Camel mastitis in Southern Algeria

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Abstract

In Algeria, camel breeding participates in the national milk production. However, this breeding faces significant health problems, including mammary pathologies. This disease is a major public health threat, due to the existence of human pathogens in milk. Several species are associated with this mastitis. Unlike cow's milk, goat and camel milk are most often consumed in the fresh raw state, thus escaping any official control. The present study aimed to determine the nature and frequency of mastitis, and the nature and frequency of the responsible bacteria in each type of mastitis in southern Algeria. A total of 62 camels were subjected to clinical examination and screening for subclinical mastitis and the presence of *Brucella* using the California Mastitis Test (CMT) and ring test, respectively. CMT positive samples were then further subjected to bacteriological analysis. Clinical and subclinical mastitis were present with frequencies of 4.44% and 95.55%, respectively. Bacteriological analysis isolated a total of 73 samples of 45 seeds. Staphylococci were most commonly isolated, with a frequency of 63.01%. Among these, *Staphylococcus aureus* were at the top of the list, with 35.61%, while *Staphylococcus* SCN (coagulase negative staphylococci) represented only 27.39%. Streptococci were the second most isolated group, with 28.77%. Gram-positive bacilli were in third place, accounting for 6.85% of all isolates. Gram-negative bacilli (enterobacteria) were isolated with a frequency of 1.36%. Brucella was present with a frequency of 4.44%. Finally, an extended study on a larger sample of camels is required in the future.

Key words: *camel milk; bacteria; CMT; Algeria; Mastitis*

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Introduction

In Algeria, 80% of milk production comes from cattle, the rest is provided by sheep, goats and camels. However, the milk demand is not covered by the local production. Indeed, Algeria ranks third in the world for the import of milk and dairy products. In 2006, more than US$ 500 million were spent on the import of milk powder, to make up for the production deficit (Msaddak et al., 2019). Production covers only 40% of the population demand, due to the fact that the majority of breeders do not own land, which increases the costs of breeding. Another reason is linked to the price of livestock feed, which has risen sharply. At the same time, areas reserved for fodder crops have been markedly reduced. Further, the aging of the cattle population has considerably reduced production, and predisposed animals to develop diseases such as mastitis (Saidi et al., 2013, 2015, 2018).

An alternative to this low milk production is to encourage milk production of other animal species, particularly camel. However, much work remains to be done, especially in the mastery of breeding techniques, since these species is confronted with certain health issues, including mastitis, which can be significant. There has long been a lack of interest in camel mastitis on the grounds that clinical mastitis in this species is infrequent. However, the "subclinical" form of mastitis goes unnoticed and its prevalence is therefore not well known. Several bacterial species are associated with this type of mastitis and their presence in milk can have a negative impact on consumer health (Turk et al., 2017; Benić et al., 2018). The milk of animals with clinical and subclinical mastitis can represent a hygienic threat due to the pathogenic or potentially pathogenic species it contains on the one hand, and by the

Table 1. Characteristics of the selected farms

Criteria	Variables	Number	%
Purpose of breeding	Fattening	4	57.14
	Milk	3	42.85
Type of breeding	Camels only	3	42.85
	Presence of other species	4	57.14
Type of food	Fodder only	1	14.42
	Fodder and concentrate	6	85.71
	Concentrate only	0	0
Destination of milk	Self-consumption	4	57.14
	Sale to dairies	3	42.85
	Informal market	/	/
Observation of clinical mastitis	Yes	2	3.22
	No	/	/
Knowledge of subclinical mastitis	Yes	/	/
	No	0	/

consumption of residues of antibiotics used in treatment on the other (Cvetnić et al., 2016; Burović, 2020). However, in most African countries including Algeria, unlike cow's milk, camel milk is directly consumed most often raw or sold to the informal market thus escaping any quality control.

The objective of this study was to determine the prevalence of clinical and subclinical mastitis in camels and the nature and frequency of the bacteria responsible for this pathology.

Materials and Methods

Study area and selected farms

The study was conducted during the period from February 2016 to May 2017 on seven farms in the Laghouat and Djelfa regions of southern Algeria. Table a summarizes the main characteristics of the farms.

Methods

The study included 62 camels, all from an indigenous race, mainly from the Sahrawi population. Breeding management followed an extensive and sometimes intensive mode and milking was performed manually.

Animals

Animals included in the study were selected randomly within herds according to the accessibility of the breeders. Age, lactation stage, lactation number were not taken into account in the choice of animals. Milk samples were taken from lactating females that may or may not show visible signs of mammary infection to exclude any cases of clinical mastitis.

