SUBCLINICAL MASTITIS AMONG LACTATING BUFFALOES IN THE MIDDLE OF NILE DELTA OF EGYPT

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ABSTRACT

The incidence of subclinical mastitis among 175 lactating buffaloes was studied in three governmental buffalo farms located in the middle of Nile delta of Egypt. Individual quarter's milk samples (699) was randomly collected and investigated for CMT, SCC, conductivity, chemical composition and mastitis causing microorganisms. The CMT positive quarters represented 21.6% of the total investigated quarters however; the front quarters were less susceptible (44.2%) to mastitis than the rear ones (55.8%). The mastitic milk resulted in a dramatic increase of SCC (19.3 x 10^5) compared to the normal one (1.0 x 10^5) with higher SCC in the left front and rear udder quarters (23.9 and 18.3%) than the right one (7.8 and 13.1%), respectively. Compared to the normal milk, the mastitic milk had significantly higher concentrations of sodium and lower concentrations of lactose with no significant differences in milk yield and pH. The mastitic milk tended to have lower solids nonfat, potassium, calcium and ferrous and higher content of magnesium and zinc than the normal milk. The microbiological analysis of the mastitic milk samples showed the incidence of *Corynbacteria* (29.2 %), *S. dysgalactiae* (24.7%), coliforms (12.0%), *Staph. aureus* (10.3%), *P. aeruginosa* (7.4%), *B. cereus* (6.7%), *S. agalactiae* (6.4 %), *S.. uberis* (2.8 %), Mycoplasma (0.3 %) and yeast and mould (0.2 %). The contagious pathogens were the prominent microorganisms in the mastitic milk samples (71.6%) however, the environmental pathogens represented 28.4% of the total identified microorganisms.

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INTRODUCTION

Mastitis, or inflammation of the mammary gland, is the most common and the costly disease of dairy farm animals in many countries. Although stress and physical injuries may cause inflammation of the gland, infection by invading bacteria or other microorganisms is the primary cause of mastitis. On the basis of clinical data, subclinical mastitis was defined as a form of the disease with no detectable change in the udder and no observable abnormalities in the milk. The impact of mastitis goes with the milk beyond the gate of the farm. It causes changes in milk composition i.e. reduction in calcium, phosphorus, protein and fat, and increases in sodium and chlorine leading to reduce milk quality. In addition, the antibiotic used in treating mastitis is an important industrial and public health concern.

The buffalo has been traditionally considered less susceptible to mastitis than cattle (Wanasinghe, 1985). Only few reports were conducted to describe the subclinical mastitis in buffaloes (Zaitoun, 2000, Varshney and Naresh 2004; Dhakal 2006). Compared to cows, buffaloes have a powerful defense against mastitis due to the anatomy of the teat. However, some researchers observed similar mastitis frequencies for the 2 species (Kalra and Dhanda, 1964; Badran, 1985; Bansal *et al.*, 1995).

Bacteria that frequently cause mastitis can be divided into two main groups based on the source of the bacteria: contagious pathogens and environmental pathogens. The primary contagious pathogens are *Corynbacteria*, *S. agalactiae*, *Staph. aureus*, *S. dysgalactiae* and Mycoplasma species (Harmon & Langlois, 1986, Eberhart *et al.*, 1987, Fox & Gay, 1993). Primary environmental pathogens include coliform bacteria, *B. cereus* and *P. aeruginosa* and species of streptococci other than *S. agalactiae*, (Schiefer, *et al.*, 1976, Smith, *et al.*, 1985, Erskine, *et al.*, 1987, El-Khodery and Osman, 2008). The sources of contagious mastitis are infected udders during the milking process through contaminated teat cup liners, milker's hands and paper or cloth towels used to wash or dry more than one udder. Environmental mastitis bacteria are defined as those usually transferred from the environment to the cow rather than from other infected quarters. The source of environmental pathogens, however, is the surroundings in which a cow lives (Blowey and Edmondson, 1995). Therefore, methods of control

developed for the contagious pathogens are not effective against environmental pathogens.

