

REPORT

Prevalence and antibiotic trials against *Salmonella enterica* isolated from diarrheic lambs and kids

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Abstract: *Salmonella enterica* (*S. enterica*) is the major zoonotic threat for small ruminants and humans responsible for huge economic losses and high mortality in Pakistan. Lambs and kids of Lahore district were examined to determine the prevalence, hematology and chemotherapy of *S. enterica*. A total of 200 diarrheic samples (n=100 lambs; n=100 kids) were collected and examined; 59 (29.50%) were found positive for *S. enterica*. Lambs had lightly greater prevalence (31%) than kids (29%). The frequency analysis (OR=1.16 [reciprocal =0.87]) showed non-significant difference in both the lambs and kids. The significant decrease (P<0.001) in hemoglobin, pack cell volume and total erythrocyte count was observed in infected lambs and kids. Results of *in-vitro* antibiotic susceptibility test revealed that *S. enteric* isolated from both lambs and kids were susceptible to levofloxacin, ciprofloxacin, ofloxacin, gentamicin, azithromycin, tobramycin, amoxicillin, ampicillin and nalidixic acid. Where as the results of *in vivo* antibiotic trials showed that isolates from both lambs and the kids with diarrhea were susceptible to levofloxacin and ciprofloxacin.

Keywords: *S. enterica*, antimicrobial susceptibility, lambs, kids, prevalence, hematology.

INTRODUCTION

Agriculture sector plays a fundamental role in the economy of Pakistan, sharing 11.4% in national gross domestic product (GDP). Livestock shared approximately 55.4 percent to the agricultural value added during 2012-13. There are 28.8 and 64.9 million heads of sheep and goats respectively, in Pakistan (Anonymous, 2013-14). Infectious diseases have been a serious threat for livestock (Elhaig *et al.*, 2016; Qayyum *et al.*, 2016; Yilmaz *et al.*, 2016). *Salmonella sp.* are Gram-negative, non-lactose fermenting, facultative anaerobes, motile and rod shape bacteria belonging to Enterobacteriaceae family. The infection is transmitted through ingestion of contaminated water and food (Mastroeni *et al.*, 2001). The paratyphoid group includes all flagellated serovars such as *Salmonella enterica* serovar Enteritidis, which causes food borne infectious diseases. Due to the capacity to infect a wide range of hosts, including humans and mammals, these enteric pathogens has emerged as a significant public health problem in the developing countries. Food borne infections caused by *Salmonella* serotypes occur at high rate in industrialized nations and developing countries, usually occurring as localized or widespread outbreaks causing high losses due to the health finance problems and discarding of contaminated food products (Selvarajet *al.*, 2010). In the United States alone, it is expected that

there are about 1.4 million cases of *Salmonella* infections resulting in 17,000 cases being hospitalized and 585 deaths each year. *Salmonella* is the cause for an estimated 26% of all infections caused by food borne pathogens in United States (Voetsch *et al.*, 2004). Antimicrobial resistance to drugs is a zoonotic health threat. There has been a limited work done on salmonellosis in small ruminants (sheep and goat) especially in Pakistan where the population of sheep and goats exceeds the population of bovine animals. Therefore, it is crucial to work on salmonellosis prevalence, antibiotic sensitivity and drug resistance by the pathogens among small ruminants. Owing to such and similar reasons, *Salmonella* was selected as a choice bacterium for *in vitro* antibiotic studies in lamb and kids.

MATERIALS AND METHODS

Collection of samples

The animals presented to various private and public sector veterinary hospitals and small ruminant farms located in district Lahore with the complaint of diarrhea was categorized into three forms including semisolid, loose or watery diarrhea. The data forms were based on species, age, sex and breed. A total of 200 diarrheic samples (n=100 lambs; n=100 kids) were examined for the presence of *Salmonella enterica*. Samples were graded after collection as semisolid, loose and watery based on fecal material consistency before their processing in

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Medicine laboratory, UVAS, Lahore. The collected samples were preserved as described by Pao *et al.* (2005).

Isolation and identification of salmonella enterica

Isolation and identification of *Salmonella enterica* was carried out by microscopy and following the standard protocols as stated by Krieg *et al.* (1994). After isolating, the pure culture of *Salmonella* on agar was subjected to biochemical tests for further confirmation. *Salmonella* isolated from lambs and kids both showed similar biochemical profile. Indole, Vogues-Proskauer and Urease tests were found to be negative for the selected isolates while Methyl red, Citrate utilization and motility test were found positive. For biochemical confirmation of *Salmonella*, triple sugar iron slants were used and *Salmonella* isolates showed yellow butt and pink slants with the production of hydrogen disulphide.

