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QUALITATIVE EVALUATION OF RAW MILK OF BUFFALO FROM THE ROMANIAN MURRAH BREED

EVALUAREA CALITATIVĂ A LAPTELUI CRUD DE BIVOLIȚĂ DIN RASA MURRAH ROMÂNEASCĂ

Mariana TĂTARU[1)], I. PAPUC[1)], Livia VIDU[3)], Camelia RĂDUCU[2)], F. CHIRILĂ[1)], S. DAN[1)], M. S. MÂRZA[1)], C.R. PURDOIU[1) *)], R. LĂCĂTUȘ[1)], Vioara MIREȘAN[2)]

ABSTRACT | REZUMAT

The purpose of this study was to evaluate the microbiological quality, the physico-chemical parameters and the degree of hygiene of raw buffalo milk from the Romanian Buffalo breed. Samples were collected between August 2017- May 2018, in total of 120, 30 samples each season. The milk collection was made from buffaloes in different lactations from the Research and Development Station for Buffalos Breeding Șercaia, Braşov County, Romania. The health of the mammary gland and milk hygiene were assessed by determining the number of somatic cells and by microbiological analysis, and the milk composition by determining physico-chemical parameters. Microbiology analysis show increased TNG during winter ($3.4x10^4$) compared with autumn where is registered the lowest TNG ($1.1x10^4$) and a mean somatic cell count (SCC) of 1026.5 x 10^3. Pathogenic bacteria were isolated and identified from *Bacillus licheniformis* species 4.6±0.32; *Staphylococcus spp*. 2.95±0.53; and *Trueperella pyogenes* 1.43±0.26. The physico-chemical parameters had the following average values: fat 7.9%±0.38; protein 4.2%±0.23; lactose 4.0%±0.19; ash 0.57%±0.57 and average density at 18ºC of milk was 1.029. Microbiological determinations correlated with the composition of buffalo milk are the main criteria for estimating losses due to the existence of subclinical or clinical mammals in the herd and provide the necessary framework for the implementation of preventive and control measures for these diseases. The correlation between the somatic cells and the milk composition is taken into account when determining the price of raw buffalo milk and this is also the element on which predictions are made as to how milk will behave in the different stages of the processing scenario and how it will influence finished products.

Key words: Romanian Buffalo breed, raw milk, physico-chemical parameters, TGC, SCC

Scopul acestui studiu a fost de a evalua calitatea microbiologică, parametrii fizico-chimici și gradul de igienă a laptelui crud de bivoliță din Rasa de bubaline românească. Probele au fost colectate în perioada august 2017 - mai 2018, în număr total de 120, câte 30 de probe în fiecare anotimp. Colectarea laptelui s-a făcut de la bivolițe aflate în lactații diferite de la Stațiunea de Cercetare Dezvoltare pentru Creșterea Bubalinelor Șercaia, jud. Brașov, România. Starea de sănătate a glandei mamare și igiena laptelui au fost evaluate prin determinarea numărului de celule somatice și prin analize microbiologice, iar compoziția laptelui prin determinarea parametrilor fizico-chimici. Analizele microbiologice au evidențiat o creștere a numărului total de germeni (NTG) în timpul iernii ($3,4x10^4$) comparativ cu toamna, când s-a înregistrat cel mai scăzut NTG ($1,1x10^4$) și o valoare medie a numărului de celule somatice (NCS) de 1026,5 x 10^3. S-au izolat și identificat bacterii patogene din speciile *Bacillus licheniformis* 4,6±0,32; *Staphylococcus spp*. 2,95±0,53; și *Trueperella pyogenes* 1,43±0,26. Parametrii fizico-chimici au avut următoarele valori medii: grăsimea 7,9%±0,38; proteina 4,2% ±0,23; lactoza 4,0%±0,19; cenușa 0,57%±0,57, iar densitatea medie la temperatura de 18ºC a laptelui a fost de 1.029. Determinările microbiologice corelate cu compoziția laptelui de bivoliță reprezintă principalul criteriu prin care se estimează pierderile cauzate de existența unor mamite subclinice sau clinice în efectiv și oferă cadrul necesar de implementare a unor măsuri de prevenție și de control al acestor afecțiuni. Corelația dintre celulele somatice și compoziția laptelui este luată în calcul când se stabilește prețul laptelui crud de bivoliță și constituie, totodată, elementul pe baza căruia se fac previziuni cu privire la modul în care se va comporta laptele în diverse faze ale procesului de prelucrare și cum va influența produsele finite.

