frequencies among the districts (Table-2). However, there was no significant association between different districts and the occurrence of Salmonella at both the animal and household levels. Salmonella-positive dogs were found in households across all districts in the Kumaon region, except in Udham Singh Nagar, with the highest prevalence (25%) observed in Nainital (Table-2).

Risk factors for Salmonella in dogs

Table-3 presents the results, indicating a significantly higher prevalence of Salmonella in female dogs (10.1%) compared to males (4.3%), as well as in diarrheic dogs (15.2%) compared to apparently healthy ones (5.5%). Female dogs were found to have 2.5 times higher faecal shedding of Salmonella than male dogs ($P<0.05$). Thin and fat body-

conditioned dogs had 2.8 and 1.5 times higher odds of Salmonella shedding, respectively, compared to dogs with medium body conditi -on. Furthermore, dogs fed uncooked preparat -ions had 2.0 times higher odds of harbouring Salmonella than those fed cooked reparations. However, no significant differences were observed regarding breed, age, feeding, feed treatment, body condition score (BCS), and educational status of dog owners (Table-3). The study also revealed a relatively higher prevalence (Table-4) of Salmonella shedding in households that primarily used raw food as the main feed for their dogs (23.1%), compared to those using leftover food (10.1%) or practicing mixed feeding (17%).

Antimicrobial susceptibility profiles

Out of the tested isolates (n = 24), all showed susceptibility to gentamicin, while varying degrees of resistance were observed for the other antimicrobials tested. Notably, a relatively high resistance rate was observed for ampicillin (41.7%), followed by tetracycline (21.2%), amoxicillin-clavulanate (12.5%), and trimethoprim-Sulfamethoxazole (4.2%). The control isolate remained suscepti -ble to all tested antimicrobials. The study found that 58.3% of Salmonella isolates exhibited resistance to at least one of the tested antimicrobials (Table-5). The dominant isolates showed resistance solely to ampicillin accounting for 20.8% of the isolates. Additionally, among the total examined Salmonella isolates, 2 (8.3%) demonstrated resistance to two classes of antimicrobials, specifically ampicillin, tetracycline, and amoxicillin-clavulanate. Furthermore, the study revealed that resistant isolates were evenly distributed across the assessed risk factors for canine salmonellosis (Table-6).

Dog handling practices in relation to Salmo-nella control

Dog handling, feeding, and hygiene practices varied among households, as shown in Table 7. A majority (71.3%) of dog owners relied on leftover food as dog feed, while none of them used commercial diets for their dogs. Regarding feed treatment, the majority (90.9%) of households occasionally cooked the dog's food. Additionally, it was noted that most dog owners (72.7%) were unaware of the risk of zoonotic dog salmonellosis. Furthermore, all owners stated that they cleaned their dog's kennel using bare hands.

Table-1: Prevalence of *Salmonella* based on clinical status of sampled dogs

Clinical state	Number of dogs examined	Number positive for *Salmonella*	Prevalence in % (95% CI)
Apparently healthy	382	21	5.5 (3.4–8.3)
Diarrheic	33	5	15.2 (5.1–31.9)
Total	415	26	6.3 (4.1–9.0)

Table-2: Prevalence of *Salmonella* in Kumaon region, Uttarakhand,, India

Districts	Total No. of dogs examined	Number of dogs positive for *Salmonella* (%)	Total household examined	Number of household positive For *Salmonella* (%)
Almora	101	10 (9.9)	72	10 (13.9)
Bageshwar	142	10 (7.0)	66	10 (14.9)
Champawat	61	1 (1.6)	23	1 (4.3)
Nainital	30	3 (10)	12	3 (25)
Pithoragarh	59	2 (3.4)	29	2 (6.9)
Udham Singh Nagar	22	0 (0)	7	0 (0)
Total	415	26 (6.3)	209	26 (12.4)

Table-3: Results of analysis on potential risk factors for *Salmonella* shedding by dogs in Kumaon region of Uttarakhand, India

