



Occurrence of fungal agents in mastitis in dairy goats

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1 ABSTRACT

Intramammary infections (mastitis) in goats constitute an enormous animal health problem, alter milk composition, lower the hygienic value of milk, impair the processing properties of milk and cause high economic losses in dairy farming today. The objective of the present study was to identify the fungal agents in milk samples of hair goats. A total of 170 milk samples were collected from the goats with clinical and subclinical mastitis and clinically healthy animals. The samples were collected from the beginning stages of lactation on goats located in Van region, Turkey. The samples (approximately 10 ml) were obtained in sterilised sample bottles and transferred immediately to the laboratory and California mastitis test was carried out. Milk samples were centrifuged at 4000 rpm for 10 min and sediments were cultivated on sabouraud dextrose agar (SDA) by sterile cotton swabs. Petri dishes were incubated at both 25°C and 37°C for 5 weeks. Fungal agents were isolated from 19 (11.1%) of 170 goat milk samples. In culture, 3 (1.7%) milk samples were positive for *Candida (C.) albicans*, 2 (1.1%) for *C. lusitaniae*, 1 (0.5%) for *C. parapsilosis*, 1 (0.5%) for *C. glabrata*, 2 (1.1%) for *Cryptococcus neoformans*, 1 (0.6%) for *Nocardia* spp., 4 (2.3%) for *Penicillium* spp., 3 (1.7%) for *Scopulariopsis brevicaulis*, 2 for (1.1%) *Aspergillus fumigatus*. All clinical mastitis samples were found to be negative for fungi. In conclusion, bacteria are believed to be the major cause of mastitis, but present study indicated that fungi are responsible as primarily aetiological agents of mastitis in goats.

2 INTRODUCTION

Geographical conditions deteriorating due to global warming, the goat industry is probably further increase the importance of the future (Yangilar, 2013). The adaptation of the goat species to its environment as well as its traditional anchoring, are strong foundations on which could be based new development initiatives such as dairy and meat production (Raynal-Ljutovac *et al.*, 2008). Because some features of goat milk such as fat, protein, casein, vitamins, lactose,

specific taste, texture and its natural and healthy image is differ from cow milk (Jenness, 1980; Raynal-Ljutovac *et al.*, 2008). The use of goat milk as an excellent food source is undeniable. It has beneficial effects for health maintenance, physiological functions, in the nutrition of children and elderly people, and according to some authors, can be consumed without negative effects by people suffering cow milk allergy (Yangilar, 2013). The ingestion of goat milk from



healthy animals is very important for the nutrition. Mastitis in goats constitute an enormous animal health problem, alter milk composition, lower the hygienic value of milk, impair the processing properties of milk and cause high economic losses due to reduction in milk yield, decreased milk quality and treatment costs (Stuhr and Aulrich, 2010). This disease can be classified into three major types: clinical mastitis, sub-clinical mastitis and chronic mastitis (Bergonier *et al.*, 2003; Contreras *et al.*, 2007; Quinn *et al.*, 2011). This multifactor disease in dairy small ruminants is mainly of bacterial origin (Bergonier *et al.*, 2003; Contreras *et al.*, 2007; Stuhr and Aulrich, 2010). However, other agents also cause mastitis and the mammary infection

diagnosis consists of underlining the germ responsible for the infection. Fungi are important causative agents of mastitis in ruminants (Quinn *et al.*, 2011) and several yeasts and moulds have been isolated from the cases of mycotic mastitis in goats (Al-Majali and Jawabreh, 2003; Bourabrah *et al.*, 2013). The mastitis studies have been focused on bovine infection (Costa *et al.*, 1993; Guler *et al.*, 2005; Karahan and Cetinkaya, 2007; Ekin *et al.*, 2015), and to a lesser extent on caprine and ovine mastitis (Bergonier *et al.*, 2003; Aydın *et al.*, 2009; Tel *et al.*, 2012; Najeeb *et al.*, 2013). The aim of the present study was to identify the mycotic agents responsible for clinical and subclinical mastitis in goats.

