The Journal of Animal & Plant Sciences, 24(1): 2014, Page: 98-102
ISSN: 1018-7081

PREVALENCE OF *SALMONELLA* IN DIARRHEIC ADULT GOATS IN FIELD CONDITIONS

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ABSTRACT

An active surveillance was undertaken to determine the prevalence of *Salmonella* from the diarrheic feces of adult goats, in field conditions. *Salmonella* is a principal cause of food-borne diseases in the world, and the most common zoonotic bacterial disease of adult goats. In this long study of one year, a total of 744/2029 cases of diarrhea were identified, which revealed an overall prevalence of diarrhea in goats to be 36.7% and salmonellosis 0.1%, with the help of commercially available API 20E kits. A significant association of a number of cases of diarrhea with the environmental temperature and humidity level of the area was established but there was no effect of season, temperature or humidity levels with the prevalence of salmonellosis in the area. Open grazing was a common practice and the animals would eat garbage, grass, leaves and drink standing water, available to them. The detection of *Salmonella* species in feces, from field conditions, needs special attention to prevent an outbreak in animal and human population in the area.

Keywords: Prevalence, *Salmonella*, diarrhea, adult goats

INTRODUCTION

Salmonella, a principal cause of food-borne diseases in the world has been isolated from the feces, lungs and liver of slaughtered goat and prevalence of 0.7% has been recorded (Molla *et al*. 2006; Ziino *et al*. 2009; Cheila *et al*. 2011). It is transmitted mainly through contaminated food and characterized clinically by septicemia and enteritis. Garg *et al*. (1979) has reported the possibility of transfer through upper respiratory tract of animals as well. The detection of these organisms in meat may constitute a serious public health concern (Adesiji *et al*. 2011). Not all the food animals, which contract *Salmonella*, develop salmonellosis, rather such animals become carriers, along with the recovered ones from the disease and such animals are constant source of zoonosis (Pao *et al*., 2005; Ekperigin and Nagaraja, 1998). In a study on 100 human patients suspected from food-borne disease, from four different hospitals of district Lahore, on an average 14% and 10% stool samples were found positive for *Salmonella enteritidis* and *S. typhimurium* (Muhammad *et al*., 2011).

Amongst the common zoonotic bacterial diseases of adult goats characterized by diarrhea, the most frequent one is salmonellosis (Hungerford, 1990; Radostits *et al*. 2007; Smith and David 2009; Kahn *et al*. 2010). On the other hand, Bulgin and Anderson (1981), has reported bloody diarrhea in young calves and adult cattle but not in goats. Diarrhea, a complex, multi-faceted illness, remains an alarming threat and a leading cause of mortality and morbidity both in humans and animals (Reid, 1976; Hungerford, 1990).

Capra hircus, the domestic goat of Pakistan, is primarily raised for mutton by millions of poor and landless communities (Yasmin, 2003; Khan *et al*., 2008). According to the livestock census 2006, Pakistan had the third largest goat population, and a third of all goats in Pakistan are reared in the province of Punjab (Khan *et al*., 2008). Keeping in view the importance of goat as a vital source of meat, and a potential zoonotic threat, the present study was designed to conduct an epidemiological survey of salmonellosis in adult goats, from the field, under different environmental and managemental conditions and husbandry practices.

MATERIALS AND METHODS

Geo-location of Study: An active surveillance was undertaken to determine the prevalence of a salmonellosis, associated with diarrhea in adult goats (meant for human consumption) in Lahore, Punjab, Pakistan, which is located between 31°15′—31°45′ N and 74°01′—74°39′ E. The weather of Lahore is extreme during the months of May, June and July, when the temperature reaches up to 40–48 °C (104–118 °F). From June till August, the monsoon seasons starts, with heavy rainfall throughout the province. The study was conducted over a 12-month period from January to December, 2010 and the data regarding climatic conditions (temperature and relative humidity) during the study period was retrieved from Regional Meteorological Centre, Lahore, Pakistan.