Cuvinte cheie: bivolul românesc, lapte crud, parametrii fizico-chimici, NTG, NCS

1) University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj-Napoca, Romania
2) University of Agricultural Sciences and Veterinary Medicine, Faculty of Zootechnics and Biotechnologies, Cluj-Napoca
3) University of Agronomical Sciences and Veterinary Medicine, Faculty of Animal Productions Engineering and Management, Bucharest, Romania
*) *Corresponding author: robert.purdoiu@usamvcluj.ro*

Milk is the product of mammary gland secretion, which due to its high nutritional properties, is the main source of food for newborns and the elderly. The quality of milk is defined by physical and chemical parameters

and hygiene (14, 27, 34, 40). Due to its rich nutrient content, buffalo milk has received a lot of attention lately and has become an important topic in research (1). Compared to cow's milk, buffalo milk has a more pleasant taste due to its different content of fat, protein, lactose, dry matter, vitamins and minerals. These properties make it used as raw material in the manufacture of dairy products such as cheese, butter and ice cream (10, 39). In the European Union, raw milk is given special attention because its hygiene limits set out in Regulation (EC) 853/2004 refer to this product (9). The measured parameter values indicate the health of the mammary gland and are always evaluated from raw milk. If these parameters are not in compliance, they increase the risks to consumer health and can cause some eating disorders (4). Raw milk can be commercialized directly to the final consumer only if it complies with the applicable sanitary veterinary legislation on the total number of germs (maximum permissible 100,000/ml) and somatic cells (max. 400,000 / ml) (35). In order to meet these quality parameters, the milk intended for processing or consumption must meet three main conditions, such as: to be integral, which means to have a normal structure and composition; absence of a large microbiological load which may damage its integrity; not to come from specimens diagnosed with mastitis in accordance with European and Romanian rules and regulations (9, 22, 23). In raw buffalo milk, microorganisms can multiply rapidly due to their rich nutrient content. Studies on the microbiological qualities of buffalo milk (14, 15, 32, 33) have revealed and isolated bacterial strains of the genus *Escherichia coli*, acidophilic bacteria, fungi and *Staphylococcus* spp. Saprophytic microorganisms in milk may deteriorate its quality, and the presence of pathogenic bacteria may pose a potential health threat to consumers (6, 14, 15).

In Romania, average productions of buffalo milk ranging from 1200 to 1700 kg per lactation are recorded with an average fat content of 7.49% (12). Research on buffalo milk in Transylvania revealed a fat content of 7.40%, and in the Murrah breed the percentage of average fat was 8.35% (41). In another Romanian study realized in 2009, on Romanian buffaloes from different areas, the bacteriological examination revealed that the coagulanzo-positive staphylococci was absent in most of the samples studied, except for the two samples in which they were identified (2 germs / ml) respectively (4 germs / ml), and *Salmonella* was absent in all samples (28). In the same study, the values of some physico-chemical parameters were compared for the buffalo milk of Romanian Buffalo breed with the Nili-Ravi breed at which protein percentages were lower and the percentage of lactose higher. Coliform bacteria from buffalo milk of the Romanian Buffalo breed had values between 3.95 ± 0.07, *Escherichia coli* 1.80 ± 0.23, *Streptococcus aureus* 1.80 ± 0.23, fungi 1.33 ± 0, 46; and in the Nili-Ravi breed, coliform bacteria were between 2.16 ± 0.30, *Escherichia coli* 1.80 ± 0.23, *Streptococcus aureus* 1.95 ± 0.36, and the fungi had values of 1.33 ± 0.46 (28).

The purpose of this study was to investigate the microbiological quality and hygiene of buffalo milk at Research and Development Station for Buffalos Breeding Şercaia, Braşov County, Romania and to identify and isolate the bacterial species to see if they fall within the admissible parameters established by the European and national legislation in the field. At the same time, the research aimed at identifying a direct correlation between the total number of germs (TNG), the number of somatic cells (NSC) and the existence of clinical or subclinical mastitis in the herd.

MATERIALS AND METHODS

The research consisted of collecting 120 milk samples raw material from 30 buffaloes from the Romanian buffalo breed. The milk collection was made seasonally between August 2017 and October 2018: 30 samples (50 ml/sample) per each season. Sample collection was done in sterile containers under aseptic conditions, and the transport to the microbiology laboratory at USAMV Cluj-Napoca was done at 4 ºC ± 1 ºC, then the samples were processed immediately. Of the total number of buffaloes from which the milk was collected for analysis, a number of 13 buffaloes were primiparous and 17 multiparous buffaloes.

