PREVALENCE OF SUBCLINICAL MASTITIS IN SHEEP CAUSED BY BACTERIAL SPECIES IN TANDOJAM, SINDH

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ABSTRACT

A total of 200 milk samples of sheep were collected from surroundings of Tandojam, Sindh to determine bacterial pathogens responsible for subclinical mastitis in sheep. The bacterial species identified were *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus*, *Corynebacterium pyogenes*, *Citrobacter* species, *Proteus Vulgaris* and *Streptococcus uberis* with prevalence of 37.14%, 18.57%, 12.85%, 11.42%, 8.57%, 5.71%, 2.85% and 2.85%, respectively. Whereas, from 200 milk samples examined through culture media, 70 (35%) were found positive with bacterial growth, while 130 (65%) had shown no growth and were recorded to be free from any bacterial infection. A total of 70 samples were positive from both udder halves. In right halves, 37 were found with subclinical mastitis, the prevalence was observed as 52.85 % while in the left halves 33 were positive with prevalence of 47.14%. The most common bacterial pathogen detected in both udder-halves of the sheep was *Staphylococcus aureus.* From 70 positive subclinical mastitic milk samples, 55 (78.57%) were recorded as pure infection, whereas 15 (21.14%) samples were found with mixed bacterial species. Among the positive samples 12 (6%) were found with strong gel formation with somatic cell/milliliter of 8,100,000.

Key-words: Prevalence, Subclinical mastitis, Sheep, *Staphylococcus aureus*, Somatic cell count

INTRODUCTION

The word mastitis comprises of two words: mastos means breast and itis means inflammation. Mastitis especially subclinical mastitis is a severe problem in ewes. Ewes having no clinical signs of inflammation in the udder and giving normal milk but are considered as positive for subclinical mastitis having somatic cell count (SCC) of $\geq 500 \times 10^3$ cells/mL (Bergonier and Berthelot, 2003).

Mastitis can be clinical, subclinical and chronic mastitis. Subclinical mastitis is very difficult to be diagnosed due to no obvious clinical signs, so somatic cell count of the milk is done for its identification (Radostits *et al.*, 2007). In subclinical mastitis, no visible changes in milk or the udder of the animal but it can be characterized by decreased milk production, altered milk composition and appearance of inflammatory components and bacteria in the milk (Leitner *et al.*, 2004). Normally 200,000 somatic cells/ mL in milk are considered as normal whereas, somatic cell count of 300,000 or more is considered as inflammation in the udder (Hillerton, 1999).

Prevalence of subclinical mastitis is relatively higher than clinical mastitis due to reduced milk production as well as altered physiochemical properties of the milk (Hamed *et al.*, 1993; Dario *et al.*, 1996; Urech *et al.*, 1999). Udder infection is one of the important diseases in dairy production not only in dairy cows but also in dairy ewes. Reduced milk yield and deteriorated raw milk quality is mainly caused by mastitis of lactating ewes (Jaeggi *et al.*, 2003; Bianchi *et al.*, 2004). Subclinical mastitis is of significant value due to its 15 to 40 times more prevalence than the clinical mastitis. Economic losses due to mastitis are higher in Pakistan than developed countries because preventive measures are not so far being carried out (Shakoor, 2006; Baloch *et al.*, 2011; Baloch *et al.*, 2013). Subclinical mastitis in sheep in different countries ranges from 5-30% whereas; clinical mastitis is less than 5% (Gonzalo *et al.*, 1994; Contreras *et al.*, 1995; Bergonier and Berthelot 2003; Contreras *et al.*, 2007). No any worker, to our knowledge has conducted research on prevalence of sub clinical mastitis in sheep in Sindh province of Pakistan. So the present experimental study was planned to record the prevalence and incidence of bacterial species responsible for subclinical mastitis in sheep.

MATERIALS AND METHODS

Two hundred milk samples of sheep from surrounding of Tandojam were collected in sterilized specimen bottles and brought to laboratory of the department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam and Central Veterinary Diagnostic Laboratory,

Tandojam. Before collection of samples the tips of sheep teats were cleaned with an antiseptic agent. Then milk samples were collected in bottles and capped properly to avoid any contamination. The specimen bottles containing milk samples were kept in refrigerator at 4ºC for few hours. Somatic cell counts were done in milk sample according to the method described by (Dohoo and Meek, 1982). The milk samples were cultured on different culture media for the isolation and identification of bacterial species. The cultural media were Brain Heart Infusion agar, blood and MacConkey's agars etc. The Coagulase, Catalase, Oxidase and TSI were used during investigation for the confirmation of the species specific characteristics.

