

Sensitivity of various methods (CMT, CE and Indicator Paper) of subclinical cattle's mastitis diagnostic in some dairy cows breeding in east of Algeria

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*Article History:

Received: 05/02/2018

Revised: 22/02/2018

Accepted: 22/02/2018

DOI: <https://doi.org/10.7439/ijasr.v4i2.4615>

Abstract

The aim of present work is to look for the prevalence of subclinical mastitis in dairy cows of different regions in eastern Algerian; the prevalence of subclinical mastitis has called for three non-specific methods and by the use of bacteriological analysis. On all teats are 416; the first non-specific test is California mastitis test (CMT) indicating polymorphonuclear witness infection of the udder. The second test indicates presence of ions (Na, Cl) in milk and carries the appellation of the electrical conductivity of milk (EC) and the last test is the one that revealed the pH of milk while using paper pH containing pH indicator and changes color to green or blue if the udder is infected. It is the first component of the study; the second component is to get the bacteriological status of each udder and passing to the study of the sensitivity and specificity of non specific tests. The present study gave values of mastitis prevalence between 6.7 to 64.7% of cows and 10 to 20% of udders tested by (CMT, pH papers and EC), with an infection rate of 9% of districts and 17% of cows. Bacteriological examination of positive areas showed the prevalence of the following pathogens: 6 species of Staph – *Saprophyticus*= 15%, 6 species staph – *epidermidis*=15%, 4 species staph – *cohnii*=10%, 9 Staph +=22.5%, 2 *Micrococcus*. Spp=5%, 4 *E. coli*=10%, 2 species of *klebsielle* =5%, 2 *Proteus vulgaris*=5%, 2 *Citrobacter freundii*=5%, 1 *Streptococcus* spp=2.5%, 1 *Streptococcus uberis*=2.5%. At 5% others specie of *bacterias* for specificity and sensitivity we have these values r: CMT = 71% and 77%; for pH paper had values of 15% and 60%. In latter puts the EC with values of 13% and 67% respectively. So the CMT remains the most accessible and reliable tset; and we advise our farmers and our veterinarians and technicians in the field.

Keywords: dairy cattle; subclinical mastitis, CMT, Electrical Conductibility; indicator papers; bacteriological analysis.

1. Introduction

As Algeria consumes on average and by citizen a quantity of milk bordering 100 liters/year which lists her in first consumer row of the countries of this product (Anonyme1 = the French agency for the international development of companies bulletin. She is grateful to import every year, a quantity of powdered milk against hundreds and millions of dollars; for example: in 2014, the invoice of import of dairy products, which 90 % in the form of milk powder intended for the transformation knew an unpublished figure by reaching 1,91 billion dollars,

(Anonyme 2 = According to the Algerian Customs, Assessment 2013), while in 2013, these imports were of the order of 1,13 billions of dollars, the same source reports (Anonyme 2; Anonyme 3 = the Ministry of Trade assessment 2015) and reveals an enormous figure that the invoice of import of the milk costs 1.4 billion dollars for 2015, the mastitis which are clinical or sub-clinical engender more and more increasing economic and medical losses, concerning milk, glands, cows either of the cost of medicine used to deal with this formidable pathology.

Klastrup *et al* [1] estimate that 25 % of the susceptibility in the infections are bound to the environmental factors, 20 % to the genetic one and 50 % to the control herd. In Algeria, the situation of the dairy bovine herds is often considered as worrisome, the frequency of the sub-clinical mastitis is brought up [2]. Several studies took this theme as problem by looking for their prevalence and their risk factors in order to put appropriate plans to fight it.

In this qualitative study, we are going to sample takings of the milk on a significant number of animals, which are randomly chosen, and this, to be able to put the point on the presence (existence) of this disease in our breeding and livestock, but also, to be able to choose the best of the available tests on ground, possessing the most reliable specificity and specialty.

This is what makes the study of the causes of loss of the milk one of the major priorities; in this sphere bring in the study of the sub-clinical mastitis, which requests the search of witnesses of the inflammation, which deviate it from the pH of the milk. Thus, we use papers indicator pH of mastitis or we look for the presence of the somatic cells in the milk by means of the test California Mastitis Test (CMT), of the electric conductivity of the milk and/or the pathogenic agents (bacteriology) in the milk [3,4].