Hematological studies

Five milliliters blood were collected from 14 infected animals (n=7 lambs; n=7 kids) positive for *Salmonella enterica* and 14 healthy animals (n=7 lambs; n=7 kids) directly from the jugular vein into gel-clot activator containing vacutainer. The blood samples were analyzed for the estimation of hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC) and total leukocyte count (TLC) using hematological analyzer (Bio-Rad California USA).

Antibiotic trials

In vitro sensitivity of *Salmonella enterica* against various antibiotics like gentamicin (GM) 10ug oxoid, azithromycin (AZM) 15ug oxoid, tobramycin TM10ug oxoid, levofloxacin (LVX) 5ug oxoid, ofloxacin (OFX) 5ug oxoid, ciprofloxacin (CIP) 10ug oxoid, amoxicillin (A) 30ug oxoid, ampicillin (AP) 10ug oxoid and nalidixic acid (NA) 30ug oxoid was determined on Muller Hinton medium using Kirby-Bauer antibiotic sensitivity test. The assessment of antibiotics was against 30 identified isolates of *Salmonella* (n=15 lambs; n=15 kids) was based on zone of inhibition (ZI) as described by Shah and Korejo, (2012). Based on *in vitro* antibiotic results, two highly effective antibiotics were further evaluated for *in vivo* trials. Each antibiotic was administered in 14 diarrheic animals (n=7 lambs; n=7 kids) positive for *S. enterica*. Efficacies of antibiotics were evaluated based on disappearance of clinical signs e.g. diarrhea.

STATISTICAL ANALYSIS

The data regarding prevalence of *S. enterica* was analyzed by frequency analysis using chi-square test. The percentages and 95% confidence limit for some parameters were determined and where appropriate odd ratio was also computed, whereas data on hematology and antibiotic sensitivity were analyzed by Student's *t*-test, using SPSS (statistical package for social science), *P*<0.05 was considered significant (Thrusfield, 2007).

RESULTS

Prevalence of *S. enterica* in lambs and kids

A total 200 fecal samples (n=100 lambs; n= 100 kids) suspected for *S. enterica* were tested microbiologically. The overall prevalence of *S. enterica* in lambs and kids was recorded 29.50% (59/200) as given in table I. Overall prevalence of *S. enterica* in score-1 (semisolid fecal material), score 2 (loose feces) and score-3 (watery feces) were recorded as 17.5%, 43.18% and 52.77%, respectively. The prevalence of *S. enterica* in lambs was 31% while prevalence of *S. enterica* in lambs in Score-1, Score-2 and Score-3 was recorded as 20.63%, 40.00% and 58.82%, respectively. The overall prevalence of *S. enterica* in kids was 28% while prevalence in Score-1, Score-2 and Score-3 was recorded as 14.04%, 45.83% and 47.37%, respectively. Frequency analysis using chi-square test showed significant (*P*<0.001) difference among different score (table I).

Hematological studies

Hematological studies showed that hemoglobin level, total erythrocyte count and packed cell volume decreased significantly (*P*<0.001), while total leukocyte count increased significantly (*P*<0.001) in lambs. Similarly, this study showed that hemoglobin level, packed cell volume and total erythrocyte count decreased significantly (*p*<0.05), while total leukocyte count increased significantly (*p*<0.05) in kids. Average values for below mentioned hematological parameters are presented in table II.

Antibiotic trials

The isolates capable of causing salmonellosis in lambs and kids were selected for testing the *in vitro* antibiotic susceptibility profiles against 9 antibiotics using the Kirby Bauer method. The diameter (mm) of zone of inhibition around *S. enterica* isolates are given in table III. It indicates the interpretation of the results according to the standards of diameter of the zones of inhibition of different antibiotics against the 15 disease causing isolates of *S. enterica*. Efficacy of drugs was analyzed according to the standard zones of inhibition of antibiotics on media as provided by Andrews, (2009). According to the results, most of the antibiotics were susceptible to *S. enterica* exhibiting zones of inhibition around the antibiotic discs. Data showed that *S. enterica* was the most sensitive to levofloxacin followed by ciprofloxacin, ofloxacin, gentamicin, azithromycin, tobramycin, amoxicillin, ampicillin and nalidixic acid in both lambs and kids.

