

ACUTE BUFFALO MASTITIS CAUSE BY MIXED INFECTION OF *Enterobacter cloacae* AND *Proteus mirabilis* AT BASRAH, IRAQ

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ABSTRACT

Mastitis caused by *Proteus mirabilis* and *Enterobacter cloacae* has been investigated and diagnosed. Milk samples carried-out from fifteen local buffalo breeds reared in Basrah, Iraq. Ten clinically healthy local buffalo breeds were considered and controls. Diseased buffalo show signs of pain, swelling, redness of the udder, watery consistency with the light red color of the milk, with high systemic reactions including significant increase ($p < 0.05$) in body temperature, respiratory and heart rate. Furthermore, a significant ($p < 0.05$) reduction of ruminal contractions also resulted. Hematological changes of diseased buffalo and the controls, reveals Leucocytosis due to a significant increase of Neutrophils. All diseased animals are clinically examined and the results reveals that *Proteus mirabilis* and *Enterobacter cloacae* are the common causes of mastitis which confirmed by VITEK 2 System. The gram stain from the milk smears show a clear Gram-negative rods, Moreover, swarm forming and pale colony character was also indicated of the swab culture of the causative *Proteus mirabilis*. It has been concluded that acute buffalo mastitis could be of adverse effect, Nevertheless, the knowledge of the causative agents of mastitis are very useful for the fast treatment of the disease.

INTRODUCTION

Mastitis is believed to be an important big problem in dairy animals life . The disease reflect an inflammation of mammary gland mostly caused by bacterial organisms which contaminated and got entrance thorough teat orifice and multiply (1). Several infectious agents,

including those caused by microorganism such as (*Coli* form bacteria, *Brevibacterium erythrogenes*, *leptospira* spp, *Micrococcus cerasinus*, *Serratia marcescens*, *Micrococcus hromidrogenes*, *Micrococcus roseus*, *Sarcina rubra* *Lactorubefaciens gruber*, and so on .) could be the causative agents of mastitis (2). Although, some viruses and some species of yeast such as red yeast (*Monascus purpureus*) has been cause the disease in the same matter (3). Most of the above microorganism are present in the animals environment (soil, water, food, bedding, and even the manure). Those causative organisms can create udder inflammation and a clinical disease in which an udder abnormality or secretions is detected and notes , Moreover, according to the severity of clinical manifestations, clinical mastitis can be mild, moderate or severe, Therefore, animals suffer from the mild type of the disease will have abnormal milk contains suck as , flakes , clots with abnormality of the inflamed udder like hot and swollen udder, However, systemic reaction might also detected(1). On the other hand, the acute or severe clinical inflammation will suddenly started accompanied by acute local inflammatory signs, abnormalities of milk contains and sever systemic manifestations, Moreover diseased animal may also show signs of depression, weakness and dehydration (2).

It has been documented that mastitis can have unsatisfactory results and bad consequences, due to buffalo producing milk which had some abnormal coloration such as red or pink due to the presence of red blood cells, As, the animal owners could not afford the economic losses because of the abnormalities of the diseased milk which will always refused by the consumers , in addition that a premature culling of affected animals (4). It has been documented that the most important causative agents of mastitis are *Enterobacter* and *Proteus mirabilis*, Since, they are widely distributed in soil, water and faces(5), However, The general protection could done via good hygienic measure and reducing udder contamination and trauma(3). The aim of the present work was to investigated the special bacterial causes of mastitis in inflamed udders of diseased buffalo at Basrah, Iraq.

MATERIALS AND METHODS

The study area and examination of animals:

Fifteen local buffalo breeds was show clinical signs of mastitis .Animals are of different ages reared in Basrah, Iraq. Ten clinically healthy buffalo breeds was considered as controls. Complete clinical examinations has been applied for all animals. With special privacy

developed for udder examination, Since, The animals were properly restrained, and identified, Moreover, the abnormalities of the diseased udder including, secretions, size, consistency and udder temperature was examines clinically by inspection and palpation, Furthermore, animal reaction such as pain during palpation, changes of milk contains was also taken in consideration as an clinical indications of the mastitis.

