Study of bacteria associated with mastitis in dairy sheep farms in the highlands of eastern Algeria

Z. Gabli[1, *], Z. Djerrou [1,2] and A. Gabli[3]

[1]*Department of Nature and Life Sciences, Faculty of Sciences, University of 20 Août 1955, Skikda, Algeria*
[2]*Laboratory of Pharmacology and Toxicology, Institute of Veterinary Sciences, University of Mentouri Constantine, Algeria*
[3]*Institute of Veterinary Sciences, University of Mentouri Constantine, Algeria*

Corresponding author email: zahracom@gmail.com

The aim of this study was to investigate the mammary infections present in dairy sheep farms through a study based on observation and palpation of the udder, as well as by milk cytology and bacteriological analysis. Clinical examination of 1006 lactating ewes revealed 11 (1.1%) with inflammatory signs. Screening the 995 ewes without inflammatory signs by the California Mastitis Test (CMT) for the prevalence of subclinical mastitis showed 59 (5.9%) as positive, with 68 udders affected. The bacterial species were identified from the 11 ewes with clinical mastitis and the 68 udders with subclinical mastitis. Among the bacteria isolated from the 11 ewes with clinical mastitis, *Staphylococcus aureus* was identified in six cases, *Streptococcus uberis* was identified in three cases and *Eschérichia coli* was identified in the other two cases. Among the bacteria isolated, coagulase negative staphylococci (CNS) were the most dominant of the organisms isolated (82.28%). It is suggested that due to the prevalence of mastitis, sheep farmers should be made aware of the problem in order to institute prevention and control measures to reduce the losses due to this disease in dairy ewes.

Keywords: Algeria, bacteriological analysis, CMT, dairy sheep, mastitis

In Algeria, dairy sheep farming has not benefited from any development policy such as the programmes implemented for the benefit of dairy cattle farming. It is only recent that national authorities and veterinary services have realised the importance of sheep farming in milk production and it is now recognised as an indispensable and promising path for the development of unfavourable regions in order to improve the standard of living of rural populations. For this, it is necessary to master the control of certain pathogens of udder infection.

Mastitis is considered to be the most important, frequent, costly and penalising pathologies for dairy farms. In sheep, mastitis is a multifactorial health problem, with many bacterial strains identified as causal agents and many factors accounting for potential predisposition (Gelasakis et al. 2015; Fthenakis and Gonzalez-Valerio 2017; Vasileiou et al. 2018).

Udder infections may or may not be associated with clinical signs; this is why we distinguish between clinical mastitis and subclinical mastitis. Unlike clinical mastitis, subclinical mastitis (SCM) has no observable symptoms in the milk or infected animal. Therefore, SCM and intramammary infection can only be accurately diagnosed through bacterial screening of milk (Fragkou et al. 2014; Knuth et al. 2019). The objective of the present study was to determine the prevalence of clinical and subclinical mastitis in dairy sheep farms in the highlands of eastern Algeria, and to determine the responsible germs associated with the mastitis.

Materials and methods

Study area and animals

The study region occupies an essential place in the field of sheep farming in the highlands in the east of Algeria. This region is characterized by a semi-arid climate and an altitude of over 1200 m. The annual rainfall varies between 200 - 350 mm. There is a large temperature

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range (winter minimum temperatures of -2 to 4°C and high summer heat 33 to 38°C). The study was carried out for 21 months, from March 2019 to December 2020, in dairy sheep farms. The number of animals that were the subject of this study was 1006 lactating ewes, from 21 semi-intensive dairy sheep farms of Ouled Djellal breed, age 2 - 6 years and between the second and the sixth month of lactation; the number of ewes examined per dairy sheep farm visited varied from 40 to 50 ewes. The selection criterion for the farms was the availability of the farmers for obtaining samples. Milk is intended for suckling lambs and for family consumption and milking was manually done in all the farms and no animal was treated with any antibiotic during the period of investigation.

