Contribution to the study of mastitis in camels in southeastern Algeria

Contribuição para o estudo da mastite em camelos no sudeste da Argélia

Contribución al estudio de la mastitis en camellos en el sureste de Argelia

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ABSTRACT
Mastitis is an important disease on camel farms in Algeria, affecting camels at different stages of lactation. This study includes several camel farms in the wilaya of Oued Souf and Ouergla in southeastern Algeria, where the consumption of raw camel milk is important, but it can expose consumers to the risk of zoonotic agents, which are currently underestimated. The objective of the study is to estimate the prevalence of the disease and detect the bacteria involved. The number of animals in the herds surveyed was 56 dromedaries.The CMT test revealed that mastitis had a prevalence of 26%; it was most prevalent in females aged between 14 and 35 years (57%), and the majority of cases occurred in summer with a prevalence of 38% compared with 19% in spring. Bacteriological analysis showed that staphylococcus aureus was the main

cause of subclinical mastitis (79%), followed by Escherichia coli (21%). However, in the clinical form we found that *E.coli* is the most involved, with a prevalence of 75%, followed by *staphylococcus aureus* in 25% of cases. The bacterial strains isolated were resistant to certain antibiotics such as streptomycin and ofloxacin, but were also sensitive to amoxicillin and sulphamethoprim.

Keywords: mastitis, camel, prevalence, *Staphylococcus*, *Escherichia coli*.

RESUMO
A mastite é uma doença importante nas fazendas de camelos na Argélia, afetando os camelos em diferentes estágios da lactação. Este estudo abrange várias fazendas de camelos nas wilayas de Oued Souf e Ouergla, no sudeste da Argélia, onde o consumo de leite cru de camelo é significativo, mas pode expor os consumidores ao risco de agentes zoonóticos, atualmente subestimados. O objetivo do estudo é estimar a prevalência da doença e detectar as bactérias envolvidas. O número de animais nos rebanhos pesquisados foi de 56 dromedários. O teste CMT revelou que a mastite tinha uma prevalência de 26%; foi mais prevalente em fêmeas com idades entre 14 e 35 anos (57%), e a maioria dos casos ocorreu no verão, com uma prevalência de 38% em comparação com 19% na primavera. A análise bacteriológica mostrou que *Staphylococcus aureus* era a principal causa de mastite subclínica (79%), seguido por *Escherichia coli* (21%). No entanto, na forma clínica, encontramos que *E. coli* é a mais envolvida, com uma prevalência de 75%, seguida por *Staphylococcus aureus* em 25% dos casos. As cepas bacterianas isoladas eram resistentes a certos antibióticos, como estreptomicina e ofloxacina, mas também eram sensíveis à amoxicilina e ao sulfametoxazol.

Palavras-chave: mastite, camelo, prevalência, *Staphylococcus*, *Escherichia coli*.

RESUMEN
La mastitis es una enfermedad importante en las granjas de camellos en Argelia, que afecta a los camellos en diferentes etapas de la lactancia. Este estudio incluye varias granjas de camellos en las wilayas de Oued Souf y Ouergla en el sureste de Argelia, donde el consumo de leche cruda de camello es importante, pero puede exponer a los consumidores al riesgo de agentes zoonóticos, actualmente subestimados. El objetivo del estudio es estimar la prevalencia de la enfermedad y detectar las bacterias involucradas. El número de animales en los rebaños encuestados fue de 56 dromedarios. La prueba CMT reveló que la mastitis tenía una prevalencia del 26%; era más prevalente en hembras de entre 14 y 35 años (57%), y la mayoría de los casos ocurrieron en verano con una prevalencia del 38% en comparación con el 19% en primavera. El análisis bacteriológico mostró que *Staphylococcus aureus* era la principal causa de mastitis subclínica (79%), seguido por *Escherichia coli* (21%). Sin embargo, en la forma clínica encontramos que *E. coli* es la más involucrada, con una prevalencia del 75%, seguida por *Staphylococcus aureus* en el 25% de los casos. Las cepas bacterianas aisladas eran resistentes a ciertos antibióticos como la estreptomicina y la ofloxacina, pero también eran sensibles a la amoxicilina y al sulfametoxazol

Palabras clave: mastitis, camello, prevalencia, *Staphylococcus*, *Escherichia coli*.

