Evaluation of Milk in a Healthy Goat : A case study



By: Md. Rabbi Rahul Roll No: 17/36 Reg No: 01863 Intern ID: 28 Session: 2016-17

Faculty of Veterinary Medicine Chattogram Veterinary and Animal Sciences University Khulshi, Chattogram – 4225, Bangladesh

Evaluation of Milk in a Healthy Goat : A case study



Approved by:

Bara

Dr. Md. Mizanur Rahman Professor Department of Medicine and Surgery

Faculty of Veterinary Medicine Chattogram Veterinary and Animal Sciences University Khulshi, Chattogram – 4225, Bangladesh

Table of contents

Content	Page No.
Abstract	iii
Introduction	1-2
Materials and Methods	3-6
Results	7-8
Discussion	9
Conclusion	10
References	11-14

List of tables

Table		Page No.
Table-1	Interpretation of CMT score	4

List of figures

Figure		Page No.
Fig-1	Milk sample collection from healthy goat.	3
Fig-2	Somatic cell counting using automatic counter.	4
Fig-3	Plate count agar media preparation, serial dilution of sample and bacterial colony on media surface.	5
Fig-4	Inoculation of milk sample on blood agar and bacterial colony identification.	6
Fig-5	California mastitis test of milk.	7
Fig-6	No growth on EMB agar surface.	7
Fig-7	No growth on MacConkey agar surface.	8

Abstract

Goat is an important livestock in the livestock sector of Bangladesh. Goat milk contributes a significant role for meeting the demands of milk for smallholder farmers and households which rear goats for milk. But intramammary infections cause hamper in milk production and also possess public health risk. This study aimed to evaluate the health condition of udder of an apparently healthy goat and also evaluate the microbiological quality of the milk obtained from the goat. Milk was collected aseptically from each quarter separately and evaluated separately using different methods like Somatic Cell Count, California Mastitis Test (CMT) and Microbiological evaluation by Total Bacterial Count (TBC) and isolation of bacteria in Blood agar, EMB agar and MacConkey agar culture media. The somatic cell count was 115,000 ml⁻¹ for left quarter and 60,000 ml⁻¹ for right quarter. CMT score was N(negative), somatic cell range 0-200,000 for both quarters. Total bacterial count (TBC) for left quarter was 9,455 CFU ml⁻¹. Growth of *Escherichia coli* and *Salmonella sp*. was absent on EMB agar and MacConkey agar surface. There was no evidence of intramammary infection in the goat and the milk was free from contamination and free from risk of public health.

Introduction

On a global level, milk is part of the diet of 6 billion people, of which the majority live in developing countries (FAO, 2019). Among milk from other species, goat milk has special characteristics; it can be easily digested because its fat globules are smaller with more short-chain fatty acids and it is less allergenic than other milk and safe to be consumed by people with lactose intolerance (Park et al., 2007; Damunupola et al., 2014). Goat milk production has been growing steadily over the past 20 years due to the recognition of its nutritional values and nutraceutical properties (Kumar et al., 2016). The microbiological qualities of goat milk are determined by its composition and hygienic practices during milking and condition during storage and distribution (Mohamed et al., 2017). Microbial contamination of milk can occur from direct contact between fresh milk and contaminated equipment surfaces during milking (Oliver et al., 2005). Improper handling or storage of fresh milk can represent a transmission hazard for bacterial pathogens that are responsible for foodborne illness (Ismiarti et al., 2019).

Mastitis, or intramammary infection (IMI; Menzies and Ramanoon, 2001; Sar et al., 2018), is primarily caused by bacterial intramammary infection. It is the most relevant small ruminant disease, causing severe economic losses to the dairy industry worldwide (Oikonomou et al., 2014; Dore et al., 2016). This is also a risk to public health due to the presence of pathogens and toxins released, as well as antimicrobial residues (Contreras et al., 2007). Several bacterial pathogens were previously reported to be responsible for milk-borne illness and includes Shiga toxin-producing Escherichia coli (STEC) (Bander et al., 1997) and Salmonella sp. (Zeinhom and Abdel-Latef, 2014).

