Bacteriological and pathological studies on mammary gland affection in Cattle and buffaloes

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Abstract
Milk samples were collected from 989 and 276 cow`s and buffalo`s quarters respectively. Sixty udder tissues from amputte udder were collected from Bastian abattoir. The result revealed that clinical mastitis were 24 (9.6%) and 7 (10%), Sub acute mastitis were 27 (10.8%) and 6 (8.5%), Chronic mastitis were 9 (3.6%) 5 (7.1%) for cows and buffalo respectively. Recurrent mastitis was recorded in 10 (6 cows and 4 buffaloes). California mastitis test revealed two hundred and seventy six quarters represent 69 animals were suspected to be affected by subclinical mastitis for 60 cows (31.5%) and19 buffaloes (27.1%). The degree of quarter attack to positive CMT result was varied from grade 1 to grade 3. Microorganisms isolated from subclinical mastitis were *Staph. aureus* as a single infection in all 3 scores of California mastitis test (25%, 35% and 20%) respectively in cows and 33.3%, 26.5 % and 35% respectively in buffalos). Bacterial isolated from clinical mastitis was *Staph. aureus* as a single infection , in subacute mastitis 29.6% and 29., in acute mastitis 17.9% and 28.6% in recurrent chronic mastitis 69.2% and 50% (in cows and buffaloes respectively). Bacterial isolates from mastitic amputee quarter were *Staphylococcus aureus* (4 isolates) and *Escherichia coli* (2 isolates)**.** The predominant bacterial isolates from all collected samples were. *Staphylococcus aureus* (166 from cows and 70 from buffaloes), followed by *E.coli* and streptococcus species. In vitro antibiogram studies conducted on randomly chosen bacterial isolates were varied. The hematological finding raveled decrease in the erythrocytic count, packed cell volume hemoglobine concentration normocytic normochromicanemia. The result of Leucogram showed significant increase in total leucocytic count due to significant increase in neutrophils and lymphocytes count. The biochemical finding was significant. Decrease serum total protein, albumin and significant increase in Y-globulin and total globin also there were significant increase in ALT, AST and AlP enzyme .Activity urea and creatinine showed non-significant The pathological change in the mammary gland and lyphm node for hitopathological microscopically examination of supramamary lymph node showed severe infiltration of Esenophil cell thickened trabeculae. Thickening of the trabeculae, infiltration of inflammatory cells with debilitation of lymph cells in the lymphoid follicular (subcapsuler edema, thickening of the capsule with infiltration of inflammatory cells there are depletion of lymphoid cells in lymph floccules. There are infiltrations of red blood cells in acini, complete necrosis in cells lined some acini and other cells showed vacculation.

Key words: *E.coli, Staphylococcus aureus, California mastitis.*

Introduction

Mastitis is an inflammation of the mammary glands of dairy animals accompanied by physical, chemical and usually bacteriological changes in milk as a result of pathological changes in glandular tissues of the udder. (**Radostits *et al*., 2000; Sharma *et al.*, 2012**). It is also defined as inflammation of mammary gland parenchyma which is caused by microorganisms usually bacteria that invade the udder multiply and produce toxins which are harmful to the mammary gland **(Sharma *et al.*, 2007).** An important factor in mastitis is the high cost of control due to antibiotic treatment, discarded

milk, financial penalties and reduced lactation yield, often resulting in culling, this often amounts to significant annual losses **(Mungube *et al.,* 2005)**. Several factors such as poor hygiene and high incidence of infectious diseases among others have been shown to predispose ruminant livestock to mastitis **Haltia *et al.,* 2006).**

The inflammation severity depends on the causative agent and the host response (**Bannerman *et al*., 2004; Barkema *et al*., 2006; Petzl *et al*., 2008**). Resident and recruited cells together play an essential role in immediate defense against local infection **(Rainard and Riollet, 2006).**

The general objective of the study is to determine the prevalence of clinical and subclinical mastitis, relatively somatic cell count (SCC) and detection of causative pathogens with regards to the recurrent and chronic mastitis, study current antibiogram trend of mastitic bacteria, hematological and clinicopathological change in amputee udder.

Materials and Methods

Collection of clinical history data.

The detail history recorded include, the production performances, hygienic condition of the houses, color and consistence of the milk, sedimentation in the milk, health status, recurrences, amount and quality of feed and water, previous disease condition, if any, was recorded and the characteristic clinical signs were studied systematically. The presented information and the development of signs were correlated and characterized according to the degree of severity of the disease**.** The lesion was examined carefully; and categorized clinically and was accepted for the treatment following amputation of the affected quarter (s) considering all capable measurements.

Animals.

A total of 250 cows and 70 buffaloes from different farms at El Giza and El. Sharkia are used in this study. The age of cows and buffaloes ranged from 5 - 8 years. Also six udder tissues of amputee udder were collected from Bastian abattoirs

Methods.

Physical examination.

Visual observation and palpation of the mammary gland quarter and macroscopic examination of the milk was recordedinclude: age, clinical state (acute, subacute and chronic), acute mastitis: severe inflammation of the mammary gland without any marked systemic reaction. Sub acute mastitis: Inflammation of the mammary gland with abnormalities in milk. Chronic mastitis: Inflammation of the mammary gland, with little change in milk where the gland was either enlarged or fibrosed.

Sampling.

Milk samples

Samples were collected under strict hygienic condition as described by **Sheikimwer *et al*., 1998)** it should be taken after the teat has been washed with disinfectant and water, dried and swabbed with alcohol .Foremilk should be discarded and approximately5ml of milk collected into a sterile container held at an angle to preclude contamination of the sample with hair and kept on ice. Samples were collected from 989 and 276 from cow`s and buffalo`s quarters respectively were examined from different herds in different farm, 11 quarters were atrophied (Seven from cows and four from buffaloes) had lost a quarter.

