Subclinical mastitis in cattle in Algeria: Frequency of occurrence and bacteriological isolates

Authors:
Radhwane Saidi[1]
Djamel Khelef[2]
Rachid Kaidi[3]

Affiliations:
[1]Department of Agronomy, University Telidji Amar, Algeria

[2]Laboratory of Animal Health and Production, National Higher Veterinary School of Algiers, Algiers

[3]Department of Veterinary Sciences, University SaadDahleb, Algeria

Correspondence to:
Radhwane Saidi

Email:
saidi.radhwane@yahoo.fr

Postal address:
PO Box 37G, 03000 Laghouat, Algeria

Dates:
Received: 01 Sep.2012
Accepted: 02 Apr. 2013
Published: 23 May 2013

How to cite this article:
Saidi, R., Khelef, D. & Kaidi, R., 2013, 'Subclinical mastitis in cattle in Algeria: Frequency of occurrence and bacteriological isolates', *Journal of the South African Veterinary Association* 84(1), Art. #929, 5 pages. http://dx.doi.org/10.4102/jsava.v84i1.929

The present study was carried out to determine the prevalence of subclinical mastitis in cattle in eighteen herds in the center region of Algeria. Milk samples were collected from 560 quarters of 140 cows free of clinical mastitis. The samples were subjected to California Mastitis Test (CMT) and the positive samples were analysed by bacteriological culture and Speed Mam® Color. The overall quarter prevalence was 28.77% whilst animal prevalence was 28.57%.Bacteriological analysis showed that there was a wide range of bacteria that cause these infections. *Staphylococcus aureus* (40%) was found to be the most prevalent organism followed by *Streptococcus* spp. (12.5%), *Enterobacteriaceae* (2.5%), *Pseudomonas* spp. (2.5%), *Staphylococcusaureus* + *Streptococcus* spp. (12.5%), *Streptococcus* spp.+ *Escherichia coli* (7.5%), *S. aureus* + *Mycoplasma* spp.(7.5%), and *S. aureus* +*Streptococcus* spp.+ *E. coli* (5%).

Introduction

Bovine mastitis is one of the most problematic diseases and continues to have a major economic impact on the dairy industry throughout the world (Bachaya *et al.* 2011). It is one of the most prevalent, important and costly diseases of dairy animals worldwide, with losses of over 1.7 billion dollars a year in the USA alone (Sahoo *et al.* 2012). It is characterised by an increase in somatic cells, especially leukocytes, in the milk and by pathological changes in the mammary tissue (Ranjan *et al.* 2010).

Various forms of clinical and subclinical mastitis occur in bovines. In clinical mastitis all the five cardinal signs of udder inflammation (redness, heat, swelling, pain and loss of milk production) are present, whilst in the sub-clinical form there are no obvious manifestations of inflammation. Subclinical mastitis is 3–40 times more common than clinical mastitis and causes the greatest overall losses in most dairy herds (Bachaya *et al.* 2011). It is responsible for 70% of economic losses (Heleili *et al.* 2012) and has a prominent place amongst the factors that limit milk production. This disease also poses a risk for the transmission of major zoonotic diseases like tuberculosis, brucellosis, leptospirosis and streptococcal sore throat to human beings (Bachaya *et al.* 2011). Mastitis has therefore become a major area of concern in the field of veterinary clinical practice worldwide.

The detection of mastitis is difficult. Clinical mastitis is confirmed by observation of clinical signs by the farmer (direct detection) (Hokmabad *et al.* 2011). Subclinical mastitis is recognised by indirect detection: the somatic cell count in milk (Hokmabad *et al.* 2011) or by animal-side milk tests (Bachaya *et al.* 2011), but most of the farmers in Algeria are not familiar with these techniques. In both cases, detection is usually late and no attempt is made to isolate and identify the causative organism. Therefore it is essential to identify and quantify pathogens to assess the adequacy of the therapeutic arsenal, avoid further complications and adapt management practices for the implementation of effective control of mastitis.

The purpose of this survey was to investigate the incidence and the microbial populations associated with subclinicalmastitis.The study was conducted on 140 cows in the Blida and Ain Defla governorates in central Algeria.