Most cases of IMI are chronic, making them difficult to treat and prone to resurgence (Polveiro et al., 2020). Frequently, they are accompanied by long-lasting cost-intensive antibiotic treatment and premature culling (White and Hinckley, 1999; Menzies and Ramanoon, 2001; Schukken et al., 2011; Grunert et al., 2018); occasionally, the animals die if not properly medicated. In dairy animals, IMI can manifest itself in clinical forms of varying levels of severity, according to symptoms, otherwise, with a total absence of visible macroscopic signs of the disease, in the form of a subclinical infection (Côté-Gravel and Malouin, 2019). Several bacterial pathogens can cause IMI, but Staphylococcus spp. are the most frequently diagnosed causal microorganisms in goats and sheep (Contreras et al., 2007). Other pathogens, such as Streptococcus spp., the Enterobacteriaceae family, Pseudomonas aeruginosa, Mannheimia haemolytica, Corynebacterium spp., and fungi, can produce IMI in small ruminants, albeit with lower occurrence rates (Polveiro et al., 2020). The high diversity of microorganisms, mainly IMI-causing bacteria, makes treatment and control in human and veterinary medicine difficult (White and Hinckley, 1999; Contreras et al., 2007). Microbiome studies of goats (McInnis et al., 2015; Zhang et al., 2017; Polveiro et al., 2020) in several situations have demonstrated the complexity of the interactions of pathogens and commensals present in situations of health and illness.

We know more or less the organisms involved in the IMI in goats. To fulfill the requirement of DVM degree the objectives of this case study were to evaluate the microbial quality of milk in a healthy goat and to detect if there is any evidence of subclinical mastitis found with existing facilities in the Department of Medicine and Surgery CVASU.

Materials and Methods

- Sample collection:
 - Animal selection: The goat from which milk sample was collected was selected from CVASU sheep and goat farm. The breed of the goat is Black Bengal. The age of the goat was 3 years and 4 months and it was in 3rd parity had three kids at the last parity. The daily milk yield was about 1kg. It was vaccinated against PPR and boosted every year and deworming was done about 8 months ago. The rearing system is semi-intensive and is fed green grass by grazing and pea husk(0.25 kg) and wheat bran (0.25 kg) as concentrate. There was no observable external symptoms of disease or disease condition. There was no external sign of mastitis or abnormality in the udder. Rectal temperature of the goat was 101.3^o F.



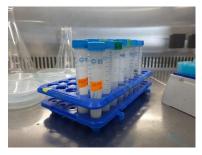


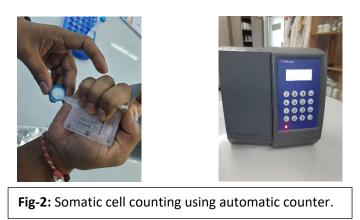
Fig-1: Milk sample collection from healthy goat.

Milk collection: Milk sample was collected from the goat at 30th August, 2022. Milk sample was collected from left and right quarters separately by hand milking. Before milking hand was washed properly using soap and water and the teats were cleaned using 70% ethyl alcohol for aseptic milk collection. Milk from first few attempts were discarded and mid stream milk was collected in falcon tube as clinical sample. From both quarters 5 ml milk sample was collected for further evaluation.

• Analysis of goat milk

Quality of milk and condition of udder was tested using two parameters.

- 1. Somatic cell analysis: Somatic cell analysis was performed using two methods;
 - A. Somatic Cell Count: To measure the number of somatic cell present in the milk. Somatic cell number from the milk sample was counted separately for left and right quarter using automatic somatic cell counter (DeLaval Group, Stockholm, Sweden; Sensitivity: 88% and Specificity: 80%). DeLaval Cell Counter counts the somatic cells optically and automatically. A digital camera takes a picture of the somatic cells' nuclei, which is stained in the cassette with a DNA- specific fluorescent reagent and counts the cells' nuclei one by one.



B. **California Mastitis Test (CMT):** To detect sub-clinical mastitis by scoring the milk based on the result obtained from the test. California mastitis test was performed for two quarters separately by mixing equal amount of CMT reagent (DeLaval Group, Stockholm, Sweden) and milk sample in Four-well plastic paddle and checked for level of thickness formed after mixing to determine the presence of sub-clinical mastitis. After mixing milk and the reagent the result is read as traces, 1, 2, 3, and negative depending up on the gel formation in the milk sample (Indian council of agricultural research, 2011).

CMT Score	Somatic Cell Range	Interpretation
N (Negative)	0-200,000	Healthy Quarter
T (Trace)	200,000-400,000	Subclinical Mastitis
1	400,000-1,200,000	Subclinical Mastitis
2	1,200,000-5,000,000	Serious Mastitis Infection
3	Over 5,000,000	Serious Mastitis Infection

Table-1: Interpretation of CMT score.