The working methods used were aimed at physico-chemical, microbiological and statistical analysis. The physico-chemical parameters represented by the non-fat dry substance (NFDS), fat, protein, lactose, density, mineral salts, sample temperature, freezing point, total dry substance (TDS), conductivity and pH were determined using the analyzer Lactoscan MCCW milk whose principle of operation is based on ultrasound technology. The milk analyzer determines the freezing point of each sample and the amount of added water. Conductivity or electrolytic conductivity depends on the concentration of milk ions, the average value of which depends on the geographic, species and breed region. In buffaloes the milk's conductivity is between 2.5 and 5 mS / cm at a temperature of 18 ºC. Microbiological analyses

were performed to determine the total germ count (TGC) and identify some bacterial strains of the genus: *Staphylococcus spp., Streptococcus spp., Bacillus licheniformis, Salmonella spp., Escherichia coli; Listeria monocytogenes*; mushrooms and fungi indicating the degree of milk hygiene. The number of somatic cells in raw milk indicates the health of the mammary gland and milk quality and it was obtained using Lactoscan SCC analyzer based on the fluorescence microscopic technique. The statistical analysis was done using SPPS.

RESULTS AND DISCUSSIONS

Quality milk has a good taste, a composition that fulfills sanitary and veterinary standards and is free from pathogenic bacteria, harmful toxic substances, sediments and foreign bodies (17).

Physico-chemical parameters. In the view of several researchers, the physico-chemical parameters of buffalo milk can be influenced by several factors such as the season, lactation period, diet, milking frequency and milking technique (6, 32, 33, 36, 37). The average values of physico-chemical parameters differ depending on the season.

Statistical Analysis. For evaluation of buffalo milk, twelve parameters where taken in consideration. Eleven parameters where measured in each season. Statistical evaluation was performed using SPSS program, for each parameter ANOVA test was perform in order to measure the difference between the seasons.

The analyze of variance was performed, if $p>0.05$ then the assumption of homogeneity of variance is met and ANOVA test was performed, when $p<0.05$ the assumption of homogeneity of variance is violated and Welch ANOVA test was performed.

The null hypothesis was that there are no significant differences in parameters between seasons. Tukey HSD and Games-Howell Post-Hoc test were used to measure the average differences of parameters between seasons. The parameters analyzed where represented by fat, somatic cells, protein, lactose, ash, pH, dried substance, mineral substance, water, density, conductivity and microbiological analysis.

Fat. The homogeneity of variance is violated $F(3,116)=6.179$; $p<0.05$, variance of the fat in the buffalo milk between seasons do not present statistical significance as shown by Welch ANOVA ($p>0.05$).

Somatic cells. In case of somatic cells statistical evaluation the analysis of variance is met $F(3,116)=0.256$; $p>0.05$. The result of the ANOVA test do not show statistical significance of somatic cells in the buffalo milk between seasons, $F(3,116)=0.757$; $p>0.05$.

Protein. The homogeneity of variance is met for $F(3,116)=1.118$, $p>0.05$, the result of the ANOVA test show statistical relevance for protein between seasons for $F(3,116)=17.113$, $p<0.05$. Tukey HSD Post-Hoc test revealed that the protein is significantly higher during summer (4.360.16, $p<0.05$) and significantly lower during spring (4.0070.20, $p<0.05$) compared with autumn and winter season. No significance was found for protein during winter and autumn season ($p=0.94$).

Lactose. The homogeneity of variance is violated for $F(3,116)=3.703$, $p<0.05$, result of Welch ANOVA test show statistical relevance for lactose between seasons, $p<0.05$. Games-Howell Post-Hoc test revealed that the lactose present statistical significance ($p<0.05$) during winter and summer compared with autumn and spring. The highest lactose value is seen during spring (4.160.14) and autumn (4.090.13) and the lowest are registered during summer (3.850.19) and winter (3.940,12).

Ash. The homogeneity of variance is met for $F(3,116)=1.534$, $p>0.05$, the result of the ANOVA test show statistical relevance for ash between seasons for $F(3,116)=15.960$, $p<0.05$. Tukey HSD Post-Hoc test revealed that the ash is significantly higher during autumn (0.620.04, $p<0.05$) compared with the other seasons.

pH. The homogeneity of variance is violated for $F(3,116)=5.801$,$p<0.05$, the result of the Welch ANOVA test show statistical relevance for pH between seasons, $p<0.05$. Games-Howell Post-Hoc test revealed that the pH present statistical significance ($p<0.05$) during spring compared with summer, autumn and winter. The highest pH value is registered in the spring season (6.640.04) and the lowest value is registered in winter (6.540.03).

Dried substance. The homogeneity of variance is violated for $F(3,116)=7.995$, $p<0.05$, the result of the Welch ANOVA test show statistical relevance for dried substance between seasons, $p<0.05$. Games-Howell Post-Hoc test revealed that the dried substance present statistical significance ($p<0.05$) during winter compared with spring, autumn and summer. The lowest dried substance value is registered in the winter season (8.860.01) and the highest value is registered in summer (9.020.03).

Mineral substance. The homogeneity of variance is violated for $F(3,116)=5.748$, $p<0.05$, the result of the Welch ANOVA test show statistical relevance for mineral substance between seasons, $p<0.05$. Games-Howell Post-Hoc test revealed that the mineral substance present statistical significance ($p<0.05$) during

autumn and winter ($p<0.05$) compared with spring, summer. The lowest mineral substance value is registered in the winter season (0.150.004) and the highest value is registered in autumn (0.180.009).