Blood samples

Two blood samples were taken from jugular vein at the farm from 60 apparently healthy and diseased cows and buffalo. The first sample was collected into a clean dry bottle containing EDTA anticoagulant for hematological studies **(Jain, 2000)** The second blood sample was placed into a clean centrifuge tube to separate serum by centrifugation at 3000 rpm for 15 minutes.

Udder tissues.

Sixten udder tissues of amputee animals were collected from el Basatin abattoir. The samples were divided in two portions. First portion for bacteriological examination and the second one was preserved in formalin (10%) for histopathological studies.

Field test.

California mastitis test.

A portion of each apparently mastitis free quarter, milk sample was inspected for clots

discoloration or wateriness before adding the California mastitis (CMT) reagent .California Mastitis Test (CMT) and the method were used alongside the physical examinations and the test was carried out as described by **Schalm, and Noorlander (1957)**. The reactions were graded 1, 2, 3 according to **Klastrup, (1975)**. The test should be read as positive when at least a trace reaction is apparent. The rest of the milk was immediately transported cooled (4ºC) to the microbiology Laboratory research, for bacteriological examination.

Table (1). Interpretation of California mastitis test.

CMT	Visible Reaction	SCC range (cell/ml)	Approximate SCC mid-point
Negative	Mixture remains liquid – no evidence of precipitate	0 – 200,000	100,000
Trace	Slight precipitate, best seen by tipping, disappears with continued movement	150,000 – 500,000	400,000
1	Distinct precipitate but no tendency toward gel formation	400,000 - 1,500,000	800,000
2	Mixture thickens immediately, moves toward center	800,000 – 5,000,000	1,600,000 3,200,000
3	Gel forms and surface becomes convex	>5,000,000	6,400,000

Grams staining

Milk samples impression smears was stained with Gram's stain **(McLeod *et al*., 1981)** to Study the morphological and staining characteristics of bacteria to provide information about the presumptive bacterial identification as per recommendation of **Cowan (1985**).

Bacteriological studies.

Isolation and identification (Quinne *et al.*, 2005).

The milk samples of California mastitis test (CMT) positive were inoculated on blood agar and MacConkey agar plates, which were divided into four sections. A disposable culturing loop was dipped into each quarter milk sample (was thus streaked in one section of the plate). Samples were cultivated under aerobic conditions for24-72 hrs at 37oC and examined for bacterial growth.Pure cultures were further examined for morphological, staining and cultural, characteristics and for biochemical reactions.

b- Antimicrobial susceptibility Testing.

Disc diffusion susceptibility test was used to analyze the susceptibility of *Staph.aureus* to twelve different antimicrobials namely Amoxicillin (30 mcg), Ampicillin (25mcg), Ceftriaxone (30 mcg), Cephotaxim (30 mcg), Chloramphenicol (30 mcg), Cloxacillin (10 mcg), ciprofloxacin (30 mcg), (10 mcg), Enrofloxacin (10 mcg), Gentamycin (30 mcg), Oxytetracycline (30 mcg) Doxycyclilin. The interpretation was made according to **NCCLS (1999**) depending on the diameter of zone of inhibition of bacterial growth.

6- Biochemical and pathological examination.

A-Biochemical.

Electrophoretic pattern of serum proteins were determined according to **Epstein and Karcher (1994)** using Helen protein Electrophoresis and titan III cellulose acetate. Total protein was assayed according to **Dounas *et al* (1981),** Albumin according to **Drupt (1974),** AST and ALT activities according to **Reitman and Frankel (1957),** urea according to **Tabucco (1979),** Creatinine.

B-Histopathological examination.

Udder and supermamary lymph nodes of cows

and buffaloes were fixed in 10% neutral buffered formalin then processed by the standard paraffin methods and sectioned at 5ml thickened, stained with Haematoxylin and Eosin H&E (**Bancroft *et al.*, 1994)** The in vivo and in vitro gross pathological characteristics were recorded during clinical examination.

Result.

Prevalence of mastitis.

Clinical mastitis.

The data analysis showed that the age of all recorded cases ranged in between 5 to 8 years. Clinical examination of 250 cows and 70 buffalos revealed 7 cows and 4 buffaloes had lost a quarter. Sixty cows (24%) and 18 (25.7%) buffaloes displayed evidence of mastitis. **The acute mastitis cases:**were 24 (9.6%) and7 (10%)for cows and buffloes respectively, characterized by severe inflammation of the mammary gland without any marked systemic reaction),**Sub acute mastitis cases**: were 27 (10.8%)and6 (8.5%)for cows and buffalo respectively **.The acute mastitis cases:** were 24 (9.6%) and 7 (10%)for cows and buffloes respectively, characterized by severe inflammation of the mammary gland without any marked systemic reaction), **Sub acute mastitis cases**: recorded in 27 (10.8%) cows and 6 in 9 (3.6%) cows and 5 (7.1%) buffalo characterized by inflammation of the mammary gland, with little change in milk where the gland was either enlarged or reduced in size. The number of quarters affected for 60 cows were: left fore, 46 right fore, 52, right hind73, left hind 69. From 18 buffaloes, the number of quarters affected was: left fore 15 (5.4%); right fore, 13 (4.7%), right hind 23 (8.3%), left hind 21 (7.6**%)(Table 2.**). **Finally, recurrentmastitic cases** recorded in 10 (6 cows and 4 buffaloes) from the chronic cases showed fibrosed udder causes indurations recognized by palpation, with a history of repeated infection and past treatment sent to the slaughter house for histopathological studies after collection of milk samples and blood samples.

Fig. (1). Abnormalities of milk samples
Serous exudate and bloody milk from mastitic udder in cow (left), in comparison to normal milk (right).

