Some Biochemical , Haematological And Bacteriological Studies Associating Bloody Milk In Buffalo Cows And Trials For Treatment

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ABSTRACT

In the present study, sixty samples of milk from buffalo cows secreting bloody milk and ten samples from clinically healthy one animals were examined. The total number of bacterial isolates from the diseased animals was (60 isolates). *Staphylococcus aureus* (35%), *streptococcus agalactia* (20%) *corynbacterium pyogenes* (15%), *E.coli* (26.67%) and *pseudomonas aeruginosa* (3.33%). Pure infection were *staph. aureus* (25%), *strept. Agalactia* (16.7%), *coryn. Pyogenes* (5%), *E.coli* (15%) and *pseudomonas aeruginosa* (3.33%). Antibiogram study revealed that most isolated bacteria were highly sensitive to Gentamycin and Enrofloxacin and less sensitive or resistant to Penicillin, Streptomycin and Oxytetracycline. Treatment of mastitic buffalo cows caused by E.coli infection with intramuscular injection of Gentamycin (5 mg/Kg.B.wt.) for five successive days resulted in a recovery of most diseased animals, however, administration of antioxidant (Vit. E & Selenium) and antihistaminic in addition to Gentamycin resulted in a better response. Regarding the haematological picture of diseased animals, a significant decrease in erythrocytic count, platelet and haemoglobin concentrations coupled with a significant ($P<0.05$) increase in total leucocytic count were detected. Moreover, the biochemical studies revealed a significant ($P<0.05$) increase of serum globulins and decrease of serum albumin. In addition, a significant decrease of serum calcium and increase of AST were noticed.

In conclusion, identification of the causative agent and sensitivity tests beside correction of haematological and biochemical alterations caused by the infection are important factors for achieving successful treatment in mastitic buffalo cows secreting bloody milk.

INTRODUCTION

The presence of blood in milk (bloody milk) is an obvious problem since the milk will be unfit for human consumption. The alteration of the colour of milk is related to mastitis and is associated with haemodynamic changes *(1)*. Milk is pale and yellowish in colour in *E.coli* infection and bloody or tinged with blood in *Staphylococcus Aureus* infection *(2)*. The main causative agents of mastitis are *Staph. aureus* followed by *E.coli, strept.* Species and *coryn. Pyogenes (3)*. Economic losses are due to discarding abnormal milk, degrading of milk due to high bacterial or somatic cell count and the cost of therapeutic agents *(4)*. Several studies discussed bovine mastitis, however, little were directed to study different aspects of mastitis in buffalo cows *(5, 6)*. Mastitis associated with bloody milk in buffaloes is due to different factors but the most important is the invading microorganisms as *Staph. aureus, streptococci and E.coli (7,8)*.

Improper treatment of mastitis ignoring the causative agents or correction of the biochemical alterations led to the development of resistant bacteria and failure of treatment. Hence the present work was directed for isolation and identification of bacterial agents from mastitic milk of buffalo cows. Moreover, haematological and serum biochemical alterations were studied. Based on *in vitro* sensitivity test, trial for obtaining an effective treatment was conducted.

MATERIAL AND METHODS

Animals

A total number of seventy buffalo cows 3 – 5 years old from different localities at Sharkia province were used in this study. Animals were examined clinically and case history was obtained.

Sampling

Sixty milk samples containing blood from buffalo cows secreting bloody milk were aseptically collected in clean sterile tubes. In addition ten samples from apparently clinically

healthy buffalo cows housed in the same environment were also collected. California mastitis test was applied on milk samples of each quarter of udder of clinically healthy animals and those secreted bloody milk. Examination of milk was conducted by drawing first streams of milk over a black surface for detection of color changes *(9)*.