Examination procedure

The udder and milk of the lactating camels were examined to determine clinical mastitis. The California Mastitis Test (CMT) was also performed to detect subclinical mastitis. Calves were allowed to suckle to stimulate milk. The milk from all quarters of the camel udders was screened with the CMT test, and quarters that responded positively to the test were sampled for subsequent bacteriological analysis to determine the nature of the bacteria involved in this type of mastitis.

Californian Mastitis Test (CMT)

This is a sensitive and rapid method of detecting abnormally cell-rich milk. The principle consists of a mixture of milk and teepool (detergent) in equal quantity to burst the cells, whose nuclear DNA gels on contact with the latter. The size of the gel is directly proportional to the cellular level of the milk (Schalm and Noorlander, 1957).

For detection, in addition to the coloration obtained by the milk/teepool mixture that signals an infection of the udders, the formation of a gel is observed that provides information on the concentration of somatic cells in the milk. In the present study, we were limited to observing the colour of the mixture obtained and the presence or absence of the formation of a gel. Bromocresol purple (pH indicator) is often mixed with the reagent for easy reading. The intensity of the reaction is noted from - to +++ or from 0 to 4. A reaction is considered positive when the assigned score is greater than 1.

Milk collection

Samples for bacteriological analysis were carried out while respecting the aseptic conditions specified in the literature (Abdi et al., 2013). The nipple of the quarter concerned was thoroughly washed with water, dried and the end of the teat was disinfected with cotton wool soaked in 70% alcohol. About 10 mL milk was collected aseptically after the first milk flow was discarded. Sample tubes were labelled and immediately placed in a cooler (4–8 °C) and taken to the bacteriology laboratory.

Bacteriology

Upon arrival at the laboratory, samples were analysed for searching brucella. For this, we applied the Ring Test to milk.

- *Brucellosis test (Ring test)*

The ring test is an immunological precipitation test in a liquid medium conventionally used for the detection of the presence of brucellosis (Saidi et al., 2015).

Study of the microbiological characteristics of collected camel milk

Bacteriological analysis was carried out following the standard method of Noireterre (2006).

- *Seeding and isolation*

Columbia agar with 5% sheep blood was inoculated with 50 to 60 µL milk. This medium made it possible to isolate the majority of bacterial species potentially responsible for mastitis. The medium was placed in an oven at 37 °C. Two readings were carried out respectively at 24 and 48 hours, with some colonies only becoming visible after 36 to 48 hours of incubation (Noireterre, 2006). Table 2 showed the evaluation method of samples.

Table 2. Evaluation of the quality of the sample

Number of types of isolated colonies	Conclusion
0	Sterile sample
1	proper sampling
2	bi-microbial infection
3	contaminated sampling

- *Identification*

Identification to the genus level was conducted by the appearance of colonies on agar and a Gram stain, with detection for catalase for Gram + bacteria and oxidase for Gram - bacteria.

Staphylococci appeared as cocci, Gram + and catalase +. The affinity factor for fibrinogen or "clumping factor" or bound coagulase was sought using the rapid slide test. Free coagulase was not detected by this test. At the end of this test, all the bacteria producing bound coagulase were identified as *S. aureus*. If the bacteria was β-haemolytic and did not bind coagulase, free coagulase was tested using the tube test. At the end of this second test, bacteria showing a positive response were definitively identified as *S. aureus* (Bes et al., 1999).

The genera Streptococcus and Enterococcus were identified as Gram +, catalase - and oxidase – cocci, and colonies were subcultured on agar by flooding. *Enterobacteriaceae* were identified as Gram -, catalase + and oxidase - bacilli (Joffin and Leyral, 2006).

The activity of *S. aureus* coagulase on human plasma was the main criterion to differentiate the coagulase-positive *Staphylococcus* species from other coagulase-negative *Staphylococcus* species (BNA) (Bendimerad, 2010).

Results and Discussion

1. RING test results

The Ring test was conducted on 45 samples of camel milk, and the results are summarized in Table 3.

Table 3. Prevalence of brucellosis in the samples

Number	MRT +	MRT -	Total
	2	43	45
Prevalence %	4.44	95.56	100

The prevalence of brucellosis in the collected samples was 4.44%, a low rate compared to Salman and El Nasri (2012) (32.5%). This could be explained by differences in the study areas and herd management. In this study, camel

Table 4. Prevalence of mastitis in camels and quarters according to CMT results

Sample	CMT				
	Number tested	Positive number	Prevalence %	Negative number	Prevalence %
Camels	62	24	38.71	38	61.29
Udder quarter	239	45	18.83	194	81.17

farms were of the mixed type, where camels were constantly mixed with other animals such as goats. The latter is a potential source of contamination.