Materials and Methods

Study area

A cross sectional study was conducted in the central region of Algeria. Two zones, namely Blida and Ain Defla, were selected based on dairy cow population, trend in dairy investment and the existence of different husbandry practices. These temperate zones are considered to be a dairy basin. This area is characterised by an average rainfall of 500 mm – 600 mm per year, with a progression from an arid climate in the valley to a humid climate on the reliefs.

According to the agricultural services (Direction des Services Agricole de Blida 2009), Blida had a total of 18 920 cattle, with 9500 dairy cows, of which 4920 were imported dairy cattle that include the Holstein Friesian breed. The *wilaya* of Ain Defla had an estimated cattle population of 37 730, including 6205 imported dairy cows and 15 685 local cows and crossbred cows.

Inclusion criteria

The selection criteria were ease of access to farms and the availability of breeders and their receptiveness to such studies. A cross sectional survey was conducted on 18 cattle farms between March 2012 and July 2012, which correspond to the seasons of spring and summer, during which forage availability promotes optimal milk production.

To be included in the study, a cow had to present at least one quarter positive on California Mastitis Test (CMT) and to have received no systemic or local treatment of any nature within the last 15 days.

Characteristics of the study population

The study animals included 46 local breed cows, 50 imported breed cows and 44 of their crosses. Local dairy cows were managed under traditional and extensive husbandry systems. They were relatively smaller in size with small udders and short teats. Milk production was poor with an average of 4 L – 5 L per cow per day. Imported cows were managed under modern and intensive husbandry systems, whilst crossbred cows were often managed under a small-scale, semi-intensive management system. They were often provided with some supplementary diet in addition to the natural pasture and agricultural by-products and were usually maintained in separate stalls a short distance from each other in a stable. This type of dairy husbandry system is increasingly becoming an important source of milk for households and a means of income generation in urban and peri-urban areas of both the Blida and Ain Defla zones. Manure is removed daily.

All milking was done by hand except for one farm, where it was done by a milking machine. Pre-milking and post-milking hygienic procedures such as udder washing was practised on 77.78% of farms, whilst udder drying was never practised. All cows were allowed to dry off in latelactation by abrupt cessation of milking. During the drying period, with an average duration of one to four months, only one of 18 farms included in this study administered an intramammary antibiotic as prevention. On the other 17 farms no preventive antibiotic treatment was given.

Collection of milk samples

A total of 560 milk samples were collected from cows with subclinical mastitis, based on the absence of the five cardinal signs of udder inflammation (Bachaya *et al.* 2011). At the time of each examination, information about breed, age, parity, stage and rank of each cow, degree of quarter attack, type of husbandry system and the village site were recorded. The information about the animal being examined and tested was attached to every sample collected. Before examination the udder was thoroughly washed, dried with a clean towel and the teats were sprayed with 70% ethanol. Then the first few jets of milk were discarded and a small quantity was used to perform the CMT.

Screening for mastitis with California Mastitis Test

Five hundred and fifty-six milk samples from individual quarters of 140 cows (four quarters proved non-functional) were analysed by the CMT, a qualitative measurement of the Somatic Cell Count (SCC) in milk. This is a screening test for subclinical mastitis that can be easily used in the milking shed (Bafitan *et al.* 2008). The principle of the test is that detergent causes rupture of somatic cells when added to a milk sample and DNA and other cell contents are released. DNA and detergents unite to form a gel, the consistency of which depends upon the number of somatic cells (Bosse 1982; Fadrig 1988; Oaki 1990).A change in the consistency of the milk indicated mastitis, whilst no change in consistency indicated healthy samples. The intensity of the mastitis was graded into categories from 0 to 4, based on the severity of disease.

From the quarters that tested positive, 25 mL of milk was collected in a sterile bottle, kept at 4 ºC and transported immediately to the Regional Veterinary Laboratory, Laghouat, in an insulated container and analysed in the microbiology unit.

Laboratory analyses

CMT positive samples were subjected to bacteriological analysis to isolate pathogens. Two methods of bacteriological analysis were used: Speed® Mam Color and bacteriological culture. The Speed Mam® Color test allows identification of bacteria that cause mastitis and offers sensitivity (Manner, Pellerin & Papierok 1999).

The samples were inoculated onto nutrient agar, blood agar and Chapman's agar. The plates and broths were incubated under aerobic conditions at 37 °C for 18 h – 24 h.