Variables	No. of Animals examined	No.of Animals with *Salmonella* (%)	χ2 value (p-value)	Univariable LG analysis	
				Odds ratio (95% CI)	p-value
Sex					
Female	139	14 (10.1)	5.158 (0.023)	2.5 (1.1–5.5)	0.027
Male	276	12 (4.3)		*	
Breed					
Local	306	20 (6.5)	0.146 (0.732)	1.2 (0.5–3.1)	0.703
Cross	109	6 (5.5)		*	
Age					
Young	189	11 (5.8)	0.117 (0.732)	*	
Old	226	15 (6.6)		1.2 (0.5–2.6)	0.732
BSC					
Medium	284	14 (4.9)	3.600 (0.135)	*	
Fat	97	8 (8.2)		1.5 (0.4–5.3)	0.543
Thin	34	4 (11.8)		2.8 (0.8–8.3)	0.115
Feeding					
Leftover	288	15 (5.2)	2.596 (0.262)	*	
Raw meat	27	3 (11.1)		1.6 (0.6–4)	0.312
Both	100	8 (8)		1.4 (0.4–5.8)	0.612
Feed Rx					
Uncooked	31	1 (3.2)	0.527 (0.404)	2.0 (0.3–1.6)	0.478
Mixed	384	25 (6.5)		*	
Diarrheic					
No	382	21 (5.5)	4.821 (0.045)	-	-
No	382	21 (5.5)	4.821 (0.045)	-	-
Educational status					
Bellow Graduate	251	17 (6.8)	0.279 (0.597)	1.3 (0.5–2.9)	0.598
Graduate	164	9 (5.5)		*	

No. - Number, LG - Logistic regression, CI - Confidence Interval, BCS - Body condition score, Rx – Treatment, * - Explanatory variables

Table 4: Owner's awareness on the risk of zoonotic transmission of dog *Salmonella* among households of Kumaon region of Uttarakhand, India (n=209).

Variable items	Category	No. of HH respondents	No. positive	Prevalence in % (95% CI)	Chi-square (p value)
Feed type	Leftover food	149	15	10.1 (5.7–16.1)	3.026 (0.220)
	Raw meat	13	3	23.1 (5–53.8)	-
	Mixed	47	8	17 (7.6–30.8)	-
Feed treatment	Uncooked	19	1	5.3 (0.1–26.0)	0.988 (0.320)
	Mixed	190	25	13.2 (8.7–18.8)	-
Educational status of dog owners	Bellow Graduate	119	17	14.3 (8.5–21.9)	0.864 (0.238)
	Graduate	90	9	10 (4.7–18.1)	-
Knowledge on transmission of *Salmonella* to human	Yes	57	10	17.5 (6.1–16.5)	1.874 (0.171)
	No	152	16	10.5 (8.7–29.9)	-

Table 5: Drug resistance patterns of *Salmonella* isolates (n=24)

Resistant to	Name of the	Resistant isolates

	antimicrobial	Number	%
None	-	10	**41.7**
One antimicrobial	AMP	5	**20.8**
	TTC	4	**16.8**
Two antimicrobial	AMP, TMS	1	**4.2**
	AMP, AMC	1	**4.2**
	AMP, TTC	1	**4.2**
Three antimicrobial	AMP, AMC, TTC	2	**8.3**
Overall	--	**24**	**100**

Key: n - Number, AMP - Ampicillin, TTC - Tetracycline, TMS – Trimethoprim sulfamethoxazole, AMC - Amoxicillin-clavulanate

Table 6: Antimicrobial susceptibility profiles of *Salmonella* isolates based on risk categories

Categories	Number (%) of isolates:		
	Resistant to TTC	**Resistant to AMP**	**Susceptible to all**
Age			
Young (n=10)	3 (30)	4 (40)	5 (50)
Old (n=14)	4 (28.6)	6(42.9)	5 (35.7)
Feed			
Leftover food (n= 13)	2 (15.9)	4 (30.8)	6 (462)
Raw meat (n=11)	5 (45.5)	6 (54.5)	4 (364)
Sex			
Male (n=11)	3 (37.3)	6 (54.5)	3 (27.3)
Female (n= 13)	4 (30.8)	4 (30.8)	7 (53.8)
Breed			
Cross (n=5)	2 (40)	2 (40)	3 (60)
Local(n=19)	5 (26.3)	8 (42.1)	7 (36.8)

N= Number of Salmonella isolates tested from each variable category,
AMP Ampicillin, TTC Tetracycline

Table 7: Summary of dog management practices and dog owner's awareness on the risk of zoonotic transmission of Salmonella (n =209)