By the using these methods, the purposes of this research are to esteem, the frequency of the sub-clinical mastitis in the randomly visited breeding, while using these various not specific methods and by the bacteriologies, secondly to test the reliability of every method to detect sub-clinical mastitis on glands and on cows for each teat. Finally, to give advice to our breeders to reduce this pathology.

2. Materials and methods

2.1 Place of this study

The breeders of 18 exploitations who participated in this study belong to various regions from the east of Algeria, they were randomly chosen.

2.2. Animals and breeding

Animals included in this study belong to various breeding, which were randomly chosen, we made our samples of the survey on 104 dairy cows representing 416 glands.

2.2.1. Samples and their preservation

Within the framework of our follow-up and of the passage on our breeders, we made samples to realize this investigation on this disease. Then we made only a single passage. The taking of the samples of milk was realized before the evening draft.

Every sample was separated in four prizes; each was used for the realization of a type of analysis.

- 1) Sample serving to detect the sub-clinical mastitis by the method CMT.
- 2) Sample using to determine the electric conductivity of the milk.
- 3) Sample for testing to Ph papers.
- 4) Sample intended for the bacteriological analysis which was realized in a aseptic way according to the recommendations of de [5,6].

Milk samples were transported under refrigeration up to the laboratory. They have been kept (preserved) throughout duration of less than 48 hours up the realization of the isolation.

2.3 Analysis methods

We applied four methods for the screening of the sub-clinical mastitis.

2.3.1 Californian Mastitis Test

In our case, the product was never a subject of a detailed research. Its principle rests on the use of substances witnesses His principle rests on the use of substances showing the presence of somatic cells; it is the cleaner, which causes the explosion of cells and the haste of their DNA. The used product was Raidex ND. It is used by mixing 2 ml of milk and 2 ml of reactive. The mixture becomes more or less liquid, viscous, or gelatinous according to the number of cell by ml of milk (**manufacturer**).

2.3.2 Test of the electric conductivity (EC)

An electronic detector of sub-clinical mastitis Draminsky is in the market.

The interpretation of results supplied by this device is such as it is mentioned on the note of the device as follows:

$x < 250$ are positive, between 250 and 300 are doubtful and ≥ 300 are negative.

Cows and glands the values of which are < 250 units are considered affected by the sub-clinical mastitis [7,8].

2.3.3 pH paper indicators

The pH paper is a blotting paper presenting four zones for four districts. Every zone is handled with two color indicators; the first one is the blue of Bromothymol and the second is the nitrazine. The first one turns of the yellow to the blue in a pH oscillate of pH from 6,6 to 7,6. And the second of the yellow to the green in a pH which varies from 6,4 to 6,8. [9]

2.3.4 Bacteriological analyses

They were made according to the classic methods of isolation of the most frequent bacteria in the cow's milk.

The sowing was made on:

- * Environment of Chapman for the staphylococci research;
- * The blood agar for the streptococci and hemolysis research;

* The environment of Hektoen for the research Enterobacteriaceae. The determination of the kind and the species in cause is according to; the aspect of colonies, State cool, Coloring of Gram, Catalase (to differentiate the Staphylococci of Streptococci), Coagulated (to make the difference between Staphylococci of coagulated positive and Staphylococci of negative coagulated one, Oxydase (for Enterobacteriaceae), the determination of the species called upon the galleries API. [3,5,6]

2.3.5 Reliability of the electric conductivity of the milk of the device

Draminsky plus pH indicator papers of the milk plus the CMT with the bacteriolo

The objective was to compare the indications supplied on these methods; with the results of the bacteriology,

The evaluation of the sensibility and the specificity was realized according to the method of [4,10].

Sensibility = $VP/(VP+FN)$ where VP is the positive truths; FN are the false negatives

Specificity = $VN / (VN+FP)$ where VN is negatives truths; FP is the false positive.

2.3.6 Static tests

As the presented study is descriptive, we resorted to the test of the chi² (threshold 5 %) for the comparison between the obtained prevalence by the various methods in both States.