Water. The homogeneity of variance is violated for $F(3,116)=12.871$, $p<0.05$, the result of the Welch ANOVA test show statistical relevance for water content between seasons, $p<0.05$. Games-Howell Post-Hoc test revealed that the water present statistical significance ($p<0.05$) during spring ($p<0.05$) compared with summer, autumn and winter. The lowest water value is registered in the spring season (3.940.09) and the highest value is registered in summer (4.050.06).

Density. The homogeneity of variance is violated for $F(3,116)=13.317$, $p<0.05$, the result of the Welch ANOVA test show statistical relevance for density between seasons, $p<0.05$. Games-Howell Post-Hoc test revealed that the density presents statistical significance ($p<0.05$) during autumn and winter ($p<0.05$) compared with spring and summer. The lowest density value is registered in the autumn season (1027.36 1.03) and the highest value is registered in summer (1031.42 0.30).

Conductivity. The homogeneity of variance is violated for $F(3,116)=4.269$, $p<0.05$, the result of the Welch ANOVA test show statistical relevance for conductivity between seasons, $p<0.05$. Games-Howell Post-Hoc test revealed that the conductivity presents statistical significance ($p<0.05$) during autumn compared with spring ($p=0.006$) and winter ($p=0.43$). The lowest conductivity value is registered in the autumn season (4.85 0.04) and the highest value is registered in spring (4.88 0.03).

Microbiological analyses. The microbiological quality of raw milk is influenced by milking, shelter, agricultural system (organic or conventional) and seasonal farming. Various medium crops were used to identify different types of bacteria for each strain studied. Samples of raw milk were diluted 1:10 (1 ml milk in 9 ml physiological saline). The sowing of the samples was done under aseptic conditions by the flooding technique. From each diluted sample was taken 1 ml which was plated into Petri dishes with selected culture media for each type of bacteria traced. To determine the total germ count (TGC) we used as glucose-added agar culture medium (TNAMG); for enterobacteria, the Mac Conkey special environment was used; for *Salmonella spp*., the special Xylose Lysine Deoxycholate (XLD) medium was used; for *Listeria monocytogenes* and *Bacillus licheniformis*, the Palcam medium; for *Staphylococcus aureus* and Manito-positives pathogenic staphylococci the Chapman medium, and for the identification of fungi, the Sabouraud medium. The special Brain heart infusion (BHI) medium supplemented with 5% bovine blood was used to identify the *Trueperella pyogenes* germ, which is a pretentious germ that does not grow on usual environments until after enrichment with serum, blood or glucose. Isolation of germs of this species is easily done after sampling and incubation in a CO_2 enriched atmosphere, at 37ºC. In the liquid medium, the serum broth, the presence of the *Trueperella pyogenes* germ is identified by the appearance of a low turbidity, and at the bottom of the tube forms a fine, granular deposit that may have a tendency to climb the walls of the tube. On the sheep-bred agar, the colonies of *Trueperella pyogenes* are small, punctuated in size between 0.1-0.2 mm, surrounded by a β-hemolysis area whose diameter may be 2 and 3 times longer bigger than the colony. After 48:00 h - 72:00 h of incubation, colonies grow in size and reach up to 0.5-1.5 mm. They have a round shape, a convex profile, a smooth, luminous, whitish or grayish-white surface (29). After sowing, the samples were incubated at 37ºC for 24:00 h to 48:00 h. After incubation, samples were read, only plates that increased between 30 and 300 colonies on the basis of which the macroscopic and microscopic characters of the bacterial colonies were established. From colonies grown on culture media, smears were made which were stained by the Gram technique, and then examined under a microscope. The microbial contamination of milk can come from various sources, both inside and outside of the udder, from bedding, flooring, water supply, milker and the environment, or it can be enabled by insects and rodents (17, 24, 26). Microbiological analyses performed on samples of raw buffalo milk taken in this study revealed the presence of several bacterial species (Table 1).

Microbiology analysis show increased TNG during winter ($3.4x10^4$) compared with autumn where we registered the lowest TNG ($1.1x10^4$). High percentage of *Staphylococcus spp.* and *Bacillus Lichiniformis* are recorded during winter. In the autumn season *Trueperella pyogenes* was identified in the buffalo milk.