Table (2). Prevalence of clinical state of the udders

state	cow	buffalo
acute	24 (9.6%)	7(10%)
subacute	27 (10.8%)	6(8.5)
chronic	9 (3.6%)	5(7.10)1
Total	60 (24%)	18(25.7%

*: percentage is calculated according to the total number of animals

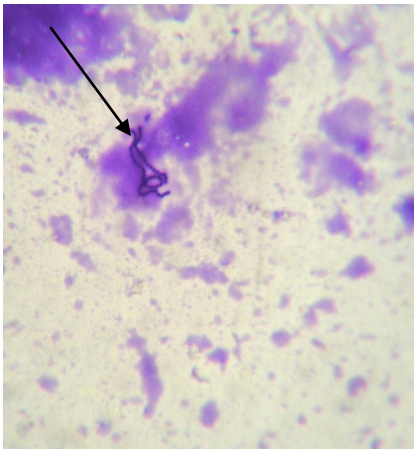
Diagnosis of subclinical mastitis using California mastitis test.

California mastitis test revealed two hundred and seventy six quarters represent 69 animals were suspected to be affected by subclinical mastitis 60 (31.5%) and19 (27.1%)for cows and buffalo respectively CMT score 3 was observed with higher percentage in cow`s quar-

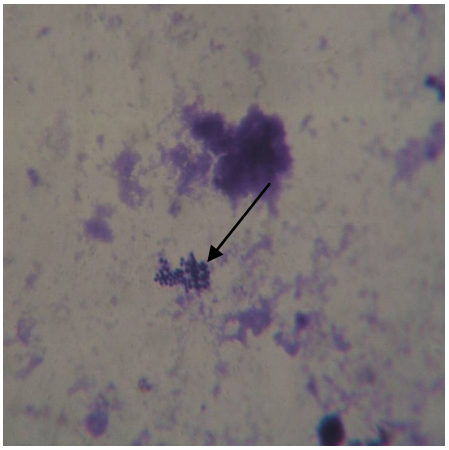
ters (13.1%) than buffalo`s quarters (8.1%). The degree of quarter attack to positive CMT was varied 100 (13.1 %) in cows and 20 (8.1%) in buffaloes showed degree 3 to 60 (7.9%) in cows and 34 (13.9%) in buffaloes showed degree 2while for degree 1 were 80 (10.5%) 18 (7.4%) in cows and buffalos, respectively.

Direct microscopically examination

A



B



(A) Streptococcus (chain like appearance)

(B) Staphylococcus (grapes like cocci)

Table (3). CMT scores of milk samples from 760 quarters in the apparently healthy dairy cows and 244 in the dairy buffalo.

	SCC mid point	N0. & (%) of cows samples	No. & (%) of buffalo samples	Total No. of sub-clinical mastitic quarter	No. & (%) ** of subclinical mastitic milk in cows & buffalo	
Negative	100 000	520 (68.4%)	172(70.5%)	0	0	0
Trace	400 000	0	0	0	0	0
1	800 000	80 (10.5%)	18 (7.4%)	98	20 (8)	5 (7.1)
2	1600000 - 3200000	60 (7.9%)	34 (13.9%)	94	15 (6)	9 (12.9)
3	6400000	100 (13.1%)	20 (8.1%)	120	25 (10)	5 /(7.1)
Total	-	760	244	267	60 (24)	19(27.1)

*: Percentage is calculated according to the total number of quarters (760 and 244 respectively)
**: percentage is calculated according to the total number of animals.

Identification of bacterial isolates.
Bacterial isolates were identified by their cultural, morphological and biochemical characters, Tables **(4 and 5)** illustrated the frequency of different microorganisms isolated from subclinical mastitis *Staph. aureus* was isolated as a single infection in all 3 scores (1,2 and 3) of California mastitis test (25%, 35% and 20%) respectively in cows and 33.3%, 26.5% and 35% respectively in buffalo .Tables **(6 and7)** illustrated the frequency of different microorganisms isolated from clinical mastitis .*Staph.aureus* was isolated as a single infection in subacute mastitis 29.6% and 29.2. In

acute mastitis 17.9% and 28.6% (in cows and buffaloes respectively) in recurrent chronic mastitis 69.2% and 50% and in chronic mastitis for the 1st time was 58.3% and 50% (in cows and buffaloes respectively).

As regarding to the (16) mastitic amputee quarter, table (8) illustrated that *Staphylococcus aureus* (4 isolates) and *Escherichia coli* (2 isolates) were the predominant bacterial isolates from all collected samples. Table (8) revealed that *Staphylococcus aureus was* the predominant bacterial isolates (166 from cows and 70 from buffaloes), followed by *E.coli* and streptococcus species. Some mastitis milk was negative for bacterial isolates .

Table (4). The frequency of bacterial species cultured from subclinical mastitic cow`s**.**

CMT score	No.of affected quarters	Type of isolated strains	Number of isolates		Single infection		Mixed infection		Quarter free from pathogens	
			No	%	No.	%	No.	%	No.	%
1	80	*Staph.aureus.*	25	31.2	20	25	5	6.2	20	25
		E. coli	13	16.3	3	3.8	10	12.5		
		Strept. spp.	12	15	6	7.5	6	7.5		
		Actinomyces pyogen.	10	12.5	8	10	2	2.5		
TOTAL	80		60	75	37	46.3	23	28.7	20	25
2	60	*Staphus aureus*	28	46.7	21	35	7	11.7	2	3.3
		E.coli	12	20	5	8.3	7	11.7		
		Strept. spp.	10	16.7	6	10	4	6.7		
		Actinomyces pyogens	8	13.3	2	3.3	6	10		
TOTAL	60		58	96.7	34	56.7	24	40	2	3.3
3	100	*Staph.aureus.*	23	23	20	20	3	3	10	10
		E. coli	30	30	17	17	13	30		
		Strept. spp.	27	27	15	15	12	12		
		Actinomyces pyogen	10	10	4	4	6	6		
TOTAL of score 3	100		90	90	56	56	34	34	10	10
Total	240		208	86.7	127	52.9	81	33.8	32	13.3

Table (5). The frequency of bacterial species cultured from subclinical mastitic buffalo`s milk.