Microbiological examination:

Milk samples were incubated overnight , then loopfuls were streaked onto plates of sheep blood agar , MacConkey agar and Edwards medium. Plates were incubated aerobically at 37°C for 24 – 48 hours *(10)* . The isolated microbial colonies were purified and identified *(11,12).*

Sensitivity tests

Sensitivity tests were carried out using disc diffusion method *(13).*

Haematological and biochemical analysis

Heparinized blood samples from all animals were collected for blood picture *(14)*. Serum samples were analyzed for estimation of aspartate aminotransferase (AST), Alanine aminotransferase (ALT) *(15)*, alkaline phosphatase (ALP) after *(16)*, total bilirubin *(17)*, total proteins *(18)* and albumin *(19)*. Serum globulins were calculated by substraction of albumin from serum total proteins. Serum calcium *(20)*, inorganic phosphorus *(21)* and magnesium *(22)* were determined.

Treatment

Twenty affected buffalo cows secreting bloody milk due to mastitis caused by E.coli were classified into four equal groups (A, B , C and D). Group A was treated by intramuscular injection of Gentamycin (5 mg/Kg.B.wt.) for 5 successive days. Group B was treated with Gentamycin in addition to antioxidant (Vit. E & Selenium (Viteselen ®) at a dose level of 5 ml/head once by I/M route). Group C was treated by I/M injection of Gentamycin, antioxidant and antihistaminic (Avil at a dose level of 10 ml ,each ml contains 22.75 mg phenaramine maleate) for five successive days. All groups (A, B and C) were also injected with vitamin K (Phytomenadione, 50mg onece/ head). Group D (five diseased non-treated buffalo cows) was kept as a control group. Statistical analysis was conducted *(23)*.

RESULTS

The present work revealed that *Staphylococcus aureus* followed by *E.coli* (35 and 26.67% respectively) were the most common causative agents of mastitis associated with bloody milk in buffalo cows (Table,1). Moreover, frequency distribution of isolated bacteria was shown in Table 2. Antibiogram showed that Gentamycin followed by Enrofloxacin were the most effective antibacterial agents against E.coli infection (Table 3). Some haematological and biochemical alterations were detected (Tables 4 , 5).

Table 1. Number and percentage of isolated bacteria from mastitic buffalo-cows secreting bloody milk .

No.	Bacterial isolates	No.	Percentage
1	*Staphylococcus Aureus*	21	35 %
2	*Streptococcus Agalactia*	12	20 %
3	*E.Coli*	16	26.67 %
4	*Corynbacterium pyogenes*	9	15 %
5	*Pseudomonas Aeruginosa*	2	3.33 %
	Total	60	100.0 %

Table 2. Frequency distribution of isolated bacteria from mastitic bloody milk samples.

Pure isolates			Mixed isolates		
Bacterial isolates	**No.**	**%**	**Bacterial isolates**	**No.**	**%**
Staphylococcus Aureus	15	15 %	*Staphylococcus Aureus (6) + Streptococcus Agalactia (2)*	8	13.3 %
Streptococcus Agalactia	10	16.7 %			
Corynbacterium pyogenes	3	5 %	*Corynbacterium pyogenes (6) + E.Coli (7)*	13	21.7 %
E.Coli	9	15 %			
Pseudomonas Aeruginosa	2	3.3 %			
Total	39	65 %	Total	21	35 %

Table 3. Sensitivity of bacterial isolates from mastitic bloody milk in buffalo-cows.

Drug / Isolates	*Coryn. Pyogenes* (N =9)		*E.coli* (N=16)		*Pseudo. Aeruginosa* (N=2)		*Staph. Aureus* (N=21)		*Strept. Agalactia* (N=12)	
	S %	R %	S %	R %	S %	R %	S %	R %	S %	R %
Ampicillin (20 ug)	33	67	6	94	0	100	10	90	8	82
Amoxycillin (30 ug)	11	89	6	94	50	50	19	81	66	34
Ceftiofur sodium	77	23	6	94	0	100	14	86	8	82
Danofloxacin (5 ug)	55	45	54	46	100	0	90	10	58	42
Enrofloxacin (10 ug)	88	12	56	44	50	50	86	14	83	17
Gentamycin (10 ug)	66	34	100	0	100	0	95	5	75	25
Penicillin (10 ug)	55	45	0	100	0	100	0	100	58	42
Streptomycin (10 ug)	11	89	18	82	50	50	9	91	16	84
Tetracycline (30 ug)	11	89	0	100	0	100	22	78	11	89

• S: Sensitive , R : Resistant

Table 4. Haematological picture in clinically healthy and mastitic buffalo cows secreting bloody milk (N=5) .

