

Full Length Research Paper

Study of the leucocytic formula of milk in the ewes of race ouled-djellal in the east of Algeria

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The study was carried out in a private ovine exploitation of Ben M' Hidi (wilaya of El-Tarf), located at northeast of Algeria during the period going from June 2000 to May 2002. It related to 13 nursing ewes of race Ouled Djellal including six first calf cows and seven multipares. The diagnosis of the subclinic mammites was made according to California Mastitis Test (CMT) without bacteriological study. The counting of the somatic cells was carried out using an optical microscope on cell of Malassez and the various types of distinguished cells (polynuclear, macrophages, and lymphocytes). The results of the CMT made it possible to distinguish three ewes reached from subclinic mammites on a total of 13 examined ewes. The given leucocytic formula at the beginning of lactation in the healthy ewes showed a prevalence of the lymphocytes (44 to 47%); the rate the polymorphonuclear ones and macrophages varies from 21 to 25%. In the batch of the ewes reached, that is, subclinic mammites, the rate of lymphocytes range between 23 and 29%, the rate the polynuclear ones are 28 to 34 %; the macrophages represent the highest rate (40 to 47%).

Key words: mastitis - subclinical - ewes - leucocytic formula - milk

INTRODUCTION

The subclinical mastitis is defined by quantitative and qualitative modifications of the milk without general clinical signs neither macroscopic buildings nor modifications of milk. The infections mammaires of the ewe involve a reduction in the production dairy (Bedo et al., 1995; Fthenakis et al., 1990b; Gonzalo et al., 1994a) and of the modifications of the biochemical composition of milk which result in a reduction in the income of the stockbreeder and outputs cheese-making (Bufano et al., 1994; Pelligrini et al., 1994; Pirisi et al., 1994). Somatic countings of cells constitute the principal indicator of health of the udder, used in routine in the milch cow. The detection of the subclinic mammites using somatic countings of cells was validated since the years 1980 in the milch cow (Serieys, 1985; Dohoo and Leslie, 1991). For the dairy ewe, work has been in hand for several years, but the thresholds of counting of somatic cells (CCS) making it possible to rule on the medical state of the udder of ewe were not fixed yet (Van Tuinen, 2001). We pro

propose in this work by using the counting of the somatic cells, to study the leucocytic formula of the ewe's milk healthy and reached subclinic mammites with the aim of defining a physiological threshold translating the number of polynuclear, macrophages and lymphocytes.

MATERIALS AND METHODS

Choice of the exploitation

The study was carried on a private ovine exploitation of Ben M' Hidi (wilaya of El-Tarf), during the period from June 2000 to May 2002. The choice of the exploitation is based on the accessibility, the availability and especially the co-operative spirit of the stockbreeder. The total staff complement of the exploitation includes a hundred heads (ewe, rams, lambs and ewe-lambs) of race Ouled Djellal. 13 ewes' gestantes of race Ouled Djellal were used in the present study.

Identification and selection of the animals

The identification of the ewes was initially, carried out according to the information sheets corresponding to the numbers related to loops at the level of the ears of the animals. The ewes gestantes were selected after a veterinary examination. The second stage

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consisted of making a clinical examination of the udders of the ewes gestantes; we carried out two types of taking away for our study at the laboratory: taking away of milk or mammary secretions for counting of the somatic cells and the establishment of the leucocytic formula of milk in the healthy ewes and the ewes reached of subclinical mastitis. We considered for the clinical examination that the ewes were healthy as of the time of experiment because of the absence of clinical signs and macroscopic modifications of milk. The ewes only have clinical mastitis, when there is presence of clinical signs and/or the macroscopic modifications of milk. For the CMT (California Mastitis Test) we considered the healthy ewes, when all the values lie between 0 and 1. The ewes reached of subclinical mastitis when at least a value is equal to + 2, only one value are higher than + 2, two values are higher or equal to 3 (Ziv et al., 1968).

