Isolation and Identification of *Streptococcus Uberis* in Lactating Cows of Haramaya University Dairy Farm

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Abstract*:* A cross-sectional study was carried out in 40 lactating dairy cows of Haramaya University dairy farm from November 2014 to April 2015 to isolate *Streptococcus uberis* and assess risk factors. A checklist, farm inspection, and clinical examination of cattle were employed to collect data before laboratory examination of milk samples. Lactating animals were examined for the presence of clinical signs of mastitis. Physical examination of milk samples and California Mastitis Test (CMT) were conducted. Milk samples collected from clinically mastitic cows and CMT positive samples were subjected to microbiological examinations. Isolation and identification of *Streptococcus uberis* were carried out according to standard microbiological procedure. From 40 cows udders examined, 17 (42.5%) and 3 (7.5%) were sub-clinically and clinically affected, respectively. *Streptococcus uberis* was isolated from 2 (5%) of these cows. Out of 160 quarters examined, 11 (6.88%), 20 (12.5%) and 50 (31.25%) of the quarters were blind, clinically mastitic and sub-clinically mastitic, respectively. *Streptococcus uberis* was isolated from hind quarters of two sub-clinically mastitic dairy cattle. In conclusion, the present study revealed low isolation rate, however, it's potential to spread and negative impact on quality milk production should not be neglected. Therefore, emphasis should be given to the control of mastitis due to this pathogen by improving hygienic and sanitation management measures.

Keywords: *CMT, Isolation, Mastitis, Microbiological examination*, *Streptococcus uberis*

Introduction

Ethiopia is endowed with the largest livestock population in Africa with an estimated total cattle population of 53.99 million (CSA, 2013). However, this population size is not commensurate with its potential benefit to the country due to different constraints, among which animal diseases takes the top rank. The livestock production sector, particularly, dairy production, has not been fully exploited and promoted in the country (MOA, 2012).

Mastitis is one of the most important threat and highly prevalent problem in dairy cattle affecting the world's dairy industry (Viguier *et al*., 2009). Mastitis is an inflammation of the parenchyma of the mammary gland characterized by changes in the milk appearance and pathological alterations in the glandular tissue in clinical cases (Radiostits *et al*., 2007). However, in subclinical mastitis, there is no visible change in the milk or udder which makes it difficult to detect, even though milk production decreases and composition is altered due to bacteria. Subclinical mastitis is 3 to 4 times more common than the clinical mastitis (Mungube *et al*., 2005) and it results in severe economic losses from reduced milk production, treatment cost, increased labor, milk being withheld following treatment and premature culling (Viguier *et al*., 2009; Abureema, 2013).

Environmental mastitis is associated with bacteria that are transferred from the environment to the cow rather than from other infected quarters. The most common environmental mastitis causing bacteria are coliforms and environmental streptococci (Garcia, 2004; Radiostits *et al*., 2007). Among environmental streptococci, *Streptococcus uberis* is one of the most common mastitis pathogens found in dairy herds throughout the world and responsible for a significant proportion of clinical and subclinical mammary gland infections (Rambeaud, 2002; Tillman, 2006).

Streptococcus uberis is a gram-positive, facultative anaerobic and catalase negative bacteria which hydrolyzes esculin. It has complex and variable nutrition requirements, which reflect its adaptation as a commensal or pathogen and explain its high percentage as environmental mastitis causing pathogen in dairy cattle (Hossain *et al*., 2015). It is also ubiquitous in the cow's environment and found in manure and other organic matter, including bedding. Although its main source is the environment, a contagious cow-to-cow transmission may also occur (Celia *et al*., 2008).

The high infection rates of *Streptococcus uberis* in the dry period and the failure of post milking teat disinfection to control disease emphasize the independence of milking and transmission. Although the organism is sensitive *in vitro* to a range of antibiotics, intramammary therapy often is ineffective and chronic infections are common in some herds. Under these circumstances cow-to-cow transmission may become more important. More importantly, *Streptococcus uberis* can sometimes be

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associated with somatic cell count problems at low bacterial count (Andrews, 2004).