Parameter	Control	Mastitic buffalo cows
R.B.Cs/ul	$6.08 \times 10^6 \pm 0.03$	$4.90 \times 10^6 \pm 0.07$**
Hb (gm/dl)	15.5 ± 0.72	9.3 ± 0.44 ***
PCV (%)	$34.1\% \pm 0.28$	28.4 ± 0.37 ***
MCV (fl)	55.4 ± 0.40	58.2 ± 0.59 **
MCH (pg)	25.2 ± 0.56	19.2 ± 0.31 ***
MCHC (gm/dl)	45.5 ± 0.39	33.3 ± 0.56 ***
Platelet count/ul	$600.8 \times 10^3 \pm 5.39$	$300 \times 10^6 \pm 4.7$ ***
Total leucocytic count/ul	$5.8 \times 10^3 \pm 0.43$	$11.4 \times 10^3 \pm 0.51$ ***
Neutrophils %	$38.2\% \pm 0.2$	$60.3\% \pm 0.67$ ***
Lymphocytes %	$59.4\% \pm 1.03$	$37.7\% \pm 0.64$ ***
Monocytes %	$0.83\% \pm 0.08$	$2\% \pm 0.05$ ***
Eosinophils %	$2\% \pm 0.002$	$1\% \pm 0.001$
Basophils %	0 %	0 %

** Highly significant at P<0.01
** Very highly significant at P<0.001

Table 5. Some biochemical values in serum of clinically healthy and mastitic buffalo cows secreting bloody milk (N=5) .

Parameter	Control	Mastitic buffalo cows
Total bilirubin (mg/dl)	0.083 ± 0.006	0.074 ± 0.005
Direct bilirubin (mg/dl)	0.03 ± 0.002	0.029 ± 0.023
Indirect bilirubin (mg/dl)	0.064 ± 0.005	0.055 ± 0.004
ALT (u/l)	75.8 ± 3.40	70.2 ± 2.8
AST (u/l)	112 ± 1.02	138 ± 1.52 **
Alkaline phosphatase (u/l)	112.8 ± 1.85	72.4 ± 1.03 **
Total proteins (gm/dl)	7.2 ± 0.097	7.2 ± 0.13
Albumin (gm/dl)	3.5 ± 0.12	2.9 ± 0.1 *
Globulins (gm/dl)	3.6 ± 0.16	4.2 ± 0.15*
A/G ratio	1.02 ± 0.04	0.7 ± 0.03 ***
Calcium (mg/dl)	10.34 ± 0.24	8.4 ± 0.25 *
Phosphorus (mg/dl)	5.36 ± 0.15	7.71 ± 0.68
Magnesium (mg/dl)	3.07 ± 0.08	2.38 ± 0.17

* Significant at P<0.05
** Highly significant at P<0.01
*** Very highly significant at P<0.001

Treatment

The results revealed that groups A,B and C were effectively treated when compared with group D . However , group C was treated faster and better than all other groups.

DISCUSSION

Many infective agents have been implicated as causes of mastitis. In majority of cases where milk was tinged with blood, bacteria are usually isolated. In the present work, the main total bacterial isolates were *staphylococcus aureus* (35%), *streptococcus agalactia* (20%) *corynbacterium pyogenes* (15%), *E.coli* (26.67%) and *pseudomonas aerugenosa* (3.33%). These findings were nearly similar to that previously mentioned ***(2,3,7)*** , While *staphylococcus aureus* and *E.coli* were isolated ***(24)*** from mastitic milk at a lesser percentage (23.56 and 6.32% respectively).

