

Prevalence of Mastitis Infection and Identification of Causing Bacteria in Cattle in the Oran Region West Algeria

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Abstract: Mastitis is a common disease that decreases milk yield and quality in lactating cows. Because of higher treatment cost and higher culling rate, mastitis causes economical losses. The CMT test was used to detect infection of mastitis in the farms; the infection is detected in 55.16% of cattle herd. The responsible infections bacteria suspected mastitis were determined. The bacteriological analysis revealed the presence of varied dominant bacteria belonging to the species of *Staphylococcus aureus* (30.76%), *Streptococcus* sp. (30.76%) and *Escherichia coli* (23.07%). These bacterial species were responsible for the infection of the cow in the different samples. The milk acidity was variable so that almost half of the samples were at pH (6.65-6.90). The establishment of a guide to monitoring and surveillance is essential for control of pathogenic bacteria.

Key words: Mastitis, Streptococcus, Staphylococcus, Enterobacteria, raw milk, cow, health quality, hygiene

INTRODUCTION

Milk for human consumption was defined in 1909 by the International Congress of Food milk is the full product of the total deals and interrupted a female dairy healthy, well fed and not overworked. It must be collected cleanly and does not contain colostrums. Milk is a rich substrate providing the young man and an almost complete food mammal. Proteins, carbohydrates, lipids, minerals and vitamins are present in concentrations quite satisfactory for growth and cell multiplication. Economic losses due to mastitis are recognized worldwide as a major problem on dairy farms. The microorganisms found in milk an ideal substrate for their development several study indicate that Milk ecosystem favorable for microbial growth (Vachee *et al.*, 2009). In the current situation in Algeria, the data of the etiology of clinical and subclinical mastitis in cattle, their prevention, detection and treatment are very important in several field such as social, economic, regulatory and health.

Mastitis is a state of inflammation of the mammary gland resulting from the action of different microorganisms. These attack and damage tissues secretory often react against aggression by the mobilization of neutrophil leukocytes in the area of infection (Gambo and Etchike, 2001). Mastitis infection occurs commonly in lactating cows and causes, firstly, the decline in milk production and secondly, the decrease in nutritional and hygienic quality of milk and its derivatives. From an

economic standpoint, clinical and subclinical mastitis leads to a reduction in the amount of milk produced and changes in its composition might degrade his ability to cheese processing (Biffa *et al.*, 2005).

Consequently, the objectives of this study were to investigate the detection of mastitis in lactating cows on two farms selected in the Oran region by use of an economical method for early detection of mastitis in individual collected milk samples. This test method is the CMT (California Mastitis test) score gives an assessment of infection. Bacteriological analysis involves the search and identification of microorganisms responsible for mastitis in Algeria cow herd.

MATERIALS AND METHODS

Study area and sampling: On the day before sample collection, each cow was examined clinically. Particular attention was given to the condition of the mammary glands. All animals selected for this investigation were clinically healthy and showed no genital or mammary disorder. Milk samples from two different farms in the region of Oran (Collection farm. Hassi Ben Okba and Bir El-Djir). Then, the milk samples taking using aseptic technique from each quarter of all cows after cleaning the udder and expulsion of the first jets of milk. All the milk samples were brought to the Applied Microbiology Laboratory, Faculty of Science, Oran University. Samples were stocked in cold at 4°C until bacteriological analysis

(Getahun *et al.*, 2008). The 24 milk samples from 6 cows at the two farms were screened by the CMT for the assessment of infection mastitis (Quinn *et al.*, 1994).

Tests (California Mastitis test): The California Mastitis Test (CMT) was carried out on all samples collected using the method described by Schalm. Scores represented four categories: 0, negative or traces; 1, positive (+); 2, positive (+ +) and 3, positive (+ + +). Schalm test principle is based on the use of a body surface (the teepol 10%) which causes the breakdown of cells and precipitation of DNA and a solution bromocresol purple to 10-5 which acts as a pH indicator. About 2 mL of milk to be tested are mixed with 2 mL of reagent at room temperature. The reaction was numbered from 0-4 based on the level of infection (Gambo and Etchike, 2001) and scoring CMT1 is doubtful results were not taken into account. Infected samples resulted in bacteriological analysis to identify bacteria causing the infection (Roy *et al.*, 2009).

The bacteriological analysis: The first milk samples were brought to Applied Microbiology Laboratory, Faculty Science, University of Oran, Algeria. Samples were inoculated onto one quadrant of typical agar plat, the methods used in this research and identification of bacteria present in milk are usual methods detailed (Quinn *et al.*, 1994; Aggad *et al.*, 2009). The isolate of total aerobic bacteria were sowed on PCA agar staphylococci on Chapman agar, streptococci on Rothe and Litsky medium and enterobacteria on Drigalski agar. All plates were incubated at 37°C for 48 h. After incubation plates were examined for the colonies enumerated were tentatively identified based on morphologic features, pattern of hemolysis, Gram strain reaction and catalase test. A second observation was made at 48 h. *Staphylococcus aureus* was differentiated from other *Staphylococcus* species by coagulase test and *Streptococcus agalactiae* was differentiated from other mastitis causing streptococci by using CAMP test and esculin hydrolysis (Debdreil, 2008; Lakew *et al.*, 2009; Abera *et al.*, 2010). Gram negative bacilli were classified according to their microscopic morphology and the results of the catalase test, the isolates were sowed onto Kligler agar to detect lactose utilization and glucose fermentation. Voges-Proskauer test and Methyl red were performed as described by Guiraud (1998).