3. Results

3.1. The positive results with the various Methods

Numbers and frequencies of affected cows and glands by the various methods

Prevalence of mastitis detected with the CMT decreases as well for cows (68 %) as for glands (24 %), taking into account doubtful results, decreasing until 45 % for cows and until 19 % for glands, if we take that the absolutely positive results are a little raised with regard to our previous studies which are 40 % and 18 % respectively.

For pH papers, the general rates are 17 % for cows and 7 % for glands, result that is close to the study of And the IT are very close. Are 10 % for glands and 24 % for cows and which are very close to the study of The difference of the rates of cows infection by both methods (CMT and EC AND Ph papers) between States is not significant (Chi²=0.3. The bacteriology gave less important prevalence 12 % for cows and 7 % for glands.

There is no significant difference between States (Chi²=0.67)

3.5. Sensibility and specificity of the methods

1. EC

Sensibility = 15 %. (i.e. that, more of 1.5 times on 10 glands declared that are affected by the EC are also by the bacteriology) close to other authors [4,10].

Specificity = 67 %. (More of six times on 10, glands declared that are holy by the EC are also by the bacteriology). [4,10]

2. CMT

Sensibility = 71 %. (I.e. that, more of seven times on 10 glands declared that are affected by the CMT are also by the bacteriology).

Specificity = 77 %. (I.e. More of seven times on 10, glands declared that are holy by the CMT are also by the bacteriology).

Are close to the values obtained by Smith *et al*[11] and a little less with regard to Spargant *et al*[12] which are respectively between 75-85 %

3. Ph Papers

Specificity = $vp/vp+fn=20\%$. (I.e. that, more of 25 times on 10 glands declared that are affected by the Ph Indicator are also by the bacteriology).

Equal in that of Kivaria FM *et al*, this is 20 %

Sensibility = $VN/VN+F+=60\%$ (I.e. that exactly, six times on 10 glands declared that are affected by the Ph indicator papers of milk are also by the bacteriology).

Little less in that of Kivaria FM *et al*, this is 80 %.

[13]

3.6. Bacteriology Results

3.6.1. Global results

On 416 districts, thirty-six (15 %) showed themselves positive in the bacteriological examination and 380 are sterile (85 %).

Among 36 samples, which made object of culture, 32 allowed the isolation of a single germ, four with two different germs.

We were able to isolate and to identify 40 bacterial strains dividing as follows:

25 origins (62.5 %) to positive Gram and 10 origins (25 %) to negative Gram.

The germs of mammary reservoir *Staphylococcus aureus*, staphylococci with negative coagulated, and micrococcus are majority 22/40 with a 68.75 % rate.

The frequency of the germs of environment *Enterobacteriaceae*, *Streptococcus uberis*, *citrobacter*, *proteus*, *Escherichia coli* more *Klebsiella* amounts to 10 / 40=25 %.

Here is the qualitative distribution of results:

16 origins of Staph – 40%, 9 Staph +=22.5%, 2 *Micrococcus*=5%, 4 *enterobacteriaceae* (*E. coli*) =10%, 2 *Proteus vulgaris*=5%, *Citrobacter freundii*=5%, 1 *Streptococcus* spp = 2.5%, 1 *Streptococcus uberis* =2.5%, 2 *Klebsiella*=5%, 2 other =2.5%.

3.7. Correlation between results of CMT of CE of pH indicator and of bacteriology (table IV)

With the CMT, prevalence of mastitis is estimated at 48 % for cows, and at 15 % for glands. The bacteriological

culture is positive at 89 % of cows having reacted positively to the CMT against 78% glands [14]. It is 75 % for Baillargeon et al[15].

With the CMT in the region of Mila, the prevalence rate borders 38 % for cows, and 15% for glands. in agreement with Boufaida et al[14].

In the region of Constantine, the prevalence of mastitis is estimated at 40 % for cows, and at 20 % for glands. In agreement with Baillargeon et al[15].

For the region of Guelma, the rate of positivity is close to 67 % for cows and 27% for glands. Close to Rakotozandrindrainy et al[3].