2. CMT test results

The prevalence of mastitis according to the results of the CMT is summarized below. Table 4 shows the prevalence of mastitis of the total of 62 camels examined and tested by CMT.

Only 24 (38.71%) camels responded positively to this test, while 38 (61.29%) were negative. These results are not in agreement with Abdi et al. (2013), who found a prevalence of positive CMT of 25.3% and negative CMT of 24.2%. Among the 24 positive camels, 2 showed clinical mastitis (8.33%).

Regarding the prevalence of mastitis at the udder quarter level, of the 239 quarters tested, 18.83% (45 udder quarters) showed a positive response compared to 81.17% (194 udder quarters) with a negative response, which is contrary to Abdi et al. (2013) who reported a positive response of 24.2%. Table 5 summarizes the prevalence of mastitis according to the affected quarters.

Table 5. Prevalence of mastitis by affected quarters

Quarters	Number of camels	Prevalence %
Right posterior	12	26.66
Left posterior	9	20
Right anterior	12	26.66
Left anterior	12	26.66

The CMT score by quarter indicated a similarity in the right posterior, right anterior and left anterior quarters, and they were the most affected with a rate of 26.66%, while the left posterior quarter was the least affected with a rate of 20%. Saleh and Faye (2011) recorded the lowest score of 14% in the right posterior quarter.

Table 6 shows the prevalence of mastitis by stage of lactation, age and lactation rank.

The data showed that camels between the 2nd and 3rd rank were the most affected, at a rate of 51.51%, as opposed to Ahmad et al (2012) who reported that the camels were most affected between the 5th and 6th rank. Camels less affected

Table 6. Prevalence of mastitis according to the lactation stage, lactation rank and age of camels

Camels	Lactation rank		Lactation stage			Age		
	1st	2-3rd	1st month	2-4 months	≥ 5 months	1-5 years	6-10 years	≥ 10 years
Tested	9	33	25	23	14	12	33	17
CMT +	2	17	6	7	4	3	18	3
%	22.22	51.51	24	30.43	28.57	25	54.54	17.65

Table 7. Prevalence of mastitis by collection site and herd feeding

Locality	Food type	Camel at CMT +	Prevalence %	Camel at CMT -	Prevalence %
Sed Rehal (Djelfa)	Semi intensive (Mixed)	5	20.83	3	7.89
Laghouat	Semi intensive (Mixed)	19	79.16	29	74.35
Bellil (Laghouat)	Extensive (natural)	0	0	6	100

Table 8. Prevalence of clinical and subclinical mastitis according to isolated germs.

Bacteria	Clinical mastitis		Subclinical mastitis		Total
	Number	Prevalence %	Number	Prevalence %	
Staphylococcus aureus	1	3.85	25	96.15	26 (35.62%)
SCN	0	0	20	100	20 (27.39%)
Streptococci	1	4 .76	20	95.24	21 (28.77%)
Gram + bacilli	1	20	4	80	5 (6.85%)
Enterobacterium	1	100	0	0	1 (1.36%)

by mastitis were those in the 1st rank with a rate of 22.22%. This is consistent with the results of Ahmad et al. (2012), where animals between the 1st and 2nd rank of lactation were more affected with a rate of 36.23%.

Camels between the 2nd and 4th month of lactation were most affected by mastitis with a prevalence of 30.43%. Ahmad et al. (2012) found that camels in the 1st month of lactation were the most affected (54.55%). In the study, camels between the ages of 6 and 10 years were most affected, with a rate of 54.54%, while Ahmad et al. (2012) found that camels between 14 and 16 years of age were most frequently affected. Table 7 shows the prevalence of mastitis by collection site and herd feeding.

The herd in the area of Bellil (Laghouat region) did not present any clinical or sub-clinical mastitis, which can be explained by the natural diet, where camels graze only the pastures rich in plants of medicinal interest, without ever receiving supplementation.

3. Bacteriological results

Table 8 shows the prevalence of clinical and subclinical mastitis according to the bacterial species isolated from camel milk to determine the species responsible for each type of mastitis.

Bacteriological results indicated more than 45 cases of mastitis which were the subject of an initial sample to identify the bacterial species involved. We found:

- no sterile samples;
- 24 samples (53.33%) where the isolated bacteria were indeed the agent of mastitis, so these were correct samples;
- 16 samples (35.56%) containing two bacterial species;
- Only 5 samples (11.11%) contained three or more bacterial species, and were considered to be contaminated.