CMT score	No. of affected quarters	Type of isolated strains	Number of isolates		Single infection		Mixed infection		Quarter free from pathogens	
			No	%	No.	%	No.	%	No.	%
1		*E. coli*	1	5.6	0	0	1	5.6		
		Strept. spp.	2	11.1	0	0	2	11.1		
		Actinomyces spp.	2	11.1	0	0	2	11.1		
TOTAL	18		15	83.4	6	33.3	9	50.1	3	16.6
2	34	*Staphylococcus aureus*	14	41.2	9	26.5	5	14.7	7	28.6
		E.coli	2	5.9	1	2.9	1	2.9		
		Strept. spp.	5	14.7	0	0	5	14.7		
		Actinomyces spp.	6	17.6	0	0	6	17.6		
TOTAL	34		27	79.4	10	29.4	17	50	7	28.6
3	20	*Staphylococcus aureus*	13	65	7	35	6	30	3	15
		E. coli	2	10	0	0	2	10		
		Strept. spp.	2	10	0	0	2	10		
		Actinomyces spp.	0	0	0	0	0	0		
TOTAL of score 3	20		17	85	7	35	10	50	3	15
Total	72		59	81.9	23	31.9	8	11.0	13	18.1

*: 7cows had lost 1 quarter

Table (6). The frequency of bacterial species cultured from clinical mastitic cow s

Clinical cases	No.of of ex. quarters	Type of isolates	Total No. of Isolates & (%)	Type of infection		
				Single in-fection	Mixed infection	negative
				No. & (%)	No. & (%)	No& (%)
Subacute	108	*Staph. aureus*	40 (37)	32 (29.6)	8 (7.4)	
		E. coli	34 (31.5)	12 (11.1)	22 (20.4)	
		Strept. spp.	22 (20.4)	6 (5.6)	16 (14.8)	
		Actino-mycesis	2 (11.1)	1 (5.5)	1 (5.6)	
Total	108		98 (90.7)	51 (47.2)	47 (43.5)	10 (9.3)
Acute	96	*Staph. aureus*	26 (27.9)	17 (17.9)	9 (9.4)	
		E. coli	22 (22.9)	8 (8.3)	14 (14.6)	
		Strept. spp.	24 (25)	15 (15.6)	9 (9.4)	
		Actino-mycesis	10 (10.4)	3 (3.1)	7 (7.3)	
Total	96		82 (85.4)	43 (44.8)	39 (40.6)	14 (14.6)
Chronic cases	36*	36*				
Recurrent	13	*Staph. aureus*	11(84.6)	9 (69.2)	2 (15.4)	
		E. coli	2 (15.4)	0 (0)	2 (15.4)	
Total	13		13 (100)	9 (69.2)	4 (30.8)	
First infection	12	*Staph. aureus*	9 (75)	7 (58.3)	2 (16.7)	
		E. coli	1 (8.3)	0 (0)	1 (8.3)	
		Ps. aeru-ginosa	1 (8.3)	0 (0)	1 (8.3)	
Total	12		11 (91.7)	7 (58.3)	4 (36.4)	1 (8.3)
Total	219		204 (93.2)	110 (50.3)	94 (42.9)	

Table (7). frequency of bacterial species cultured from clinical mastitic buffalo`s milk.

Clinical cases	No. of ex. quarters	Type of isolates	No. of isolates & (%)	Type of infection	
				Single infection	Mixed infection
				No. & (%)	No. & (%)
Subacute	24	*Staph. aureus*	10 (41.7)	7 (29.2)	3 (12.5)
		E. coli	7 (29.2)	2 (8.4)	5 (20.8)
		Strept. spp.	4 (16.7)	4 (16.7)	0 (0)
		Actinomycesis	3 (12.5)	0 (0)	3 (12.5)
Total	24		24 (100)	13 (54.2)	11(45.8)
Acute	28	*Staph. aureus*	14 (50)	8 (28.6)	6 9 (21.4)
		E. coli	6 (21.4)	0	6 (21.4)
		Strept. spp.	8 (28.6)	6 (21.4)	2 (7.2)
		Actinomyces pyogen	0 (0)	0 (0)	0 (0)
Total	28		28 (100)	14 (50)	14 (50)
Chronic cases	5 cases				
Recurrent	12*	*Staph. aureus*	7 (58.3)	6 (50)	1 (8.3)
		E. coli	5 (41.7)	4 (33.4)	1 (8.3)
Total	12		12 (100)	10 (83.3)	2 (16.7)
First infection	4	*Staph. aureus*	2 (50)	2 (50)	0 (0)
		E. coli	1 (25)	0 (0)	1 (25)
		Ps. aeruginosa	1 (25)	0(0)	1 (25)
Total	4		4 (100)	2 (50)	2 (50)
Total	68		68 (100)	39 (57.4)	29 (42.6)

*: 4 buffalos had lost 1 quarter

Table (8). Total number of isolated bacteria from cows and buffaloes

Animals	Cases	Number of isolates				
		Staph. aureus	*E.coli*	*Strep. Spp*	*Actinomycespyogen*	*Ps.aeruginosa*
Cows	subclinical	76	55	46	28	0
	clinical	86	59	46	12	1
	Udder tissues	4	2	0	0	0
Total		166	116	92	40	1
Buffaloes	subclinical	37	5	9	18	0
	clinical	33	19	12	3	1
Total		70	24	21	21	1

Antimicrobial susceptibility Testing.
The in vitro antibiogram studies were conducted on 73 out of 166 *Staphylococcus aureus* strains were chosen randomly. Table (9) illustrated that most of isolates were highly resistant to Cloxacillin (98.2%), Ampicillin (97.3%), Streptomycine (95.9%) and Oxytetracycline (93.2%).in percentage of 98.2%, 97.3%, 95.9% and 93.2% Only 60.3 % was sensitive to Ciprofloxacin followedbyGentamycine(58.7%) Enerofloxacin(50%).Fig.(3) Table (9).