The type of bacteria most frequently isolated in milk samples of buffaloes, with mastitis in previous studies, has been coagulase negative staphylococci (CNS) followed by *corynebacterium* spp. and *streptococcus* spp. (Chander and Baxi, 1975; Paranjabe and Das, 1986; Saini *et al.*, 1994; Costa *et al.*, 1997 and Naiknaware *et al.*, 1998). Also, *Staph. aureus* was the most important microorganism responsible for mastitis in buffaloes in another study (Jaffery and Rizvi, 1975). In the bovine, somatic cell count (SCC) is usually used as an indicator to diagnose mastitis. The SCC also has been used for mastitis diagnosis in buffaloes. According to Dhakal (2006), Singh and Ludri (2001) and Caraviello (2004) it seems probable that a SCC >200,000/mL is an indicative value of udder infection. The results obtained by Cerón-Muñoz *et al.*, (2002) showed that elevated SCC has a negative effect on milk and lactose yield in buffaloes. The aim of the present study is to investigate the incidence of mastitis in three farms of lactating buffaloes and its impact on milk composition. The environmental and contagious mastitis causing pathogens were also identified.

MATERIALS AND METHODS

Farm management and milk sampling

The present work was done on 699 individual milk samples collected from 175 lactating buffaloes. The animals were randomly selected from 3 governmental farms (F1, F2 and F3) located in the middle of Nile delta of Egypt. As a common practice in the studied farms, the milking animals were kept restrained under sheds from the morning (7.00 a.m.) till the evening (4.00 p.m.) thereafter they were kept loose in the shed during the rest of the day. Buffaloes were milked by hand twice daily at 7.00 a.m. and 4.00 p.m. throughout the lactation period, and milk production was recorded. The experimental animals were fed in feeding groups according to their milk production. The animals were fed on concentrate feed mixture (CFM) along with Egyptian clover hay (*Trifolium alexandrinum*) and wheat straw or rice straw. The CFM was given twice daily before milking at 8 a.m. and 4 p.m., wheat straw was offered once daily after milking, whereas clover hay was offered at

11 p.m. Animals were allowed to drink water three times a day and multi mineral licking blocks were available for animals in the stalls.

Prior to milking, the udder and teat ends were scrubbed with 70% ethanol. About 30 ml of milk, 15 ml foremilk and 15 ml of stripping, was taken under aseptic conditions from each quarter into sterile tube for microbiological and chemical analysis. All samples were kept in an ice box (at about 6°C) and quickly transported to the laboratory for analysis.

Physical and chemical parameters

Individual milk samples were screened by CMT test according to the APHA, (1993) using CMT reagent (Dairy Research Products, INC., Spencerville, USA). Buffaloes were considered positive if one or more of their quarters scored two or three. Milk SCC were determined by the fluoro-opto-electronic method using Fossomatic 5000; Foss Electric apparatus, 3400 Hillerod, Denmark. The samples were analyzed after warming up at 40°C in a water bath for 15 min. Basically, the Fossomatic counter contains ethidium bromide dye that penetrates the cell and forms a fluorescent complex with the nuclear (DNA). Each cell produces electrical pulse, which is amplified and recorded as number of cell ($x10^3$)/ml milk. The pH value of milk was measured using pH-206 (Lutron co. LTD. USA). The electrical conductivity (EC) of milk samples was measured using a hand-held electronic mastitis detector instrument according to the manufactures instruction (C.T.A.S.R.I. Costruzioni tecnologiche applicate. Via Ugolani Dati, 16, 2604 Pugnolo di Cella Dati (CR), Italy).