Identification of the isolates was done on the basis of colony morphology, microscopic examination of Gram-stained smears, catalase production and biochemical propertiesaccording to the methods cited in literature (Waage *et al.* 1999). A sample was considered contaminated if it contained more than two bacterial species.

Results

The CMT positivity rate for all the samples was low (28.57%) (Table 1).

Bacteria isolated from California Mastitis Test-positive samples

Amongst the 40 samples that tested positive for subclinical mastitis, 95% yielded bacterial growth. No growth was

TABLE 1: Incidence of sub clinical mastitis per quarter.

Total of animals	Number of examined quarters	Scores of CMT test				Total positive		Normal quarters	Positive animals	
		1	2	3	4	*n*	%		*n*	%
140	556	51	44	39	26	**160**	**28.77**	396	40	28.57

CMT, California Mastitis Test.

evident in 5% of samples (Table 2). The most prevalent pathogens isolated were *Staphylococcus aureus* (40%) followed by *Streptococcus* species (12.5%) (Table 3). Other bacteria were isolated at variable and low frequency.

Discussion

The CMT positivity rate of 28.57% is similar to the previous finding reported in Egypt (Abdel-Rady & Sayed 2009), Jordan: 31.4% (Azmi *et al.* 2008), France: 25% (Longo *et al.* 1994), and in Spain: 33.5% (Ares *et al.* 1995).

In other Maghreb countries, including Morocco, the frequency of subclinical mastitis was 50% (Bouaziz 2005). The difference in prevalence of subclinical mastitis observed in the present and the previous studies may be due to differences in management practices, use of different methods of diagnosing of subclinical mastitis (CMT, bacteriological examination, SCC, modified Whiteside test, pH, chlorine and catalase tests). Breeds of the animals, immune responses, climatic conditions and the definition of infection, which is variable according to published information can also explain this difference (Eberhart, Natzke & Newbould 1986). Other factors that could influence the prevalence of subclinical mastitis could be attributed to variation in hygienic standards of the dairy environment and milking conditions, as well as genetic variation in disease resistance amongst the breeds maintained in the systems. Previous studies confirmed that the Holstein Friesian breed is more susceptible to udder infection, particularly in areas where hygienic conditions are poor and treatment of mastitis cases is not well managed (Girma 2001). In pens in Tanzania, traditional animals were reported to be more resistant than dairy animals (Mdegela *et al.* 2005). The occurrence of mastitis may be influenced by some heritable characteristics such as milk production capacity, teat structure, and udder conformation (Schutz 1993).

The results of this study show that certain major pathogens are predominant in subclinical mastitis. Staphylococci or streptococciaccount for almost 60% of bacterial isolates. Culture results of positive samples reveal a higher incidence of staphylococcal mastitis with a frequency of 40%, which is in agreement with other findings in the east of Algeria (Heleili *et al.* 2012), Jordan (Lafi *et al.* 1994) and Italy (Moroni *et al.* 2006). A survey conducted in the Netherlands highlighted the importance of staphylococcal and streptococcal contagious mastitis (Miltenburg *et al.* 2006). The findings in Ontario, with a very significant isolation of *S. aureus* (40%), are in accordance with the findings reported here (Sargeant *et al.* 1998). In Jordan, it was reported that the most common organisms isolated from clinical and sub clinical cases were *Staphylococcus* spp. 30% (Lafi *et al.* 1994). It is of interest to note that the organisms isolated in clinical and subclinical mastitis in Zebu cattlein Sudan were*Staphylococcus* spp. (50%) and *Streptococcus* spp. (68.7%) (Bagadi 1970).

TABLE 2: Number of bacterial species isolated by sample.

Number of samples	Number of bacterial species	
	n	%
2	0	5.0
23	1	57.5
13	2	32.5
2	3	5.0

TABLE 3: Frequency of bacterial strains isolation from subclinical Mastitis.