RESULTS AND DISCUSSION

During the present study, overall percentage prevalence of subclinical mastitis caused by bacterial species was recorded in sheep and results are given in Table 1. Of the 200 samples collected and examined on various culture media, 70 (35%) were found positive, while 130 (65%) showed no growth on culture media and were recorded as negative. Sixty three (31.5%) of the samples were negative from right havles and 67(33.5%) were negative from left halves. The results obtained regarding the prevalence of subclinical mastitis in sheep during present study are similar to authors (Batavani *et al.,* 2003) who recorded the prevalence of 39% subclinical mastitis in sheep. Gebrewahid *et al.* (2012) observed the prevalence of subclinical mastitis in sheep as 28.14%. Eight bacterial species were recognized from subclinical mastitic milk samples of sheep in our study (Table 2) which were: *Staphylococcus aureus* (37.14%), *Bacillus cereus* (18.57%), *Escherichia coli* (12.85%), *Micrococcus lutes* (11.42%), *Corynebacterium pyogenes* (8.57%), *Citrobacter* species (5.71%), *Proteus vulgaris* (2.85%), *Streptococcus uberis* (2.85%). Similarly, Hartman *et al.* (2009) isolated coagulase negative *Staphylococci* (41%), *Bacillus cereus* (33%), *Staphylococcus aureus* (22%) and *Streptococcus* spp. (4%) from mastitic milk. From 70 positive subclinical mastitic milk samples, 55 (78.57%) were recorded as pure, whereas, 15 (21.14%) samples were found with mixed bacterial species (Table 3). Present study, revealed that pure infection (78.57%) is higher than mixed infection (21.14%) in subclinical mastitis in sheep.

Table 1. The overall percentage prevalence of subclinical mastitis caused by various bacterial species in sheep.

Total No. of samples	No. of positive samples	% of positive samples	No. of negative samples			% of negative samples		
			Right halves	Left halves	Total	Right halves	Left halves	Total
200	70	35%	63	67	130	31.5%	33.5%	65%

Table 2. The number and percentage incidence of individual bacterial species in subclinical mastitic milk samples of sheep.

Bacterial species	No. of samples occurring	Percentage (%)
Staphylococcus aureus	26	37.14
Bacillus cereus	13	18.57
Escherichia coli	9	12.85
Micrococcus luteus	8	11.42
Corynebacterium pyogenes	6	8.57
Citrobacter species	4	5.71
Proteus vulgaris	2	2.85
Streptococcus uberis	2	2.85

Table 3. The number and percentage incidence of pure and mixed bacterial species isolated from subclinical mastitis milk samples of sheep.

Animal species	Total No. of milk samples	No. of positive samples	% of positive samples	No. of pure samples	%of pure samples	No. of mixed samples	%of mixed samples
sheep	200	70	35	55	78.57	15	21.14

Table 4. The mean number of somatic cell counts from subclinical mastitis in sheep.

Gel formation	No. of samples	Percentage	CMT score	Mean SCC (cell/milliliter)
Negative	130	65%	0	100,000
Trace	24	12%	1	300,000
Weak +ve	20	10%	2	400,000
Distinct +ve	14	7%	3	2,700,000
Strong +ve	12	6%	4	8,100,000

In right udder halves, 37 (52.85%) samples were found positive, while in the left udder halves 33(47.14%) samples were detected with subclinical mastitis. Thirty five percent of samples (70/200) were found positive for sub clinical mastitis (Table 4). Among these positive samples, 24 samples (12%) showed trace gel formation and somatic cell per milliliter was counted as 300,000, whereas, 20 (10%) showed a weak gel formation, the somatic cell per milliliter was counted as 400,000 while 14 (7%) were found with distinct gel formation, the somatic cell per milliliter was determined as 2,700,000. Further that 12 (6%) samples were found with strong gel formation, the somatic cell per milliliter was counted as 8,100,000. The major factor affecting SCC is an infection of the mammary gland (Dohoo and Meek, 1982). A value of SCC 300,000 or above is considered to be abnormal and an indication of inflammation in the udder (Hartman *et al.*, 2009). In our study 35% milk samples showed more than 300,000 SCC, hence they were considered positive for SCM. Hartman *et al.* (2009) carried out study on somatic cell counts in relation to infection status of the sheep udder and revealed that the somatic cell count was higher in subclinical mastitis so our results are in agreement with these results.

CONCLUSION

It was concluded from the present study that 35% cases of subclinical mastitis in sheep were caused by bacterial organisms. *Staphylococcus aureus* was the most dominant pathogen and caused 37.14% subclinical mastitis in sheep either alone or in association with other bacterial species. *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli* are highly pathogenic bacterial species capable to cause subclinical mastitis in sheep. Right udder halves had higher incidence of bacterial infection than left udder halves during subclinical mastitis in sheep.

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