Concentration of milk fat, protein, lactose and total solids (TS) was determined by infra-red spectroscopy using Milko-Scan 133B N (Foss Electric, Denmark). Five ml of milk samples were used for mineral determination. Samples were placed in a heat-resistant glass digestion tubes (25x240 mm, calibrated to 50 and 100 ml, Spektrum 3D, Hungary). To each milk sample, 10 ml of 65% nitric acid (Scharlau Chemie, Spain) were added and heated at 60°C for 30 min. in a dry block (Labor MIM OE 718/A, Hungary). Three ml of 30% hydrogen peroxide (Merck, Germany) was added and digestion was continued at 120 °C for 90 min and then cooled. Samples were diluted with Milli-Q water (18 $M\Omega cm^2$ Milipore Co.,

USA) up to 50 ml and filtered using MN 619 G1/4 filter (Macherey-Nagel, Germany). Mineral content of the digested samples was determined by inductively coupled plasma optical emission spectrophotometer (ICP-OES) (Perkin Elmer Ltd., Optima 3300 DV, USA) as described previously (Prokisch, *et. al.*, 2006).

Microbiological analysis

Selective and differential media for the enumeration of mastitis pathogens microorganisms were obtained from Oxiod, Hampshire, England. The microbiological analysis was performed according to standard methods for examination of dairy products as described by Collins and Patricia, (1976) and APHA, (1993).

The presumptive coliform bacterial count was counted using MacConkey agar media. Streptococcci groups causing mastitis (*agalactiae*, *dysglactiae* and *uberis*) were enumerated on blood-added-modified Edward's media for identification of blood hemolytic bacteria. *Staph. aureus* was counted on Barid-Parker agar media with Egg yolk Tellurite emulsion (SR54). *Bacillus cereus* (*B. cereus*) was enumerated in Polymixin pyruvate egg yolk mannitol bromothymol blue agar media added with Oxoid *B. cereus* supplement (SR99) and egg yolk emulsion. *Pseudomonase aeruginase* (*P. aeruginase*) was enumerated on Pseudomonas agar base media with Pseudomonas C-N- selective supplement (SR102). Mycoplasma species were counted on Mycoplasma agar base media fortified with Mycoplasma selective supplement–G (SR59C). *Corynebacterium* species were enumerated on blood agar media (Nutrient agar plus sheep's blood) with potassium tellurite. Yeast and mould were enumerated on potato dextrose agar media.

Statistical analysis

Data are expressed as the mean, mean and range in parenthesis or mean ± SE. Statistical differences were determined by student's t test according to Fisher (1970) and Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The California mastitis test (CMT) is widely utilized to determine the mastitis disease status of lactating animals, as it is simple, inexpensive and rapid screening test. Observed frequencies

of milk samples from apparently unaffected buffaloes, which were negative or positive for mastitis using the CMT are displayed in Table 1. Out of 699 inspected quarters, the infected quarters were 151 which represent 21.6% of the total quarters. Among the investigated 3 farms, one farm (F1) had almost twice infection cases (34.9%) compared to the others (15.2% and 14.4%) which could be traced to poor farm management and hygienic control. Our data also showed that, the front quarters were less suspected (44.2%) to mastitis than the rear quarters (55.8%).

Table (2) shows a comparison between normal and mastitic milk samples collected from the investigated farms. The basic patterns of milk were similar whether buffaloes were healthy or mastitic (SCC >3 x 10^5 cells/ml milk).

Table (1):Incidence of sub-clinical mastitis in individual buffalo quarters from different farms as detected by CMT* test

	F1 (58)[a]		F2 (89)[b]		F3 (28)[c]		Total (175)	
Quarters	232		356		111		699	
	No.	%	No.	%	No.	%	No.	%
Negative	151	65.1	302	84.8	95	85.6	548	78.4
Positive	81	34.9	54	15.2	16	14.4	151	21.6

CMT, California Mastitis Test

[a, b, c] numbers in parenthesis are numbers of the investigated animals.