Sampling

Blood samples: Blood was take out from jugular vein from each animal mixed with EDTA for complete blood picture using the automatic cell counter from Beckman, (USA).

Milk samples: Diseased milk was collected under aseptic precautions and the clinical data was listed from diseased animals, ten(10) ml of milk was aspirated and applied in sterile plastic vials for further analysis (3).

Staining:Milk smears stained with Gram's stain. Was used for the primary identification of the causative bacteria

Culture media: Suspected samples of milk was cultured in Nutrient broth, then incubated for about 24 h . Furthermore, the sample was transfer to subcultured from nutrient broth medium into blood agar medium, MB and MacConky agar and incubated for about 24 h. In order to obtain pure cultures. Moreover, the colony size, shape, consistency and the color was recorded ,On the other hand, a microscopic examination has been applied for the gram stain smears of the bacterial isolates (6).

Biochemical tests: VITEK 2 systems (Biomerrieux /France) which uses Advanced Colorimetry™ , was used in this study to confirm the diagnosis of the isolated bacteria.

The statistics: The statistical difference between diseased buffalo and the controls was done and The significance of variations was analyzed statistically using student t-test (7).

RESULTS

Diseased local buffalo breeds show different clinical manifestations including the local inflammatory signs which include hotness, redness, swelling and pain of the infected udder (100%), Partial or complete loss of appetite (80%), Lameness and unable to recumbency (53.3%), However, fluid exudation, and crepitating was detected on infected quarter area

(46.6%). Moreover, Infected milk was watery in its consistency with light red color (46%) and grinding of teeth, table No.1.

Table1: Clinical manifestations of Acute buffalo mastitis.

Clinical manifestations	% n=15
Local inflammatory signs which include (hotness, redness, swelling and pain of the udder)	100
Partial or complete loss of appetite	80
Lameness and unable to recumbency	53.3
Fluid exudation, and crepitating	46.6
Watery milk in its consistency with light red color	46.6
Grinding of teeth	33.3

Significant increase ($p<0.05$) has been detected in the vital signs including (animal body temperature , respiratory rates and also the heart rates compared with controls, Furthermore, the contractions rates of the rumen was significantly lowered ($p<0.05$) in diseased buffalo than in controls table No.2.

Table 2: The vital signs and ruminal contractions of diseased buffalo and controls

The parameters	Control buffalo n=10	Diseased buffalo n=15
Animal body temperature C°	39.3± 0.28	41.4 ± 0.7*
Respiratory rate/ min	23.2± 1.82	58.6± 7.2*
Heart rate/ min	65.8±1.72	95.7± 11.83*
Ruminal contractions / 5 min	4± 0.54	2± 32 *

*(Values are mean ± standard error of mean. * (P<0.05).*

Concerning the blood change parameters, results indicated a significant increase ($p < 0.05$) in total leukocyte count which occur due to a significant ($p < 0.05$) Nutrophelia, table No.3.

Table 3:Hematological changes of diseased buffalo and control animals

Hematological values	Control buffalo n=10	Diseased buffalo n=15
TRCx 10⁶	7.92±1.47	7.94±0.33
Hb mg/dL	13.22 ± 1.77	13.4±0.2
PCV %	32.62 ± 3.63	33.94±1.09
TLC x 10³	11.23±1.66	15.74±1.08*
Nutrophiles %	47.22 ± 2.34	53.11± 4.56*
Lymphocytes %	45.56 ± 1.78	41.42± 5.12
Eosinophiles %	3.34 ±0.64	3.54±0.06
Monocytes%	4.46± 0.23	5.61±0.14
Basophiles %	0.56± 0.07	0.32± 5.42

(Values are mean ± standard error of mean. * ($P < 0.05$).