Previously, environmental mastitis constituted less than ten percent of total mastitis cases, but more recently there has been an increase in the incidence of environmental mastitis, particularly associated with *S. uberis* infection (Tiwari *et al*., 2013). Isolation of *S. uberis*, as a cause of bovine mastitis has come under increased scrutiny in dairy cattle, which were previously considered as minor pathogens associated with a mild inflammatory reaction but they are now known to cause bovine mastitis (Hussein, 2012). In fact, a high incidence of *S. uberis* as significant agents of mastitis in New Zealand and USA draw huge attention to this micro-organism as cause of clinical and subclinical mastitis (Rossitto *et al*., 2002; McDougall *et al*., 2004).

Although an increasing isolation of *S. uberis* mastitis has been reported throughout the world including Ethiopia, it still is relevant and important to study the recent status of environmental mastitis pathogen like *S. uberis*. Therefore, the present study was conducted with the objectives of isolating and identifying *S. uberis* in Haramaya University Dairy Farm.

Materials and Methods

Study Area

The present study was conducted in Haramaya University Dairy Farm where there was no regular and systematic detection of mastitis pathogens. Haramaya University is located at 09^{o} N and 42^{o}E at an altitude of 1950 meters above sea level. The area receives a bimodal rainfall; long rainy season (July to September) and short rainy season (March to June). The average rainfall is about 790mm. The mean maximum and minimum temperature are 23.6^{o}C and 10.1^{o}C, respectively (HADB, 2014).

Study Population

During the study period a total of 40 lactating cross bred (Holstein Friesian X Zebu) cows were present in the farm and all the cows were included in the study. The cows were kept under intensive husbandry practice and milked twice daily using a milking machine.

Study Design

A cross-sectional study was employed from November 2014 to April 2015. Clinical examination and laboratory test were conducted to isolate and identify *S. uberis* in lactating dairy cows of Haramaya University. Checklist, personal observation and farm records were used to collect data including husbandry system, age, parity, hygienic condition, lactation stage, production level, milking practices, barn drainage and milking personnel hygiene.

Study Methodology

Physical examination of udder and milk: The udder was examined visually and thorough palpation for detection of injury, blindness, presence of cardinal signs of inflammation, tick infestation and swelling. Viscosity and appearance of milk secreted from each quarter was examined for abnormalities in color, consistency, presence of clot, blood, flakes, and any other visible abnormalities. Depending on the clinical inspection findings, cases were categorized as clinical mastitis positive or negative**.**

After physical examination of the udder, milk samples were screened by California Mastitis Test (CMT) according to Quinn *et al.* (2002). A squirt of milk sample from each quarter of the udder was placed in a separate cup on the CMT paddle and an equal amount of CMT reagent was added and mixed well. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane for few seconds. The CMT results were read immediately and scored based on the amount and thickness of gel formed. Milk samples from animals with CMT positive were used for microbiological analysis.

Milk samples collection and transportation for bacteriological examination was conducted as follows: Udder washing was performed only when it was found with paste of dung. Teats were thoroughly cleaned with soap and water and dried with clean towel before milk collection. The teats were disinfected with cotton wool moistened with 70% ethanol and air dried before sampling. From each quarter an approximately 10ml of milk sample was collected into sterile universal bottle. All samples were labeled using the cow's identification number and quarter using permanent marker, the samples were placed in icebox and transported (Quinn *et al*., 2002) to Haramaya University College of Veterinary Medicine Microbiology Laboratory for bacteriological examination.

The milk samples were cultured to isolate *S. uberis* according to procedures recommended by Quinn *et al.* (2002). A loop full of milk was taken after mixing by swirling and inoculated onto blood agar enriched with 5% sheep blood. The inoculated plates were labeled and given numbers corresponding to the milk sample. Plates were incubated at 37^{o}C and reading was made initially after 24 hours then repeated after 48 hours of incubation. Identification of the bacteria on primary culture was done on the basis of colony morphology, hemolytic characteristics, and Gram stain reaction including shape and arrangements of the bacteria.