Isolates	Number of isolates	%
Staphylococcus aureus	16	40.0
Streptococcus spp.	5	12.5
Enterobacteriaceae	1	2.5
Pseudomonas spp.	1	2.5
Staphylococcus aureus + *Streptococcus spp.*	5	12.5
Staphylococcus aureus + *Mycoplasma spp.*	3	7.5
Streptococcus spp. + *E. coli*	2	5.0
Staphylococcusaureus + *E. coli*	3	7.5
Streptococcus spp. + *Staphylococcus aureus* + *E. coli*	2	5.0
Sterile collection	2	5.0
Total	**40**	**100**

E.coli, Escherichia coli.

Bacterial culture in other countries of the Mediterranean basin has shown varying results. Similar results were reported in France: 39.0% (Bouchot *et al.* 1985), in Jordan:' 44.7% (Azmi *et al.* 2008), and 29.0% (Ben Hassen *et al.* 2003; Fabre *et al.* 1997; Fallet 1999; Harini & Sumathi 2011). In Egypt, it was found that the most frequently isolated major causative agents were *S. aureus*, *Streptococcus agalactiae* and *Escherichia coli* from the positive CMT samples with prevalence of 52.5%, 31.25% and 16.25%, respectively (Abdel-Rady & Sayed 2009). An earlier study in Egypt showed that 29.1% of mastitis was caused by *S. aureus* (Saddek, Abd-Elkader &Abd-Elhaffez 1996). Although *S. aureus* and *Streptococci* represent more than half (52%) of pathogens involved in subclinical mastitis, other bacterial species were found but in smaller proportions. Similar results were reported in Italy (Moroni*et al.* 2006).

The high rate of isolation of *S. aureus* may be attributed to the fact that the principal reservoirs of *S. aureus* are the skin of the udder and milk of the infected gland. In addition, *S. aureus* has the capacity to penetrate into the tissue, producing deep-seated foci protected by a tissue barrier (Ranjan *et al.* 2010). The high frequency of staphylococcal mastitis is considered to be due to the existence of inadequate hygiene in the dairy industry, poor animal health services, and lack of proper attention to the health of the mammary gland in general. The hygiene at milking is of paramount importance in control of these infections because they are spread during the milking process (Harmon 1994).

The third category of bacteria highlighted was *Pseudomonas* spp., which represents 2.5% of bacterial isolates, high for a group of bacteria deemed minor pathogens. This result is similar to those reported previously in eastern Algeria, with an infection rate with *Pseudomonas* of 3.03% (Heleili *et al.* 2012).

The failure of some pathogens to grow *in vitro* may be due to the fact that certain microorganisms (such as *Mycoplsma* spp.) require specific culture media (Ranjan *et al.* 2010). It could also be explained by the possible premedication of the animals with antibiotics (Azmi, Al-Dabbas & al-Dabbas 2008) because the withdrawal time may not have been respected.

Systematic records regarding the epidemiology of bovine mastitis including status of infection, distribution, prevalence, treatment patterns and microbiological and antibiogramstudies would provide useful management information to the producer, farmer and veterinarian. This has been evident from countries where information has been documented regularly (Shitandi *et al.* 2004). Thus, there is a real need to routinely investigate and record the epidemiology of bovine mastitis in various parts of Algeria and in others countries of Mediterranean basin.

Conclusion

The findings of the present study are in accordance with the observations of previous studies, with mainly minor variations possibly attributable to different geographical climates and individual variations in susceptibility.

Subclinical mastitis is a dominant disease on dairy cattle farms in the central region of Algeria with a prevalence of 28.57%. The major pathogens isolated were *S. aureus* and *Streptococcus* spp. In order to prevent mastitis it is recommended that the following measures are adhered to:

- The application of good sanitary and hygienic measures.
- Adequate housing with proper sanitation.
- Regular screening for early detection and treatment.
- Regular bacteriological monitoring to adapt the prophylactic treatment plan and follow up chronic cases.
- Culling of older cows with repeated attacks.
- Prompt treatment of teat or udder injuries.

Acknowledgements

The authors acknowledge the staff of the Veterinary Regional Laboratory of Laghouat and Laboratory of biotechnology related to animal reproduction, where the work was carried out. Authors acknowledge outside reviewers of their drafts.

Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article.

Authors' contributions

K.R. (University of Blida) was the project leader, S.R. (University Laghouat) was responsible for experimental and project design, prepared the samples and performed most of the experiments. K.D. (National Higher Veterinary School of Algiers) made conceptual contributions. K.R., S.R. and K.D. wrote the manuscript.

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