3 MATERIALS AND METHODS

3.1 Samples: A total of 170 milk samples were collected from the 4 commercial hair goats herds in Van Region located Eastern Anatolia, Turkey. The samples were collected from the beginning stages of lactation between May 2014 and April 2015. Before sampling, the teat ends were cleaned by 70% alcohol and dried. The first streams of foremilk were discharged and then 10 ml of milk was collected aseptically in sterile tubes. The samples were immediately transported to the laboratory for California mastitis test (CMT) and mycological examination. All samples were cooled on ice during transport to Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Microbiology.

3.2 Assessment of clinical and sub-clinical mastitis: Clinical mastitis was diagnosed by palpation, visualization of the udder and diagnosed if the udder was swollen red, hard, or hot to touch, a visibly abnormal secretion (Figure 1, some animals were nearly complete cessation), including clots, revealed by the use of a strip-cup test (Coles, 1986; Islam *et al.* 2012). Sub-clinical mastitis was assessed by the California mastitis test (CMT) using CMT reagent (CMT-Test, 100366, Denmark). Reactions were graded as negative, trace and +1,+2,+3 for positive reactions (Larsen, 2000).



Figure 1: A hair goat with clinical mastitis

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.3 Mycological examinations: For the determination of ideal plating method in goat milk, swabs from the cream and pellet (10 min at 4000 rpm) inoculated on two series of Sabouraud dextrose agar (SDA; DM200, Mast Diagnostics, Merseyside, UK). One series supplemented with chloramphenicol (0.05 mg/mL; 220551, Calbiochem, Darmstadt, Germany) and cycloheximide (0.5 mg/mL; C7698, Sigma-Aldrich, Steinheim, Germany), other series without supplement. Petri dishes were incubated aerobically both at 25°C and 37°C for 5 weeks. The isolates were examined macroscopically and microscopically after staining with lactophenol

cotton blue (LFPM; 1.13741, Merck, Germany). Identification of fungi based on gross (colony colour, textures, reverse colour) (Figure 2-5) and microscopic morphology (colour, shape and arrangement of spores along the sporophore, number of septa in the spores etc.) (Figure 6, 7). In addition, carbohydrate assimilation and fermentation tests, urease activity on urea agar (CM52; Oxoid, Basingstoke, UK), pigment production on corn meal agar (CMA; 7350A, Acumedia, Michigan, USA), growth at 37°C on SDA and other biochemical tests were assessed (Larone, 2011; Quinn *et al.*, 2011).



Figure 2: *Cryptococcus neoformans*. Sabouraud dextrose agar, 25°C, 5 days, surface of colony



Figure 3: *Cryptococcus neoformans*. Sabouraud dextrose agar, 25°C, 5 days, reverse of colony



Figure 4: *Scopulariopsis brevicaulis*. Sabouraud dextrose agar, 25°C, 7 days, surface of colony



Figure 5: *Scopulariopsis brevicaulis*. Sabouraud dextrose agar, 25°C, 7 days, reverse of colony