Out of the 9 drugs, levofloxacin and ciprofloxacin which showed higher *in vitro* antibiotic sensitivity against *S. enterica* were selected for *in vivo* therapeutic trials in 14 live animals (n= 7 lambs; n= 7 kids). The results for *in vivo* antibiotic trials against *S. enterica* in diarrheic lambs and kids are given in table IV and V respectively. Among

Table 1: Prevalence of *Salmonella enterica* in diarrheic lambs and kids

Species/Score	No. of Animal	Positive		95% CI	Odd Ratio/ reciprocal	MH Chi-sq P-value
		N	%			
Species						
Lambs	100	31	31.0	22.53-40.56	1.16/0.87	-
Kids	100	28	28.0	19.87-37.39		
Total	200	59	29.5	23.49-36.10		
Overall Fecal Score						
Score-1	120	21	17.50	11.47-25.08	-	P 0.001
Score-2	44	19	43.18	29.21-58.02		
Score-3	36	19	52.77	36.57-68.55		
Total	200	59	29.50	23.49-36.10		
Lambs						
Score-1	63	13	20.63	11.99-31.93	-	P 0.007
Score-2	20	08	40.00	20.61-62.11		
Score-3	17	10	58.82	34.97-79.89		
Kids						
Score-1	57	08	14.04	6.74-24.91	-	P 0.003
Score-2	24	11	45.83	26.96-65.66		
Score-3	19	09	47.37	26.13-69.36		

[Score-1 (Semisolid feces), Score-2 (Loose feces), Score-3 (Watery feces)]

Table 2: Influence of *Salmonella enterica* on various hematological parameters in Lambs and Kids (Mean $\pm$ SD)

Hematological parameters	Lambs			Kids		
	Healthy	Infected	P-Value	Healthy	Infected	P-Value
Hemoglobin (g/dl)	11.33 $\pm$ 0.56	7.83 $\pm$ 0.61*	0.001	9.96 $\pm$ 0.55	7.62 $\pm$ 0.38*	0.001
PCV (%)	33.89 $\pm$ 0.84	31.25 $\pm$ 0.41*	0.001	31.93 $\pm$ 0.60	29.09 $\pm$ 0.43*	0.001
TEC (10 ¹² /l)	16.68 $\pm$ 0.53	13.60 $\pm$ 0.43*	0.001	14.12 $\pm$ 0.59	12.14 $\pm$ 0.35*	0.001
TLC (10 ⁹ /l)	11.98 $\pm$ 0.78	14.18 $\pm$ 0.42*	0.001	10.17 $\pm$ 0.67	13.05 $\pm$ 0.50*	0.001

(*Indicates the significant difference from the healthy)

lambs levofloxacin and ciprofloxacin were effective for treatment of all (100%) animals. After 2nd day 2(28.57%) animals were recovered, 6(85.71%) after 3rd day 7(100%) and after 4th day making 100% overall efficacy of levofloxacin. Ciprofloxacin recovered 3(42.86%) animals after 2nd day of treatment, 4(57.14%) after 3rd day, 6(85.71%) after 4th day and 7(100%) after 5th day, so overall efficacy of ciprofloxacin was 100% in lambs. Among kids, levofloxacin and ciprofloxacin both cured 7 out of 7(100%). After 2nd day 3(42.86%) were recovered, 5(71.43%) after 3rd day and 7(100 %) after 4th day making 100% overall efficacy of levofloxacin. Ciprofloxacin was found effective for 2(28.57%) cases after 2nd day, 5(71.43%) after 3rd day, 6(85.71%) after 4th day and 7(100%) after 5th day, so overall efficacy of ciprofloxacin was 100% for kids.

DISCUSSION

The findings of current study regarding prevalence congruent with the findings of Bonke *et al.* (2012) who reported 43% prevalence of *Salmonella* in the feces of sheep and goats at slaughterhouse. Climatic conditions might be a factor in the difference between the two studies. Similarly, current findings are in close resemblance with the findings of Chandra *et al.* (2006)

who reported 17.6% prevalence of salmonellosis in goats. Woldemariam *et al.* (2005), reporting 12.1% salmonellosis in apparently healthy slaughtered sheep and goats at debrezeit abattoir, Ethiopia. Current findings are not in agreement with the findings of Keen *et al.* (2007) who found 11.1% prevalence of salmonella. Molla *et al.* (2006) reported 11.5% and 3% salmonellosis in apparently healthy slaughtered sheep and goats.