Moreover, Microbiological findings of the cultured media was confirmed by VITEK 2 systems and the results indicated the causative agents which are *Enterobacter cloacae* and *Proteus mirabilis*, figure 1 and 2.

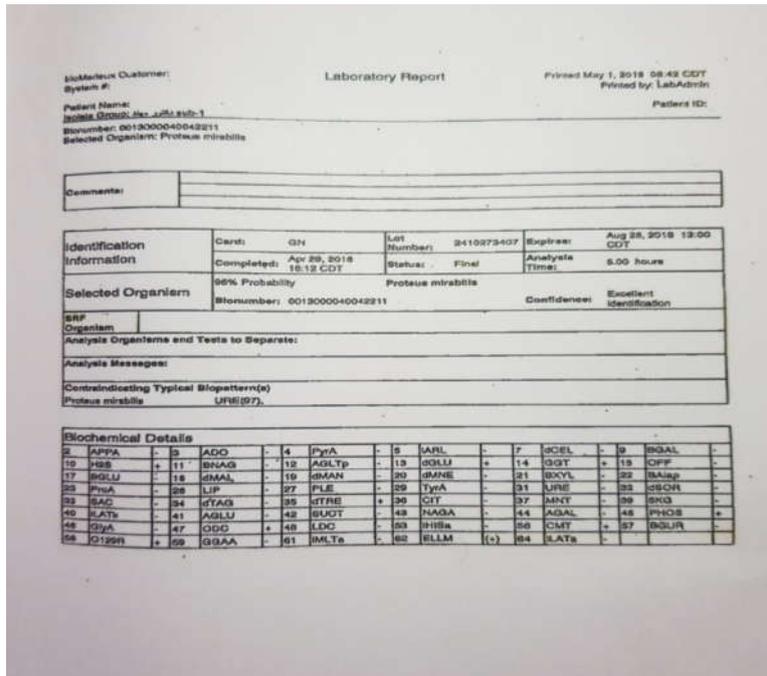


Fig1: VITEK 2 systems for *Proteus mirabilis*

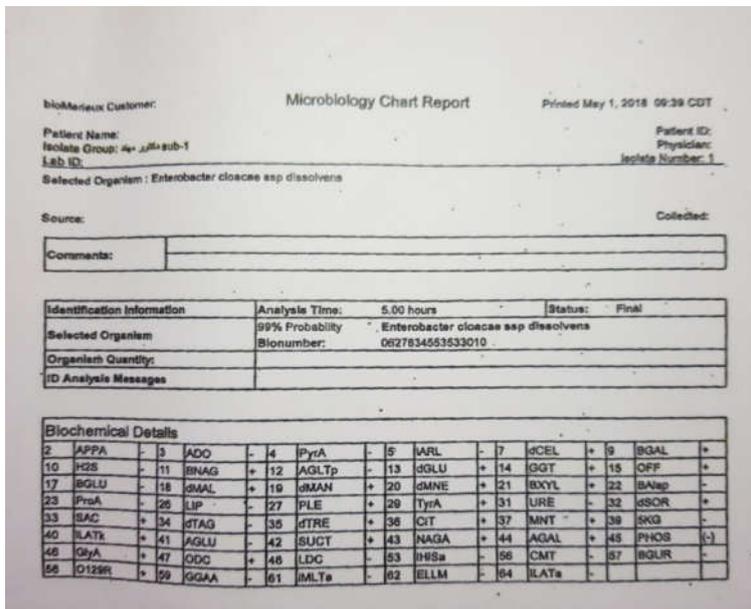


Fig2 : VITEK 2 systems for *Enterobacter cloacae*

Furthermore, the gram stain of the milk smears showed a clear Gram-negative rods figure 3. However, swarm forming and pale colony character was also indicated of the swab culture of the causative *Proteus mirabilis*, figure 4. Moreover, the characteristic feature of cultural media of was also indicated figure 5.