Clinical examination of the udder

A thorough visual examination of each of the 1006 ewes was followed by individual palpation of each udder. The aim was to identify the inflammation which manifests itself in local signs (redness, heat, edema, voluminous, gangrene, asymmetry, sclerosis, and abscess) and functional signs (qualitative and quantitative changes in milk production). This clinical examination revealed 11 (1.1%) ewes with inflammatory signs.

Screening for subclinical mastitis by the California Mastitis Test (CMT)

CMT was performed on milk samples from the 995 animals with presumably healthy udders. Milk from each teat was drawn just before the morning milking, after washing and drying the udder. A quantity of 2 mL of milk to be tested was mixed with the same quantity of surfactant reagent based on Teepol (sodium alkylauryl sulfate). The CMT score ranges from 0 - 4 depending on the appearance of the mixture (Faroult et al. 2003). This test is considered positive when it is greater than or equal to 2. If at least one quarter is positive, the ewe is declared positive.

Samples and bacteriological analyses of milk

Milk samples from ewes with inflammation and those positive for CMT were collected for bacteriological examination. An additional volume of 25 mL of milk from each teat of these animals was collected in a sterile bottle, after disinfection of the teats with 70% alcohol and the elimination of the first spurts (National Mastitis Council 1999). The samples were identified and sent within 24 hours to the laboratory of the Institute of Veterinary Sciences, El Khroub under strict refrigeration at 4°C (Ben Hassen et al. 2003). Each milk sample was inoculated on gloss with sheep blood (5p.100) and incubated at 37°C for 24 - 48 hours. The bacteria were identified by conventional methods (appearance of the colonies, gram stain, catalase test, oxidase test associated with coagulase). The biochemical characters were studied using API galleries (Bio Mérieux) allowing characterisation of bacterial species within the same genus: if at least five bacterial colonies are present, the isolated germ is held responsible for the mastitis (Rakotozandrindrainy and Foucras 2007).

Results

Clinical examination of the udder

As mentioned above, individual clinical examinations of the udder of 1006 ewes revealed inflammations on 11 ewes of the ewes. Figures 1, 2, 3 and 4 show some of the infections revealed. Each infected ewe had only one infected udder.

Figure 1: Gangrenous mastitis

Figure 2: The left half-udder abscess (caseous abscess)

Figure 3: Udder asymmetry with the left half udder hypertrophy

Figure 4: Udder asymmetry with enlarged right half-udder

Subclinical mastitis and the CMT test

The 995 ewes with presumably healthy quarters were analysed by the CMT. The results showed 68 udders belonging to 59 ewes which were positive for infection.

Bacteriological analyses of milk for clinical mastitis

Bacteriological analyses of milk samples from the 11 udders with clinical mastitis indicated three different bacterial species. *Staphylococcus aureus* was found in six cases, *Streptococcus uberis* in three cases and *Eschéricia coli* in two cases (Table 1).

Bacteriological analyzes of milk from subclinical mastitis

The bacteria of subclinical mastitis are coagulase negative staphylococci (CNS); The isolated CNS species were *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus simulans*, *Staphylococcus chromogenic*, *Staphylococcus xylosus*, *Staphylococcus warneri* and *Staphylococcus caprae. Staphylococcus aureus* was also found in three cases in subclinical mastitis (Table 1). All milk samples analysed in this study were mono bacterial with only one dominant species whether there was one or two infected udders.