Variable items	Response	Number of respondents	%
Feed types	Commercial diet	0	0
	Leftover food	149	71.3
	Offal	11	5.3
	Mixed	49	23.4
Feed treatment	Uncooked	19	9.1
	Always cooked	0	0
	Sometimes cooked	190	90.9
House cleaning	Use glove	0	0
	Bare hand	209	100
	No clean	0	0
Water source	Tap water	209	100
	Groundwater	0	0
Addition of drug to feed	Yes	0	0

	No	209	100
Knowledge on transmissionof Salmonella tohuman	Yes	57	27.3
	No	152	72.7

N=Number of households examined

Results and Discussion

Out of the 415 dogs surveyed, 26 (6.3%) tested positive for Salmonella. The study indicated that the estimated prevalence of Salmonella in healthy dogs was 5.5%, while in diarrheic dogs, it was 15.2%. Please refer to (Table-1) for the corresponding confidence intervals.

Our study focused on determining the prevalence of Salmonella carriage in both apparently healthy and diarrheic dogs through bacteriological culture and biochemical identification. Additionally, an *in vitro* antimicrobial test was conducted using the disc diffusion method to assess the resistance profiles of Salmonella isolates against five antimicrobials used in both veterinary and human medicine. The study also aimed to identify potential risks for the transmission of salmonellosis in dogs and humans by employing a questionnaire. Our findings revealed a 6.3% faecal shedding prevalence of Salmonella among pet dogs in the Kumaon region. Significantly higher prevalence rates were observed in diarrheic dogs (15.2%) compared to apparently healthy ones (5.5%).This finding is within the range of 0 to 44% subclinical carriage of *Salmonella* in dogs (Sanchez *et al*., 2002). The higher Prevalence of *Salmonella* in diarrheic dogs is supported by previous studies of some workers Leonard *et al*., (2012) and Polpakdee *et al*., (2012).

Overall sub-clinical *Salmonella* shedding in our study (5.5%) is in accordance to the report of Amadi *et al*. (2018). Prevalence is influenced by factors such as pet sanitary practices, feeding habit, difference in public awareness and socioeconomic status of the owners. Despite the above facts, season of study, geographical areas, and diagnostic methods employed might have also accounted for the observed difference.

There was no significant difference between feeding of leftover, raw meat and both (leftover and raw meat). But the Prevalence ~~is~~ was higher in dogs fed on raw meat (11.1%) as compared to dogs fed on household leftover food (5.2%) and mixed diet (8%) as also observed by Finley *et al*. (2015). opined that feeding raw meat and other uncooked diets were risk factors for presence of *Salmonella* in dogs. Public Health Agency of Canada (PHAC) reported that raw meat and meat products were frequently contaminated with *Salmonella*, and consequently, homemade raw diets were considered as a potential source of *Salmonella*.

Our study showed that *Salmonella* shedding was significantly higher in female than male dogs. There was no significant ~~ce~~ difference between medium, fat and thin body condition score of the dogs, which is in accordance with the reports of Jajere *et al*. (2014)

Our results showed that all *Salmonella* isolates were susceptible to gentamic, may be due to the fact that gentamicin is not commonly used in veterinary sector in Kumaon. Meanwhile, some isolates have shown resistance against ampicillin (41.7%), tetracycline (21.2%), amoxicillin-clavulanate (12.5%), and trimethoprim-sulfamethoxazole (4.2%) because their frequent use in veterinary medications. Similarly, a previous study in Taiwan showed resistant isolates to tetracycline (77.5%) and sulfamethoxazole/ trimethoprim (37.5%) as also reported by Tsai *et al.,* (2007). These findings indicated that *Salmonella* drug resistance can vary from country to country and even from one area to another area in the same country.

Conclusions

Our study findings indicated a higher occurrence of non-typhoidal Salmonella in

diarrheic dogs compared to apparently healthy dogs across various districts in the Kumaon region.. The prevalence of Salmonella was particularly elevated in households that predominantly fed raw meat to their dogs, in contrast to those using leftover food or practicing mixed feeding. This suggests that dogs could serve as significant carriers of the organism, posing a risk for transmission to humans and other animals. Additionally, the presence of Salmonella isolates exhibiting resistance to multiple antimicrobials commonly used in veterinary and human medications poses a significant threat to both public health and veterinary sectors. This resistance limits the effectiveness of antimicrobial drugs for controlling Salmonella infections. Regular monitoring of circulating serotypes and assessment of multi-drug resistance profiles are crucial for the control of zoonotic salmonellosis, especially in areas where a substantial number of households keep dogs as pets.

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