There was a lack of literature regarding reasons for variation in the hematological parameters during *S. enterica* infections in lambs and kids. Our results coincide with the results of the Dangana *et al.* (2010) who found a decrease in PCV and Hb and hence support findings of the current study but the TLC values did not match with this study as it increases in the case of salmonellosis that may be providing protection to animal to avoid the disease.

Our results regarding antibiotic trials match with the findings of Ali *et al.* (2010) who found that 33% *Salmonella* isolates were resistant to ampicillin. Current study results also match with the findings of Tajbakhsh *et al.* (2013) who found that *Salmonella* isolates from raw cow milk offered highest 78.57% resistance against nalidixic acid. Shah and Korejo (2012) found similar

Table 3: In-vitro antibiotic sensitivity against *Salmonella enterica* in lambs and kids

Antibiotics Sensitive Discs	No. of samples	Remarks					
		Sensitive		Intermediate		Resistant	
		No. of isolates	ZI (mm)	No. of isolates	ZI (mm)	No. of isolates	ZI (mm)
Lambs							
Levofloxacin	15	14	>16	01	14-16	-	<13
Ciprofloxacin	15	13	>19	01	17-19	01	<16
Ofloxacin	15	11	>28	02	26-28	02	<25
Gentamicin	15	10	>19	03	17-19	02	<16
Azithromycin	15	10	>16	03	14-16	02	<13
Tobramycin	15	11	>20	02	18-20	02	<17
Amoxicillin	15	09	>14	04	12-14	02	<11
Ampicillin	15	10	>14	04	12-14	01	<11
Nalidixic acid	15	08	>17	04	16-17	03	<15
Kids							
Levofloxacin	15	12	>16	03	14-16	-	<13
Ciprofloxacin	15	13	>19	01	17-19	01	<16
Ofloxacin	15	12	>28	02	26-28	01	<25
Gentamicin	15	11	>19	02	17-19	02	<16
Azithromycin	15	10	>16	03	14-16	02	<13
Tobramycin	15	11	>20	02	18-20	02	<17
Amoxicillin	15	09	>14	03	12-14	03	<11
Ampicillin	15	11	>14	03	12-14	01	<11
Nalidixic acid	15	08	>17	04	16-17	03	<15

ZI= zone of inhibition

Table 4: In vivo antibiotic trial of Levofloxacin & Ciprofloxacin against *Salmonella enterica* in diarrheic Lambs

Antibiotics	Treatment Group	Drug efficacy (%)				P-Value
		2nd day	3rd day	4th day	5th day	
Levofloxacin	n=7	2 (28.57%)	6 (85.71%)	7 (100%)	7 (100%)	0.555
Ciprofloxacin	n=7	3(42.86%)	4 (57.14%)	6 (85.71%)	7 (100%)	

Table 5: In vivo antibiotic trial of Levofloxacin & Ciprofloxacin against *Salmonella enterica* in diarrheic Kids

Antibiotics	Treatment Group	Drug efficacy (%)				P-Value
		2 nd day	3 rd day	4 th day	5 th day	
Levofloxacin	n=7	3(42.86%)	5 (71.43%)	7 (100%)	7 (100%)	0.392
Ciprofloxacin	n=7	2 (28.57%)	5 (71.43%)	6 (85.71%)	7 (100%)	

results accordingly, 55.9% *Salmonella* isolates from poultry meat were resistant to nalidixic acid while no resistance was offered against ciprofloxacin. These results match with the results of the current study, as ampicillin resistance was found in 60% of ETEC isolated from diarrheic lambs and kids. Fatma *et al.* (2012) found similar results with reference to *Salmonella* isolates from hatcheries. These authors found highest sensitivity to enrofloxacin, lower

REFERENCES

Ali, NH, Farooqui A, Khan A, Khan AY and Kazmi SU (2010). Microbial contamination of raw meat and its

environment in retail shops in Karachi, Pakistan. *J. Infect. Dev. Ctries.*, **4**(6): 382-388.

Andrews JM(2009). BSAC standardized disc susceptibility testing method (version 8). *J. Antimicrob. Chemother.*, **64**: 54-489.

Anonymous, Pakistan Economic Survey, 2013-14. sensitivity against gentamicin and 100% resistance to nalidixic acid.

Bonke R, Wacheck S, Bumann C, Thum C, Stuber E, Konig M, Stephan R and Fredriksson-Ahomaa M (2012). High prevalence of *Salmonella enterica* subsp. *diarizonae* in tonsils of sheep at slaughter. *Food Res. Int.*, **45**(2): 880-884.

