Contribution to the study of staphylococcus contamination of cows' milk on a number of farms in Algiers: its impact on human health

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Summary

The authors describe a survey and screening programme for staphylococcus. The study covers 14 dairy farms in the Algiers region, from which 203 samples of cows' milk were taken for bacteriological testing. The survey results show that poor husbandry conditions are the main cause of staphylococcus in cows' milk. Staphylococcus was found in the milk of 30% of the cows sampled. These results were influenced by a variety of factors, in that: the contamination rate rose with the number of pregnancies, age, and volume of milk output of the cow, as well as the bedding thickness; the milk contamination rate was greater when milking occurred outside a milking parlour and when it was performed by machine; higher rates of staphylococcus infection were found in the milk of cows at the end of lactation, in red and white breeds, and in those with cylindrical teats. Identification of the bacteria found (staphylococcus) showed that coagulase-negative staphylococci were present in 67.21% of samples, whereas coagulase-positive staphylococci were present in only 32.79%. The average count for the latter was equal to 0.54×10^4 colony-forming units per ml of *Staphylococcus aureus.* Seventy percent of the milk analysed was free from staphylococci and most of the bacteria identified were not pathogenic to consumers (coagulase-negative staphylococci); nevertheless, consuming fresh milk still presents a degree of risk.

Keywords

Algeria – Bacteriological testing – Coagulase-negative – Coagulase-positive – Milk – Prevalence – Public health – Staphylococcus.

Introduction

Milk is one of the main sources of animal protein necessary for human nutrition and helps to increase family incomes and food security. The dairy sector creates jobs at every level, from livestock farming to selling the finished product, with processing, distribution and technical support in between.

The cows' milk-producing organ, the udder, is highly prone to staphylococcus infection, which is one of the costliest pathologies for the dairy-farming sector.

To obtain a satisfactory volume and quality of milk output and reduce Algeria's dependence on imports, udder health needs to be improved. This is what prompted the authors to conduct their investigations.

Materials and methods

Milk sampling and testing

The study was conducted during the period 2008 to 2009. The milk samples were taken aseptically. All four quarters

1036

Rev. sci. tech. Off. int. Epiz., **33** (3)

of each cow udder were sampled, using the same sterile container.

Sampling equipment and reagents

The following equipment was used: sterile plastic containers (jars); cotton wool and absorbent paper; hydrogen peroxide; 70% alcohol; soap and water; coolers and ice packs.

Sampling protocol

The Mialot recommendations for sampling (1) were followed:

1. Wash hands.

2. Wash the udder with water.

3. Dry the teats with absorbent paper.

4. Disinfect the teat ends with cotton wool dipped in 70° alcohol.

5. Discard the first squirt of foremilk.

6. Fill the jar with a few squirts of foremilk from each quarter.

7. Label the jar (date, cow number), using an indelible pen.

8. Store the samples in a cooler and send them to the laboratory.

Table I
Distribution of samples by farm surveyed

Farm no.	No. of samples
I–A	5
I–B	10
II–C	18
II–D	11
II–E	10
II–F	24
III–G	6
III–H	16
IV–I	14
IV–J	15
V	32
VI	17
VII	15
VIII	10
Total	**203**

Distribution of samples

The 203 samples of cows' milk tested came from 14 herds. The distribution of samples by herd is shown in Table I.

Bacteriological analysis

The bacteriological investigation focused on screening for staphylococcus (coagulase-negative and coagulase-positive) in the milk samples.

Testing laboratories

The samples were transported in a cooler to the laboratory of Algeria's National School of Veterinary Services in Algiers (ENSV) or the Draa Ben Khedda laboratory in Tizi Ouzou.

Equipment

Standard laboratory equipment was used: an oven set to 37°C ± 1°C, a water bath set to 47°C ± 2°C, and a refrigerator set to +4°C, etc.

Culture media and reagents

The following culture media and reagents were used:

– culture media (Institut Pasteur, Algeria): Baird Parker agar, brain-heart infusion broth, mannitol-motility medium

– reagents (Institut Pasteur, Algeria): potassium tellurite, rabbit plasma, 70° alcohol, gentian violet, Lugol, fuchsin

– other (Institut Pasteur, Algeria): egg yolk emulsion, hydrogen peroxide, sterile saline, sterile distilled water.