1 INTRODUCTION

Dromedary (Camelus dromedarius) husbandry occupies an important place in the Saharan zones of Algeria ensuring the production of meat,milk, and other products such as, leather and wool (Meguellati et al 2018).Thus, camel milk is a staple food for nomadic people. It possesses valuable nutritional properties, such as a high proportion of antibacterial substances and a large proportion of the daily requirements of humans for these nutrients, as camel milk contains most of the essential nutrients (Abdurahman OA et al 1998). Several diseases have a negative impact on camel productivity, of which mastitis is a major one (Abdi H et al 2013, Alhendi et al 2000).

Mastitis in camels is responsible for major economic losses such as costs incurred in treatment and poor growth that follows the disease (Alamin et al 2013).It is often of bacterial origin including zoonotic agents (Al-Juboori et al 2013, Alhendi et al 2000), so contaminated milk is a major source of infection for susceptible subjects. It is important to point out that epidemiological and microbiological data are really rare and very few studies have been carried out on camels in our study area.

The aim of this study is to examine the epidemiology of mastitis in camels in southeastern Algeria, estimate the prevalence of the disease and its distribution as a function of a number of zootechnical parameters (age, season and region) and identify the bacteria involved and their antibiogram.

2 THEORETICAL FRAMEWORK

2.1 STUDY AREA AND PERIOD

Our work concerned extensive and semi-extensive dairy camel farms in the willayas of Oued Souf and Ouergla in the southeast of Algeria, and a few farms in the south of the wilaya of Tébessa in the east. It took place from March 2022 to July 2022.

Regarding the choice of season, we carried out our research in the two breeding seasons that are known in the study region, spring and early summer, in order to sample lactating females where they are predisposed to mastitis at this physiological stage.

2.2 TARGET POPULATION

All the analyses were carried out on 56 milk samples taken from females aged between 8 and 35 years, of which 4 camels showed clinical signs of mastitis. The study population consisted mainly of the Mehari breed and a few head of Targuie breed distributed among 6 dairy farms, fed mainly on pasture and barley mixed with wheat bran.

The source population was based on the clienteles of a few veterinary surgeons in the region who agreed to take part in our survey, so we can classify our sampling as convenience sampling (B. Toma, 2007).

2.3 COLLECTION OF SAMPLES

A clinical examination of the udder was carried out before each collection of milk samples to detect udder abnormalities, such as swelling, lesions, induration, fibrosis and congenital abnormalities. The teat was disinfected with cotton moistened with 70% alcohol. After removing the first sprays of milk, we tested the milk from each quarter using the CMT test (California Mastitits

Test). Then, milk samples were collected from the diseased teats (positive to the CMT test) in sterile plastic bottles, labelled and transported in an isothermal cooler (4 to 8°C) within 24 hours to the Animal Production Laboratory and Biotechnology and Health (PABIOS) Laboratory at the Taoura Institute of Agronomic and Veterinary Sciences (ISAV), Souk Ahras University for bacteriological examination. Each sample was accompanied by an individual information sheet containing the sample number, date, number of the farm visited, age, calving condition, clinical appearance of the animal, color and consistency of the milk.

2.4 BACTERIOLOGICAL EXAMINATION

For bacteriological analysis, we commenced by diluting 1 ml of the sample with 10 ml of sterile 0.9% physiological water. After incubation in nutrient agar for 24 hours, we proceeded with Gram staining and then inoculation in selective media using Mac Conkey and Hektoen medium for Gram-negative bacilli and Chapman agar for isolating Gram-positive cocci. Bacterial isolates were identified based on the morphological characteristics of the colonies, Gram staining reactions, haemolytic reactions, the catalase test and other biochemical tests.

2.5 ANTIBIOGRAM

In order to determine the sensitivity of the strains isolated, we used the agar diffusion method, which allows us to apply several antibiotic discs simultaneously.