Milk from mastitic animals had significantly higher concentration of sodium and lower concentration of lactose. No significant differences were found in milk yield and pH however, the mastitic milk tended to have lower solids nonfat, potassium, calcium and ferrous content and higher, magnesium and zinc compared to the normal milk. Electrical conductivity (EC) of milk is determined by sodium, potassium, calcium, magnesium, chlorine, and other ions (Barth and Worstorff, 2000 and Špakauskas *et al.* 2006). The mastitic milk resulted in higher conductivity compared to the normal one although the difference was not significant.

In the case of subclinical mastitis, pathogens don't cause enough disruption in the alveolar tissue to be noticed in the milk, but infection can be detected by an increase in the somatic cell count (SCC) per ml of milk (Caraviello, 2004). Therefore, the

determination of milk SCC is widely used to monitor udder health and, thus, milk quality. Data in table (2) show a dramatic increase in SCC in the mastitic milk (19.3 x 105) compared to the normal one (1.0 x 105). On the other hand, the SCC among the mastitic buffaloes quarters showed higher SCC for the left front and rear udder quarters (23.9 and 18.3%) than the right quarters (7.8 and 13.1%), respectively (Table 3). These findings may be explained by the position of animals during lying down on the left side which may increase the chance for microorganisms found in bedding or manure to invade open or partially open teat canal (Wattiaux, 2001).

Table (2). Properties of normal and mastitic milk from different farms.

Parameters	Normal milk	Mastitic milk
Sample number	66	157
Milk yield (Kg/day)	7.3 (4 - 12)	7.3 (4.5 - 13)
Somatic cell count x10^5	1.04^a (0.09 – 2.78)	19.3^b (3.08 - 148.3)
Conductivity (unit)	298 (120 - 560)	347 (190 - 770)
pH	6.8 (6.4 – 7.4)	6.9 (6.6 – 9.9)
Total solids (%)	15.0 (9.0- 18.8)	14.9 (10.9 – 21.9)
Solids nonfat (%)	9:63 (7.6 – 10.7)	9.17 (7.2 – 10.8)
Fat (%)	5.7 (4.0 – 9.2)	6.2 (3.7 – 13.7)
Total protein (%)	4.0 (2.3 – 5.1)	4.0 (3.1 – 5.4)
Lactose (%)	4.8^a (3.4 – 5.6)	4.4^b (2.8 – 5.6)
Minerals (mg/ kg)		
Calcium (Ca)	1578 (624 – 2581)	1543 (872 – 2303)
Potassium (K)	894 (312 – 1318)	863 (459 – 1167)
Magnesium (Mg)	141 (79 – 225)	148 (47 – 230)
Sodium (Na)	454^a (187 – 966)	527^b (210 – 1157)
Phosphorus (P)	1110 (403 – 1861)	1060 (503 – 1715)
Copper (Cu)	0.22 (0.04 – 0.97)	0.21 (0.06 - 0.95)
Ferrous (Fe)	2.00 (0.25 – 9.10)	1.48 (0.25 – 3.10)
Zinc (Z)	3.75 (3.00 – 9.36)	5.12 (2.96 – 7.19)

Data are mean and range (in parentheses). Means with unlike superscript letters are significantly different ($P<0.05$).

Table (3). Somatic cell count (SCC) among quarters of mastitic buffalo's udders.

Milk samples	(SCC X10^5)
Whole udder	19.3 (3.08 - 148.3)
Front quarters	
Right	7.81 (3.43 – 15.82)
Left	23.9 (3.08 – 148.3)
Rear quarters	
Right	13.1 (3.31 – 67.74)
Left	18.3 (3.34 – 115.8)

Data are mean and range (in parentheses) of 157 animals.