RESULTS AND DISCUSSION

Sanitary and hygienic conditions examination of dairy cows: From a total of 24 dairy cows, 6 cows had precalving suspected symptoms of mastitis were apparent

Table 1: Prevalence of mastitis, CMT test results of total milk samples from 6 dairy cows from Hassi Ben Okba and Bir El Djir farms

| Farms | Number | CMT | | | | | |
|------------|--------|----------------|---|----|-------|-------|----------------|
| | | Positive cases | + | ++ | +++ | ++++ | Negative cases |
| Farm 1 | 20 | 11.00 | 0 | 0 | 11.00 | 0.00 | 9.00 |
| Farm 2 | 4 | 2.00 | 0 | 0 | 0.00 | 2.00 | 2.00 |
| Total | 24 | 13.00 | 0 | 0 | 11.00 | 2.00 | 11.00 |
| Percentage | 100 | 55.16 | 0 | 0 | 84.61 | 15.38 | 45.83 |

Farm 1: Hassi Ben Okba; Farm 2: Commune Bir El Djir

Table 2: Bacterial isolates from clinical and subclinical mastitic milk samples from two farms in West Algeria (Hassi Ben Okba and Bir El Djir)

| Group of isolates | Type of isolates | Number | Number (%) |
|-----------------------|-----------------------------------|--------|------------|
| <i>Staphylococci</i> | <i>Staphylococcus aureus</i> | 4 | 30.76 |
| <i>Streptococci</i> | <i>Streptococcus agalactiae</i> | 2 | 15.38 |
| | <i>Streptococcus dysgalactiae</i> | 2 | 15.38 |
| <i>Enterobacteria</i> | <i>Escherichia coli</i> | 3 | 23.07 |
| | <i>Proteus</i> | 2 | 15.38 |
| Total | | 13 | 100.00 |

clinical signs of mastitis were observed. Mastitis is a disease that affects a large number of dairy cows around the world. The results of clinical mastitis on milk local signs (presence of lumps, abnormal texture, color and odor) and can sometimes lead to signs general animals, such as fever, depression, anorexia, nervous (Debdreil, 2008). A total 45.83% of quarters had precalving mastitis, the positive predictive values of CMT and were registered a rate 84.61 and 15.16% of dairy cows infected mastitis (Table 1). A examination of all major milk producing countries indicates that clinical mastitis type occurs annually from 15-20% of cows. It has been shown that half or more of the cows are infected in Canada and USA. Where in Danmark and Ethiopia, it is estimated that mastitis reached a proportion of dairy cows from 30-52% (Debdreil, 2008; Mungube *et al.*, 2005). Other results showed by Debdreil (2008) that the rate (8.3%) is significantly lowers in dairy cows in the United State and Canada.

Bacteriological analysis: The list, number and proportion of the bacterial isolates from a total of 6 dairy cows (24 quarters) are shown in (Table 2 and Fig. 1). A total of 13 isolates were found, of which (46.17%) were from milk samples collected from clinical mastitis quarters while the remaining (53.83%) were from subclinical cases. The common isolate from the clinical mastitis quarters were *Staphylococcus aureus* (30.76%) and *Escherichia coli* (15.41%) while from subclinical cases *Streptococcus agalactiae* (15.38%), *Streptococcus dysgalactiae* (15.38%) and *Escherichia coli* and *proteus* sp. (23.07%). Based on their importance, 84.62% of the isolates from the clinical and subclinical mastitic cases, respectively were major pathogens while the remaining was not

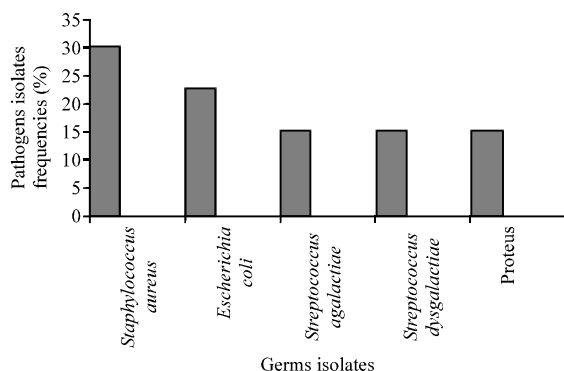


Fig. 1: Pathogens isolates from mastitic milk samples frequencies

Table 3: The importance pathogen and origin of common isolates from the clinical and subclinical mastitis cases

| Type of isolates | Gram type | Origin | Important pathogen |
|-----------------------------------|-----------|--------|--------------------|
| <i>Staphylococcus aureus</i> | + | No | Majeur |
| <i>Streptococcus agalactiae</i> | + | No | Majeur |
| <i>Streptococcus dysgalactiae</i> | + | No | Majeur |
| <i>Escherichia coli</i> | - | Yes | Majeur |
| <i>Proteus</i> | - | Yes | ? |