We used Muller-Hinton agar cast in petri dishes to a thickness of 4 mm and then dried before use. The antibiotic discs were placed on the agar with a dispenser or with the aid of a thin pair of flaming forceps. No more than 6 antibiotic discs should be placed on the culture plate, so the antibiotic discs should be spaced 24 mm apart, centre to centre, then incubate the plates in an oven at 37°C for 18 to 24 hours: Ofloxacin, Cefotaxime, Streptomycin, Gentamicin, Amoxicillin, Sulfamethoxazole-trimethoprim.

The reading was done by accurately measuring the inhibition diameters using a calliper, then we compared these results to the critical readings in the reader table, where we classified the bacteria isolated into one of the categories: sensitive, intermediate or resistant.

2.6 STATISTICAL ANALYSIS

The results were processed using an Excel spreadsheet (Exc-07) based mainly on descriptive statistics, which consisted of calculating percentages (rates) and determining the dependence between mastitis and certain zootechnical and environmental parameters, bacteria and type of mastitis by using the Chi-square test of dependence for comparison between various variable at the

α = 5% significance level.

Given that the study area is located in a Saharan region, the vast size of the area and the extensive farming methods made it particularly challenging to access all farms and animals. Moreover, the study's duration, limited to only a few seasons, did not allow for a full understanding of seasonal variations. Therefore, it would be advisable to extend the study period to a full year or more to better assess the influence of the seasons and increase the sample size for more representative results.

3 RESULTS

3.1 RESULTS OF THE CMT TEST

Out of 56 females included in our study population, 4 showed clinical signs of clinical mastitis, either a prevalence of 7.14%, while the prevalence of subclinical mastitis was estimated at 26. The prevalence of clinical mastitis was 92% with the CMT test, which revealed 14 positive cases out of a population of 52 camels, although this varied from one area to another. It was higher in the Ouargla region, with a prevalence of 40%, but lower in the Tebessa region (19.3%), although the chi-square test of dependence did not show a significant difference (p>0.05).

Table 1: Prevalence of different forms of mastitis in camels

Type of mastitis	Number of positives	Prevalence (n=56)
Clinical	4	7,14%
Subclinical	14	26,92%
Total	18	27,69%

Source: Prepared by the authors (2024)

Table 2: Prevalence of subclinical mastitis by CMT test, by region

Region	Number of Samples	Positive cases (CMT+)	Prevalence (%)
El-oued	11	4	36,36%
Ouargla	10	4	40,00%
Tebessa	31	6	19,35%
Total	52	14	26,92%

Source: Prepared by the authors (2024)
Chi-square (x2) = 1,65/ p= 0,4

3.2 SUBCLINICAL MASTITIS BY AGE

The distribution of CMT-positive cases differed significantly according to the age of the camel, with a very high incidence in females aged over 8 years.

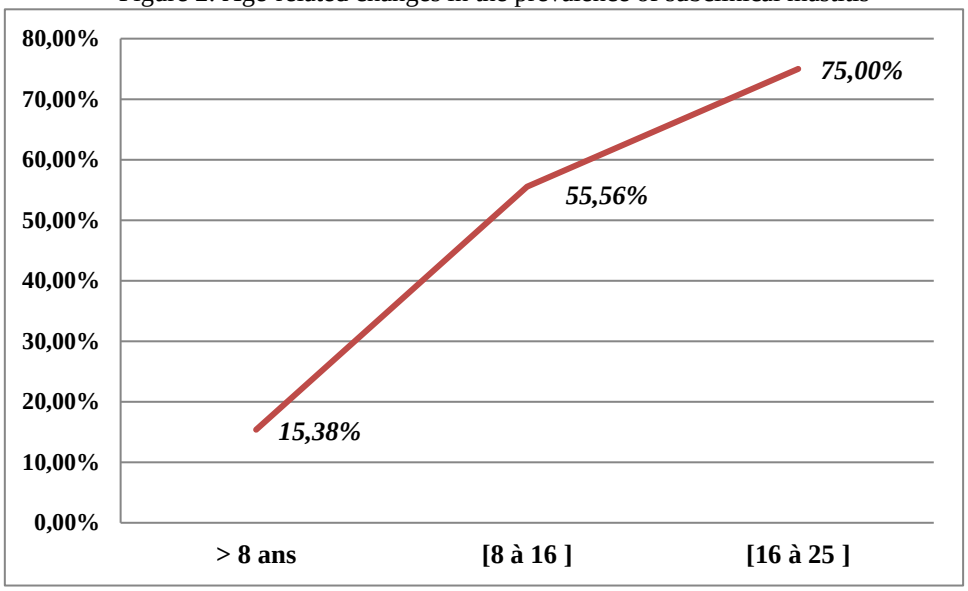
Table 3: Prevalence of subclinical mastitis according to age