Bacteriological testing protocol

The following bacteriological testing protocol was used:

– the isolation of staphylococci on Baird Parker agar

– a study of the microscopic characteristics: a Gram stain test (Gram +)

– a study of the biochemical characteristics: a catalase test and free coagulase test

– screening for mannitol motility and degradation (2).

Field survey and data collection

A field survey was conducted in parallel with the sampling. A questionnaire (oral questions put to each farmer at an interview) was drawn up for this purpose and completed by one of the authors. The questions posed concerned the volume of milk produced per cow; the lactation stage; the destination of the milk; the number of pregnancies; and feeding and hygiene practices.

Results

Epidemiological survey

The farms concerned were mainly private (85.91%). Most of the farms (78.57%) sent their milk to the factory.

Of the 14 farms surveyed, 85% had herds of 10 or more cows, while 15% had fewer.

The age of the cows varied from 2 to 13 years.

The cows presented two forms of teat: funnel-shaped or cylindrical.

The number of pregnancies varied from 1 to 10.

Milk output varied from 4 litres a day to 32 litres.

All stages of lactation were represented: the early, peak and end stages of lactation.

The cows' markings were red and white or black and white.

Some farms raised their livestock on deep litter while others did not.

Some farms milked their cows by machine while others milked by hand.

Bacteriological analysis

Overall results

The results obtained from the analysis of 203 samples can be summarised as follows:

– 69.96% (142/203) of the samples were free from staphylococcus

– 30.04% (61/203) of the samples were contaminated with staphylococcus.

The 61 samples contaminated with staphylococcus were distributed as follows:

– 41 samples (67.21% = 41/61) were coagulase-negative staphylococci

– 20 samples (32.79% = 20/61) were coagulase-positive staphylococci; the average count for the latter was equal to 0.54×10^4 colony-forming units (CFU) per ml of *Staphylococcus aureus*.

Distribution of results by sample origin

For this study, the authors analysed samples of cows' milk taken from 14 farms; the results are shown in Table II.

Enumeration of *Staphylococcus aureus* by colony counting at 37°C

The *S. aureus* count was established by counting typical colonies (2) which are blackish, shiny and convex, surrounded by a transparent area and are positive to the mannitol test.

Calculation of the number of colonies found in the test sample (cows' milk)

If the number of colonies in the dish was fewer than 15, the result was obtained using the following formula:

$N = a \times 10$.

Where:

a: was the number of colonies of *S. aureus*

× 10: because the volume spread on each dish was 0.1 ml.

If the number of colonies in the dish was greater than or equal to 15 and fewer than 150 colonies, the following formula was used:

$$N = \frac{\Sigma a}{V \times 1{,}1 \times F}$$

Table II
Results of staphylococcus screening by farm surveyed

Farm no.	I–A	I–B	II–C	II–D	II–E	II–F	III–G	III–H	IV–I	IV–J	V	VI	VII	VIII	Total
No. of positive samples	0	2	10	2	3	10	1	7	5	6	3	4	5	3	**61**
No. of negative samples	5	8	8	9	7	14	5	9	9	9	29	13	10	7	**142**
Total	**5**	**10**	**18**	**11**	**10**	**24**	**6**	**16**	**14**	**15**	**32**	**17**	**15**	**10**	**203**

1038

Rev. sci. tech. Off. int. Epiz., **33** (3)

Where:

Σa was the sum of the typical colonies identified on the two selected dishes (at a dilution of 10^{-2} and 10^{-3})

V was the volume spread on each dish (= 0.1 ml)

F was the dilution rate (dilution 10^{-2}).

Expression of results

The results of the count are given in Table III.

Descriptive and analytical study

Factors of variation

The results showed the influence of the following factors.

– The rate of infection rose with the number of pregnancies, age and volume of milk output of the cow, as well as the bedding thickness.

– The infection rate of milk was greater when milking took place outside a milking parlour (directly in the cowshed) and when the milking was performed by machine.

– The cows sampled for milk presenting the highest staphylococcus contamination rate were those at the end stage of lactation, with red and white markings and with funnel-shaped teats.