Table 1: Description of the farms and animals in the study

Cattle exploitation	Cattle breed		Milking mode		Dry treatment		Hygienic index			Teat dipping	
	Holstein 12/67= 18%	Montbeliard 6/42= 14% sig p<0.01	M 5 5/14 =36%	Mec 13 13/90 =14%	yes 3/46 =7%	No 15/58 =26%	00-2.5 2/24 =3%	2.5-4 6/30 =27%	+4 10/50 =50%	yes 2 2/20 10%	No 16 16/84 19% p<0.01
E1	3	1	m		no		2.5			yes	
E2	2	3	m		no		4.5			no	
E3	3	0	man		no		2.5			no	
E4	3	2	m		no		1.5			no	
E5	1	3	m		no		2			no	
E6	4	0	man		no		2			no	
E7	17	3	m		yes		3			no	
E8	2	0	man		no		5			no	
E9	10	6	m		yes		3.5			yes	
E10	1	1	man		no		4.5			no	
E11	3	3	m		no		4.5			no	
E12	.0	6	m		no		4.0			no	
E13	1	1	m		no		4.5			no	
E14	3	1	m		no		3			no	
E15	2	2	m		no		4.5			no	
E16	3	1	m		no		00			no	
E17	0	3	man		no		00			no	
E18	5	5	m		yes		3.5			no	

M = manual / Mec = mechanical

Table 2: Sensitivity and specificity values by region

Test	Region	Sensibility	Specificity
C.E	Mila	38	12
	Constantine	55	22
	Guelma	77	22
	Total	67	13
C. M. Test	Mila	71	77
	Constantine	77	67
	Guelma	78	71
	Total	77	71
pH indicator paper	Mila	38	12
	Constantine	55	22
	Guelma	70	22
	Total	60	15

Table 3: Effect of different risk factors on the occurrence of the disease

Factors	Breed		Treats mode		Dry treatment		Index of hygiene			Teat dipping	
	Holstein	Montbeliarde	Man	Mec	yes	No	00-2.5	2.5-4	+4	yes	No
Characteristics	12/67	6/42	5/14	13/90	3/46	15/58	2/62	6/22	10/20	2/20	16/84
Disease/Number	18%	14% sig p<0.01	36%	14%	7%	26%	3%	27%	50%	10%	19% p<0.01
The infection rate	Khi2=6.81 p<0.05Nsign p<0.01sign p<0.001sign		Khi2=2.41 p<0.05 sign p<0.01sign p<0.001sign		Khi2=10.78 p<0.05 N sign p<0.01 N sign p<0.001 sign		Khi2=3.51 p<0.05 sign p<0.01sign p<0.001sign			Khi2=1.23 p<0.05sign p<0.01sign p<0.001sign	

Here are our detailed results:

Strains of Staph – *Saprophyticus*= 15%, 6 staph Strains – *epidermidis* =15%, 4 strains of *S. cohnii* with rate of 10%, 9 Staph +=22.5%, 2 *Micrococcus*. Spp=5%, 4 *E. Coli*=5%, 2 strains of *klebsiella* =5%, 2 *Proteus vulgaris*=5%, 2 *Citrobacter freundii*=2.5%, 1 *Streptococcus* spp with rate of 2.5% and 1 strain of *Streptococcus uberis* with rate of 2.5 % and 2.5 % rate of not identified germs which are bacillus

4. Discussion

It is obvious that remarkable prevalence of the sub-clinical mastitis during the study is very close to the standards of breeding which are far from being in accordance with the rules that are recognized in theory, and it is indeed was brought back by Faye et al[16].

There has been also that there is differences of prevalence's mastitis according to the used screening test, it is reported by Boufaida et al [14] and Saidi et al[17].

However, a largest number of mastitis is highlighted by the CMT with regard to pH papers and to the EC.

The presence of somatic cells allows identifying an infection in a more sensitive way than the modification of the concentration in ions of the milk.

A good concordance between the results of the CMT and those obtained with the Bacteriology was observed for Boufaida et al[14] and Saidi et al[17].

Indeed, Casura et al[18] showed that the CMT supplied a reliable prediction

The test CMT classified correctly about 75 % of infected cows and 73 % of glands with compared with the bacteriological examination.

This result is close to that reported by Ruegg and Reinemann[19] and which varied from 75 to 80 %.

The medium sensibility (58 %) and the good specificity (94 %) are a results close to those reported by Gaudin and Billon[10] (41 % and 94 % respectively)

The Draminsky device is reliable to detect much more the healthy glands than the infected one.