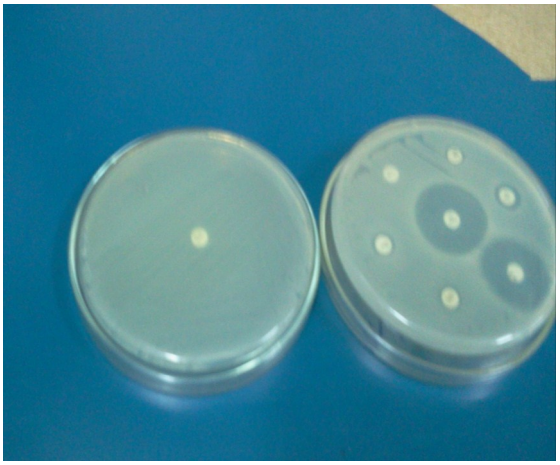


Fig. (3). sensitivity test of *Staph. aureus* against different antibiogram

Table (9). Sensitivity test of bovine mastitic agents to different antibiotics.

Antibiotic disc	Sensitive isolates		resistant	
	No.	%	No.	%
Amoxicillin	6	8.2	67	91.7
Ampicillin	2	2.7	71	97.3
Ceftriaxone	9	12.3	64	87.8
Cephotaxim	15	20.5	58	79.6
Chloramphenecol	20	27.3	53	72.6
Ciprofloxacin	44	60.3	29	39.7
Cloxacillin	1	1.4	72	98.2
Doxycyclilin	6	8.21	67	91.7
Gentamycine	43	58.7	30	41.1
Oxytetracycline	5	6.8	68	93.2
Streptomycine	3	4.1	70	95.9
Enerofloxacin	37	50	36	49.3
Ceftriaxone	9	12.3	64	87.8
Cephotaxim	15	20.5	58	79.5

1-Results of erythrogram of infected cows and buffaloe.

Examination of erythrogram revealed significant decrease in the erythrocytic count, hemoglobin concentration and packed cell volume normocytic normochromic anemia (.Table 10). Cows and buffaloes revealed significant increase in total leucocytic count due to significant increase in neutrophils and lymphocyte count. (Table 11).

Table (10). Blood haemogram of apparently healthy and mastitic cows&buffloes.

Parameter	Control	Infected cows & buffloe
RBCs(10)*	8.4±0.07	7.1±0.21
Hb(gm/dl)	14.15±0.02	12.5±0.01
PCV%	40,28±0.21	38.00±0.10
MCV(fl)	61.82± 0.55	62.00±0,66
MCH(fc)	21.00±0.19	21.11±0.12
MCHC%	33.57±0.21	34.00±0.11

Significance at p<0.001

Table (11). Total and differential leucocytic counts.

Parameter	control	Infected cows & buffloe
WBCS	8.69±0.12	11.96±0.22
Neutrophils	4.28±0.10	5.95±0.12**
Lymphocytes	4.37±0.11	4,71±0.13*
Eosinophils	0.88±0.11	0.91±0.11
Monocytes	0.15±0.0	0.19±0.22
Significance at p<0.001		

2-Serum biochemistry.

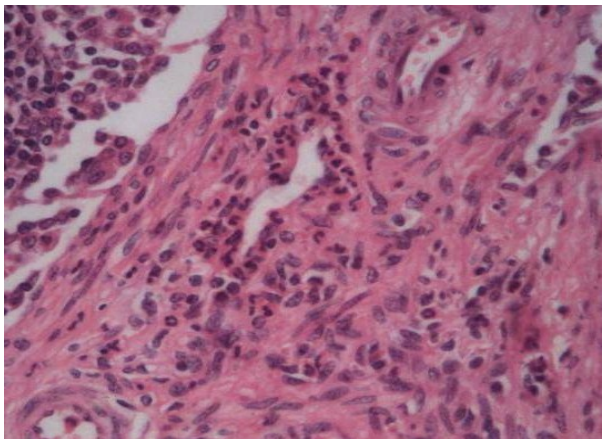
Serum total protein, albumin revealed significant decrease and significant increase in Y-globulin and total globin.) Table 12), serum enzyme activity and urea, creatinine are illustrated in Table (13). Results show significant increase in ALT, AST and AP activity and showed non-significant change in urea and creatin

Table (12). biochemistry analysis of serum

	ALTv/l u/ml	ASTμ/l u/ml	Alkaline phosphatase	Urea Mg/dl	Creatine Mg/dl
Healthy animals	35.50±1.1	63.56±1.80	101.56±1.90	42.30±1.11	1.06±0.03
Mastitic animals	44.95±1.55**	95.65±2.11**	137.21±2.95**	44.26±1.12	1.23±0.12

Gross examination:

The lymph nodes collected from amputte quarter(s); extremely swollen the cardinal signs of inflammation (redness swelling, heat, pain) were noticed. Supramamary lymph node showed severe infiltration of nutrophil cells other lympe node showe dthickened trabeculae (fig4). Thickening of the trabeculae, infiltration of inflammatory cells with depilitation of lymphocytes in the lymphoid follicles (fig 5).Some lymph node subcapsuler edema, thickening of the capsule with infiltration of inflammatory cells there are depletion of lymphoid cells in lymph floccules (fig 6). There are infiltrations of red blood cells, in acini (fig. 7a), Other showed complete necrosis in cells lined some acini fig. (7b) other cells showed vacoulation. There were complete necrosis of cell there are infiltrations of red blood cells, in acini, There were complete necrosis of cell lined acini , infiltration of inflammatory cell lumen of the acini, focal infiltration monoculear inflammatory cells in between the proliferated



Fi g(4). Lymph node showed severe infiltration of nutrophilsl cell thickened trabeculae H&E.