Age range	Number of Samples	Positive cases (CMT+)	Prevalence (%)
> 8 years old	39	6	15,38%
[8 -16 years]	9	5	55,56%
[16 - 25 years]	4	3	75,00%
Total	52	14	26,92%

Source: Prepared by the authors (2024)
Chi-square (x2) = 8,16 / p=0,01

The table above shows that the 16 to 25 age group is the most sensitive (75%), followed by the 8 to 16 age group, and despite the fact that most of the samples tested (39) came from females aged under 8 years, the prevalence was low (15%) compared with the other age groups. The chi-square test showed a significant relationship between subclinical mastitis and age ($p<0.05$).

Figure 2: Age-related changes in the prevalence of subclinical mastitis



Source: Prepared by the authors (2024)

Figure 2 shows that subclinical mastitis develops progressively with age, with females over 8 years of age being the most common and camels between 16 and 25 years of age being the most susceptible. On the other hand, animals under 8 years of age are also at risk of subclinical mastitis, but with a significantly lower prevalence.

3.3 SUBCLINICAL MASTITIS BY SEASON

During the study period, 31 samples were collected in spring and 21 samples were analysed in summer. The prevalence of subclinical mastitis was 19.35% in spring and 38.1% in summer.

Table 4: Prevalence of subclinical mastitis according to season

Season	Samples	Positive case (CMT+)	Prevalence (%)
Spring	31	6	19,35%
Summer	21	8	38,10%
Total	52	14	26,92%

Source: Prepared by the authors(2024)
Chi-square (x2) = 1,38 / p=0,2

With regard to the effect of season on mastitis in camels, we recorded 8 cases in the summer season with a prevalence of 38.1% and only 6 cases in the spring season with a prevalence of 19.35%. Although the chi-square test of dependence did not show a significant difference (p>0.05).

Regarding the effect of season on camel mastitis, we recorded 8 cases in summer, giving a prevalence of 38.1%, and only 6 cases in spring, giving a prevalence of 19.35%. The Chi-square test of dependence did not show a significant relationship between the season of the year and mastitis (p>0.05), Despite the fact that there is no statistical link with the season, the prevalence is very high in the summer, because the summer in the study region is a dry season when feed is less available and consequently it can increase the prevalence of several infectious diseases such as subclinical mastitis.

3.4 BACTERIOLOGICAL RESULTS

Of all the CMT+ samples submitted for bacteriological analysis, 11 showed the presence of a pathogen associated with mastitis, with Staphylococcus aureus dominating, isolated in 11 samples with a prevalence of 78.57% (11/14), followed by Escherichia coli (21.43%). We also observed that all E. coli positive samples (3) were infected with Staphylococcus aureus.

Table 5: Prevalence of bacterial species isolated from lactating camels

Bacterial species	Number of isolates	Prevalence (%)
Staphyloccocus aureus	11	78,57%
Escerichia coli	3	21,43%
Total	14	100%

Source: Prepared by the authors (2024)

3.5 PREVALENCE OF BACTERIA ACCORDING TO THE TYPE OF MASTITIS

On the basis of our laboratory analysis, we found that Staphylococcus aureus was the most dominant bacterium in both types of mastitis in camels, since it was involved in clinical and subclinical mastitis at a rate of 30% and 40% respectively. Escherichia coli, on the other hand, was isolated in 10% of cases of clinical mastitis and 20% of cases of subclinical mastitis.