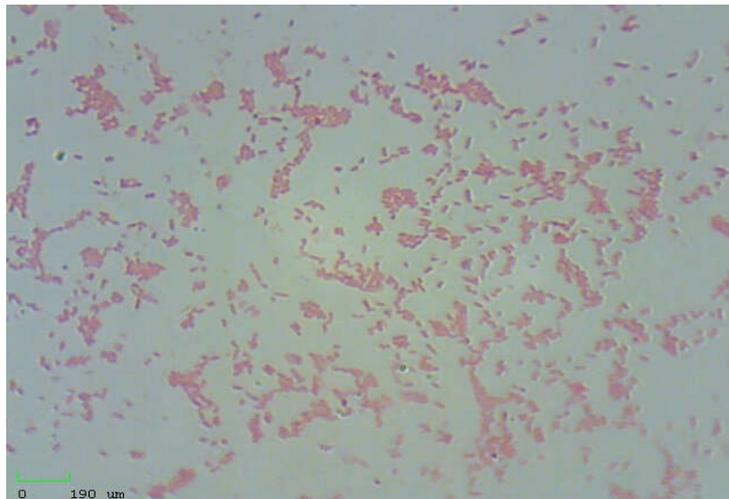


Fig 3: Milk sample :Gram stain from the milk smear show a clear Gram-negative rods



Fig 4: Swarm forming and pale colony character was indicated of the swab culture of the causative *Proteus mirabilis*(blood agar)



Fig 5:The characteristic feature of cultural media of *Enterobacter cloacae* was indicated(blood agar)

DISCUSSION

It has been shown that, the parturition mostly related to udder problems after calving in the large ruminants like cattle and buffalo which appears to be common due the animal physiological conditions around the time of parturition, Since, the lower cisternal storage of secreted milk and milk production might play good roles as a predisposition (8) On the other hand, the anatomy of the animal udder might add another factors such as , the long teat size and the thick streak milk canal which was different in both cattle and buffalo , Moreover, the edema of the udder, the presence of blood in milk and hypogalactia will also occur in cattle and buffalo with same circumstances (9) .

Mastitis, reveres to the inflammation of tissues of udder (the mammary gland) occur due to different types of microorganisms, in both cattle and buffalo , such as *Streptococcus galactiae* and *disagalactia*, *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae*, *Corynebacterium spp.* , and *Mucoplasmas*, However, some viruses and some yeast such as *Monascus purpureus* (the red yeast) might also responsible for local udder infection and also systemic invasion resulting in erythrocyte destructions due to capillary damage leading to discoloration of milk (8,10). Mastitis will affects the quality of the milk , However, that the production of the diseased animals with chemical and physical changes of milk contains, However, the extent to which different alterations will observed and detected (11).

Diseased buffalo exhibited different clinical signs which also mentioned by (1,2,4), As the local and systemic clinical signs exhibited by the diseased buffalo confirmed the acute stage of the disease in infected animals, Moreover, Clinical diagnosis of mastitis were easily confirmed by the appearance of clinical signs noticed on the inflamed udder (12). Ali and Ahmad (13) has been mentioned that, The acute type of mastitis might appears suddenly with its local and systemic reactions like, the chemical, physical, and other changes in the infected milk, However, the inflamed udder might changed to red, hot and hard palpation. Beside the animals will feel pain on touching the udder, Moreover, the diagnosis of clinical mastitis could based on the abnormal appearance of the milk. Milk may be off different colors and consistency, watery, have blood, serum like , mixed with pus and milk clots, flakes and shreds containing of cellular and fibrin debris(14).