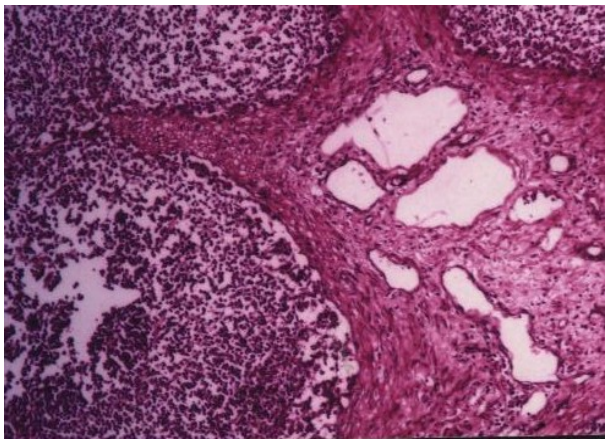
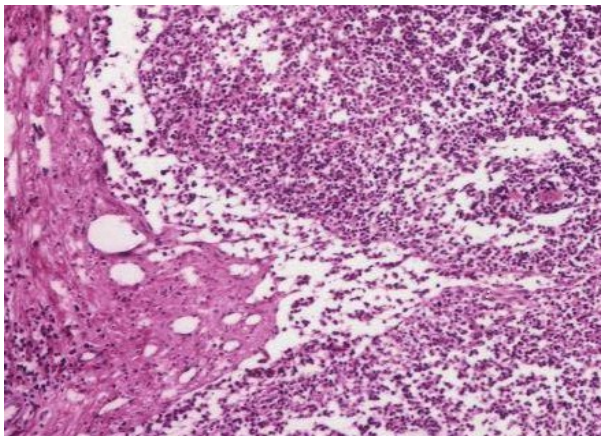


Fig. (5). Lymph node showed thickening of the trabeculae, infiltration of inflammatory cells with depilitation of white cell in the lymphoid follicular.H&E X100.

Fig (6). lymph node showed subcapsuler edema, thickening of the capsule with infiltration of inflammatory cells there are depletion of lymphoid cells in lymph follicles. H&E X100.



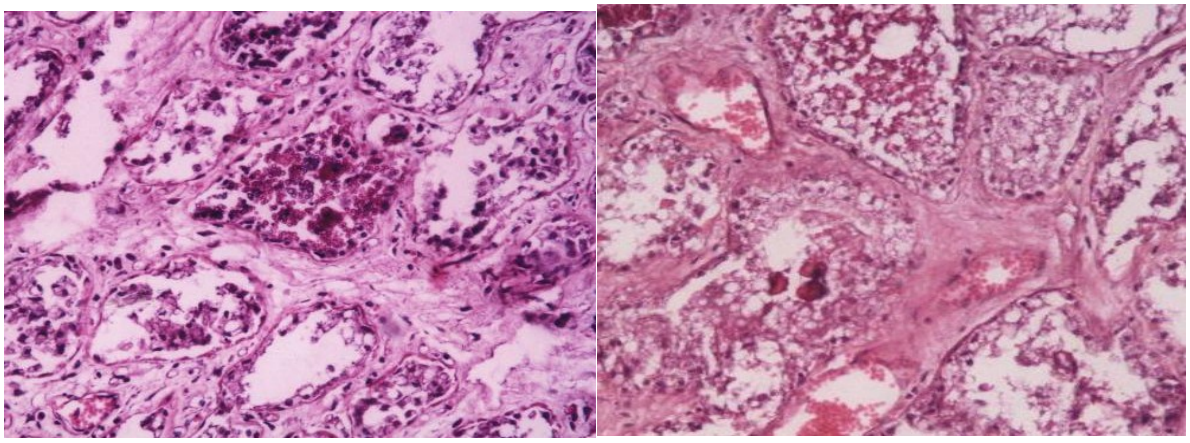


Fig. (7a &b). a-Acini of the udder tissue showed infiltrations of red blood cells. H&E X200
b- Acini of udder tissue of showed complete necrosis in cells. H&E X2.

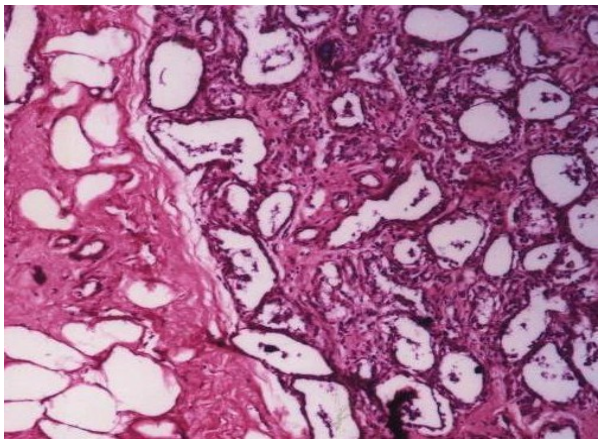
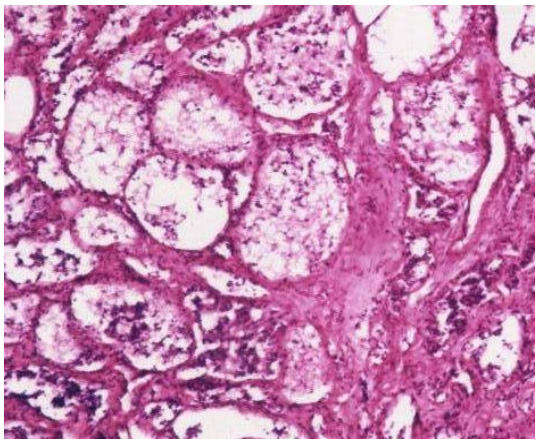



Fig. (8a &b). udder tissue showed acini cells showed vacculation, complete necrosis of the lining cell of some acini, infiltration of inflammatory cell in lumen of the acini, focal infiltration monocular inflammatory cells in between the proliferated fibrous connective tissues.H&E100.