The obtained results showed that the prevalence of single mastitogenic bacteria from bloody milk samples were *strept. agalactia* (16.7%), *Staph. aureus* (25%), *Coryne pyogenes* (5%) , *E.coli* (15%) and *pseudomonas aeruginosa* (3.3%) while double mastitogenic bacteria were *strept. agalactia with staph. aureus* (13.3%) and *coryne pyogenes with E.coli* (20%) . Similar causative bacterial agents at different percentages were reported ***(25, 26)***. The incidence of *Staph. aureus* and *E.coli* may be a result of heavy contamination of bedding, housing and increase of moisture ***(27)***.

For achieving effective treatment, it is important to carry out *in vitro* sensitivity of the isolated strains to various antimicrobial agents. The obtained results indicated that most of bacterial isolates were highly sensitive to Gentamycin and Enrofloxacin. This was not the case for the other tested antimicrobials (Streptomycin, Ceftiofur sodium, Danofloxacin, Ampicillin, Amoxycillin, Tetracycline and Penicillin) where considerable resistance was evident. Similar results were detected. ***(28, 29)*** for isolates from bovine mastitis. the low sensitivity of bacterial isolates in the present work to commonly used antimicrobials (Penicillin, Streptomycin, and Tetracycline) might be attributed to the misuse of these agents leading to the increase of resistance of tested strains ***(30 , 31)***. Based on the antibiogram, treatment of buffalo cows suffering mastitis caused by *E.coli* infection with intramuscular injection of Gentamycin (5 mg/Kg.B.wt.) for five successive days resulted in a high rate of recovery. These data were in agreement with previous reports on the susceptibility of *E.coli* isolated from mastitic milk to Enrofloxacin and Gentamycin ***(32, 33)***. In addition, ***(34)*** proved that the efficacy of Gentamycin in treatment of cattle mastitis at Sharkia Governorate (Egypt). Furthermore, intramuscular injection of Gentamycin was found to be highly effective for curing mastitis in buffaloes when compared with Enrofloxacin ***(35)***.

The incidence and severity of both clinical and subclinical mastitis are greatly influenced by the level of antioxidants in the body specially selenium and/or vitamin E ***(36)***. The infection of the udder tissues and its inflammation lead to the release of increased amounts of oxygradicals which result in denaturation of hemoglobin and disruption of red blood cell membrane ***(37)*** .

Treated mastitic buffalo cows with Gentamycin, Vit. E and selenium showed a better recovery than those treated with Gentamycin only as vitamin E supplementation has a positive effects on the function of bovine neutrophils ***(38)***.

Bovine mastitis caused by *E.coli* infection was found to be associated with an elevated level of free histamine in the circulation ***(39)***. This explains the high recovery rate of mastitic buffalo cows in the present study when treated with antihistaminic .

Regarding the haematological change , a significant decrease in erythrocytic count, platelets and hemoglobin concentration coupled with a significant increase of total leucocytic count were noticed . These findings correlate with that previously cited ***(37)*** where a significant decrease in erythrocyte GSH-P (glutathione peroxidase) activity , total erythrocytic count and haemoglobin concentration were recorded in cows suffering mastitis . The author attributed the development of anaemia to the released oxydradicals resulted in denaturation of hemoglobin and disruption of red blood cell membrane . The anaemia recorded ***(40)*** may be due to suppression of bone marrow stem cell activity by cytotoxins of *E.coli*. On the other hand, cattle suffering *Staphylococcus aureus*

mastitis showed a decrease in mean serum Cu , Zn and Fe concentrations which may be another explanation of development of anemia *(41)* . Furthermore ,. a significant decrease of serum iron and zinc in mastitic cows caused by *Staph.* or *E.coli* was recorded *(42)* . An increase of monocytes, eosiniphils and neutrophils and decrease of platelet concentration with no change in erythrocytic count , PCV and Hb concentration of mastitic cows when compared with the normal mean values were also recorded*(1)*. Moreover , a marked leucopenia in mastitic cattle was noticed *(43)*. Most researchers now accept that the polymorphonuclear (PMN) leucocytes is a key factor in the cow's defense against intramammary infection with *E.coli* , however , while PMN are phagocytosing and destroying the invading pathogens , they inadvertently release chemical mediators which induces swelling of secretory epithelium cytoplasm, sloughing of secretory cells and decreased secretory activity *(44)*.