Epidemiological Survey: A multistage probability sampling method was practiced for active surveillance,

with random selection of 8 villages from the rural unit of southern district Lahore, designated as V1, V2, and so on. A total of 744 goats (Table 1) from these villages were identified suffering from diarrhea, on the basis of clinical signs. Each homestead was visited from door to door and a questionnaire based survey was conducted, relating to the information of affected animals (morbidity, mortality and case fatality rates).

Fecal Sampling and Analyses: Fecal samples were collected directly from the rectum of each adult goat suffering from diarrhea, however more than one sampling was implied upon recurrence of diarrhea, for the detection and identification of etiologic agent of salmonellosis. The fecal samples were subjected to culture for selective isolation of *Salmonella* species and later on, ELISA was performed through commercially available API 20E kits (Biomerieux, France).

The fecal samples were suspended in test tubes in phosphate buffered saline (PBS) and mixed thoroughly and left to stand for 15 min. The supernatant collected was streaked on MacConkey Agar petri plates and incubated at 37 ºC for 24 h. The specific colonies of *Salmonella*, colourless and translucent in appearance were collected and enriched by incubating in selenite broth at 37 ºC for 24 h. The culture from the selenite broth was purified by streaking on the SS Agar and incubated at 37 ºC for 24 h and a pure culture of *Salmonella* was obtained. For the microscopic examination and identification of *Salmonella*, Gram staining was performed on the colonies grown on the SS Agar. In the API 20E biochemical testing of the *Salmonella*, described procedures by the manufacturers were employed. The colours obtained in the wells of the strip of API 20E kits were recorded to make a 7-digit profile number. The API software was used for the identification of bacterial species.

Clinical Observation and Monitoring of Husbandry Practices: Pro forma based data were collected regarding visible signs and symptoms of the cases having diarrhea. Furthermore, their husbandry/dietary practices, and generalized body conditions were also assessed in order to devise strategic and directional approach for prevention of diarrhea in goats.

Statistical Analysis: Data were analyzed using the Statistical Package for Social Science (SPSS for Windows version 12, SPSS Inc., Chicago, IL, USA). Data collected regarding morbidity, mortality and case fatality rate were subjected to Chi square analyses.

RESULTS AND DISCUSSION

In this study over a period of one year, a total of 744 cases of diarrhea were identified, among a population of 2029 heads of goats, which revealed an overall prevalence of diarrhea to be 36.7%. This is quite an alarmingly high number that could spoil the economy of the farmers. This coincides with the findings of Hunduma *et al*. (2010) who stated diarrhea as a major problem among goats with a higher prevalence of 42.2%. The village wise results of prevalence revealed to be highest (18.9%) for V1 (383/2029), which was an area with the highest population (915) as well and the lowest morbidity rate of diarrhea was recorded in the small population (21) village V8 (0.3%) and a significant difference was recorded between the villages ($P < 0.05$) (Table 1) (Asif *et al*., 2008).

In the current study the diarrhea was graded into four classes based on its consistency and it was found that 63.8% (475/744) i.e. the majority of the cases suffered from pasty diarrhea. Semi-liquid type of diarrhea was found in 24.3% (181/744) of cases, 9.3% (69/744) were having watery and only 2.6% (19/744) cases were with bloody diarrhea. A significant difference was recorded in the consistency of stool with pasty and semi-liquid type diarrhea as the most common types prevalent, in this study. According to findings of Asif *et al*. (2008) factors as overcrowding of livestock, lack of proper drainage and grazing the young and adult stock at the same place significantly accounts for maintaining the cycle of infections. Furthermore, poor nutritional value of livestock and the economy of the local people in the area attributes to the poor immunity in the animals.