The abnormal color of milk could be due to the result of changes in vascularity during acute inflammation and flow of blood from body of animal to the udder. The shape and size of udder was also changes grossly, Furthermore, Muhammad et al ,1997 (12) was added that, the presence of blood in milk have different cusses, as hemorrhage occurs when passage of erythrocytes was detected via the wall of capillaries inside the tissues in which the erythrocytes exist in the alveolar cells of the mammary tissues occurs after calving. Moreover, When there are large number of erythrocytes in the milk , then it will give the milk a pinkish or reddish colors , On the hand udder trauma seems to be an additional important reason for the presence of blood in the milk (15).

Results was also indicated that the, main cause of the acute buffalo mastitis of the current study was *Enterobacter cloacae* and *Proteus mirabilis*, which confirmed by the VITEK system 2.It have been documented that *Proteus spp.* are uncommon environmental mastitis causative agent that have been known to cause an important felid outbreaks (16). It was also shown that any new infections could occur at any time during lactation period . It was also mentioned that dairy animals especially at the early stage of lactation will be at high risk for new udder infection because of depression of immunity and the stress status which related to the postpartum period . It was indicated that *Proteus spp.* was always responsible for the great buffalo herds outbreaks, However, the infection could mostly become chronic (17).On the other hand, the *Enterobacter spp.* considered as an important cause for the udder infection which could be isolated easily from the infected milk. Moreover, it have the similar structure to other coliform type of bacteria with

an especial odor (fecal odor) when the organism grow on the blood agar, The spread of Enterobacter spp. are indicated firstly via environmental contact, especially when teats are come in contact with manure or contaminated bedding.(18,19). Infection with Enterobacter could be created from water supply, animal houses and milk equipments. The organism is thought to be more resistant to disinfectants , Furthermore The bedding used to animal house could be the primary source of environmental pathogens, but will contaminated teat dips, intra-mammary infusions, water used for udder preparation before milking, However, mud holes, skin lesions, teat trauma, and flies have all been incriminated as sources of infection, .(20). It has been postulated that, Udder infection due to coliform organisms will cause high economic losses due to the losses of bad milk quality , Moreover, high mortalities of diseased animals was also recorded(10).

التهاب الضرع الحاد في الجاموس المتسبب عن الخمج بجراثيم *Enterobacter cloacae* and *Proteus mirabilis* في البصرة، العراق

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الخلاصة

شخص التهاب الضرع الحاد في خمس عشر من الجاموس المحلي في البصرة، العراق. تم اختيار عشرة من الجاموس المحلي السوي سريريا عدت كمجموعة سيطرة. اظهرت الحيوانات المريضة علامات الالم مع تورم واحمرار الضرع، وكان الحليب الملتهب مائي القوام ذا لون احمر خفيف فضلا عن ارتفاع معنوي في درجات حرارة الجسم ، ترداد التنفس وضربات القلب وعلى العكس من ذلك فقد انخفضت تقلصات الكرش بشكل معنوي في الحيوانات المريضة بالمقارنة مع حيوانات السيطرة . اظهرت نتائج التغيرات الدموية وجود ارتفاع معنوي في العدد الكلي لخلايا الدم البيض بسبب الارتفاع المعنوي للعدلات . فحصت جميع الحيوانات المريضة سريريا ومختبريا وبينت النتائج ان المسببات الرئيسية لالتهاب الضرع في الجاموس هي *Enterobacter cloacae* و *Proteus mirabilis* اكد تشخيص الشرائح الزجاجية المصبوغة بصبغة كرام ظهور المسببات الجرثومية بشكل عصيات سالبة الكرام والتي تم تأكيد تشخيصها باستخدام برنامج VITEK 2 System. فضلا عن ملاحظة ظاهرة العج والشكل باهت اللون للمستعمرات الجرثومية . استنتج من هذه الدراسة ان التهاب

الضرع الحاد في الجاموس ذا فعل عكسي مؤذي للحيوان المصاب لذا فإن التعرف على المسببات الرئيسة تسرع من عملية الاستجابة للعلاج.

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