Discussions

The total number of germs (TNG) from the samples taken under study was within the normal limits of the legislation in force (maximum 100,000 / ml) according to EC Regulation 853/2004 (9). The bacterial species *Listeria monocytogenes spp., Salmonella spp., Escherichia coli*, fungi have not been identified in the samples studied. From the 120 samples of raw buffalo milk, the

Table 1

Results of the microbiological analysis of raw buffalo milk

Season	TNG	Staphylococcus spp. %	Salmonella spp. %	Escherichia coli spp. %	Bacillus Lichiniformis spp. %	Listeria monocytogenes spp. %	Trueperella pyogenes spp. %
Autumn n=30	$1.1x10^4$	2.8±0.42	-	-	-	-	1.43±0.26
Winter n=30	$3.4x10^4$	3.1±0.64	-	-	4.6±0.32	-	-
Spring n=30	$2.3x10^4$	-	-	-	-	-	-
Summer n=30	$1.6x10^4$	-	-	-	-	-	-
Total average value	$2.1x10^4$	2.95±0.53	-	-	4.6±0.32	-	1.43±0.26

following bacterial species were identified: *Bacillus licheniformis,*mesophilic aerobic germs*, Staphylococcus spp., Streptococcus spp*. and *Trueperella pyogenes.*

The cultural examination of the seeded samples on the Palcam medium reveals the presence of mixed micro flora represented by *Bacillus licheniformis* (Fig. 1.a.b.) which is presented in the form of rippled films and *Staphylococcus spp.* Middle sized to big colonies are flattened, mid-sized ones are bulging, creamy, glossy, and small ones have punctuated aspect.

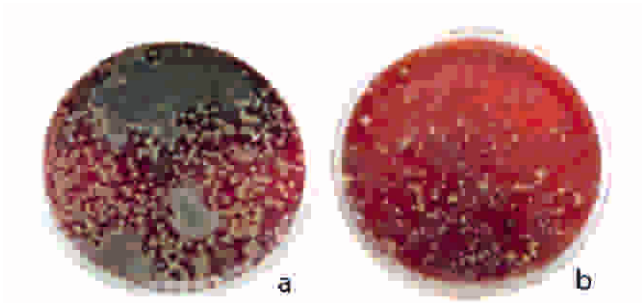


Fig. 1.a.b. *Bacillus licheniformis* spp. on Palcam medium

In the cultural exam of mesophilic aerobic germs seeded on agar with added glucose, the presence of isolated, opaque, middle-sized colonies was remarked (Fig. 2. a.b.).

For the identification of the *Staphylococcus* type germs, The Chapman solid medium was used, and in order to identify the type of colony, the sample was replanted, after confirming the presence of two types of colonies, yellow (Manito-positive) and red (Manito-negative) (Fig. 3. a.b.).

Trueperella pyogenes is a gram-positive, cocobacillary, motionless germ, which develops under aerobic conditions and partially under anaerobic conditions.

After 24:00 h – 48:00 h after incubation, they develop and form hemolytic colonies on agar supplemented with 5% sheep erythrocytes. The clear microbial identification of this germ is based on biochemical tests (11). *Trueperella pyogenes* was identified in the microscopic exam based on morphological characters.

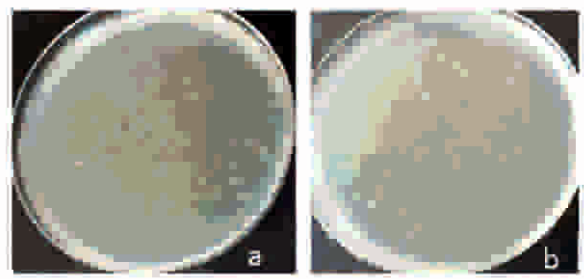


Fig. 2.a.b. Mesophilic aerobic germs on agar for TNAMG

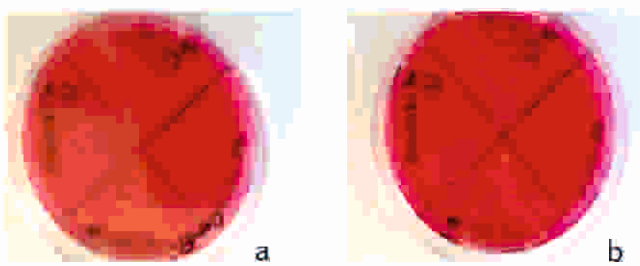


Fig. 3. a.b. *Staphylococcus spp*. cultures on Chapman medium

On the smear bacterioscopic exam, from the colonies developed on the Palcam medium stained through the Gram method, we found to be germs from the following bacterial genes: *Bacillus*, big Gram-positive bacilli, identified from the rippled film of the culture medium, which according to the morphological aspect and cultural, belong to the *Bacillus licheniformis* species

(Fig. 4.a.b.); *Staphylococcus* (Fig. 5.a.b.), identified from the flattened yellow colony, middle-sized.

Micrococcus identified from bulging, creamy, medium-sized, creamy-pink color colonies and Gram-positive coccobacillus from the *Trueperella* genus in small spotted colonies. They are unequally calibrated, isolated, grouped diplo as a V-shaped letter (Fig. 6.a) or in irregular piles with Chinese letters, aspect (Fig. 6.b).