Comparison of factors influencing the staphylococcus contamination rate of cows' milk on the farms surveyed

To compare the significance of these factors, an independence test was carried out with a threshold of 5%. The calculations were performed using Microsoft Office Excel® 2007 software.

Table III
Results of the *Staphylococcus aureus* count by colony counting at 37°C

Identification no. of samples contaminated with coagulase-positive staphylococcus	Expression of results (no. of *S. aureus*/ml)	Log$_{10}$ (of *S. aureus*/ml)
Sample no. 18	0.36×10^4	3.55
Sample no. 22	1.80×10^4	4.25
Sample no. 23	0.91×10^4	3.95
Sample no. 31	0.54×10^4	3.73
Sample no. 32	0.72×10^4	3.85
Sample no. 59	0.09×10^4	2.95
Sample no. 75	0.18×10^4	3.25
Sample no. 90	–	–
Sample no. 106	0.54×10^4	3.73
Sample no. 110	–	–
Sample no. 125	0.54×10^4	3.73
Sample no. 128	0.27×10^4	3.43
Sample no. 129	0.91×10^4	3.95
Sample no. 137	–	–
Sample no. 162	1.10×10^4	4.04
Sample no. 178	–	–
Sample no. 182	0.36×10^4	3.55
Sample no. 192	0.18×10^4	3.25
Sample no. 196	0.91×10^4	3.95
Sample no. 202	0.91×10^4	3.95
Average	$\mathbf{0.54 \times 10^4}$	**3.73**

Rev. sci. tech. Off. int. Epiz., **33** (3)

1039

The test was used to establish any correlations between a number of factors and the contamination rate; namely:

– age and milk output

– markings and teat shape

– age and number of pregnancies of the cow.

No correlation between the colour of the markings (red and white or black and white) and the volume of milk output appeared to influence the staphylococcus contamination rate of the milk.

Discussion

A total of 203 samples of cows' milk from the Algiers region were analysed at the Draa Ben Khedda laboratory in Tizi Ouzou and the ENSV laboratory in Algiers, to screen for the presence of staphylococci. The results revealed a prevalence of 30.04%.

The percentage of positive samples by herd ranged from 0% to 55.56%, with the highest rate on farm II–C, where 10 of the 18 samples (55.56%) were contaminated with staphylococcus. The main cause of contamination in this case was poor hygiene (unhygienic conditions in the cowshed, unhygienic practices during milking, poor hygiene among milking staff and inappropriate handling of the milk, all of which cause the staphylococci to spread from the environment to the milk). On the farms visited for this study, it was found that most farmers:

– threw the foremilk straight onto the ground

– used the same water container for washing the udders of all the cows

– used the same drying cloth for all the cows

– did not carry out post-milking teat dipping.

According to Bareille and Lemarchand (3), disinfecting the teats after milking can reduce the rate of new intramammary staphylococcus infections by 50% to 95%.

Variation factors

Age

According to Bouchard (4), the contamination risk for cows' milk increases with the cow's age.

This study shows an increase in the contamination rate of milk from cows aged between 7 and 15 years, peaking in the 13-to-15 age range (100%). Factors that might explain greater susceptibility to udder infections among cows aged 7 to 15 years include increased milk output and increased diameter of the teat canal.

Milk output

The results of this study showed contamination rates of 40% in high-yielding cows (a milk output of 30 to 40 litres per day) and 25.27% to 38.78% in low-yielding cows (a milk output of between 0 and 30 litres per day). There is, therefore, a correlation between higher infection rates and the volume of milk output. Despite the implementation of hygiene measures and an infection control plan, udder infection remains a major problem for dairy herds.

Stage of lactation

The most critical periods for new infections were the start of the drying-off period and the peripartum period (4).

In this study, the respective contamination rates observed during the different stages of lactation were as follows: 32.78% at the start of lactation; 13.13% at the time of peak lactation; and 54.09% before drying-off. These results have been confirmed by other authors:

– during lactation (apart from the beginning), the risk of staphylococcus contamination increases as lactation progresses (5);

– the lactation period is marked by a sharp rise in new infections associated with bacteria of mammary origin. A total of 80% of infections are found to persist until drying-off and 10% of quarters that are disinfected during lactation remain clear for the remaining lactation period (6).