- 2. **Microbiological analysis:** Microbiological qualities of milk was determined using three parameters;
 - A. **Total Bacterial Count (TBC):** To measure total amount of mesophilic aerobic microbes TBC was conducted using Plate Count Agar (PCA) media. Goat milk sample from left and right quarter was separately serially diluted from 10⁻¹ to 10⁻⁷ and then plated onto PCA media and incubated at 370C for 24 hours. Total colonies on PCA media were counted and number of bacteria was determined as CFU ml⁻¹ using the following formula:

$TBC = \frac{No. of \ counted \ colony}{1 * 1. 1 * Dilution \ factor}$







Fig-3: Plate count agar media preparation, serial dilution of sample and bacterial colony on media surface.

- B. **Isolation of specific organism:** For isolation of specific bacteria from milk sample, inoculation in Blood Agar and later in EMB (Eosin Methylene Blue) agar and MacConkey agar was performed for detection of *Escherichia coli* and *Salmonella sp.* respectively.
 - Blood Agar Inoculation: Blood agar media (Oxoid®, Oxoid Ltd, Basingstoke, United Kingdom) was prepared using commercial agar mixture and cow blood. After preparation of media it was incubated at 37°C for 24 hours to detect any contamination. Next day milk sample from separate quarters were inoculated separately onto the agar plate by streaking. Then the inoculated agar plates were incubated at 37°C for 24 hours and the type of colony formed on agar plate was identified. Two types of colony was formed in case of milk sample from both quarters; one type was small, yellowish colony and the other type was medium sized moist, whitish colony.



Fig-4: Inoculation of milk sample on blood agar and bacterial colony identification.

- E. coli isolation: The two types colony formed on the blood agar was separated and inoculated separately on EMB agar media (Oxoid®, Oxoid Ltd, Basingstoke, United Kingdom). The culture plates were incubated at 37^oC for 24 hours and checked for the presence of characteristic colony of *Escherichia coli* with metallic sheen on the colonies.
- Salmonella isolation: The two types colony formed on the blood agar was separated and inoculated separately on MacConkey agar media (Oxoid®, Oxoid Ltd, Basingstoke, United Kingdom). The culture plates were incubated at 37°C for 24 hours and checked for the presence of non-lactose fermenting colony of *Salmonella sp*.

Results

- **Somatic Cell Count:** The number of somatic cells in milk from left quarter was 115,000 ml⁻¹ and number of somatic cells in milk from right quarter was 60,000 ml⁻¹.
- California Mastitis Test (CMT): The test result was N (negative) for milk sample from both quarters. The somatic cell range was within 0-200,000 which is an indication of healthy quarters.



- **Total Bacterial Count (TBC):** The total bacterial count in milk from left quarter was 9,455 CFU ml⁻¹ and in milk from right quarter was 4,455 CFU ml⁻¹.
- **E. coli isolation:** No characteristic colony of *Escherichia coli* was formed on EMB agar media surface.

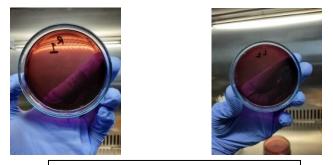


Fig-6: No growth on EMB agar surface.

• Salmonella isolation: No characteristic colony of *Salmonella sp.* was formed on MacConkey agar media surface.

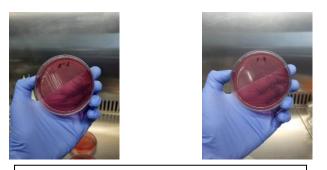


Fig-7: No growth on MacConkey agar surface.

Discussion

The somatic cell count (SCC) obtained from the milk samples were 115,000 ml⁻¹ and 60,000 ml⁻¹ in milk from left and right quarter respectively. According to Paape et al. (2001), the SCC value in healthy goats should not exceed 100,000 cells per ml for individual goats. So, the SCC value of milk from the goat was under normal standard.

The california mastitis test (CMT) result for milk sample from both quarters were negative. Therefore there was no evidence of subclinical mastitis.

The total bacterial count (TBC) obtained from the milk from left quarter was 9,455 CFU ml⁻¹ and from right quarter was 4,455 CFU ml⁻¹. Ramsahoi et al. (2011) published, that the TBCs in bulk samples range from 10³ to 10⁶ Colony Forming Units (CFU) ml⁻¹. The total bacterial count in the tested milk sample was very low which indicates healthy udder and low contamination during milking and handling of milk.