In this work , a significant decrease of albumin and significant increase of globulins and AST in serum of buffalo cows suffering mastitis with bloody milk were detected . This may be a response to the inflammatory state *(45)*. Moreover , bacterial toxins may affect the hepatic parenchyma resulting in failure of liver to synthesize albumin *(29)*. However, the decrease in serum albumin was attributed to their infiltration from the blood to milk due to increase permeability of blood vessels as a consequence of inflammation *(46)* .

Supporting these data , previous studies reported a significant decrease of albumin level *(47)* and significant elevation of globulins in serum of mastitic cows . However , no changes in total protein content and distribution of protein fractions in serum of cows manifesting inflammation of the udder were recorded *(48)*. The present study revealed hypocalcemia in mastitic buffalo cows . Similar results were recently cited *(29, 49)* in mastitic cattle. The authors attributed the decreased serum calcium level to the released endotoxins.

In conclusion, identification of the causative agent and sensitivity testing beside correction of haematological and biochemical alterations caused by the infection are important factors for achieving successful treatment in mastitic buffalo cows secreting bloody milk.

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الملخص العربي

بعض التغيرات البيوكيميائية و الهيماتولوجية و البكتريولوجية المصاحبة للبن المدمم في الجاموس و محاولات العلاج

أمينة السيد فارس و مختار عبد الحكيم سليم
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في هذه الدراسة تم فحص ٦٠ عينة لبن من جاموس يفرز لبن مدمم و ١٠ عينات لبن من حيوانات سليمة اكلينيكياً. كان إجمالي المعزولات كالتالي : الميكروب المكور العنقودي الذهبي (٣٥%) و الميكروب السبحي اجلاكتيا (٢٠%) و ميكروب كوريني باكتيرم بيوجينز (١٥%) و ميكروب القولون العصوي (٢٦,٦٧%) و ميكروب السودومونس اورجونوز (٣,٣%) أما من ناحية العدوي الفردية كانت نسبة العزل في الميكروبات السابق ذكرها بنفس الترتيب هي ٢٥% و ١٦,٧% و ٥% و ١٥% و ٣,٣% علي التوالي .و قد وجد أن معظم الميكروبات المعزولة كانت عالية الحساسية لكلاً من الجنتاميسين و الانروفلوكساسين و كانت أقل حساسية أو مقاومة للبنسلين و الاستربتوميسين و الاوكسي تتراسيكلين . تم علاج الحيوانات المصابة بالتهاب الضرع الناتج عن العدوي بالميكروب القولوني بحقن جنتاميسين في العضل بجرعة ٥ مجم/كجم وزن حي لمدة خمسة أيام متتالية و أثبت العلاج فعالية إلا أن استعمال مضاد للتأكسد (سيلنيوم ، فيتامين E) و فيتامين K ومضاد للهستامين بالإضافة إلي الجنتاميسين أثبت أنه أكثر فاعلية في العلاج .

بالنسبة لصورة الدم لوحظ نقص معنوي في عدد كرات الدم الحمراء و الصفائح الدموية و نسبة الهيموجلوبين مقرونة بزيادة العد الكلي لكرات الدم البيضاء. أثبتت الدراسات البيوكيميائية زيادة الجلوبيولينات في مصل الدم ونقص معنوى فى الألبيومين بالإضافة إلي أنه قد لوحظ نقص معنوى فى الكالسيوم وزيادة معنوية فى مستوى انزيم اسبرتيت أمينو ترانسفيريز فى مصل الدم فى الحيوانات المصابة مقارنة بالحيوانات السليمة.

و يستنتج من هذه الدراسة أن التعرف علي المسبب للمرض و إجراء اختبار الحساسية له بالإضافة إلي تصحيح الاختلالات الهيماتولوجية و البيوكيميائية الناتجة عن الإصابة تعتبر عوامل هامة لتحقيق علاج ناجح لالتهاب الضرع في الجاموس الذي يفرز لبناً مدمماً .