In this study, *S. aureus* was responsible for subclinical mastitis with a rate of 96.15% and was responsible for clinical mastitis only in 3.85%. Streptococci were responsible for 4.76% cases of clinical mastitis and Gram-positive bacilli for 20% of cases. *Enterobacteriaceae* were 100% responsible for clinical mastitis.

The rank of bacteria responsible for mastitis (clinical and subclinical) in descending order was 35.62% *S. aureus*, 28.77% streptococcus, 27.39% SCN, 6.85% Bacilli Gram positive and 1.36% enterobacteria, unlike Woubit et al. (2001) who reported the following prevalence of major pathogenic bacteria: 21.1% *S. aureus*, 43.4% SCN and 5.7% streptococcus and the rest for bacilli, enterococci and fungi. For the two results, we note that *S. aureus* are generally the most responsible pathogens for mastitis, either clinical or subclinical.

Conclusions

This study allowed us to determine the frequency of clinical and subclinical mastitis in samples taken from camel herds in southern Algeria. A total of 73 bacteria were isolated from 45 samples. Of all the isolated bacteria, Gram-positive cocci predominated with a high frequency represented mainly by *Staphylococcus*. An extended study on a larger number of camels is required in the future.

References

1. ABDI, H., H. BERIHU, H. ADDISALEM and T. ASAMENEW (2013): Prevalence of camel (*Camelus dromedaries*) mastitis in Jijiga Town, Ethiopia. Afr. J. Agric. Res. 8, 3113-3120.
2. AHMAD, S., M. YAQOOB, M. Q. BILAL, G. MUHAMMAD and L. G. YANG (2012): Risk factors associated with prevalence and major bacterial causes of mastitis in dromedary camels (Camelus dromedarius) under different production systems. Trop. Anim. Health. Prod. 44, 107-112.
3. ALAOUI ISMAILI, M., B. SAIDI, M. ZAHAR, A. HAMAMA and R. EZZAIER (2019): Composition and microbial quality of raw camel milk producedin Morocco. Journal of the Saudi Society of Agricultural Sciences 18, 17-21.
4. BENDIMERAD, A. (2010): Effet de la supplémentations en sélénium sur la réponse immune au cours de l'infection à sarm. Thesis of Master II, Molecular Biology moléculaire, University of Abou Bekr Belkaid Tlemcen, Algeria.
5. BENIĆ, M., N. MAĆEŠIĆ, L. CVETNIĆ, B. HABRUN, Ž. CVETNIĆ, R. TURK, D. ĐURIČIĆ, M. LOJKIĆ, V. DOBRANIĆ, H. VALPOTIĆ, J. GRIZELJ, D. GRAČNER, J. GRBAVAC and M. SAMARDŽIJA (2018): Bovine mastitis: a persistent and evolving problem requiring novel approaches for its control - a review. Vet. arhiv 88, 535-557.
6. BES, M., F. V. GUERIN, H. MEUGHIER, J. ETIENNE and J. FRENEY (2000): Improvement of the identification of staphylococci isolated from bovine mammary infection using molecular methods. Vet. Microbiol. 71, 287-294.
7. BUROVIĆ, J. (2020): Isolation of bovine clinical mastitis bacterial pathogens and their antimicrobial susceptibility in the Zenica region in 2017. Vet stn. 51, 47-52. (In Croatian).
8. CVETNIĆ, L., M. SAMARDŽIJA, B. HABRUN, G. KOMPES and M. BENIĆ (2016): Microbiological monitoring of mastitis pathogens in the control of udder health in dairy cows. Slov. Vet. Res. 53, 131-140.
9. JOFFIN, J. and G. LEYRAL (2006): Microbiologie technique. 4ème édition, centre régional de documentations pédagogique d'Aquitain.
10. HADEF, L., H. AGGAD, B. HAMAD, M. S. MAHMOUD and A. ADAIKA (2016): Subclinical mastitis in dairy camels in Algeria: comparison of screening tests. Acta Argic. Slov. 108/2, 85-92.
11. MSADDAK, M., J. BEN NASR and L. ZAIBET (2019): Resolving recurrent imperfections in the dairy production using gaming simulation. *New. Medit.*18, 35-49.
12. NOIRETERRE, P. (2006): Suivis de comptages cellulaires et d'examens bactériologiques lors de mammites cliniques chez la vache laitiére. Etude expérimentale au centre d'élevage Lucien Bizetde Poisy. Ecole Nationale vétérinaire de Lyon. Thèse présentée à l'université Claude-Bernard. Lyon. France I, pp. 53-55.
13. SAIDI, R., D. KHELEF and R. KAIDI (2013): Bovine mastitis: Prevalence of bacterial pathogens and evaluation of early screening test. Afr. J. Microbiol. Res. 7, 777-782.
14. SAIDI, R., Z. CANTEKIN, D. KHELEF, Y. ERGÜN, H. SOLMAZ and R. KAIDI (2015): Antibiotic Susceptibility and Molecular Identification of Antibiotic Resistance Genes of Staphylococci Isolated from Bovine Mastitis in Algeria. Kafkas. Univ. Vet. Fak. Derg. 21, 513-520.
15. SAIDI, R., F. Z. AISSAOUI, M. BEHALIL, D. KHELEF and R. KAIDI (2018): Prévalence dans le lait de mammites camelines des bactéries pathogènes: quelle réalité? Renc. Rech. Rmnts. 24, 382.
16. SAIDI, R., Z. CANTEKIN, N. MIMOUNE, Y. ERGUN, H. SOLMAZ, D. KHELEF and R. KAIDI (2021): Investigation of the presence of slime production, VanA gene and antiseptic resistance