Data in Fig. (1) show the count of the mastitis causing pathogens isolated from the mastitic buffalo's milk samples expressed as log cfu/ml milk. The relative incidence of the pathogens in descending order was *Corynbacteria* (29.2 %), *S. dysgalactiae* (24.7%), coliforms (12.0%), *Staph. aureus* (10.3%), *P. aeruginosa* (7.4%), *B. cereus* (6.7%), *Strptococcus agalactiae* (*S. agalactiae*) (6.4 %), *Strptococcus uberis* (*S. uberis*) (2.8 %), Mycoplasma (0.3 %) and yeast and mould (0.2 %). In recent study, the bacteriological examination of 80 quarter milk samples obtained from 56 buffaloes with acute mastitis collected from north Nile delta of Egypt revealed that, coliform bacteria was the most common pathogen (45 cases) followed by *Staph. aureus* (7 cases) then *S. uberis* (3 cases), and *S. agalactiae* (1 case) (El-Khodery and Osman, 2008).

In attempting to control different types of mastitis causing pathogens, it is important to consider the source and means of transmission of the pathogen. The origin and count of the pathogens causing mastitis in the investigated farms are illustrated in Fig. (2). The contagious pathogens were the prominent microorganisms in the mastitic milk samples (71.6%) however the environmental pathogens represented 28.4% of the total identified microorganisms. The occurrence of the contagious pathogens in descending order was *Corynbacteria* (43.7 %), *S. dysgalactiae* (34.8 %), *Staph. aureus* (13.4 %), *S. agalactiae* (7.4 %) and Mycoplasma species (0.7 %). On the other hand, the environmental pathogens were

coliform bacteria (37.1 %), *B. cereus* (27.8 %), *P. aeruginosa* (27.3 %), *S. uberis* (7.1 %) and yeast and mould (0.7 %).

Figure (3) show the occurrence (log cfu/ml milk) and origin of the pathogens in the individual farms. There were significant differences among the 3 farms on the incidence of the identified pathogens however; the contagious pathogens were the predominant microorganisms in all farms compared to the environmental derived one. On the other hand, the animal udders of farm 3 (F3) resulted in higher incidence of the contagious pathogens and lower content of the environmental one (80.7% and 19.3%) than the other 2 farms. The figures were (66.7% and 33.3) and (54.0% and 46%) for farm 1 (F1) and farm 2 (F2), respectively. The primary source of environmental pathogens is the surroundings in which an animal lives. The sources of contagious mastitis, however, are infected animals and transmission is from animal to animal. However, General cleanliness of animals and their housing, as well as good management procedures are effective ways of controlling the spread of mastitis.