Off some culture media from the mixed culture, there were identified both *Bacillus licheniformis* and *Staphylococcus* spp. (Fig. 7). In the smears of cultures, the *Trueperella* germs are in the form of fine and thin bacilli, 0.2-2 μm or 0.5-0.7 μm, sometimes in old cultures, they may have a cocoid look.

These germs are unsorporated, unconfined, without cilia (18), Gram -positive in young and Gram-labiled cultures in older cultures. They may be isolated, in the form of diplo groups, having the form of letters V, Y or T, or palisade form (30).

Bacillus licheniformis and *Bacillus cereus* are pathogenic species from the *Bacillaceae* family, frequently found in raw milk and in all stages of dairy processing (31). These bacteria are present everywhere: in soil, animal feed, barn garbage and unprocessed food (7, 16), and their presence depends on the geographical and areas or season, most commonly encountered during winter (3, 7, 21). Because most strains of *Bacillus cereus* and *Bacillus licheniformis* are isolated from food, especially milk, they have been proven to have pathogenic properties, and they may establish a danger for health (8, 20, 25). These microorganisms form spores that can survive the pasteurization and UHT temperatures and in this way they can be found in the finite dairy products (42).

Bacillus licheniformis develops on special medium crops, forming opaque, characteristic colonies. Microbial identification is confirmed through specific biochemical tests. This germ, gram-positive, endosporous producer, can occasionally cause abortion in cattle or other infections (7).

In association with other microorganisms, *Bacillus licheniformis* can also affect other species such as: pigs, sheep, and to humans can cause food poisoning and septicemia (11). Since the first reports of the presence of this germ in buffalo herds in Campania, Italy, *Bacillus licheniformis* has been isolated quite frequently to buffalo in Romania (5).

In the buffalo herds where the presence of this germ has been identified, a considerable number of infections are diagnosed, which shows that the buffalo is one of the most sensitive species to the action of this germ. These bacilli can cause abortion to buffaloes, especially in the winter months, during the last period of gestation. Generally, the infection is contracted by eating food that has been contaminated by the bacteria or its spores. Being an omnipresent organism, *Bacillus licheniformis* has a diagnostic significance only if it is isolated in pure crops from fetal tissues. The diagnosis is based on the bacteriological examination of the organs (11).

Trueperella pyogenes is commonly found in the oropharyngeal mucosa of ruminants where they can cause purulent lesions, pneumonia and abortion. The infection can spread through the hematogenic descending pathway or the vaginal ascendant pathway.

Abortion caused by *Trueperella pyogenes* is extremely common in buffaloes, although abortigen epidemic have not been reported to this species.

Abortion generally occurs in the final stage of gestation and can be followed by placental, endometriotic or metric retention. To buffaloes, this microorganism can cause mastitis, omphalitis, pneumonia and septicemia. The diagnosis is based on the bacteriological examination of the organs (11).

Counting somatic cells.

The number of somatic cells is an internationally recognized parameter for assessing the milk quality and the udder health (2).

Mahendra and Dang (2001) showed that a low somatic cell number from raw milk of $1x10^5$ cells/ml indicates a healthy udder, while a somatic cell number of 2.5×10^5 cells/ml indicates the presence of an infection of the udder. The number of somatic cells from raw buffalo milk was classified into three classes: low (<100,000); moderately (between 100,000- 250,000) and increased (>250,000). A smaller number than 250.000 cells / ml of milk was considered insignificant for the presence of mastitis.

The value of $223.46 \times 10^3 \pm 26.522$ cells/ml is considered the average somatic cell value in buffalo milk (19). Another indicator of milk quality and plant cleanliness is represented by bacterial flora, also called "refreshing mesophilic aerobic flora" (13).

The higher values of the somatic cell number in milk highlight the need to improve the management of practices to reduce microorganism contamination and the judicious use of antibiotics in lactating animals (38).

From the 120 samples of buffalo raw milk studied, the number of somatic cells had the average value of $1026.5x10^3$, the lowest value was $9x10^3$ cells / ml (Fig. 8, Histogram 1) and the highest was $2044x10^3$ cells/ml (Fig. 9, Histogram 2).

