

Short Communication

Outbreak of Mastitis in Sheep Flock Due to *Streptococcus Agalactiae* and Unusual Neonatal Lamb Mortality

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ARTICLE HISTORY

Received: 2013-07-31

Revised: 2013-08-02

Accepted: 2013-08-02

Key Words: Mastitis; Lamb Mortality; Antibiotic sensitivity; Sheep; *Streptococcus agalactiae*

ABSTRACT

The functioning of mammary glands is affected by clinical as well as subclinical mastitis and has a major economic impact on the sheep and goat dairy industries. In the present study, in sheep flock of 120 sheep, 12 lambs suffered from debilitated conditions leading to the death of 4 lambs without showing any clinical disease condition. The postmortem findings revealed no or minimum intake of feed/milk by lambs prior to death. However, these were suckling prior to death. On clinical examination of ewes, mastitis appeared in 17 out of 45 lactating ewes in the flock. *Streptococcus agalactiae* was recovered from the mastitic ewes in pure cultures. All the clinical isolates had identical cultural, morphological and biochemical profiles. The antimicrobial susceptibility patterns were assessed against 24 standard discs and it revealed drug resistance against the commonly used antibiotics particularly for the intra mammary preparations in mastitis viz., penicillin, ampicillin and cloxacillin. The organism was found sensitive to most of the quinolones group of antibiotics and the treatment with enrofloxacin recovered all the mastitic ewes without any complication. It appears to be the first report of its kind in India where monoculture of *Streptococcus agalactiae* was obtained from mastitic cases of sheep which led to agalactia and ultimately the death of neonatal lambs. It can be concluded that mastitis in ewes may cause high morbidity and rapid reduction in milk leading to the death of neonatal lambs. *Streptococcus agalactiae* should be considered as a noteworthy pathogen that induces mastitis and can easily be transmitted between ewes and has developed significant antibiotic resistance.

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ARTICLE CITATION: Kumar A, Verma AK, Rahal A, Sharma AK, Varshney S and Gupta MK (2013). Outbreak of mastitis in sheep flock due to *streptococcus agalactiae* and unusual neonatal lamb mortality. *Adv. Anim. Vet. Sci.* 1 (4):XXXXX.

Mastitis is one of the most devastating diseases leading to severe economic losses to livestock industry worldwide. It has become a challenge to the researchers, field veterinarian due to involvement of various etiological agents viz., bacteria, virus, fungi and protozoa etc (Deb et al., 2013). Clinical as well as subclinical mastitis directly affect the functionality of mammary gland and have a significant economic impact on the sheep and goat dairy industries (Guerreiro et al., 2013). *Streptococcus agalactiae* are beta group bacteria in Lancefield and are referred as Group B streptococci (GBS). *Streptococcus agalactiae* is one of four Beta-Hemolytic streptococci, which results in clear zone of hemolysis through complete rupture of blood cells and appear as wide and clear areas surrounding bacterial colonies on blood agar (Quinn, et al., 2002). Various species of genus *Streptococcus* viz. *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Streptococcus parasanguinis* and *Streptococcus equi* are the species frequently identified in mammary gland infections (Javed and Siddique 1999; Cheng, et al., 2010; Kumar et al., 2010). Moreover, Streptococci are the second most common group of microorganisms in importance, after staphylococci, responsible for mastitis in sheep (Chanda et al., 1989; Sarvanan et al., 2000; Cheng, et al., 2010; Kumar et al., 2010). As sheep are reared for

wool and meat production in India, mostly no shepherd look after for the lactation from sheep and it is usually supposed that milk produced from sheep is sufficient for the growth and development of lamb. In the present report, we describe an unusual outbreak of death in neonatal lambs in the age group less than one month of age due to the mastitis in sheep by *Streptococcus agalactiae*.

The incidence took place at sheep unit, Madhurikund farm of Uttar Pradesh Pandit Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura, Uttar Pradesh, India. In this sheep flock of 120 sheep, 12 lambs suffered from the conditions like weak and debilitated conditions leading to the death of 4 lambs without showing any clinical disease condition. The gross and postmortem examination findings were non-conclusive. The postmortem findings were indicative of no or minimum intake of feed/milk by lambs prior to death. It suggested that lambs were off feed prior to death or were not getting milk. When history was recorded workers reported that lambs were suckling even few hours to death. It forced to examine the milk production and quality/quantity of dam milk.

Standard aseptic procedures were followed for collecting the samples. Briefly, the udders of all the dams were cleaned

with 70% alcohol and teats were flushed with sterile phosphate buffer saline solution. The flush was collected in a sterile a sample collection vials and brought to laboratories of department of Veterinary epidemiology and Preventive Medicine, Veterinary Microbiology & Immunology for cultural examination.

All the samples were purified (Cruickshank, et al., 1975) and then were subjected to the morphological, cultural and biochemical examinations for species confirmations as per the standard procedures (Kreig and Holt; 1984). Edward's modified media (HiMedia, Mumbai) was also used for the isolation and confirmation of *Streptococcus agalactiae*.

The drug sensitivity test was performed by disc diffusion method and sensitivity for a particular drug was made as described by Bauer et al. (1966) and as per the guidelines of NCCLS (2002). All the isolates were subjected to drug sensitivity test against 24 standard discs (Hi media, Mumbai) of Amoxycillin/Clavulanic acid (50/10µg), Ampicillin (10µg), Ampicillin/Sulbactam (10/10µg), Cefaclor (30µg), Cefixime (5µg), Cefoperazone (75µg), Cefotaxime (30µg), Ceftriaxone (30µg), Cephalothin (30µg), Methicillin (5µg), Penicillin G (10 units), Amikacin (30µg), Gentamicin (10µg), Streptomycin (25µg), Oxytetracycline (30µg), Tetracycline (30µg), Chloramphenicol (30µg), Colistin (10µg), Nystatin (50µg), Ciprofloxacin (5µg), Enrofloxacin (10µg), Gatifloxacin (5µg), Norfloxacin (10µg), Pefloxacin (5µg).