Clinical examination

After application of the animal, palpation starts with milk and then generalized on the udder. The slightly drawn trayon to the bottom, in order to tighten it, is palpated between the inch and the index. The channel of the trayon, easy to perceive, can be compared with a tube of the diameter of a lead of pencil. The milk sine and the parenchyma of each district are then palpated with two hands. The fabrics being taken in the hollows of the hands, the end of the fingers depresses successively all the parts of gland. The examination ends in the palpation of the lymphatic ganglia retro mammaires, which, in a normal state, have the shape of a vertical disc from 4 to 5 cm in diameter and 1 cm thickness.

California mastitis test (CMT)

Apart from the direct methods of countings like Fossomatic and Coulter Counter (Fieten et al, 1983), there are indirect methods like the CMT. This last is founded on the notion of the stages of the intensity of the flocculation of the DNA of the cells deteriorated beforehand using a detergent (Teepol). This one being proportional to the quantity of DNA and thus to the quantity of somatic cells. According to Poutrel (1983) and Shultz (1967), the counting of somatic cells (CCS) is one of the indirect variables most representative of the medical state of the mamelle. It is thus possible, by measurement of the cellular rate of milk, to detect the subclinic mammites, dominant pathology in dairy breeding. The interpretation of test CMT is based on the content of leucocytes. The latter is a function of the formation of the flocculate (Ziv et al., 1968; Regi et al., 1991). The adopted scale is as follows: - light flocculated transitory: 1 or +/-, light flocculated persistent: 2 or +; flocculated thick adhering: 3 or ++, flocculated standard white or gelation: 4 or +++.

Taking away of milk

Each ewe was the subject of 11 taking away of the right and left worse. Three taking away were carried out before the low setting (J-10, J-6 and J-3), eight taking away after the low setting (J0) and in the course of lactation (J+4, J+16, J+23, J+26, J+28, J+30 and J+32). From the taking away, CMT was carried and a leucocytic formula for all the ewes.

Taking away for the CMT

The taking away of milk of the half udders for the CMT were carried out before the beginning of the draft of the morning, without elimination of the first jets in cups of the plate test, a cup by half udder. One fills each 2 cups of the plate with 2 ml of milk and 2 ml

of Teepol to 10% (a cup by district). One mixes the two liquids by a rotational movement of the plate in a horizontal plane. The reading is immediate.

Determination of the leucocytic formula

The preparation of the samples for cellular counting proceeded in four stages: taking away of milk, preparation of the blades, coloring and reading. The taking away of milk of half udders for the study of the leucocytic formula was also made before the draft of the morning, according to the technique described by Bind et al. (1980). After expulsion of the first jets and disinfection of the end of the trayon with alcohol at 70°C, milk is collected in sterile tubes of 25 ml. The taking away are then transported to refrigerators at a temperature of 4°C at the laboratory of the veterinary center in El Tarf where they are preserved at a temperature of 4°C until the reading. Before the low setting, one takes 02 ml of secretions mammaires of each district right and left, after low setting one takes 10 ml of milk of each district; the tubes are then marked and numbered. For the taking away that is carried out before low setting 01 ml are centrifuged (1200 g, 12 mn, 4°C). The base is directly washed after centrifugation. For the taking away carried out after low setting the cellular smears are obtained as follows 5 ml of milk are centrifuged (1200g, 12mn, 4°C), one eliminates the supernatant and the fat content. The base is washed by centrifugation (1000 g, 6 mn, 4°C) in nutritive bubble. The cellular concentration is adjusted with 2.106 cellules/ml with nutritive bubble. The blades are then coloured in May Grunwald Giemsa (MGG). Cellular counting was made under the optical microscope on cell of Malassez.

Cellular numeration

The blades smears are observed under the optical microscope. One proceeds to the enumeration of the cells (Macrophage, Polynucleaire and Lymphocyte) by progression in cascade of the fields observed. The results are expressed at a percentage compared to a total of 100 cells. The blades are then preserved at 4°C after being fixed in acetone.