The small-medium sized colonies that were hemolytic or non-hemolytic on 5% sheep blood agar and yielding gram positive cocci were sub-cultured onto nutrient agar to obtain a pure isolate for further identification and subjected to catalase test. The catalase test was performed by transferring a bacterial colony with a sterile wire loop onto a cover slip and a drop of 3% H_2O_2 was added. Any colony that showed a positive reaction was discarded. Bacterial isolates that were Gram positive and negative for catalase production were set up for aesculin hydrolysis incorporated into the primary isolation media (Edward's medium). Esculin hydrolysis

positive cocci were transferred to Mac-Conkey agar to detect growth. Bacteria which did not grow on Mac-Conkey agar were considered as *S. uberis*. Bacteria which grew on Mac-Conkey agar were considered as *Enterococcus faecalis* (Quinn *et al*., 2002).

Statistical Analysis

The data was collected and recorded on specifically designed formats for this purpose and entered on Microsoft excel spreadsheet and analyzed with STATA version 12 statistical software. Descriptive statistics including frequency and percentage were used to summarize the data generated from the study.

Results

Among the 40 lactating cows examined, three (7.5%) and 17(42.5%) were affected by clinical and subclinical mastitis, respectively and two (5%) were identified positive for *S. uberis* (Table 1).

Table 1. Isolation of *Streptococcus uberis* in relation to cow level mastitis forms

Mastitis form	Number of animal affected	Percentile	*S. uberis* positive (%)
Clinical	3	7.5%	0 (0%)
Subclinical	17	42.5%	2 (11.76%)
Overall	20	50%	2 (5%)

Isolation of *S. uberis* on the bases of animals' age groups revealed prevalence of 5.88% for young and 4.34% for adult ages. *S. uberis* isolation was observed only in multiparous cows (5.56%). On the other hand, cows in late lactation were affected at 6.7% rate while cows in early lactation had 8.3% prevalence. In addition, isolation of *S. uberis* was observed in animals with high production and low production with isolation rates of 11.1% and 5%, respectively (Table 2).

Table 2. Isolation of *Streptococcus uberis* with respect to host related factors

Factors	Categories	Number examined	Positive (%)
Age	Young adult (3-5 years)	17	1 (5.88%)
	Adult (>5years)	23	1 (4.34%)
Parity	Primiparous	4	0
	Multiparous	36	2 (5.56%)
Lactation stage	Early (≤ 4 months)	12	1 (8.3%)
	Mid (5-7 months)	13	0 (0%)
	Late (>7 months)	15	1 (6.7%)
Milk yield	Low (≤ 5 lt)	20	1 (5%)
	Medium (6-10 lt)	11	0 (0%)
	High (>10 lt)	9	1 (11.1%)

Eleven (6.88%) of the 160 quarters were blind whereas 20 (12.5%) and 50 (31.25) were positive for clinical and sub clinical mastitis, respectively. On quarters' level, S. uberis was isolated from hind quarters of two (1.25%) cows with sub clinical mastitis (Table 3).

Table 3. Quarter level mastitis form and isolation of *S. uberis*

Mastitis form	**No Mastitis + (%)**	***Streptococcus uberis* positive (%)**	
Clinical	20 (12.5%)	0 (0%)	
Subclinical	50 (31.25)	2 (4%)	
Quarter	**No of quarter examined**	***Streptococcus uberis* positive (%)**	**Blind (%)**
RF	40	0 (0%)	2 (5%)
LF	40	0 (0%)	1 (2.5%)
RR	40	1 (2.5%)	3 (7.5%)
LR	40	1 (2.5%)	5 (12.5)
Total	160	2 (1.25%)	11 (6.88%)

RF=Right Front; LF=Left Front; RR=Right Rear; LR=Left Rear.