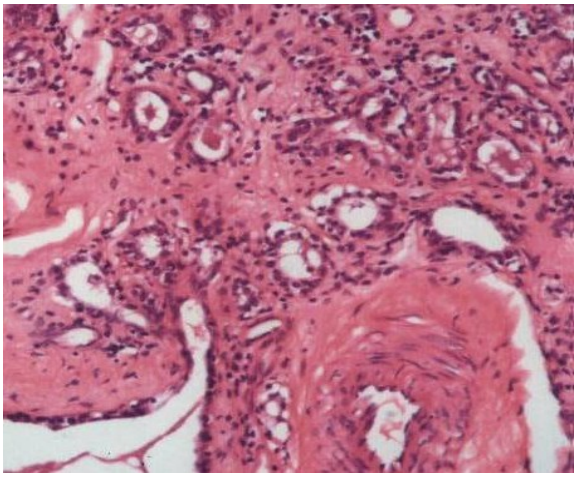
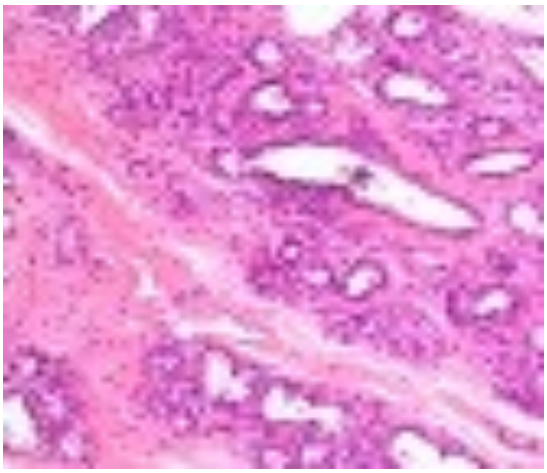



Fig. (9a). Acini showed thiknining of the wall of the blood vessles H&E X200.
Fig.(9b). lumen of acini showed infiltration of nutrophils cells H&E X400.

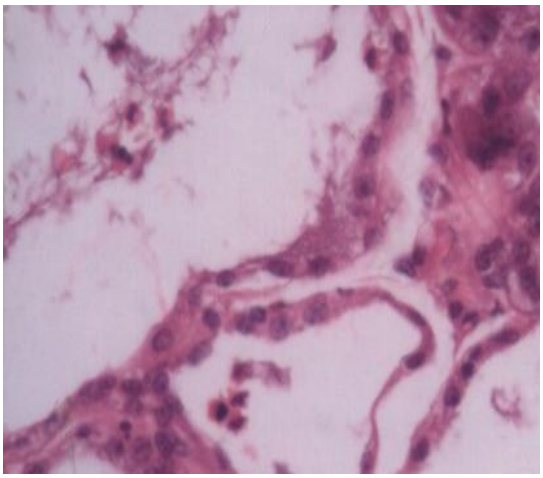


Fig (10). lumen of acini showed nutrophils cells H&E X400.

Discussion

Mastitis is produced by a variety of pathogenic microorganisms **(Hawari and Fowzi, 2008).** Although, stress and physical injuries may cause inflammation of mammary glands, infection by invading bacteria is the primary cause of mastitis. Bovine mastitis is associated with deteriorated milk quality, significant reduction in milk yield and increased costs of production. The economic losses from mastitis due to severe drop in milk production, potential health risks for other animals and human beings, increase cost of the treatment and

Culling processes, are tremendous. In addition to economic losses to farmers, effective control of mastitis is also important from the consumer's and processor's point of view, because milk from affected animals may harbor organisms potentially pathogenic to humans and the processing of such milk may result in substandard fermented products **(Dhakal et al., 2007).** It has been estimated that the mastitis alone can cause approximately 70% of all avoidable losses incurred during milk production **(Sumathi et al., 2008**). The disease also results in partial or complete damage to udder tissues and decreases the productive life span of the animal. As regarding to the result recorded, the incidence of mastitis is increase in cases ranged in between 5 to 8 years, this agreed with **Sharma *et al*.(2007) who** reported high prevalence of mastitis between 5 to7 years and above

As regarding to the result recorded, the incidence of mastitis is increase in cases ranged in between 5 to 8 years, this agreed with **Sharma *et al*., (2007)** and **Sharma and Prasad (2002) who** reported high prevalence of mastitis between 5 to7 years and above .

Clinical examination of 250 cows and 70 buffalos revealed 7 cows and 4 buffaloes had lost a quarter. Sixty cows (24%) and 18 (25.7%) buffaloes displayed evidence of mastitis. The prevalence of clinical mastitis in cattle was reported to be 18.21% while in buffaloes the prevalence of clinical mastitis was 24.60% which is congruent with the findings of **Bilal *et al.,* (2004)** and **Nooruddin *et al.,* (1997).** On the other hand the result recorded by **Thapa and Kaphle (2002)** for clinical mastitis was59% and 41% in cattle and buffaloes respectively 52.7%.This disagree with the result of **Haltia *et al.,* (2006)** 52.7%. , This difference may be attributed towards different geo-climatic conditions prevailing and different study area.