Statistical analysis

The results are expressed by the average and the standard deviation for each batch of healthy ewes and reached subclinic mammites and for each type of cellular population. The statistical analysis was made with software MINISTAT. The averages were compared using the test-T of Student.

RESULTS AND DISCUSSION

The results showed that in the healthy ewes, the percentage of the lymphocytes (Tables 1 and 9) was 47 to 48% during the pre-colostrale phase; in the colostrums, this rate was 47%, where as 16th with the 32nd day of breast feeding, it varies from 44 to 47%; the rate of the polymer-nuclear ones (Tables 2 and 7) was respectively 23 to 25% in pre-colostrale phase, between 19 and 20% in phase colostrale and from 21 to 25% between 16th and the 3rd day of breast feeding; on the other hand, the macrophages (Tables 3 and 8) varied between 25 and 31% during the pre-colostrale phase, in the colostrums this rate were 33%, between 16th and the 32nd day, it was between 30 and 32%. In the batch of ewes reached

Table 1. Rate of lymphocytes (%) in the healthy ewes (n = 10).

End of gestation			Put low	Lactation							
- 10	- 6	- 3	0	4	16	23	26	28	30	32	
40	38	56	50	44	45	51	50	52	51	55	
50	42	41	38	42	44	45	44	45	39	38	
55	49	49	40	42	41	44	42	44	49	48	
53	49	42	51	54	50	48	47	56	42	42	
53	49	42	51	54	50	48	47	56	42	42	
56	47	44	54	45	49	48	50	37	45	37	
45	41	50	38	39	41	46	45	40	49	44	
44	52	51	56	57	39	38	36	42	41	39	
56	56	52	51	48	45	48	50	52	50	50	
43	47	55	49	47	47	50	51	49	51	52	

Table 2. Rate of polynuclears (%) in the healthy ewes (n = 10).

End of gestation			Put low	Lactation							
- 10	- 6	- 3	0	4	16	23	26	28	30	32	
19	22	20	22	25	24	25	24	22	23	22	
24	21	26	23	22	20	24	20	32	30	29	
22	28	20	21	17	17	22	23	19	15	23	
19	18	22	18	16	18	21	27	22	26	27	
19	18	22	18	16	18	21	27	22	26	27	
21	24	32	20	15	17	16	17	20	22	22	
28	29	24	22	21	19	15	14	19	16	21	
32	30	28	16	14	32	33	29	28	25	25	
28	30	32	17	27	28	27	22	23	24	27	
20	21	18	19	24	23	26	23	20	21	21	

Table 3. Rate of macrophages (%) in the healthy ewes (n = 10).

End of gestation			Put low	Lactation							
- 10	- 6	- 3	0	4	16	23	26	28	30	32	
20	33	19	32	33	28	30	32	34	32	30	
28	36	30	38	37	37	38	36	35	30	32	
17	23	34	32	42	41	43	39	37	36	36	
24	27	26	27	33	32	35	32	30	31	29	
24	27	26	27	33	32	35	32	30	31	29	
21	29	25	32	34	38	38	36	34	39	31	
29	28	30	33	35	31	33	35	32	33	32	
32	30	29	31	30	31	28	25	26	27	25	
30	32	34	30	31	29	32	33	35	28	29	
30	32	34	30	31	29	32	33	35	28	29	

of subclinical mastitis, the results show that the rate of macrophages (Table 6 and 8) going from 40 to 47% was higher than that of polynuclear (28 to 34%) (Table 5 and

7); the percentage of the lymphocytes (Table 4 and 9) was lowest (23 to 29%). The statistical analysis of the cellular rate of the three populations, between the healthy

Table 4. Rate of lymphocytes (%) in the ewes reached of subclinical mastitis (n = 3).