There was no growth of Escherichia coli on EMB agar and Salmonella sp. on MacConkey agar. This is also an indicator that the milk sample was not contaminated during milking or handling and the milk possess no public health risk or health hazard for the consumer. This also indicates that the udder is not infected by coliform bacteria.

Conclusion

Based on the study it can be concluded that there was no evidence of subclinical mastitis found in the goat. The goat was apparently healthy with healthy quarters and no physiological abnormality or abnormality in milk was present. So it can be said that, there is a less chance of having subclinical mastitis in apparently healthy goats with healthy udder with normal characteristics of milk during lactation.

References

- Addis, M. F., Tanca, A., Uzzau, S., Oikonomou, G., Bicalho, R. C., and Moroni, P. (2016). The bovine milk microbiota: insights and perspectives from -omics studies. Mol. BioSyst. 12, 2359-2372.doi:10.1039/c6mb00217j.
- Bender, J.B., C.W. Hedberg, J.M. Besser, D.J. Boxrud, K.L. MacDonald and M.T. Osterholm, (1997).Surveillance for Escherichia coli O157: H7 infections in Minnesota by molecular subtyping.New Engl. J. Med., 337: 388-394.
- Bonsaglia, E. C. R., Gomes, M. S., Canisso, I. F., Zhou, Z., Lima, S. F., Rall, V. L.M., et al. (2017). Milk microbiome and bacterial load following dry cow therapy without antibiotics in dairy cows with healthy mammary gland. Sci. Rep. 7:8067. doi:10.1038/s41598-01708790-5.
- Contreras, A., Sierra, D., Sánchez, A., Corrales, J. C., Marco, J. C., Paape, M. J., et al. (2007).
 Mastitis in small ruminants. Small Rumin. Res. 68, 145–153. doi: 10.1016/j.smallrumres.2006.09.011.
- Côté-Gravel, J., and Malouin, F. (2019). Symposium review: features of Staphylococcus aureus mastitis pathogenesis that guide vaccine development strategies. J. Dairy Sci. 102, 4727-4740. doi: 10.3168/jds.2018-15272.
- Damunupola, D.A.P.R., W.A.D.V. Weerathilake and G.S. Sumanasekara, (2014). Evaluation of Quality characteristics of goat milk yogurt incorporated with beetroot juice. Int. J. Scient. Res. Pub.,4: 515-519.
- Dore, S., Liciardi, M., Amatiste, S., Bergagna, S., Bolzoni, G., Caligiuri, V., et al. (2016).
 Survey on small ruminant bacterial mastitis in Italy, 2013–2014. Small Rumin. Res. 141, 91– 93. doi:10.1016/j.smallrumres.2016.07.010.

Eckhardt, M., Hultquist, J. F., Kaake, R. M., Hüttenhain, R., and Krogan, N. J.(2020). A systems approach to infectious disease. Nat. Rev. Genet. 21, 339–354.doi:10.1038/s41576-020-02125

FAO (2019). Overview of Global Dairy market Developments in 2018. FAO Rome.

- Garira, W. (2019). The replication-transmission relativity theory for multiscale Modelling of infectious disease systems. Sci. Rep. 9, 16353–16317. doi: 10.1038/s41598-019-52820-3.
- Grunert, T., Stessl, B., Wolf, F., Sordelli, D. O., Buzzola, F. R., and Ehling-Schulz, M. (2018).
 Distinct phenotypic traits of Staphylococcus aureus are associated with persistent, contagious bovine intramammary infections. Sci. Rep. 8:15968. doi:10.1038/s41598-018-34371-1.
- Indian council of agricultural research (ICAR) (2011) Handbook of animal husbandry. 3rd (edn), New Delhi, pp. 520-522.
- Ismiarti, Yustina Yuni Suranindyah and Widodo, (2019). Microbiological Qualities of goat milk obtained under different milking systems at a smallholder dairy farm in Yogyakarta, Indonesia. Int. J. Dairy Sci., 14: 29-35.
- Kumar, H., Yadav, D., Kumar, N., Seth, R., and Goyal, A. K. (2016). Nutritional and nutraceutical properties of goat milk a review. Indian J. Dairy Sci. 69, 513–518.
- McInnis, E. A., Kalanetra, K. M., Mills, D. A., and Maga, E. A. (2015). Analysis of raw goat milk microbiota: impact of stage of lactation and lysozyme on microbial diversity. Food Microbiol. 46, 121–131. doi: 10.1016/j.fm.2014.07.021.
- Mohamed, A.F., M.K. Somda, A.E. Fourreh, A.A. Okieh, C.N. Said, A. Merito and S. Yagi, (2017).Evaluation of microbiological quality of raw milk from farmers and dairy producers in six districts of Djibouti. J. Food Microbiol. Saf. Hyg., Vol. 2. 10.4172/2476-2059.1000124.