Table 1: Frequency of isolation of bacteria found in the two mastitis types

Type of mastitis	Bacterial species		Number	% compared to isolations		% of each mastitis
Clinical mastitis	*Staphylococcus aureus*		6	7.60		11(13.92%)
	Streptococcus uberis		3	3.80		
	Eschérichia coli		2	2.52		
Subclinical mastitis	Coagulase negativeive Staphylococci	*Staphylococcus epidermidis*	20	25.32	65(82.28%)	68(86.08%)
		Staphylococcus haemolyticus	17	21.51		
		Staphylococcus simulans	8	10.12		
		Staphylococcus chromogenes	6	7.60		
		Staphylococcus warneri	6	7.60		
		Staphylococcus xylosus	5	6.32		
		Staphylococcus caprae	3	3.80		
	Staphylococcus aureus		3	3.80%		
Total			79	100		100

Discussion

Clinical mastitis

In the present study, the prevalence of clinical infections of the half-udder was 1.1%. This percentage is markedly lower than that of 5% found by Bergonier et al. 2003 on the annual incidence of clinical mastitis in small ruminants in France.

Of the 11 milk samples taken from clinical mastitis, 11 germs were isolated with a percentage of 13.92%; over 50% of these were *Staphylococcus aureus*. This value appears to be lower than the data of Fthenakis 1994a, Bergonier et al. 2002 and Viban Banah 2007, in which they are represented 31.15, 45 and 29.35% of positive bacteriologies respectively. *Streptococcus uberis* was responsible for 3.79%. *E. coli* participated with 2.53% of the isolations of major pathogens; this result is close to the 3.28% recorded in Senegal by Viban Banah 2007.

The presence of *E. coli* is probably attributed to the poor hygienic conditions of certain visited farms, or it could also be due to poor conditions of milking. Some studies reported the existence of germs that we did not isolate in our study such as coagulase negative staphylococci, *Bacillus* and *Micrococcus*. According to a study conducted by Viban Banah 2007, coagulase negative staphylococci recorded 27.89%, *Bacillus cereus* 8.19% and *Micrococcus* sp. was 4.91%.

Subclinical mastitis

The prevalence of subclinical infections was 5.9%. This value is lower than the range of 20 - 55% found by Bergonier et al. 1994c, 1998, as well as in that of Las Helas et al. 1998

which indicated 5 - 67% of half-udders affected by subclinical mastitis. Among the bacteria isolated, coagulase negative staphylococci (CNS) were the most common germs (82.27%). This value is in the range of 80 - 91.5% found by Bergonier et al. 1994a, 1998. However, other researchers have reported lower values (Smaali 2014; Cosseddu et al. 1994; Fthenakis 1994a). In our study, the CNS most often found in subclinical mastitis were *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus simulans*, which represented 25.31, 21.51 and 10.12% of staphylococcal isolates, respectively. *Staphylococcus epidermidis* was also the predominant species in other studies (Bergonier et al. 1994b; Cossedu et al. 1994; Fthenakis 1994a; Las Heras et al. 1998; Moles 2002).

The relative share of *Staphylococcus aureus* in the etiology of subclinical mastitis in dairy ewes was low, only representing 3.79% of the isolates in our study. This is in agreement with the results of studies reported elsewhere (Bergonier et al. 1994b; Cossedu et al. 1994; de la Cruz et al. 1994; Fthenakis 1994; Moles 2002; Smaali 2014). However Brolund 1985 and Fabre et al. 1997 reported values of 29 and 48% of the isolations of *Staphylococcus aureus*. Some works reported the prevalence of germs that we did not isolate in our study such as: *Streptococcus* sp, *Arcanobacterium pyogenes*, *Pasteurella*, *Bacillus*, and Enterobacteriaceae (Lafi et al. 1998; Beheshti et al. 2010; Smaali 2014; Ergun et al. 2009; Otto 1991; Fthenakis 1994a; Bergonier et al. 1998; Las Heras et al. 1998; Moles 2002).

Conclusion

This study conducted on dairy sheep farms revealed the prevalence of clinical mastitis (1.11%) and subclinical mastitis (5.9%). The bacteria isolated from clinical mastitis were *Staphylococcus aureus*, *Streptococcus uberis* and *E. coli*. The most common germs isolated from subclinical mastitis were *Staphylococcus epidermis*, *Staphylococcus haemolyticus* and *Staphylococcus simulans*.

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