Discussion

The overall isolation of *S. uberis* is 5% which is slightly greater than noted by Belayneh *et al.* (2014), Bitew *et al.* (2010) and Bedada and Hiko (2011) who reported prevalence of 1.2%, 2.5% and 0.9% *S. uberis*. However, the current finding is in line with the findings of Girma *et al.* (2012), G/Michael *et al.* (2013) and Kerro and Tareke (2003) who reported 5.8%, 5.2% and 5.1% prevalence of *S. uberis*, respectively. This discrepancy between different studies is probably due to the fact that environmental *Streptococcus* (*S. uberis*) infection is strongly influenced by hygienic status, poor housing conditions and sanitation problem (Radiostits *et al.*, 2007). Despite the observed poor drainage and inadequate hygienic state of the farm, the prevalence of *S. uberis* remains low. This could be due to the fact that udder infection with *S. uberis* is highly established in dry cows managed to stay in deep straw beddings, which is reported as major risk factor as it favours bacterial multiplication (Andrews, 2004).

Streptococcus uberis was isolated only from cows with subclinical mastitis. Belayneh *et al.* (2014) and Bitew *et al.* (2010) also isolated *S. uberis* only from animals with subclinical mastitis with prevalence of 1.3% and 2.63%, respectively. The present finding is also in agreement with Zadoks (2002) finding who reported *S. uberis* as a major cause of subclinical mastitis in dairy herds. On the other hands Girma *et al.* (2012), Bedada and Heko (2011) and Sori *et al.* (2005) reported higher isolation of *S. uberis* from clinical cases rather than subclinical cases. This difference could be attributed to variation in sample size and study setting among various studies.

In this study, isolation of *S. uberis* is observed in 2 (5.56%) cows that has two previous births. This finding is supported by Zadoks *et al.* (2001) who reported lower incidence of *S. uberis* in lower parity cows than higher-parity cows. Kerro and Tareke (2003) and Getahun *et al.* (2008) also showed direct relationship between parity and prevalence of mastitis. The high isolation rate in aged multiparous animals might be due to increase in teat patency and frequency of previous exposure (Ayano *et al.*, 2013). *S. uberis* isolation both in young adult 1(5.88%) and adult cows 1(4.34%) is in accordance with that noted by Pryor (2008) who reported age to have no influence on *S. uberis* isolations.

Isolation of *S. uberis* only from hind quarters concur with Pryor (2008) and Zadoks (2002) findings who reported the incidence of mastitis caused by any pathogen to vary between the quarters of the udder with the rear two quarters more likely to be infected than the front two quarters, which could be related to greater production capacity of hind quarter, likelihood of fecal accumulation, environmental contamination and difficulty of cleaning of the hind quarter (Sori *et al.*, 2005).

Higher prevalence at early lactation stage than late in the present study is in agreement with the findings of Abureema (2013) who indicated *S. uberis* to be the most common isolate at early lactation. However, Chairman *et al.* (2012) noted *S. uberis* to be dominant pathogen at all stages of lactation since *S. uberis* mastitis is mainly the result of heavy contamination of the teats and udder with water, mud and faecal matter at any stage during lactation (Radiostits *et al.*, 2007).

Isolation of *S. uberis* in both high 1(11.1%) and low 1(5%) milk production group agrees with the finding of Charles (2014) and Moges *et al.* (2012) who reported higher mastitis in cows with high milk yield. This could be due to ease with which injuries are sustained in large udders, so that foci for the entrance of microorganisms are created and stress associated with a high milk yield may weaken the defense system of the cow (Charles, 2014).

Conclusion and Recommendations

Even though occurrence of S. *uberis* can be considered low in the current study, as high as 50% of mastitis prevalence level is worrisome. It is necessary to take appropriate measures to minimize the overall mastitis problem and to prevent potentially harmful effect of *S. uberis* and its spread to other farms in the surrounding. Therefore, husbandry and sanitation management, screening of animals for subclinical mastitis and appropriate dry cow management should be employed to reduce the possible risk of *S. uberis*.

Conflict of Interests

The authors declare that they have no competing interests.

References

Abureema, S. (2013). Characterization of Streptococcus uberis from bovine milk. PhD Thesis, RMIT University, Melbourne, Victoria, Australia.