Salmonellosis: Out of the total 744 cases of the diarrheic animals, only two cases of stool were found positive for the *Salmonella* species, from two different locations in the area i.e. one from V1 and second from V3. Both the animals were in very poor health status with profuse bloody diarrhea, a finding also reported by Van *et al*. (2000). Munoz *et al*. (1996) has also reported isolation of *Salmonella* from diarrheic goat kids and a prevalence of 2.7% was recorded. But Adesiyun *et al*., (1993) reported no significant difference in prevalence of *Salmonella* between diarrheic and non diarrheic animals. The overall morbidity and mortality rate recorded in the present study was 0.1%, whereas the case fatality rate was 100% (Table 2), which matches with the findings of Bulgin and Anderson (1981), where high mortality rate was reported in salmonellosis despite treatment. Radostits *et al*. (2007) have also reported 100% mortality with salmonellosis.

In general the prevalence of *Salmonella* in goat population was very low. The finding of such a low prevalence rate in the present study was also supported by D'Amico *et al*. (2008) who surveyed 133 milk samples but *Salmonella* could not be recovered even from a single sample of goat, sheep and cow's milk. Radostits *et al*. (2007) reported its prevalence in other species but no record was available for prevalence in goats. Similarly Adesiyun *et al*., (1993) recorded highest prevalence in calves (4.8%) but non was isolated from kids. In another

study on caprine abortions Masala *et al*. (2007) could not detect *Salmonella* species through PCR. Similarly D'Souza *et al*. (1978) did not even find serological evidence of *Salmonella* in sheep and goats in their study.

Molla *et al*. (2006) isolated *Salmonella* from three goats out of a total 100 in Ethiopia, which was a high prevalence, reported in animals used for human consumption. Similarly even higher prevalence was recorded by Pao *et al*. (2005), where a total of 17 samples were positive for *Salmonella* species out of 287 tested in small ruminants.

Temperature and Humidity: A significant association ($P < 0.05$) of a number of cases of diarrhea with the environmental temperature was recorded, with the highest prevalence (256/2029) in the temperature range from 25-30 ºC, which turned out to be an ideal environment temperature for the occurrence of diarrhea (Table 3). Similarly, a significant association was also established with the humidity level of the area ($P < 0.05$). A maximum number of cases was recorded in the humidity range from 70-80% (221-2029) i.e. at the highest range of humidity level in the area (Table 4). The data indicated a rise and fall in number of diarrhea cases with a rise and fall in humidity levels of the area.Factors contributing to helminthic infestation in the area also appear to contribute to *Salmonella* infection. Vaessen *et al*., (2011) reported high association of *S. dublin* infection with a liver fluke infection. Pal and Qayyum (1993) identified a number of contributing factors for helminthic infection, suggesting factors related to agro-climatic situations as quantity and quality of feed, along with humidity and temperature of the area and the grazing pattern of the animals as vital for the development of infestation which may influence the prevalence of *Samonella* in the area, indirectly. Contrary to this, Valcarcel *et al*. (1999) has reported no effect of season, instead a high risk of infection throughout the year for parasitic infections.

As far as salmonellosis was concerned, no effect of season, temperature or humidity levels were established in the study. Supported by the findings of Zibilske and Weaver (1978) who could not establish any correlation with the environmental factors in a designed experiment. However, Rowbury (1995) established the effect of temperature, pH and nutrient level on the acid tolerance and sensitivity of the *Salmonella* species.

Husbandry Practices, Strategic Prevention and Control Measures in the Area: Illiteracy was high in the area and the living standards of the people were also not up to the satisfactory levels. Farmers were not technically aware of the latest scientific standards of hygiene and feed for their animals. Most of animals were vaccinated for foot and mouth disease and enterotoxemia but no other vaccine was in practice. The majority of the farmers were also familiar with lactic acidosis and Peste des petits ruminants, however only in their native names but had no awareness of salmonellosis. Deworming was known to all the farmers and they kept deworming their animals irrationally and usually at low doses. Concomitantly, the drinking water for the animals was substandard, on account of lack of proper sewerage system and the drains were open and broken. Delhalle *et al*., (2008) stressed that effective washing and disinfection are key factors playing vital role in controlling *Salmonella* and even routine procedures adapted in kitchen for disinfection of *Salmonella* were declared unsatisfactory by Cogan *et al*. (1999) and stressed over better hygiene and procedures, whereas Adesiyun *et al*., (1993) recorded no significant difference in its prevalence between semi-intensively and intesively kept animals.