Examination of dam udder revealed agalactia or almost negligible volume of mastitic milk in all four ewes which lost the lamb. Washing of teat canals of mastitic ewes produced shiny gray-white colonies with a narrow zone of hemolysis on sheep blood agar (SBA). Morphological examination of culture through Gram staining revealed bacteria in pairs or short chains. These further appeared encapsulated, non spore forming, non motile and catalase free cocci. When monoculture from the blood agar was inoculated on selective Edward's modified media, it produced specific blue color colonies indicating the presence of *Streptococcus agalactiae*. Similarly, all the ewes suffering with mastitis revealed the involvement of monoculture of *Streptococcus agalactiae*. These findings are in concurrence of the findings of Zdragas et al., (2005), who reported an outbreak of mastitis in ewes due to *Streptococcus agalactiae*. Moreover, in the studies performed in different parts of world *Streptococcus spp* have been reported second to *Staphylococcus spp* in mastitis (Ashfaq and Muhammad, 2008; Hawari and Fawzi, 2008; Cheng, et al. 2010; Kumar et al., 2010). Ashfaq and Muhammad (2008) isolated *Streptococcus agalactiae* from the cases of clinical and sub clinical mastitis in cattle and buffaloes of Pakistan and recorded prevalence 27.78 % and 21.15%, respectively. While in China, Cheng, et al., (2010) isolated *Streptococcus agalactiae* from 27% cases of bovine mastitis. In contrast to these some other workers reported lower prevalence rate of *Streptococcus spp*. in mastitic milk samples as Javed and Siddique (1999) in Pakistan and Seyoum et al., (2003) in Ethiopia, isolated *Streptococcus spp*. from 3.01% and 8.34% cases only, respectively. The prevalence rate of *Streptococcus spp* has been reported to be very high in the cases of bovine mastitis in India, where Chanda et al., (1989), Prabhakar et al. (1995), Sarvanan et al., (2000) and Kumar et al., (2010) observed *Streptococcus spp*. in the cases of sub clinical and clinical bovine mastitic milk samples with the isolation rate of 35.04%, 14.47%, 25.17% and 11.76%, respectively. However, there are no such studies on the prevalence rate of bacteria in mastitis of ewes in India.

Drug sensitivity of all the isolates of *Streptococcus agalactiae* against 24 commonly used antibacterials revealed similar drug

sensitivity patterns. All the isolates were resistant to penicillin, ampicillin and cloxacillin, commonly used antibiotics in intra mammary preparations. The drugs like amoxycillin/clavulanic acid, ampicillin/sulbactam, cefaclor, cefixime, cefotaxime, cephalothin, methicillin, streptomycin, oxytetracycline, tetracycline, colistin, nystatin, norfloxacin, pefloxacin appeared to have intermediate response. While the antibiotics as cefoperazone, ceftriaxone, amikacin, gentamicin, chloramphenicol, ciprofloxacin, enrofloxacin, and gatifloxacin produced good zone of inhibition and appeared effective against the isolates. Almost similar findings were reported by Bhattacharya (2002), who reported that most of the Streptococcal isolates most sensitive to ciprofloxacin and chloramphenicol with the least sensitivity to penicillin, ampicillin and cloxacillin. The resistance against these antibiotics might be because of continuous and indiscriminate use of all these antibiotics (Kumar et al., 2010) in intramammary preparations. Many times irrespective of stage of mastitis and type of organism involved the similar easily available preparations are used by laymen without any veterinarian prescription. Similarly, development of antibiotic resistance due to injudicious use of antibiotics has been reported by various workers in *Salmonella* (Singh et al., 2005; Verma, 2005; Verma et al., 2007; Lambey et al., 2009; Kumar et al., 2011), *Mycoplasma* (Kumar et al., 2012a), *Campylobacter* (Kumar et al., 2012b), *E. coli* (Malik et al., 2013; Anita et al., 2013; Kumar et al., 2013a, 2013b and 2014). Developments of antibiotic resistance in bacteria have forced the scientist to develop a collaborative strategy to protect the health of man, animals and environment (Dhama et al., 2013). Moreover, the antibiotic resistance in *Streptococcus* is very prevalent due to encapsulation tendency (Kumar et al., 2014) as in an outbreak of *Streptococcus agalactiae* mastitis in ewes antibiotic therapy was proved to be ineffective, even though antibiotics were selected and administered after the sensitivity test (Zdragas, et al., 2005). Thus, the presence of mastitis due to these resistant *Streptococcus agalactiae* is a matter of great concern which produced mastitis and led to agalactia. This agalactia seems to be a major cause of neonatal lamb casualties in sheep flocks existing in rural areas of India, where shepherds are not well aware of these problems and veterinary facilities are not up to the mark.

CONCLUSION:

From the study, it can be concluded that mastitis in ewes may cause high morbidity and rapid reduction in milk leading to the death of neonatal lambs. *Streptococcus agalactiae* should be considered as a noteworthy pathogen that induces mastitis and can easily be transmitted between ewes and has developed significant antibiotic resistance.

ACKNOWLEDGEMENT:

The authors are highly thankful to Head, Department of Veterinary Epidemiology and Preventive Medicine, Department of Veterinary Microbiology and Immunology, Dean, College of Veterinary Sciences and Animal Husbandry, and Hon'ble Vice Chancellor, Uttar Pradesh Pandit Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishvidhyalaya Evum Go-Anusandhan Sansthan (DUVASU), Mathura, India; for providing all the necessary support and facilities for conducting this study.

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