genes in Staphylococci isolated from bovine mastitis in Algeria. Vet. stn. 52, 57-63.
17. SALEH, S. K. and B. FAYE (2011): Detection of subclinical mastitis in dromedary camels (*Camelus dromedaries*) using somatic cell counts, california mastitis test and udder pathogen. *Emirates Journal of Food and Agriculture* 23, 48-58.
18. SALMAN, A. M. A. and H. A. EL NASRI (2012): Somatic cell count, total bacterial count and acidity properties of milk in Khartoum State, Sudan. J. Cell. Anim. Biol. 5, 223-230.
19. SCHALM, O. W. and D. O. NOORLANDER (1957): Experiment and observation leading to the development of the CMT. Amer. J. V. Res. 25, 75-90.
20. TURK, R., M. KOLEDIĆ, N. MAĆEŠIĆ, M. BENIĆ, V. DOBRANIĆ, D. ĐURIČIĆ, L. CVETNIĆ and M. SAMARDŽIJA (2017): The role of oxidative stress and inflammatory response in the pathogenesis of mastitis in dairy cows. Mljekarstvo 67, 91-101.

Mastitis u deva u južnom Alžiru

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U Alžiru, uzgoj deva sudjeluje u nacionalnoj proizvodnji mlijeka. Međutim, taj uzgoj suočava se sa zdravstvenim problemima, uključujući patologiju mliječnih žlijezda koja zauzima znatno mjesto. Ova bolest predstavlja glavnu prijetnju javnom zdravlju uslijed prisutnosti patogena opasnih za ljude u mlijeku. Naime, nekoliko bakterija povezano je s mastitisom. Za razliku od kravljeg mlijeka, kozje mlijeko i mlijeko deva često konzumiraju sami uzgajivači svježe i sirovo, čime se izbjegava službena kontrola. Ova studija imala je za cilj ustvrditi svojstva i učestalost mastitisa, kao i svojstva i učestalost opasnih bakterija u svakoj vrsti mastitisa u južnoj regiji Alžira. Ukupno 62 deve podvrgnute su kliničkom pregledu vimaa i prisutnosti brucele uporabom kalifornijskog testa za mastitis (CMT), odnosno ring testa. Pozitivni uzorci CMT testa podvrgnuti su bakteriološkoj analizi. Klinički i supklinički mastitis bili su prisutni s učestalošću od 4,44 %, odnosno 95,55 %. Bakteriološka analiza omogućila je izolaciju ukupno 73 uzorka 45 sojeva. Stafilokoki su najčešće izolirani s učestalošću od 63,01 %. Među njima, *Staphylococcus aureus* bio je najprisutniji s 35,61 %. *Staphylococcus* CNS (koagulaza negativni stafilokoki) predstavljali su 27,39 %. Streptokoki su bili druga najčešće izolirana skupina s 28,77 %. Gram-pozitivni bacili bili su na trećem mjestu sa 6,85 % izolata. Gram-negativni bacili (enterobakterije) izolirane su s učestalošću od 1,36 %. Brucela je bila prisutna s učestalošću od 4,44 %. Zaključno, u budućnosti će biti osmišljena proširena studija na većem broju deva.

Ključne riječi: *mlijeko deva, bakterije, CMT, Alžir, mastitis*