- Chandra M, Singh BR, Shankar H, Agarwal M, Agrawal RK, Sharma G and Babu N (2006). Study on prevalence of Salmonella infection in goats. *Small Rumin. Res.*, **65**(1): 24-30.
- Dangana A, Ajobiewe J and Nuhu A (2010). Haematological changes associated with Salmonella typhi and Salmonella paratyphi in humans. *Int. J. Biomed. and Health Sci.*, **6**(4): 219-222.
- Elhaig MM, Selim A, Mahmoud MM and El-Gayar EK (2016). Molecular confirmation of Trypanosoma evansi and Babesia bigemina in cattle from lower Egypt. *Pak. Vet. J.*, **36**: 409-414.
- Fatma AG, Gohary AH, Bably MA and Mohamed AA (2012). In vitro antibiotic sensitivity of isolated strains of salmonella and E.coli from poultry farms. *Int. Sci. Conf., Mansoura.*, pp.191-199.
- Keen JE, Durso LM and Meehan TP (2007). Isolation of Salmonella enterica and Shiga-Toxigenic Escherichia coli O157 from Feces of Animals in Public Contact Areas of United States Zoological Parks. *Appl. Environ. Microbiol.*, **73**: 362-365.
- Krieg NR, Holt JG, Sneath PHA, Staley JT and Williams ST (1994). *Bergey's Manual of Determinative Bacteriology*, 9th Ed. Williams & Wilkins, Baltimore, Md, USA. pp.1-8.
- Mastroeni P, Chabalgoity JA, Dunstan SJ, Maskell DJ and Dougan G (2001). Salmonella: Immune responses and vaccines. *Vet. J.*, **161**(2): 132-164.
- Molla W, Molla B, Alemayehu D, Muckle A, Cole L and Wilkie E (2006). Occurrence and antimicrobial resistance of Salmonella serovars in apparently healthy slaughtered sheep and goats of central Ethiopia. *Trop. Anim. Health Prod.*, **38**: 455-462.
- Pao S, Patel D, Kalantari A, Tritschler JP, Wildeus S and Sayre BL (2005). Detection of Salmonella strains and Escherichia coli O157: H7 in feces of small ruminants and their isolation with various media. *Appl. Environ. Microbiol.*, **71**: 2158-2161.
- Qayyum A, Khan JA, Hussain R, Awais M, Ahmad N and Khan MS (2016). Investigation of milk and blood serum biochemical profile as an indicator of sub-clinical mastitis in Cholistani cattle. *Pak. Vet. J.*, **36**: 275-279.
- Selvaraj R, Das R, Ganguly S, Ganguli M, Dhanalakshmi S and Mukhopadhyay SK (2010). Characterization and antibiogram of Salmonella spp. from poultry specimens *J. Microbiol. Antimicrob.*, **2**(9): 123-126.
- Shah A and Korejo N (2012). Antimicrobial Resistance Profile of Salmonella Serovars Isolated from Chicken Meat. *J. Vet. Anim. Sci.*, **2**: 40-46.
- Tajbakhsh F, Tajbakhsh E, Rahimi E and Momenii M (2013). Determination of Antibiotic Resistance in Salmonella Spp Isolated from Raw Cow, Sheep and Goat's Milk in Chaharmahal Va Bakhtiyari Province, Iran. *Global Veterinaria.*, **10**(6): 681-685.
- Thrusfield M (2007). *Veterinary Epidemiology*. Blackwell Science, Oxford UK.
- Voetsch AC, Van Gilder TJ, Angulo FJ, Farley MM, Shallow S, Marcus R, Cieslak PR, Deneen VC and Tauxe RV (2004). Food Net estimate of the burden of illness caused by nontyphoidal Salmonella infections in the United States. *Clinic. Infec. Dis.*, **38**(3): 127-134.
- Woldemariam E, Molla B, Alemayehu D and Muckle A (2005). Prevalence and distribution of Salmonella in apparently healthy slaughtered sheep and goats in Debre Zeit, Ethiopia. *Small Rumin. Res.*, **58**(1): 19-24.
- Yilmaz R, Cangul IT, Onat K, Akkoc A, Ozyigit MO and Akdesir E (2016). Histopathological, immuno-histochemical and bacteriological characterization of Mycoplasma bovis pneumonia in cattle. *Pak. Vet. J.*, **36**: 316-321.