Andrews, A. H. (2004). Mastitis. In: Edmondson, P.W. and Bramley, A.J. (Eds.) Bovine Medicine Diseases and Husbandry of Cattle. 2nd ed. Blackwell Science Ltd, 9600 Garsington Road, Oxford OX4 2DQ, UK, pp: 333-334.

Alemu Aylate, Fikiru Hiriko, Alemante Molla Simyalew & Aster Yohannes (2013). Prevalence of subclinical mastitis in lactating cows in selected commercial

dairy farms of Holeta district. *Journal of Veterinary Medicine and Animal Health*, 5: 67-72.

Bedada, B. A. & Hiko Adem. (2011). Mastitis and antimicrobial susceptibility test at Asella, Oromia regional state, Ethiopia. *Journal of Microbiology and Antimicrobials*, 3: 228-232.

Redeat Belayneh, Kelay Belihu & Asamenew Tesfaye (2014). Microbiological study on bacterial causes of bovine mastitis and its antibiotics susceptibility patterns in East Showa zone, Akaki district, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, 6, 116-122.

Molalign Bitew, Arega Tafere & Tadele Tolosa (2010). Study on bovine mastitis in dairy farms of Bahir dar and its environs. Journal of Animal and Veterinary Advances, 9: 2912-2917.

Viguier, C., Arora, S., Gilmartin, N., Welbeck, K., & O'Kennedy, R. (2009). Mastitis detection: Current trends and future perspectives. Trends in Biotechnology, 27: 486-493.

Celia, K. L., Nelson, D. & Kerr, D. E. (2008).Characterization of a bacteriophage lysin (Ply700) from Streptococcus uberis. Veterinary Microbiology, 130 (1-2): 107-117.

Chairman, N., Dyson, R., Hodge, A., Robertson, N. & Chaplin, S. (2012). A survey of mastitis pathogens in the South eastern Australian dairy industry, Parkville, Australia.

Charles, M.K. (2014). Prevalence, control and antimicrobial susceptibility patterns of bovine mastitis causing bacteria in Mityana district. MSc Thesis, Makerere University, Kampala, Uganda.

CSA (2011). Central Statistical Agency Agricultural sample survey, report on livestock and livestock characteristics volume II, private peasant holdings. Addis Ababa, Ethiopia.

CSA (2013). Central Statistical Agency Agricultural sample survey, report on livestock and livestock characteristics, private peasant holdings. Addis Ababa, Ethiopia.

Lidet G/Michael, Benti Deressa, Feyissa Begna, & Abebe Mekuria, (2013). Study on prevalence of bovine mastitis in lactating cows and associated risk factors in and around Areka town, Southern of Ethiopia. *African Journal of Microbiology Research*, 7: 5051-5056.

Garcia, A. (2004). Contagious vs. environmental mastitis. South Dakota state university, Brookings, USDA, pp: 1-4).

Getahun, K., Kelay Belihu, Merga Bekana & Fikre Lobago (2008). Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, Central Ethiopia. *Tropical Animal Health and Production*, 40: 261-268.

Sisay Girma, Alo Mammo, Ketema Bogele, Teshale Sori, Fanos Tadessse, & Tariku Jibat (2012). Study on the prevalence of bovine mastitis and its major causative agents in Western Hararghe zone, Doba district, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, 4: 116-123.

HADB (2014). Haramaya woreda Agricultural Development Bureau, Haramaya district, Ethiopia.

Hossain, M., Egan, S. A., Coffey, T., Ward, P. N., Wilson, R., Leigh, J. A., & Eme, R. D. (2015). Virulence related sequences; insights provided by comparative genomics of Streptococcus uberis of differing virulence. *BMC Genomics,* 16: 334.

Hussein, S. A. (2012). Prevalence and bacterial etiology of subclinical mastitis in dairy cows in Al Sulaimaniyah district. *Kufa Journal of Veterinary Medicine Science*, 3: 190-203.