Table 6: Prevalence of bacteria according to type of mastitis

Bacteria	Clinical mastitis		Subclinical mastitis		Total
	Number	Prevalence (%)	Numbre	Prevalence (%)	
Staphylocoque aureus	1	25%	11	78,57%	66,66%
Escherichia coli	3	75%	3	21,43%	33.33%

Source: Prepared by the authors(2024)
Chi-square (x2) = 9,09/ p= 0,002

The prevalence of pathogenic bacteria isolated from camel milk with or without clinical signs of mastitis is shown in Table 6. In our study, we found that Staphylococcus aureus was responsible for subclinical mastitis with a prevalence of 78. In our study we found that Staphylococcus aureus was responsible for subclinical mastitis with a prevalence of 78.57% and that it was involved in 25% of the cases of clinical mastitis. Escherichia coli was the main cause of clinical mastitis with a prevalence of 75%, although it was isolated in 21% of the cases of subclinical mastitis. The chi-squared test showed a significant difference ($p<0.05$). Overall, Staphylococcus aureus was the most commonly isolated germ (66%) in camel milk from the farms included in our study.

3.6 ANTIBIOGRAM

In our study, all isolates obtained from clinical and subclinical mastitis cases were subjected to an antibiotic susceptibility test using six (6) antibiotics. The results of antimicrobial susceptibility and resistance are shown in the prevalence table below.

Table 7: In vitro susceptibility of mastitis pathogen isolates (n=56)

Antibiotics	Susceptible (%)		Intermediate (%)		Resistant (%)	
	S.aureus	E.coli	S.aureus	E.coli	S.aureus	E.coli
Cefotaxime CTX	17%	22%	30%	26%	65%	83%
Ofloxacine OFX	13%	17%	9%	13%	91%	86%
Amoxicilline AML	87%	78%	17%	16%	17%	26%
Nitrofurantoine F300	78%	91%	14%	13%	26%	30%
SulfamethoprimSXT	26%	30%	15%	13%	78%	89%
Streptomycine STR	17%	26%	9%	11%	87%	96%

Source: Prepared by the authors(2024)

Most strains of Staphylococcus aureus and E.coli were sensitive to amoxicillin and nitrofurantoin (>78%). However, all these isolates were resistant to streptomycin, cefotaxime, ofloxacin and sulphamethoprim.

4 DISCUSSION

The results obtained concern 56 samples from lactating camels aged between 8 and 25 years from four different herds to ensure that our results are representative of the herd in the study region. The target population included 4 camels (7.14%) that showed signs of clinical mastitis, and the CMT

test detected subclinical mastitis in 14 females aged 8 years and older, a prevalence of 26%. 92%, our result is similar to that of Woubit et al in 2001 in south-western Ethiopia who found prevalences of 28, 8% and 1.7% for subclinical and clinical mastitis respectively, while in the same area subclinical mastitis was found with a prevalence of 18. Similarly, the prevalence found in the present study is lower than that observed by Saidi et al. in 2021 in the Leghouat and Djelefa region (south-central Algeria), who found a prevalence of 38.71%, i.e. 24 positive cases in a study population of 62 camels, and significantly higher than that shown by Al-Juboori et al. in 2013 in the Abu Dhabi region (24.7%). We also found that females over 14 years of age were the most affected, because the number of lactations and lactation play a very important role in the appearance of lesions in the udder. This could also be explained by the onset of a decline in immunity in older animals. On the other hand, the 6-10 year age group was the most susceptible, with a prevalence of 51% in 2nd and 3rd year lactating females in the study by Saidi et al 2021.

In our study, we showed that the season can influence the prevalence of mastitis in camels, as the majority of positive cases were detected in summer (38.1%). This could be explained by the lack of fodder, and especially of grasses, due to the drought conditions in the Saharan zone. With regard to the type of rearing, several authors have shown the importance of the type of rearing in the occurrence of mastitis, such as (Saidi et al 2021) who only detected positive cases in females reared semi-intensively. This is in contrast to what we found in the present study, where all samples were taken from animals reared extensively.