End of gestation			Put low	Lactation						
- 10	- 6	- 3	0	4	16	23	26	28	30	32
25	26	27	30	32	26	25	25	26	25	26
24	24	25	26	27	27	26	26	27	25	26
22	25	28	31	29	29	28	29	27	31	30

Table 5. Rate of polynuclears (%) in the ewes reached of subclinical mastitis (n = 3).

End of gestation			Put low	Lactation						
-10	- 6	- 3	0	4	16	23	26	28	30	32
27	25	27	30	31	32	34	34	32	30	32
30	36	34	32	34	35	35	34	36	35	34
29	30	31	32	34	34	32	34	36	34	32

Table 6. Rate of macrophages (%) in the ewes reached of subclinical mastitis (n = 3).

End of gestation			Put low	Lactation						
-10	- 6	- 3	0	4	16	23	26	28	30	32
48	49	46	40	37	42	41	41	42	40	42
46	40	41	42	39	38	39	41	39	38	40
49	45	41	37	37	37	38	38	37	39	38

Table 7. Average percentages of the rate the polynuclear ones in the ewes healthy (m ± S, n=10) and reached of subclinical mastitis (m ± S, n = 3).

lactationstage (days)	Polynuclears ewes healthy (n = 10)	Polynuclears subclinical mastitis (n = 3)	P
-10	23,40 ± 4,62	28,66 ± 1,528	0,0004
-6	23,40 ± 4,67	30,33 ± 5,51	0,0048
-3	25,00 ± 4,74	30,67 ± 3,51	0,0021
0	19,800 ± 2,573	31,33 ± 1,155	0,0001
4	20,20 ± 4,61	33,00 ± 1,73	0,0004
16	21,90 ± 4,89	33,66 ± 1,528	0,0004
23	23,20 ± 5,29	33,66 ± 1,528	0,0001
26	22,30 ± 4,72	34,00 ± 0,00	0,000
28	24,00 ± 4,88	34,67 ± 2,31	0,0001
30	24,30 ± 4,19	33,00 ± 2,65	0,0001
32	25,00 ± 3,23	32,66 ± 1,15	0,0031

ewes and reached subclincic mammites, reveal a highly significant difference ($p < 0,001$) between the rate of lymphocytes, the polynuclear ones, and macrophages during the three studied phases.

Apart from any infectious, general factor or mammary, we can distinguish three categories of variations from the counting of the somatic cells. First of all, the factors of

physiological variations, related to the operation of the udder and acting in a comparable way for all the animals. The second category gathers the zootechnical factors of variations corresponding to the conditions of breeding, which also act in a similar way for all the animals. The third category factors of genetic variations, clean with each animal. Among the physiological factors, the varia-

Table 8. Average percentages of the rate the macrophages ones in the ewes healthy ($m \pm S$, $n = 10$) and reached of subclinical mastitis ($m \pm S$, $n = 3$).

Lactation stage(days)	Macrophages ewes healthy (n = 10)	Macrophages subclinical mastitis (n = 3)	P
-10	25,50 \pm 5,04	47,66 \pm 1,528	0.0001
-6	29,70 \pm 3,71	44,67 \pm 4,51	0.001
-3	28,70 \pm 4,83	42,67 \pm 2,89	0.0001
0	31,200 \pm 3,15	39,67 \pm 2,52	0.0001
4	33,90 \pm 3,51	37,66 \pm 1,15	0.0021
16	32,80 \pm 4,37	39,00 \pm 2,65	0.0006
23	34,40 \pm 4,40	39,33 \pm 1,52	0.0004
26	33,30 \pm 3,71	40,00 \pm 1,73	0.0021
28	32,80 \pm 3,29	39,33 \pm 2,52	0.0001
30	31,50 \pm 3,75	39,00 \pm 1,00	0.0021
32	30,200 \pm 2,86	40,00 \pm 2,00	0.0001