Markings

The results of the bacteriological testing conducted for this study showed that 50.82% of contaminated milk samples came from red and white dairy cows and 49.18% from black and white cows.

Guérin (5) believes that high-yielding red and white cattle are exposed to a higher number of infections. The contamination rate is linked to the volume of milk output.

Number of pregnancies

In this study, the samples most frequently found to be contaminated were those of cows which had had multiple pregnancies (5 to 11), with a contamination rate ranging from 46.15% to 55.56%, peaking in cows with 9 to 11 pregnancies (55.56%). The contamination rate fell as the number of pregnancies decreased. These results could be explained by:

– lowering of the immune response linked with a rise in the number of pregnancies

– the shape of the teat, with very developed pendulous udders more sensitive to infections because they are more exposed to dirt and injury.

1040

Rev. sci. tech. Off. int. Epiz., **33** (3)

Teat shape

The results of this study showed that the staphylococcus contamination rate of milk varied depending on the shape of the teat, as follows: 57.38% (35/61) in cows with cylindrical or bottle-shaped teats and 42.62% (26/61) in cows with funnel-shaped teats. The latter shape prevents the milking machine teat cups from 'climbing'.

Milking method

The results of the study showed a higher contamination rate in the milk of machine-milked cows (68.85%) than in hand-milked cows (31.15%). The factors affecting these results include the following:

– the milking machine works the papillary duct and gradually leads to hyperkeratosis of this canal. This hyperkeratosis appears to encourage the onset of mastitis. Falkenberg (5) identified a positive correlation between the degree of hyperkeratosis of the teat duct and the prevalence of udder infections with *S. aureus*. The morphological aspects of the udder and the teats are therefore increasingly factored in to breeding systems;

– the milking method and the functioning of the milking machine affect mastitis in two ways: teat injuries and milk reflux or the impact phenomenon (7);

– the milking equipment transmits staphylococci mechanically.

Bedding

This study identified an increase in the staphylococcus contamination rate of milk on farms using deep-bed systems: 54.10% (33/61), compared with 45.90% on farms not using deep-bed systems. Contamination increases in line with the thickness of the bedding. These results could be explained as follows:

– bedding is clearly a source of contamination as it is regularly sown with staphylococcus. Where there is sufficient straw, its surface provides ideal temperature, humidity and oxygenation conditions for the spread of the bacteria. Contamination occurs outside the milking stage;

– a Serbian study showed that there were 27% fewer cases of staphylococcus contamination of milk under extensive herd systems, when compared with intensive herd systems (8).

Type of staphylococcus

The results of this study showed that the contamination rate of milk varied, depending on the type of staphylococcus, as follows: 67.21% for coagulase-negative staphylococci and 32.79% for coagulase-positive staphylococci. The average counts for the latter were 0.54×10^4 CFU/ml of *S. aureus*.

This could be explained as follows:

– according to Guérin (5), coagulase-negative staphylococci are the primary bacteria involved in udder infections of cows during their first lactation;

– the high number of coagulase-negative staphylococci isolated on farm II–C was due to poor hygiene during milking. Moreover, fewer coagulase-negative staphylococci were found on farms III–G, V and VI, which disinfected the teats after milking. Several studies show that teat disinfection after milking helps to reduce the prevalence of coagulase-negative staphylococci;

– according to Rekartozandrindrainy and Foucras (9), the rate at which coagulase-negative staphylococci is found in the subclinical udder infections of dairy cows varies from country to country. In developed countries, the rate of coagulase-negative staphylococcus isolation in cows' milk has declined steeply. Conversely, in developing countries, the rate of staphylococcus isolation in cows' milk is very high.

Conclusion

Although 69.96% of the milk analysed as part of this study was free from staphylococci, and most of the bacteria identified were not pathogenic to consumers (coagulase-negative staphylococci), the consumption of fresh milk nevertheless presents a degree of risk.

It would be very useful to conduct systematic screening of all dairy herds in Algeria. This would help to control the disease, as well as making the country less dependent on foreign milk imports, thereby procuring long-term benefits for the Algerian economy.

Only laboratory tests can confirm the diagnosis of staphylococcus. Prevention must take place as early as possible.

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Rev. sci. tech. Off. int. Epiz., **33** (3)

1041

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