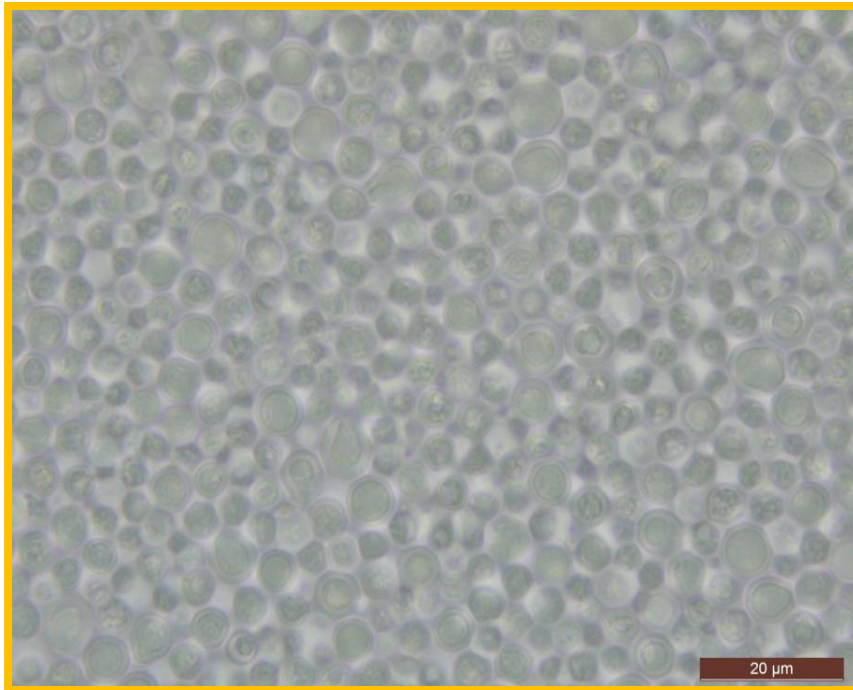


Figure 6: *Cryptococcus neoformans* (40 x 10)

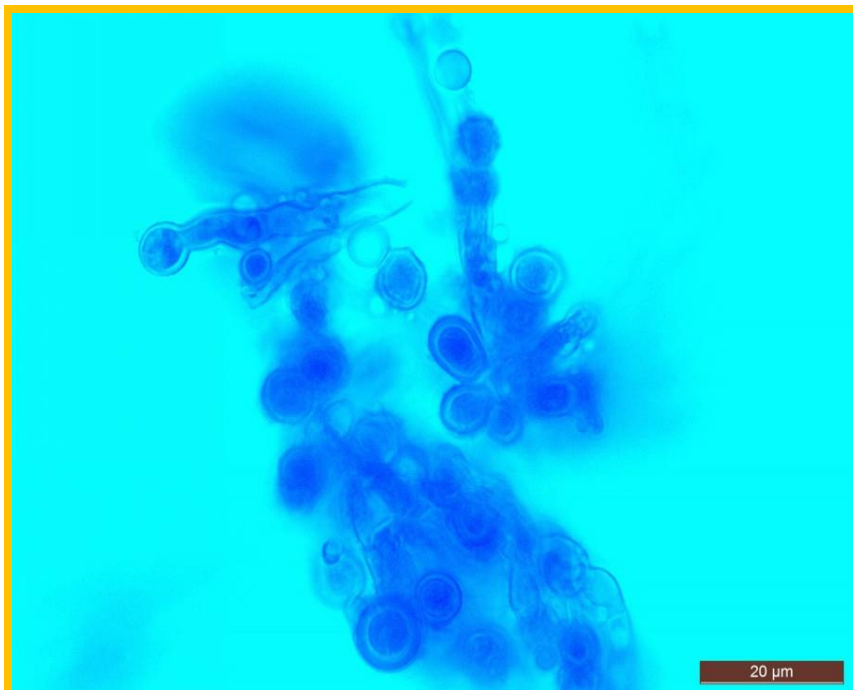


Figure 7: *Scopulariopsis brevicaulis* (40 x 10)

4 RESULTS AND DISCUSSION



Of the 170 goats, 9 (5.2%) were diagnosed as clinical mastitis, 3 (33.3%) of them bilateral and 6 (66.7%) unilateral clinical mastitis, according to clinical diagnosis and strip-cup test. A total of 70 (41.1%) goats were evaluated as subclinical mastitis by CMT and the rest of them (53.5%) were evaluated as healthy. Out of 170 milk samples 19 (11.1%) were found to be positive for fungi whereas 10 (52.7%) were cultured from subclinical mastitis and 9 (47.3%) from healthy

goats. All clinical mastitis samples were found to be negative for fungi. Nineteen yeast and mould isolates belonged to 5 genera (Table 1). In culture, 3 (1.7%) milk samples were positive for *Candida (C.) albicans*, 2 (1.1%) for *C. lusitaniae*, 1 (0.5%) for *C. parapsilosis*, 1 (0.5%) for *C. glabrata*, 2 (1.1%) for *Cryptococcus (C.) neoformans*, 1 (0.5%) for *Nocardia* spp., 4 (2.3%) for *Penicillium* spp., 3 (1.7%) for *Scopulariopsis (S.) brevicaulis*, 2 (1.1%) for *Aspergillus (A.) fumigatus*.

