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Detection of *Streptococcus agalactiae* Existence within Milk Samples of Hair Goats Grown in West Anatolia Region

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Abstract: This study is performed to identify existence of *Streptococcus agalactiae* being mastitis factor in hair goats grown in Cine district of Aydin province in West Anatolia region. Material of the study consists of samples of 232 goat milk. Samples have been received from 116 goats of 9 herds grown in Kavsit, Tatarmemisler, Catak, Ibrahimkavagi towns of Cine district. Samples have been delivered to routine diagnosis laboratory of Adnan Menderes University, Faculty of Veterinary Science, Microbiology Departments to analyze them under cold chain and in terms of *S. agalactiae*. For *S. agalactiae* isolation purposes, double inoculation of sheep blood agar of each milk sample to be analyzed has been applied. Inoculated milk samples have been left for inoculation during 24-48 h at 37°C in both aerobic and micro aerobic medium. After incubation, gram staining has been applied to milk samples shaped, gram positive cocci have been sorted and catalyze test has been applied to gram positive cocci. Catalyze negative colonies have been separated to which other chemical tests have been applied. At the end of analysis, *S. agalactiae* has been identified in 12 of 116 goats samples (10.3%).

Key words: Mastitis, *S. agalactiae*, identification, hair goat, raising condition, Aydin

INTRODUCTION

An increasing rate in goat existence and increasing demand towards goat products can be analyzed in worldwide (Morand-Fehr *et al*., 2004). In Turkey, raising milk goat has been rapidly increasing in recent years. One of the reason is increasing demand towards goat milk and products.

According to the studies applied on goat milks, it has been concluded that nutritional value of goat milk is higher than that of cow milk and goat milk is similar to human milk due to its higher rate of mineral materials and biological values (Belewu and Aiyegbusi, 2002; Morales *et al*., 2005). Another study provided the result that food based pathogens were less in goat milks (Gutta *et al*., 2009). In addition, recent studies attached higher importance to isolation and identification of microorganisms that may exist within milk and having adverse impacts on human and animal health (Andersen *et al*., 2003; Celik *et al*., 2009).

Mastitis is one of the important factors causing significant economical losses in milk industry (Erksine, 1992). Causing clinical and subclinical mastitis, bacterial contamination of udder glands affect milk quality (Contreras *et al*., 1999). *S. agalactiae* and *S. aureus* are worldwide known clinical or subclinical mastitis factors. In goats, mastitis generally represent a subclinical pattern (Contreras *et al*., 1999). Subclinical mastitis caused due to *S. agalactiae* has essential qualitative and quantitative impacts of bulk tank milks (Keefe, 1997).

Economical losses caused due to mastitis can be counted as disposal of milk, treatment costs, veterinary surgeon costs, lactation in which mastitis is shaped and decrease of milk efficiency in following lactation. Many foreign studies exist regarding wide dissemination area of *S. aureus* and *S. agalactiae* among herds. Researchers found that prevalence of *S. agalactiae* in tank milks is 6-70% (Schlegelova *et al*., 2002; Fox *et al*., 2003; Andersen *et al*., 2003; Khaitsa *et al*., 2000; Schoonderwoerd *et al*., 1993; Kelton *et al*., 1999). In addition, some researches applied regarding existence of *S. agalactiae* among cattle and sheep and goats exists in Turkey (Bastan *et al*., 2008; Celik *et al*., 2009). However, any study regarding search of *S. agalactiae* in goats does not exist in Turkey. Routine method used in identification of *S. agalactiae* in milks is bacteriological culture. Other methods such as Latex agglutination, slide coaglutination, Enzyme Linked Immunosorbent Assay (ELISA) and Immunoflorescence Assay (IFA) are also developed. Bacterial colony cannot be easily obtained in isolation

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studies (Keefe, 1997). Adequate rate of studies about goat milk does not exist in Turkey. Particularly, insufficient number of studies have been applied on hair goats constituting >95% of goat population. This study searches existence of *S. agalacatiae* among hair goat herds grown in in-forest and side towns of Cine district of Aydin province and aims to reflect its prevalence.

Aim of this study was to identify the pathogens responsible for subclinical mastitis in hair goats and to determine their percentage of milk samples. The results are discussed according to results of previous studies.

MATERIALS AND METHODS

Material of the research composes of 232 units of milk samples received from right and left udders of 116 goats in 9 hair goat herds of Kavsit, Tatarmemisler, Catak and Ibrahimkavagi towns being in-forest or side towns of Cine district of Aydin province in West Anatolia region. Milk samples have been taken at the end of lactation (on 6th and 7th month).

Samples taken have been delivered to routine diagnosis Laboratory of ADU, Faculty of Veterinary Science, Microbiology Department under cold chain. Milk samples have been numbered with ear tag of hair goats. Respective towns have an altitude between 500-700 m on piedmonts of Madran Baba mountain.

For isolation purposes, sheep blood agar inoculation has been applied from each milk sample to be analyzed. Milk samples inoculated have been left for incubation for 48 h under 37°C.

After incubation, gram staining has been applied to bacterial colonies. Catalyze test has been applied to gram positive cocci obtained as a result of gram staining. Bacitracin sensitivity, Sulfometoksazol-Trimetoprim sensitivity, Optochin sensitivity, CAMP test, PYR (Pyrrolidonyl-β-naphthylamide) hydrolyze test, Esculin hydrolyze test, production at 40% bile, production at 6.5% NaCl, melting at bile test have been applied to gram positive, catalyze negative cocci and identification of *Streptococci* have been applied in accordance with these tests (Koneman *et al*., 1997). Strains identified as *S. agalactiae* have also been classified with Streptococcal grouping set (Oxoid, DR0585A).