The CMT is very precise and less expensive among all the alternatives, it is realized by the breeder and gives an immediate answer.

It is still the best practicable test in the side of the animal to specify the infectious status of cows [15].

The rate of positive samples (17 %) of glands is comparable to that testified by Baillargeo and Wallas[15].

89 % of milk takings contained a single bacterial species. This result is comparable to the data brought back in other studies: 73,3% for Sargeant et al[12], 74,6% for Bouchot et al [20] and 76,7% for David et al[21].

In 11 % of the takings, there is association of two species at the same time. This association is described; 7,9% for Fabre et al[22] 6,2 % for Smith et al [11], and 11,1% for Ramiisse et al[23]. On the other hand, Fabre and al[22] bring back report a very weak rate of 1,3 %.

In the present study, the germs of mammary reservoir are majority (75 %) compared with germs of environment (25 %).

Staphylococci of negative coagulase with prevalence of 40 % occupy the second position among the isolated germs. The prevalence of these germs in the sub-clinical mammary infections of the dairy cow is variable from a study to another one: 26% for Bouaziz [29], 33%, 34% for Rakotozandrindrainy et al[3], 41 % for Fabre et al[22] and 49% for Beroual [24] and 27 % for Shyaka et al[25]. The incidence of these bacteria considered as minors thus is not to be neglected.

Staphylococci of negative coagulase are at the origin of the moderate increase of the somatic cellular concentration of the milk, it thus seems necessary to take into account the impact of these germs [22].

The high number of isolated SNC in the exploitations would be due to the bad conditions of hygiene of the milking.

Several works showed that the application of a disinfection of teat after milking contributes to the decrease of prevalence of SNC [26].

Our study reveals the ascendancy of *Staphylococcus epidermidis*

This result is in line with Bigerson and al [27] and Ben Hassen et al [28] data.

In the present study, the bacteriological results place *Staphylococcus aureus* as the most frequent etiological species regarding sub-clinical mastitis hospitals. Indeed, it was isolated in 22.5 % cases that confirm a dominant place among the major germs.

This result is in agreement with the frequencies of 31 %, 27 % and 30 % brought back respectively by Bouaziz [29] in Constantine's region, Beroual [24] in Rakotozandrindrainy et al[3] in Madagascar's highlands.

The rates of *Proteus* with 2.5 % as well as *Micrococcus* with 5 % in our study were also isolated in other studies such as Shyaka et al[25].

The frequency (15 %) Enterobacteriaceae recorded in our study is close in that brought back (21 %) by Beroual [24].

The rate of 10 % *por escherichia coli* is very in agreement with 7 % of Fabre et al [2]. This germ is rather at the origin of clinical mastitis. The poor maintenance of the litter, the bad hygiene of the stalling and the animals generally could explain the high frequency of *Escherichia coli* isolated in this study.

It should be noted that cows being the object of this study have never received a treatment in the drying up, effective measures against *Staphylococcus aureus*. The poor hygiene conditions of milking (on-milking and under milking and our distribution casting of food after milking and the manipulation of teat us milk with naked hands could explain this high frequency of germs of mammary reservoir responsible of mastitis of milking.

For the antibioresistance of germs to peni g+peni A + erythromycine + oxytetracycline. These results are comparable to those identified by Bouaziz [29], Mammache et al [30] and Saidi et al [17].

5. Conclusion

At the end of our study can inferred importance of this disease and its impact on our sample, with a positivity rate 15% of teats and 48% of the cows tested by California Mastitis Test (CMT), and with an infection rate of 9% of teats and 17% of the cows objects of studies from the bacteriological test, which makes the CMT test as a very reliable test for the detection of Subclinical Mastitis.

Our study gave us an idea about germs that take place within these farms poorly maintained and badly followed; to know *Staphylococcus aureus*, *Enterobacteriaceae*, *Streptococcus uberis*, *Streptococcus* spp, *Micrococcus Klebsiella*.

But certainly there are other germs which mean that we have been lacking prevented us to go look for them, and in future, we're going more expand the scope of research to track other kinds.

Acknowledgements

Thanks for all employees and breeders who have agreed to join the plan of screening and fight against this pathology.

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