Open grazing was a common practice in the area, feeding commonly on green fodder, for which the animals were set free in the morning. The animals would eat garbage, grass and leaves from dirtier sources and drink contaminated, standing water available to them. Vaessen *et al*. (2011) reported more infections when the feed consisted of grass and identified new entrant as a risk factor associated with spread of *Salmonella*, an association was also revealed between herd size and water surface.

Table 1. Prevalence of diarrhea in the goat population in district Lahore, 2010

Villages	Goat Population 2010	Number of Diarrheic Cases	Prevalence % ($P < 0.05$)
V1	915	383	18.9
V2	494	212	10.5
V3	443	64	3.2
V4	38	23	1.1
V5	21	19	0.9
V6	69	19	0.9
V7	22	17	0.8
V8	27	7	0.3
Total	**2029**	**744**	**36.7**

All the species of animals owned by the farmers such as hens, sheep, goats, cattle, buffaloes, mules, horses and dogs were kept in the same vicinity, feeding and defecating in the same area, which was an additional factor, which maintained the cycle of infectious pathogens from one species to another and contributed adversely to the extinction of diseases. Zibilske and Weaver (1978) reported that approximately 10% of the cattle excrete *Salmonella* in their manure in routine.

The quality of fodder was also on an average poor and varied with the economic status and manpower of individual farmers. There was also no proper arrangement for the animals for providing protection

against the extremes of temperature. The mortality rate in young animals was also high.

The present study has revealed that the temperature range from 25-30 ºC, humidity range from 70-80%, open grazing, substandard hygiene, uncontrolled movement of animals, keeping all the species in one vicinity and irrational use of anthelmintics, proved to be the favourable factors for the development of diarrhea. The detection of *Salmonella* species in feces of adult goats, from field conditions, is a silent threat to human health. Its presence instigates the steps required to control such a disease of zoonotic potential, which may lead to dire consequences if not addressed. Special attention should be paid to the identification of possible sources of *Salmonella* and measures be adapted for its prevention in the food animals in the area, to ensure the supply of safe and healthy meat in the market for human consumption. The literacy rate of the people should be improved and short courses on improving hygiene and livestock management should be provided.

Table 2. Monthly cases of salmonellosis recorded and their morbidity, mortality and fatality rates

Months	Temp. ºC	Humid. %	No. of cases of Diarrhea	Morbidity (P > 0.05)		Mortality		Fatality	
				No.	%	No.	%	No.	%
Jan	11.6	75	80	0	0	0	0	0	0
Feb	17.2	61.9	80	0	0	0	0	0	0
Mar	25.2	53.2	57	0	0	0	0	0	0
April	29.5	37.4	44	0	0	0	0	0	0
May	34	32.8	34	0	0	0	0	0	0
June	33.9	41.2	33	0	0	0	0	0	0
July	31.3	67.8	35	0	0	0	0	0	0
Aug	30.2	77.1	60	0	0	0	0	0	0
Sep	28.8	71.7	81	0	0	0	0	0	0
Oct	26.9	59.1	74	1	0.05	1	0.05	1	100
Nov	21.1	52	77	0	0	0	0	0	0
Dec	14.5	62.2	89	1	0.05	1	0.05	1	100
Total	**25.4***	**57.6***	**744**	**2**	**0.1**	**2**	**0.1**	**2**	**100**

* Average Values N=2029

Table 3. Association of temperature with number of diarrheic cases

S. No.	Temperature Range ºC	Total Number of diarrheic cases recorded (P < 0.05)
1	11-15	169
2	15-20	80
3	20-25	77
4	25-30	256
5	30-35	162

Table 4. Association of humidity with number of diarrheic cases

S. No.	Humidity Range %	Total Number of diarrheic cases(P < 0.05)
1.	30-40	78
2.	40-50	33
3.	50-60	208
4.	60-70	204
5.	70-80	221

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