Regarding the bacterial agent responsible for clinical and subclinical mastitis, our results are in agreement with those of Saidi, Al-Juboori, Geresu and Woubit (2021,2013,2021,2001), to this effect it is staphylococci which are the first responsible for subclinical mastitis in camels especially S.aureus followed by streptococci on the other hand clinical mastitis was often caused by streptococci and E. coli, in our study Staphylococcus aureus was detected in 78.57% of CMT + samples and in the remaining CMT positive cases we isolated Escherichia coli (21.42%), this is higher than the results of Geresu (2021) which are 11.9% and 10% for Staphylococcus aureus and Escherichia coli respectively. samples were taken from extensively reared animals.

However, Woubit (2001) found Staphylococcus aureus and Staphylococcus hyicus with a prevalence of 21.1% and 25.3%, respectively, and Escherichia coli with a low prevalence (0.4%) in cases of subclinical mastitis, adding that Aljuboori et al. (2013) showed that Staphylococcus is the main pathogen of mastitis in camels with a prevalence of 40.67%, followed by Streptococcus spp (21.67%). Our result is close to that of Saidi, who detected Staphylococcus aureus in 96% of cases of subclinical mastitis, which explains the involvement of the contaminated environment in the transmission of infection, according to Kula et al. (2017) and El Tigani (2020), several other

infectious agents of bacterial origin can also be isolated, such as Corynebacterium spp, Mycoplasma spp, Actinomyces pyogenes, Micrococcus, Pasteurella haemolytica and Bacillus spp, as well as fungal pathogens, which may also be involved in camel mastitis.

In humans, Staphylococci and E.coli are responsible for a number of respiratory, digestive and urinary tract infections. Staphylococci are bacteria involved in a variety of pathologies of varying degrees of severity. They are one of the main causes of nosocomial infections.

Enterohaemorrhagic E. coli (EHEC), strains of EHEC regularly cause food poisoning through the consumption of animal products (meat or dairy products).

The strains isolated from Staphylococcus aureus and Escherichia coli were resistant to olfloxacin and streptomycin in the antibiograms performed in our study. This agrees with the findings of Kant et al 2003 who reported that strains isolated from Staphylococcus aureus were resistant to penicillin and streptomycin. However, these strains are susceptible to certain antibiotics such as amoxicillin, sulphamethoprim and nitrofuran, the latter of which was reported by Kan et al 2003 to be effective with tetracyclines and oxytetracyclines.

Antibiotic resistance is caused by self-medication and the uncontrolled use of antibiotics, especially in desert areas where it is difficult for veterinary surgeons to get around.

Finally, our results are always linked to the survey context, such as the study region, the number of samples analysed, which was not representative of the Camlin population in the region, but this is due to the difficulty of accessing the farms, which are mainly extensive, and therefore difficult to move around in the Saharan zones. In addition, the duration of the study is somewhat limited to just a few seasons, so it would be preferable to extend the study over the whole year in order to better study the seasonal effect.

5 CONCLUSION

In the camel farms of south-eastern Algeria, mastitis is a common disease. Several risk factors are involved, including age, season, lactation stage, farm type, immune status and general animal condition. This infection can be caused by a number of bacterial pathogens. In the case of our research, it appears that Staphylococcus aureus is most commonly involved in subclinical mastitis, with less involvement in clinical mastitis. Escherichia coli was also isolated in both types of mastitis. The bacterial strains isolated in our study were resistant to certain antibiotics such as streptomycin and olfloxacin, but sensitive to amoxicillin and sulfonamides. Given the importance of this pathological entity, further research is needed to determine the prevalence of the different bacteria responsible for this pathology. Furthermore, to ensure the accuracy of the results, our further research requires an increase in the number of samples, a staggering of the study period and

would include several areas of breeding concerned. It is also of interest to use other methods (ELISA, PCR...) in the laboratory analysis. As well as the practices responsible for the appearance of cases of antibiotic resistance, in order to implement control measures capable of reducing the economic impact of these pathologies and also to detect any zoonotic agents.

Finally, given the risk of the presence of zoonotic agents, raw milk consumption should be avoided and all hygiene rules should be respected. Antibiotic therapy must be monitored by veterinary surgeons and farmers should avoid self-medication in order to reduce antibiotic resistance, which can also be developed in humans through the consumption of milk and dairy products containing antibiotic residues.

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