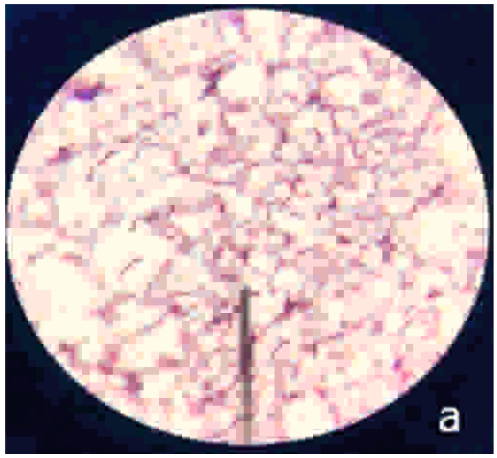


Fig. 4.a. Big-sized Gram positive bacilli *Bacillus licheniformis* spp. from Palcam medium (col. Gram; ob.100x)

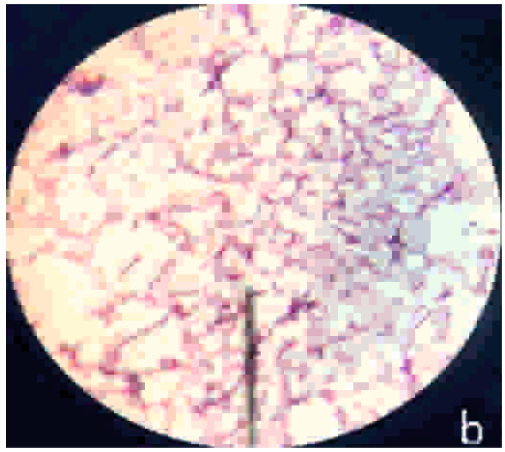


Fig. 4.b. Big-sized Gram positive bacilli *Bacillus licheniformis* spp. from Palcam medium (col. Gram; ob.100x)

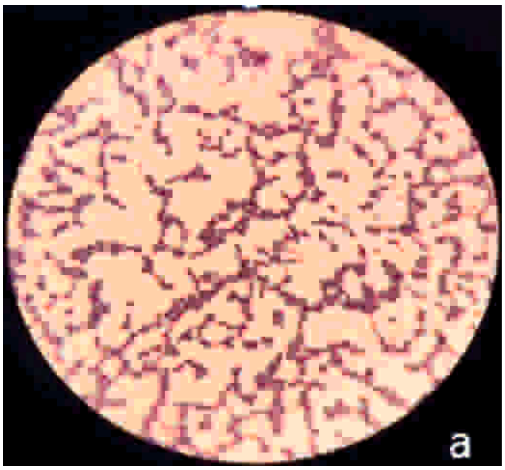


Fig. 5.a. Gram positive cocci characteristic grouped in a bunch of grapes form, *Staphylococcus spp*. Genus on agar TNAMG (col. Gram; ob.100x)

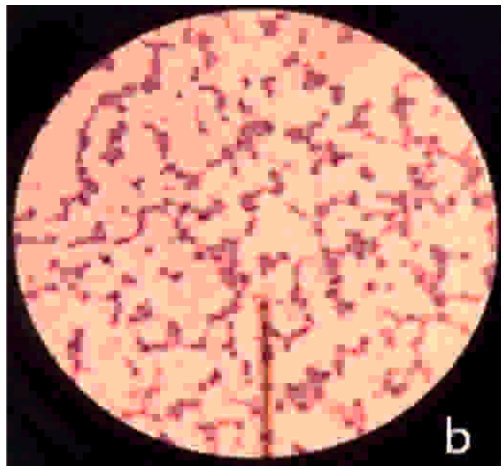


Fig. 5.b. Gram positive cocci characteristic diplo grouped or in the form of chains, *Staphylococcus spp*. Genus on agar TNAMG (col. Gram; ob.100x)

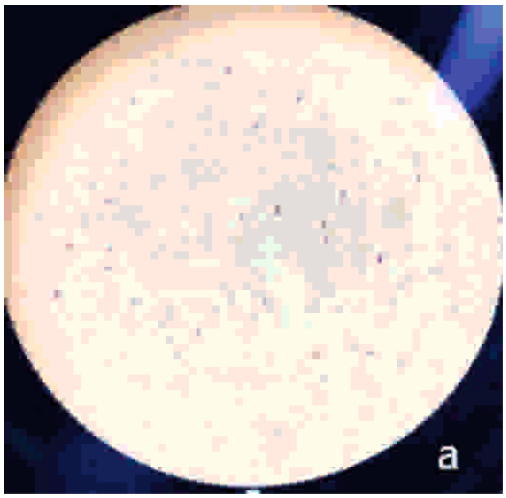


Fig. 6.a. 48:00 h on agar glucose culture (unequally calibrated, isolated and diplo V-shaped letter coccobacillus Gram positive) (col. Gram; ob.100x)

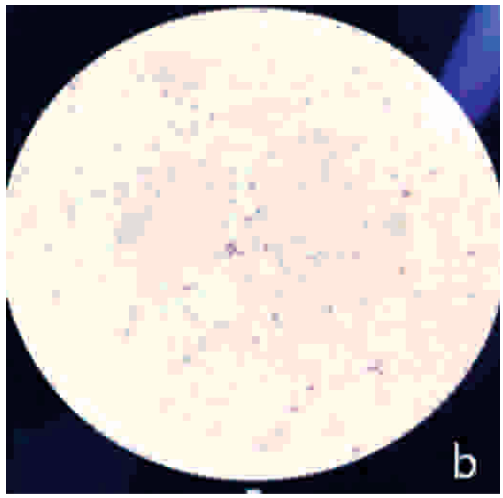


Fig. 6.b. 48:00 h on agar glucose culture (unequally calibrated, isolated and diplo irregular piles coccobacillus Gram positive) (col. Gram; ob.100x)