Table 1. Fungi isolated from the milk samples of hair dairy goats

Fungi	Clinical mastitis (n:9)		Subclinical mastitis (n:70)		Clinically healthy (n:91)		Total (n:170)	
	n	%	n	%	n	%	n	%
<i>C. albicans</i>	-	-	2	2.8	1	1.1	3	1.7
<i>C. lusitaniae</i>	-	-	1	1.4	1	1.1	2	1.1
<i>C. parapsilosis</i>	-	-	1	1.4	-	-	1	0.5
<i>C. glabrata</i>	-	-	1	1.4	-	-	1	0.5
<i>C. neoformans</i>	-	-	2	2.8	-	-	2	1.1
<i>Nocardia</i> spp.	-	-	-	-	1	1.1	1	0.5
<i>Penicillium</i> spp.	-	-	1	1.4	3	3.2	4	2.3
<i>S. brevicaulis</i>	-	-	1	1.4	2	2.1	3	1.7
<i>A. fumigatus</i>	-	-	1	1.4	1	1.1	2	1.1
Total			10	14.2	9	9.8	19	11.1

Mastitis is a global health problem of lactating animals and it considered one of the most important diseases of domestic animals, caused by several etiologic agents (Bergonier *et al.*, 2003; Stuhr and Aulrich, 2010; Quinn *et al.*, 2011). Although bacteria are the major microorganisms involved in this disease, fungi have also been incriminated and it should always be born in mind when determining the aetiology of chronic cause of udder infection. The studies of mycotic mastitis have been focused on bovine infection (Costa *et al.*, 1993; Guler *et al.*, 2005; Ekin *et al.*, 2015), and only a few reports have dealt with infection in goats and sheep (Al-Majali and Jawabreh, 2003; Bourabah *et al.*, 2013). Therefore, in this study special attention was paid to fungi in goat mastitis besides bacterial agents. Many studies have been conducted to determine the prevalence of mastitis in small ruminants. Overall

prevalence of goat mastitis including both clinical and subclinical cases has been reported as 36%, 36%, 33% and 18% in the United States, UK, France and Spain, respectively (Poutrel and Lerondelle, 1983; Manser, 1986; Contreras *et al.*, 1995; White and Hinckley, 1999). Moroni *et al.* (2005) found an average prevalence in individual samples of two goat herds as 49.8%. Vasiu *et al.* (2008) diagnosed the prevalence of subclinical mastitis in individual goat milk samples taken from flocks with somatic cell counts as 70.2%. Recently, Bourabah *et al.* (2013) indicated that an average prevalence was 33.9%. In this study, milk samples were collected from mastitis, subclinical mastitis and apparently healthy animals. Overall prevalence was determined as 46.4% whereas 5.2% were clinical and 41.1% were subclinical mastitis. This may be explained, as mastitis is a complex disease involving interactions of