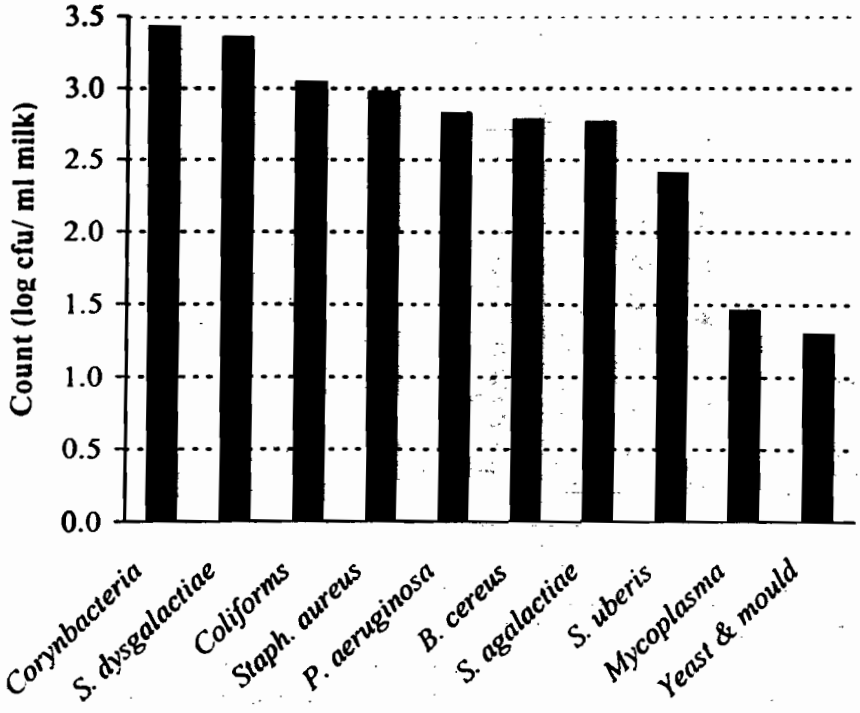

Fig. 1. Incidence of mastitis pathogens in mastitic buffalo's milk from different farms. (Data are means for 151 samples).

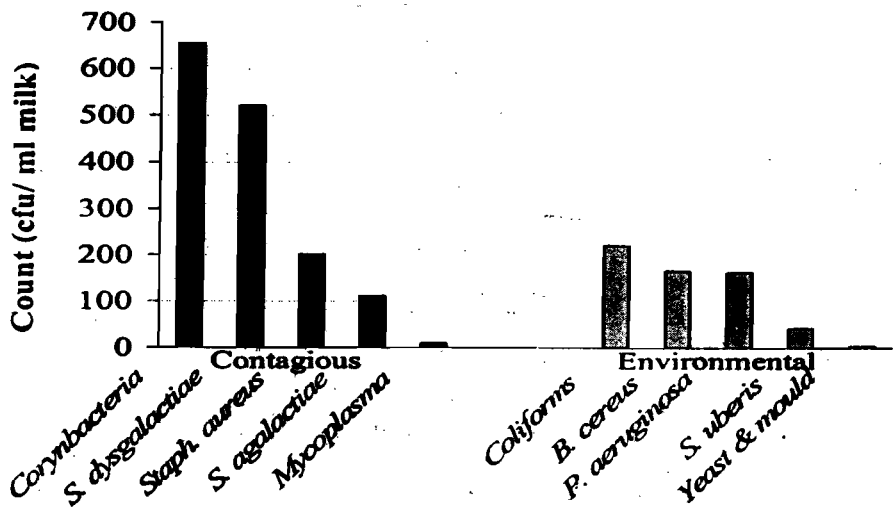

Fig. 2. Contagious and environmental pathogens count in mastitic buffalo's milk from different farms. (Data are means for 151 samples).