RESULTS AND DISCUSSION

In this study, *S. agalactiae* has been identified in 12 of 116 heads of hair goat (10.3%) whose milk samples have been received. Results obtained are shown in Table 1. According to these results, existence of *S. agalactiae* can be observed within milks of hair goats in Tatarmemisler and Kavsit towns whereas *S. agalactiae* does not exist in milks obtained from Ibrahimkavagi and Çatak towns (Table 1). Existence of *S. agalactiae* in milks of hair goats has been analyzed for the first time with this study. In addition, *Streptococcus pyogenes* (8.6%) has been isolated and identified from 10 heads of goat, *Streptococcus pneumoniae* (0.86%) from 1 head of goat, *Escherichia coli* (1.72%) from 2 heads of goat and *Plesiomonas shigelloides* (1.72%) 2 heads of goat.

Studies are applied to gain information about *S. agalactiae* based mastitis within dairy cattle at herd level as well as eliminate this matter (Andersen *et al*., 2003). According to a study applied in Mississippi in 1982, 39.5% of dairy cattle has been infected with *S. agalactiae* (Sears *et al*., 1982).

In another study, applied in Massachusetts between 1976-1982, it has been obtained that 44.7% of dairy cattle was infected (Oliver and Mitchell, 1984). A decrease has been analyzed in rate of infection according to the results of studies applied during following years. While a study performed in 1990 provided the rate of 7.9% for infected animals within herd (Godkin and Leslie, 1990), study of 1992 analyzed respective rate as 10% (Bartlett *et al*., 1992).

A study performed in Elazig notified that 9 of 146 isolates have been reflected *S. agalactiae* (6.16%). In addition, this study obtained following rates; *Staphylococcus aureus* 39.04%, *Staphylococcus epidermidis* 17.81%, *Actinomyces pyogenes* 14.38%, *E. coli* 8.9% and *Streptococcus uberis* 4.11%, *Streptococcus dysagalactiae* 3.42%, *Corynebacterium bovis* 1.37%, *Pasteurella haemolytica* 1.37% and *Bacillus cereus* 1.37%. In a study performed in Iran, 42 *Streptococci* has been isolated from 148 milk samples and following rates have been obtained; *S. dysagalactiae* 35%, *S. agalactiae* 26%, *S. uberis* 18%, *Enterococcus* sp. 4% (Ebrahimi *et al*., 2008). A study applied in Tanzania obtained following rates in herds analyzed between 1971-2002; *Staphylococcus aureus* 25.7%, *S. agalactiae*

Table 1: Distribution of *S. agalactiae* identifications in milks of hair goats in accordance with towns included in sampling

Study centers	No. of goats sampled	Total no. of samples	Right udder *S.agalactiae* positive sample rate (%)	Left udder *S. agalactiae* positive sample rate (%)	Both udders positive sample rate (%)	Total no. of positive goats (%)
Kavsit town	40	80	4 (10)	3 (7.5)	0 (0)	7 (17.5)
Ibrahimkavagi	32	64	0 (0)	0 (0.0)	0 (0)	0 (0.0)
Tatarmemisler	23	46	3 (13)	2 (8.0)	0 (0)	5 (21.0)
Catak	11	22	0 (0)	0 (0.0)	0 (0)	0 (0.0)
Total (4 towns)	116	232	7 (6)	5 (4.3)	0 (0)	12 (10.3)

15.4%, *Klebsiella pneumoniae* 14.3% and *Esherichia coli* 14.1%, *Pseudomonas aeruginosa* 7.5%, *Streptococcus dysagalactiae* 5.2% and *Streptococcus uberis* 4.2%. Contagious mastitis pathogens have been isolated as 45.6% of positive cultures, environmental pathogens as 48.2% and various pathogens have been insulated at the rate of 5.7%. In addition, this study obtained following rates; *Candida albicans* 33%, *Candida guillermondi* 29%, *Candida tropicalis* 19%, *Candida pelliculosis* 19% and *Trichophyton verrucosum* 7% (Kivaria and Noordhuizen, 2007). Ergun *et al.* (2009) in their study performed in South regions of Turkey analyzed 1458 milk samples received from 729 awassi sheep and concluded following values; *S. epidermidis* 35.7%, *Staphylococcus xylosus* 1.2%, *Staphylococcus saprophyticus* 10.2%, *Staphylococcus warneri* 9.2%, *Staphylococcus intermedius* 7.1% and *S. agalactiae* 2%.

In a study realized in Lebanon, subclinical mastitis factors have been investigated in samples taken from 3472 udder lobes of 1736 awassi sheep and following values have been obtained; coagulase negative Staphylococci 17.8%, *E. coli* 13.6% and *S. aureus* and *S. agalactiae* 6.8% (Lafi *et al*., 1998). Generally, conventional isolation and identification as well as California Mastitis Test (CMT) have been used in these studies. *Streptoccus* sp. level has been identified in studies applied by goats in foreign countries and any *S. agalactiae* based mastitis has not been observed (Contreras *et al*., 1995; White and Hinckley, 1999).

If results of this study are compared, it may be observed that a correlation exists between *E. coli* (1.72%) results and results of White and Hinckley (1999) (1.6%). *Streptococcus pyogenes* (8.6%) and *Streptococcus pneumoniae* (0.86%) and *Plesiomonas shigelloides* (1.72%) have been first identified in goat milks in Turkey. Values for *S. agalacatiae* (10.3%) have been obtained more than that of Lafi *et al*. (1998).

CONCLUSION

Identification of *S. agalacatiae* based mastitis in hair goat herds of in West Anatolia region rating to 10.3% seems to be essential problem. Results indicate that existence of *S. agalacatiae* in these herds can cause important production losses. Controlling factors that provide emergence of mastitis disease increasing application of udder disinfection before and after milking shall provide decrease of economical losses arisen.

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