causative organisms, environmental factors and management. Several species of yeast or yeast-like microorganisms from the genera *Candida*, *Cryptococcus*, *Rhodotorula*, *Curvularia* and *Aspergillus* have been isolated from goat's mastitis (Singh *et al.*, 1994; Singh *et al.*, 1998; Chhabra *et al.*, 2004; Hassan *et al.*, 2014). Hassan *et al.* (2012) stated that a total of 25 milk samples were collected from different farms including diseased and apparently healthy cases of sheep and goats. In culture, 6 (24%) milk samples were positive for *C. albicans*, 2 (8%) for *C. krusie*, 1 (4%) for *C. parapsilosis* and 3 (12%) for *Rhodotorula (R.) rubra* in goat milk samples from clinically diseased animals. Two (8%) milk samples were positive for *C. albicans*, 1 (4%) for *Geotrichum (G.) candidum*, 1 (4%) for *R. rubra*, and 1 (4%) for *Trichosporon (T.) cutanium* in apparently healthy goats. In this study, 3 (1.7%) milk samples were positive for *Candida albicans*, 2 (1.1%) for *C. lusitaniae*, 1 (0.5%) for *C. parapsilosis*, 1 (0.5%) for *C. glabrata*, 2 (1.1%) for *C. neoformans*, 1 (0.5%) for *Nocardia* spp., 4 (2.3%) for *Penicillium* spp., 3 (1.7%) for *S. brevicaulis*, 2 (1.1%) for *A. fumigatus*. These isolation rates are lower than indicated by Hassan *et al.* (2012). These differences might be due to the different isolation techniques or different status of immune defensive mechanisms of animals. A majority of yeast isolates from mastitic milk samples belonged to *Candida* genus (Singh *et al.*, 1998). In a study out of 25 milk samples were cultured, 7 (28%) milk samples were positive for *C. albicans*, 2 (8%) for *C. krusie* and 1 (4%) for *C. parapsilosis* (Hassan *et al.*, 2012). In this research, from the 7 candida isolates, 3 (42.8%) were *C. albicans*, 2 (28.5%) *C. lusitaniae*, 1 (14.2%) *C. parapsilosis*, and 1 (14.2%) *C. glabrata*. These findings indicated the importance of *Candida* spp. in the aetiology of mastitis in goats. Cryptococcosis has been described in a many species of animals. *C. neoformans* as a causative agent of mastitis has been isolated from the different mammals (Pal and Randhawa, 1976; El-Far *et al.*, 1987; Menzies and Ramanon, 2001). In this study, *C. neoformans* was isolated from 2

(1.1%) goats suffering from mastitis. This is believed to be the first instance of goat mastitis in which *C. neoformans* has been isolated as the etiologic agent in Turkey. Different saprophytic fungi were isolated from the milk samples of goats (Chhabra *et al.*, 2004; Bourabah *et al.*, 2013). A total of 298 milk samples were collected from each half-udder from 149 goats and evaluated by culture. It was stated that *A. niger* and *A. nidulans* was isolated with a percentage of 24.7% in samples (Bourabah *et al.*, 2013). *Scopulariopsis* spp. is common soil saprophytes and has been isolated from a wide variety of substrates. Among the genus *Scopulariopsis*, *S. brevicaulis*, *S. brumptii*, *S. acremonium*, *S. fusca* and *S. koningii* are frequently related to human infections. Most human infection caused by *S. brevicaulis* is onychomycosis, although there are several reports of other infections including skin infection, endocarditis and endophthalmitis in patients with impaired immunity, trauma or surgery (Lee *et al.*, 2012). In this study, *Penicillium* spp. (2.3%), *S. breviaries* (1.7%) and *A. fumigatus* (1.1%) were isolated from the milk samples obtained from goats with subclinical mastitis and clinically healthy animals. It was stated that these opportunistic fungi are frequently affects immunocompromised patients (Lee *et al.*, 2012). In this research, two samples were positive for *S. brevicaulis* and it can be evaluated as important finding for caprine mycotic mastitis and for public health. It was speculated that goat's milk and dairy products consumption would increase in the future all over the world (Yangilar, 2013). Nevertheless, raw milk provides all necessary nutrients and conditions for growth of many fungal species. The aetiology of mastitis involves multiple infectious agents. Some of the mastitogens have public health significance. Among the fungi (*C. albicans*, *C. tropicalis*, and *C. neoformans*) isolated in this study are frequently isolated agents from the mastitic milk of dairy goats (Mahendra and Vijay, 2013). In conclusion, the present study indicated that dairy goats with subclinical mastitis and apparently healthy



animals might harbour pathogenic fungi, the matter that has a great epidemiological importance as it may cause severe harm for milk consumers. In addition, the prevalence of the

mycotic mastitis will probably increase in future, because of contamination of animal ration as well as the extensive indiscriminate use of antibiotics.

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