Table 9. Average percentages of the rate the lymphocytes ones in the ewes healthy ($m \pm S$, $n = 10$) and reached of subclinical mastitis ($m \pm S$, $n = 3$).

lactation stage (days)	Lymphocytes ewes healthy (n = 10)	Lymphocytes subclinical mastitis (n=3)	P
-10	49,50 \pm 5,99	23,66 \pm 1,52	0.0001
-6	47,00 \pm 5,37	25,00 \pm 1,00	0.0001
-3	48,20 \pm 5,57	26,66 \pm 1,52	0.0001
0	47,80 \pm 6,63	29,00 \pm 2,65	0.0021
4	47,20 \pm 6,01	29,33 \pm 2,52	0.0021
16	45,10 \pm 3,93	27,33 \pm 1,52	0.0001
23	46,60 \pm 3,69	26,33 \pm 1,52	0.0021
26	46,20 \pm 4,66	26,67 \pm 2,08	0.0001
28	47,30 \pm 6,68	26,66 \pm 0,57	0.000
30	45,90 \pm 4,61	27,00 \pm 3,46	0.0001
32	44,70 \pm 6,24	27,33 \pm 2,31	0.0004

tions related to the stage of lactation are prevalent (Moles, 2002). The distribution of the cellular types was discussed a long time because of the difficulty of differentiating these cells (Lepage, 1999). Nevertheless, the approach is different, holding account of the presence or absence of infection of the udder.

The cellular types of the milk of the ruminants in normal conditions gather two main categories of cells (Linzell et al., 1970; Shalm et al., 1971; Paape et al., 1991). The first includes/understands cellular elements of epithelial nature, which come from the exfoliation of the secretory or milk epithelium. The presence of these cells is physiological: it corresponds to the normal renewal of epithelial bases. The second category of cells includes/understands three pennies populations of blood origin: macrophages, the polymorphonuclear ones (mainly neutrophils) and lymphocytes. The presence in the milk of these cells is inflammatory and immunizing.

With the ovine and bovine species, the beginning and

the end of lactation are marked by a stronger polymorphonuclear proportion neutrophils (PMN) and a smaller percentage of lymphocytes (Bergonier et al., 1994b). With the approach of the low setting, the colostrum stages in the ewe have rarely been dealt with except for Fruganti et al. (1985) who noted that, the CCS of the colostrum can be higher than in the first months of lactation. Gonzalo et al. (1986) compared the contents of milk cell and the given colostrum using a microscopic method. They obtain average values of 750.10 c/ml in colostrum phase against 134.10 c/ml into milk towards the 23rd day. Our results reveal lymphocytary prevalence to 47%, followed by the polymorphonuclear ones between 21 and 25% and one rate of macrophage of 33%; whereas Lee and Outteridge (1981) observe an important proportion of neutrophils polymorphonuclear: 41 to 84%, 8 to 49% of macrophages and 6 to 11% of lymphocytes. On the other hand, they observe only little or no epithelial cells. Pathogenic of the values from 80 to 83.10 c/ml at 35 postpartum of

days. Owing to lack of data concerning the cellular proportions at the beginning of lactation in the sheep, we will compare our results with those of the milch cow, contrary to Miller et al. (1986); the beginning of lactation in the ewe race, Ouled Djellal, the prevalence is lymphocytary, whereas the proportions of the macrophages and neutrophils are identical: 21 to 25%. These differences between our results and those of the bibliography will be related not only to the stage of lactation but probably with other factors.