- Oikonomou, G., Bicalho, M. L., Meira, E., Rossi, R. E., Foditsch, C., Machado, V. S., et al. (2014). Microbiota of Cow's milk; distinguishing healthy, sub-clinically and clinically diseased quarters. PLoS One 9:e85904. doi: 10.1371/journal.pone.0085904.
- Oliver, S.P., B.M. Jayarao and R.A. Almeida, (2005). Foodborne pathogens in milk and the dairy farm environment: Food safety and public health implications. Foodborne Pathog. Dis., 2:115129.
- Park, Y.W., M. Juarez, M. Ramos and G.F.W. Haenlein, (2007). Physico-chemical characteristics of goat and sheep milk. Small Rumin. Res., 68: 88-113.
- Paape MJ, Poutrel B, Contreras A, Marco JC, Capuco AV. 2001. Milk somatic cells and lactation in small ruminants. J Dairy Sci 84 (E. Suppl.): E237–E244.
- Polveiro, R. C., Vidigal, P. M. P., Mendes, T. A. D. O., Yamatogi, R. S., Lima, M. C., and Moreira, M. A. S. (2020). Effects of enrofloxacin treatment on the bacterial microbiota of milk from goats with persistent mastitis. Sci. Rep. 10:4421. doi: 10.1038/s41598-020-61407-2.
- Ramsahoi L, Gao A, Fabri M, Odumeru JA. 2011. Assessment of the application of an automated electronic milk analyzer for the enumeration of total bacteria in raw goat milk. J. Dairy Sci.94(7):3279–3287.
- White, E. C., and Hinckley, L. S. (1999). Prevalence of mastitis pathogens in goat milk. Small Rumin. Res. 33, 117–121. doi: 10.1016/S0921-4488(99)00013-9.
- Zeinhom, M.M. and G.K. Abdel-Latef, 2014. Public health risk of some milk borne pathogens. Beni-Suef Univ. J. Basic Applied Sci., 3: 209-215.

Zhang, F., Wang, Z., Lei, F., Wang, B., Jiang, S., Peng, Q., et al. (2017). Bacterial diversity in goat milk from the Guanzhong area of China. J. Dairy Sci. 100, 7812–7824. doi:10.3168/jds.201713244.

Acknowledgement

I consider it my utmost obligation to express my gratitude to the **Almighty**, the omnipresent, kind and merciful who gave me the health, thoughts and the opportunity to complete this task.

I am grateful for the generous advice and encouragement from my supervisor, **Professor Dr. Mizanur Rahman**, Professor of Department of Medicine and Surgery, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University.

I would like to express my deep sense of gratitude and thanks to Professor **Dr. Gautam Buddha Das**, honorable vice chancellor, Professor **Dr. A.K.M Saifuddin**, director, external affairs and Professor **Dr. Mohammad Alamgir Hassain**, Dean, Faculty of Veterinary Medicine, CVASU for arranging this type of research work as a compulsory part of this internship program.

I am thankful to the **Udder Health Bangladesh lab** at Faculty of Veterinary Medicine, CVASU for allowing me to perform laboratory works.

Md. Rabbi Rahul

Author, November 2022.

Biography

Md. Rabbi Rahul son of Abdur Razzaque and Rahana Bagum was born on 29th November, 1998. He passed his Secondary School Certificate Examination from BAF Shaheen College, Chattogram in 2014 (GPA 5.00). Then he passed his Higher Secondary School certificate examination from BAF Shaheen College, Chattogram in 2016 (GPA 5.00). Now he is completing his one-year long internship program for fulfilling the requirement of Doctor of Veterinary Medicine (DVM) degree in Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. During his internship period he received his clinical training on Veterinary Medicine from UVH Patuakhali, SAQTVH, CVASU, Teaching & training Pet Hospital and research Center (TTPHRC), CVASU, CVH, FV & FC, Dhaka, Chattogram and Dhaka Zoo and managemental training from Chattogram based farm and Chattogram based Pharmacy etc. His primary research interest is in public health, one health and food safety.