Iqbal, M., Khan, M.A., Daraz, B., & Saddique, U. (2004). Bacteriology of mastitic milk and in vitro antibiogram of the isolates. Pakistan Veterinary Journal, 24: 161-164.

Kerro D.O., & Tareke, F. (2003). Bovine mastitis in selected areas of Southern Ethiopia. *Tropical Animal Health and Production*, 35:197-205.

McDougall, S., Parkinson, T.J., Leyland, M., Anniss, F.M., & Fenwick, S.G. (2004). Duration of infection and strain variation in Streptococcus uberis isolated from cow's milk. *Journal of Dairy Science*, 87, 2062-2072.

MOA (Ministry of Agriculture) (2012). Ministry of Agriculture, Ethiopia animal health year book. Federal Ministry of Agriculture Animal and Plant Health Regulatory Directoriate (APHRD), Ministry of Agriculture, Addis Ababa, Ethiopia.

Nibret Moges, Tekle, Hailemariam, Tewodros, Fentahun, Mersha, Chanie, & Achenef, Melaku (2012). Bovine mastitis and associated risk factors in smaller holder lactating dairy farms in Hawassa, Southern Ethiopia. *Global Veterinaria*, 9: 441-446.

Mungube, E. O., Tenhagen, B. A., Fikru Regassa, Kyule, M. N., Shiferaw, Y., Kassa, T., & Baumann, M. P. (2005). Reduced milk production in udder quarters with subclinical mastitis and associated economic losses in crossbred dairy cows in Ethiopia. *Tropical Animal Health and Production*, 37: 503–512.

Pryor, S. M. (2008). Bovine mastitis and ecology of Streptococcus uberis. PhD Thesis, Waikato University, Waikato, New Zealand.

Quinn, P. J., Carter, M. E., Markey, B., & Carter, G. R. (2002). Bacterial causes of Bovine Mastitis. *Veterinary*

Microbiology Microbial Diseases, 8th Ed. Mosby international Limited, London, pp: 465-475.

Radiostits, O. M., Gay, C. C., Hinchcliff, K. W., & Constable, P. D. (2007). A textbook of the diseases of cattle, sheep, goats, pigs and horses, 10th Ed. Ballier, Tindall, London, pp: 674-762).

Rambeaud, M. (2002). Dynamics of leukocytes and cytokines during experimentally-induced Streptococcus uberis mastitis. MSc Thesis, Tennessee University, Knoxville.

Rossitto, P. V., Ruiz, L., Kikuchi, Y., Glenn, K., Luiz, K., Watts, J. L., & Cullor, J. S. (2002). Antibiotic susceptibility oat terns for environmental *Streptococci* isolated from bovine mastitis in Central California dairies. *Journal of Dairy Science*, 85: 132-138.

Hundera Sori, Ademe Zerihun, & Sintayehu, Abdicho (2005). Dairy cattle mastitis in and around Sebeta, Ethiopia. *International Journal of Applied Research in Veterinary Medicine*, 3: 332-338.

Tillman, W. S. (2006). The Development of a *Streptococcus uberis* mastitis challenge protocol. MSc Thesis, North Carolina State University, Raleigh, North Carolina.

Tiwari, J.G., Babra, C., Tiwari, H.K., Williams, V., Wet, S.D., Gibson, J., Paxman, A., Morgan, E., Costantino, P., Sunagar, R., Isloor, S., & Mukkur, T. (2013).Trends in therapeutic and prevention strategies for management of bovine mastitis: An overview. *Journal of Vaccines and Vaccination*, 4: 176-187.

Zadoks, R. N. (2002). Molecular and mathematical epidemiology of *Staphylococcus aureus* and *Streptococcus uberis* mastitis in dairy herds. *Epidemiology and Infection,* 130: 335-349.

Zadoks, R. N., Allore, H. G., Barkema, H. W., Sampimon, O. C., Wellenberg, G. J., Grohn Y. T., & Schukken, Y. H. (2001). Cow and quarter-level risk factors for *Streptococcus uberis* and *Staphylococcus aureus* mastitis. *Journal of Dairy Sci*ence, 84: 2649–2663.