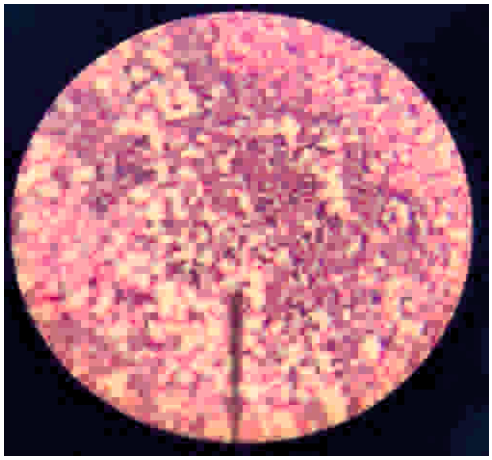


Fig. 7. *Bacillus licheniformis spp*. and *Staphylococcus spp*. from mixed culture (col. Gram; ob.100x)

CONCLUSIONS

Multiple pathogenic microorganisms such as *Bacillus licheniformis, Staphylococcus spp., Streptococcus spp.* and *Trueperella pyogenes spp.* have been identified and isolated in the milk samples that had been studied. The chemical composition of the buffalo milk provides information to the consumer about the milk hygiene and the need for nutrients as well as opportunities for the local industry development.

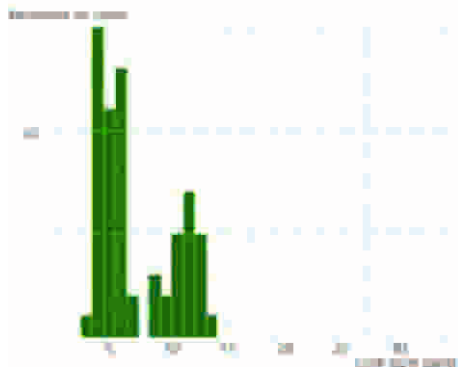
In addition, the presence of pathogenic microorganisms in raw milk and products obtained from inappropriately treated milk is a marker of quality and a threat to consumer health.

An important role in food processing is the good quality of the raw material used. The quality of the finished product cannot be obtained from a raw material of improper quality, which does not meet the hygiene standards.

Microorganisms can cause milk degradation by changing its organoleptic properties, which causes it to change its quality. Many studies have reported some epidemiological disorders caused by dirty hands of workers in milk production, use of dirty equipment, insects, and dirty water sources. The quality of the microbiological parameters of raw milk and dairy products plays an important role in quality control.

It is necessary to minimize technological and economic losses in the process of milk processing and to obtain a longer shelf life. Therefore, the study confirmed that raw milk might present a health risk to consumers, especially if the producers do not respect the recommended rules on transport, handling, storage, and hygiene in farms.

Fig. 8. Lowest somatic cell value (9x10³ cells/ml)



Histogram 1. (9x10³ cells / ml)

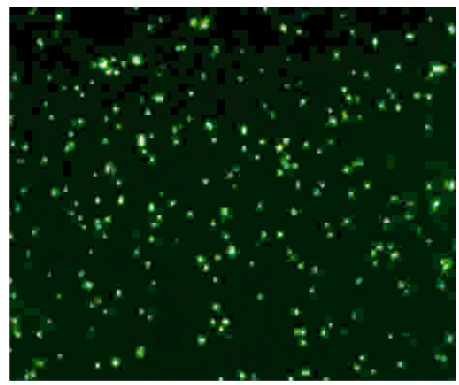
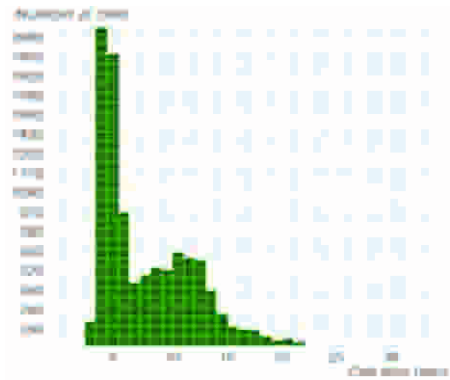


Fig. 9. Highest somatic cell value (2044x10³ cells/ml)



Histogram 2. (2044x10³ cells/ml)

The average somatic cell value of raw buffalo milk from the samples studied show that 1026.5 x 10³.

Conflicts of interest:

The authors declare that they have no conflict of interest regarding this publication.

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