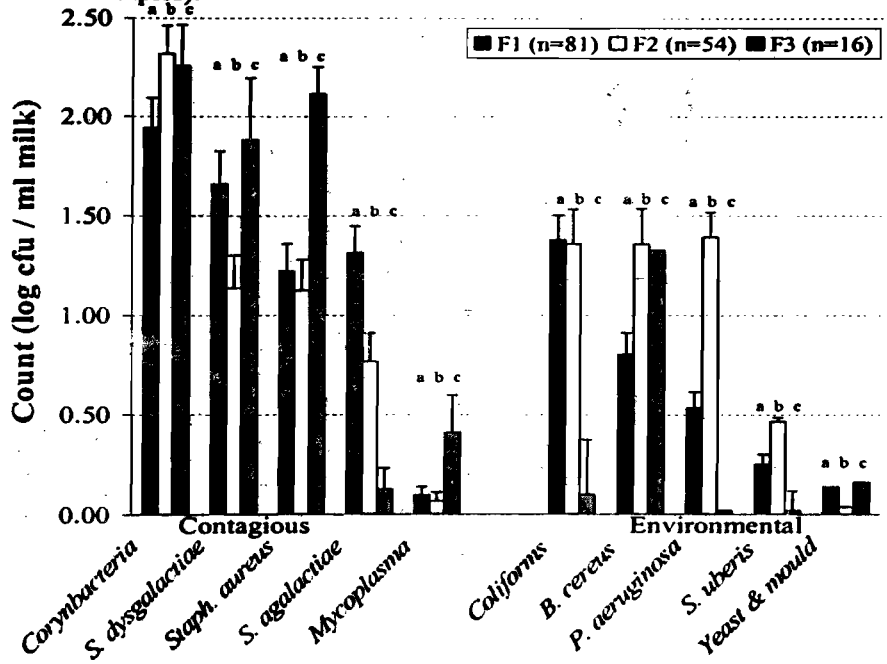

Fig. 3. Incidence of contagious and environmental pathogens in mastitic buffalo's milk samples from individual farms. (Data are means ± SE for 151 samples. ^{abc}Means with unlike superscript letters are significantly different ($P<0.05$).)

On this respect, the obtained results may give an indication to the hygienic and husbandry practice adopted in the investigated farms. In conclusion, the study shows the importance of SCC determination as a fast indication for the incidence of subclinical mastitis. It strongly emphasizes the importance of the presence of an active hygienic system in the lactating buffalo's farms to prevent animals from buses mastitis as the impact of mastitis goes with the milk beyond the gate of the farm to the milk factories and consumers as well.

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الملخص العربى
التهاب الضرع بين الجاموس الحلاب فى وسط الدلتا بمصر

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تم دراسة وجود مرض التهاب الضرع فى ١٧٥ حيوان من الجاموس الحلاب فى ٣ مزارع حكومية بوسط الدلتا بمصر. تم تجميع ٦٩٩ عينة لبن عشوائية واختبارها بواسطة اختبار كاليفورنيا لالتهاب الضرع، عد الخلايا الجسدية، التوصيل الكهربائى، التركيب الكيماوى وكذلك الفحص الميكروبيولوجى للميكروبات المسببة للمرض. أوضحت نتائج اختبار كاليفورنيا لالتهاب الضرع أن ٢١,٦% من ارباع ضروع الحيوانات التى تم فحصها كانت إيجابية لهذا الاختبار، وأن الأرباع الأمامية كانت أقل إصابة (٤٤,٢%) من الأرباع الخلفية (٥٥,٨%). أدى التهاب الضرع لحدوث زيادة كبيرة فى عدد الخلايا الجسدية فى لبن الحيوانات المصابة (١٩,٣ x ١٠^{5}) مقارنة بالحيوانات السليمة (١ x ١٠^{5})، مع تزايد الأعداد فى الأرباع اليسرى الأمامية والخلفية (٢٣,٩، ١٨,٣%) عن التى فى الجانب الأيمن من الضرع (٧,٨، ١٣,١%) على التوالى. ومقارنة باللبن الطبيعى فإن لبن الحيوانات المصابة بالتهاب الضرع احتوت على تركيزات أعلى من الصوديوم والكلاكتوز مع عدم وجود تغير ملحوظ فى انتاج اللبن وحموضته (رقم الأس الهيدروجينى). وقد لوحظ أيضا وجود انخفاض فى الجوامد الصلبة اللادهنية والبوتاسيوم والكالسيوم والحديد وزيادة فى الماغنسيوم والزنك رغم أن هذه التغيرات لم تكن معنوية.
أوضحت التحليلات الميكروبيولوجية لعينات لبن الحيوانات المصابة بتواجد انواع الـ *Corynbacteria* (٢٩,٢%)، *S. dysgalactiae* (٢٤,٧%)، coliforms (١٢%)، *Staph. aureus* (١٠,٣%)، *P. aeruginosa* (٧,٤%)، *B. cereus* (٦,٧%)، *S. agalactiae* (٦,٤%)، *S. uberis* (٢,٨%)، الميكوبلازما (٠,٣%)، الفطريات والخمائر (٠,٢%). وقد وجد أن الميكروبات المرضية المعدية تمثل النسبة الأكبر من الميكروبات المسببة لألتهاب الضرع (٧١,٦%) عن الميكروبات المرضية التى توجد فى البيئة المحيطة بالحيوانات (٢٤,٨%).