+ = Positive; - = Negative

identified. Based on their origin, 84.62% of the isolates from the clinical and subclinical mastitic cases were contagious pathogens while the remaining were environmental pathogens. Among the contagious pathogens, the predominant isolate was *Staphylococcus aureus* from clinical cases. Among the environmental pathogens, *Escherichia coli* and *Proteus* sp. (38.48%) were the most frequently isolated bacteria from subclinical mastitis cases (Table 3 and Fig. 2).

The primary objective of this study was to evaluate if it possible to select infected quarters on the basis of CMT results to have more targeted approach to prepare therapy. This test was easily usable on farm, rapid and inexpensive.

The description of the epidemiology of mastitis in milk samples collected were linked with clinically apparent. A visual study was conducted to determine the health status of dairy cows west Algeria farms. The results revealed the presence of clinical signs on the quarters of two cows. A blackish color and hyperthermia under implicate the presence of clinical mastitis, other cows observed no signs of clinical mastitis, indicating a high prevalence of subclinical mastitis from the dairy cows examined in the farms of Hassi Ben Okba and Bir El JIR.

Confirmation of the epidemiological description of mastitis infection evaluate on the basis of CMT (California Mastitis test). Results whose revealed the presence of mastitis infection was recovered from 55.16% of samples. The major pathogens, *Staphylococcus aureus*

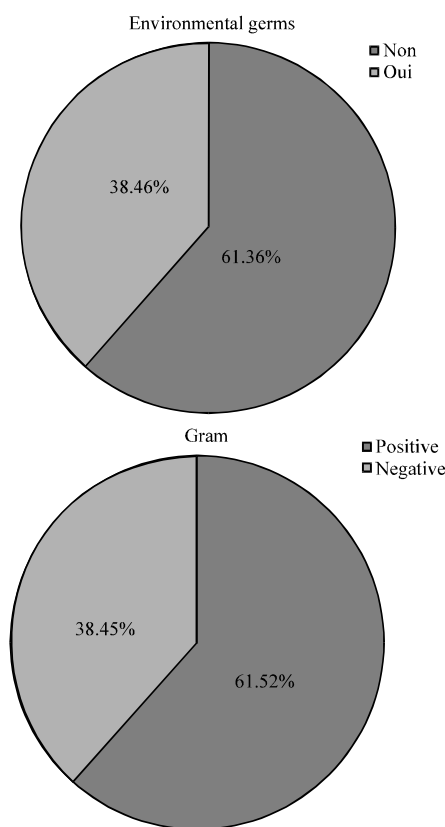


Fig. 2: The origin and gram type of common isolates from the clinical and subclinical mastitic cases

(30.76% of the samples) were responsible to clinical mastitis according to the researchers Sears (2003) and Debdreil (2008). It needs to be given more attention to the hygiene of milk, breast health and the determination of critical points and control at the farm to prevent *Staphylococcus* eruptions.

The presence of *Streptococcus* has been limited by both species *agalactiae* (15.38%) and *dysgalactiae* (15.38%) in the case of subclinical mastitis. These bacteria were isolated with considerable frequency this probably due to the environmental conditions. In fact, the conditions environmental are relatively favorable for the development of *Streptococcus agalactiae* (Serieys, 2003; Abera *et al.*, 2010).

Escherichia coli are the second agent most frequently encountered in 23.07% of samples, giving considerable environmental germs. According to studies by Jones and Ward (1990), the density of *Escherichia coli* is often higher and is the predominant agent. The abundance of Enterobacteriaceae in the samples implicates for lack of sanitary requirements during milking. The main responsible are the teat skin, soiled by feces

(Aggad *et al.*, 2009). The presence of mammary infections could be attributed to poor hygiene conditions of milking which favorite the transmission of infection from one quarter to another or from one cow to another (Fadrig, 1988; Serieys, 2003). The quality and quantity of milk are deteriorating due to various causes. The biological causes include responsible is the low genetic potential of animals, poor diet and disease prevalence. Mastitis is one of the most important diseases of dairy cattle (Atyabi *et al.*, 2006; Unnerstad *et al.*, 2009). The results of this research showed the presence of mastitis infection from 55.16% of quarters, the results observed at the two farms indicating that poor hygiene and poor conditions of milking herd were the probable risk factors.

CONCLUSION

The overall prevalence of mastitis observed in this study is lower than the results of previous findings in other countries (Biffa *et al.*, 2005; Sori *et al.*, 2005). The study identified various major pathogens *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and Enterobacteriaceae that have a close relationship with the infection of the udder samples collected.

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