The results in the cow seem contradictory; owing to the fact that, Mc Donald (1981) reports that the colostrum practically does not contain the polymorphonuclear ones. On the other hand, Lee and Brandon (1989) and Liu (1988) indicate that in the absence of any infection, the colostrum is characterized by the presence of a high number of polymorphonuclear ones, of which the number falls during the first week following the vêlage and great number of érythrocytes sometimes that can result in a hemolactation. At the beginning of lactation, in the dairy ewe and for publications the bacteriological statute of the analyzed milks was not specified in the study at the lactation stage, but treating the CCS by hêmi-udder, the average of the CCS of the first month is of 50.10^3 c/ml for Regi et al. (1991) and of 200.10^3 c/ml for Bergonier et al. (1994b). According to Mavrogenis et al. (1994), the average of the CCS of the first month varies from 100 to 500.10^3 cellules/ml. On the other hand, in milch cow Sheldrake et al. (1983) present for unscathed districts of organizations related on the conditions of breeding and the race. In our study, we based ourselves only on the CMT for the subclinical diagnosis of mastitis, without determination of the pathogenic agent. We attended in the ewes reached of subclinical mastitis an increase in the rate of macrophages between 40 and 47% followed of polynuclear with a rate of 28 to 34%; and to a reduction in the lymphocytes from 23 to 29 % the averages of cellular countings in the ewe vary according to the presence of infectious agents.

The actual values in the literature are very variable according to authors. Eitam (1994) does not find any correlation between the average of the CCS and the infectious agent responsible for the mastitis. Deinhofer et al. (1993b) conclude with a correlation between *Staphylococcus* spp., the state of private clinic of the udder and countings cellular. Bergonier et al. (1994a) notice that 70% of the CCS associated with the presence of *Staphylococcus epidermidis* are higher than 1 million/ml, the oscillating remainder around 500 000 cellules/ml. According to Mavrogenis et al. (1994), the average of the CCS of the first month varies from 100 to 500.10^3 cellules/ml. On the other hand, in milch cow Sheldrake et al. (1983) present for unscathed districts of organizations related on the conditions of breeding and the race. In our study, we based ourselves only on the CMT for the subclinic diagnosis of mastitis, without determination of the pathogenic agent. We attended in the ewes reached of mastitis

a subclinic an increase in the rate of macrophages between 40 and 47% followed of polynuclear with a rate of 28 to 34%; to a reduction in the lymphocytes from 23 to 29% the averages of cellular countings in the ewe vary according to the presence of infectious agents. The actual values in the literature are very variable according to authors. Eitam (1994) does not find any correlation between the average of the CCS and the infectious agent responsible for the mastitis. Deinhofer et al. (1993) conclude with a correlation between *Staphylococcus* spp., the state of private clinic of the udder and countings cellular. Bergonier et al. (1994a) notice that 70% of the CCS associated with the presence with *Staphylococcus epidermidis* are higher than 1 million/ml, the oscillating remainder around 500 000 cellules/ml. In the cow with respect to the infection, the relative proportion, in milk, from each type of cells (epithelial, polynuclear, macrophages and lymphocytes) varies according to the statute of the district or the udder (Weller et al., 1992; Zhang et al., 1994). The infections cause increases in the cellular concentrations, blood deteriorations (Youl and Nicholls, 1987; Ickowicz, 1985), and of the considerable modifications in the pattern of the cellular settlements in milk. The increase will amount in hundreds of thousands, while at the time of the physiological modifications the variations of the rates observed are about a few tens of thousands. One assists, in particular, with a very important rise in the number of polymorphonuclear neutrophils. Cassell (1994) and Coffey et al. (1986) bring back a percentage of polymorphonuclear, which can reach 90 to 95%; Paape et al. (1991) made this same observation in the event of clinical or subclinical mastitis. Consequently, the determination of the rate of leucocytes becomes an invaluable tool for tracking of the infection mammaire. A very weak increase in the relative percentage of the neutrophils and macrophages as well as a light reduction in the percentage of the epithelial cells are noted at the time of infection by a minor pathogenic agent such as, negative *Staphylococcus coagulase*, (Lepage, 1999). The percentage of the lymphocytes does not vary practically. In revenge, an increase in the relative percentage of the neutrophils as well as a reduction in the relative percentage of the epithelial cells is noted at the time of infection by a major pathogenic agent (Rupp and Boichard, 1999).

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