In the present study, acute mastitis cases were 24 (9.6%) and 7 (10%), **Sub acute mastitis cases** were 27 (10.8%) and6 (8.5%). While **chronic mastitis cases** were 9 (3.6%) and 5 (7.1%) for cows and buffaloes respectively. **The chronic form** showed progressive fibrosis which leads to enlargement and asymmetry of the gland. Moreover a portion of the gland may become eventually atrophy. **Du Prez (1994)** reported that according to severity, duration, nature of the exudates and primary cause mastitis can be classified into clinical and subclinical forms. Clinical mastitis is most frequently infectious and classified, according to its severity, rapidity of onset and duration, into peracute, acute, subacute and chronic forms.

Also, the present work showed that incidence of mastitis is higher in hind quarters than front, The reasons for higher hind quarters involvement might be due to more frequent exposure to dung and urine, larger capacity and mass, greater vulnerability to direct trauma and relatively more closeness to the floor as compared to fore quarters this recorded by **(Rasool *et al.,* 1985).**

Somatic cells are, in great quantity, cells of the immune system (80% in uninfected quarters, and 99% in quarters with mastitis) **(Sordillo *et al.*, 1997**) They are part of the natural defense mechanisms, including lymphocytes, macrophages, polymorphonuclear and some epithelial cells **(Pillai *et al*., 2001**). Somatic cells are therefore a reflection of the inflammatory response to an intra mammary infection (IMI.) Somatic cell counts are often used to distinguish between infected and uninfected quarters according to the general agreement between infection status and the inflammatory response to infection reflected as an increased SCC. As with any diagnostic test, errors will occur when

solely depending on a single test. To minimize error, diagnostic test parameters such as sensitivity & specificity are calculated at various cut -off values in the continuum SCC **(Schepers *et al*., 1997** Hence, to be able to distinguish between infected and uninfected quarters a cut-off of approximately 200, 000 to 250, 000 cells is accepted **(Dohoo *et al*., 1991; Laevens *et al*., 1997; Leslie *et al*, 1997;** At this cut-off value, diagnostic sensitivity is approximately 75%, and specificity approximately 90% The200,000cellscut-offis not considered a physiological cell concentration in milk able to distinguish between healthy and unhealthy udders, but it is a practical value under field conditions (minimizing diagnostic error) the SCC for an uninfected quarter is approximately 70, 000 cells. There is of course variation around this mean; its value can increase with age, **(Schepers *et al*., 1997).**

Microbial infection results in rapid accumulation of large numbers of somatic cells in the udder, mainly neutrophils **(Harmon, 1994; Kehrli and Shuster, 1994 and Östensson *et al*., 1988)**. CMT used for diagnosis of subclinical mastitis due to it is a reliable, easy, rapid and cheap tooland helping in controlling the disease because it directs attention to individual mammary quarter that is secreting milk of high somatic cell content (SCC). Programs for control of subclinical mastitis may be planned around the routine examination of all lactating cows, and consequently early treatment can be applied towards positive cases rapidly for preventing their conversion towards recurrent form among dairy cows and for protecting the herd health, milk hygiene and consequently the consumer health.

The standard methods of monitoring subclinical mastitis, routine somatic cell counts (SCC) and culture of cows with elevated SCC., The degree of quarter attack to positive CMT was varied 100 (13.1 %) in cows and 20 (8.1%) in buffaloes showed degree 3 (+++) to 60 (7.9%) in cows and 34 (13.9%) in buffaloes showed degree 2 (++) while those showed grade (+) 1 were 80 (10.5%) 18 (7.4%) in cows and buffalos respectively. The SCC ranged from 400,000-6, 400,000 at which contagious and environmental bacteria were isolated.

In the present study, 276 quarters represent 69 animals were suspected to be affected by subclinical mastitis. 60 cows (31.5%) and19 buffaloes (27.1%). CMT score 3 was observed with higher percentage in cow`s quarters (13.1%) than buffalo`s quarters (8.1%). These results revealed that buffaloes were less susceptible to be mastitis than cows **Wanasinghe, (1985)** This variation may be attributed to, greater risk of mastitis such as more pendulous udder and longer teats for cows conversely; they have a long narrow teat canal, which may be expected to prevent the invasion of microorganism **Fagiolo and Lai (2007).,** while **Sharma *et al.* (2004)** and **Maiti *et al..*, (2003)** reported subclinical mastitis incidence in buffaloes70.32%, and 70.37% in cows.**)**. Also, **Krishnaswamy *et al.* (1965)** stated that teat sphincter of buffalos have smoother muscular fiber in such a way it constitutes a better barrier to microorganism invasion than cows teat sphincter.

Staph. aureus was isolated as a single infection in all 3 scores of 25%, 35% and 20% respectively in cows and 33.3%, 26.5 % and 35% respectively in buffaloes. *Staph. aureus* as a single infection, in subacute mastitis 29.6% and 29.2, in acute mastitis 17.9% and 28.6%, In recurrent chronic mastitis 69.2% and 50% (in cows and buffaloes respectively) and in chronic mastitis for the 1st time was 58.3% and 50% in cows and buffaloes respectively These revealed that *Staph. aureus* isolated in higher incidence in chronic mastitis than acute and subacute mastitis and its role in recurrent mastitis.

All subclinical and clinical mastitis cases were of bacterial origin (86.7% and 81.9% from cows and buffalos respectively). *Staphs. aureus* was the most frequently isolated from milk samples(166 and 70 isolates) followed by *E.coli* (116 and 24 isolates) and streptococcus species (92 and 21 isolates) from cows and buffalos respectively These results were nearly reported by **Shem *et al.*, (2001),** many workers have been reported that *Staphylococcus* spp. is

the chief etiological agent of mastitis in cattle and buffaloes (**Sharma *et al.*, 2007; Sharma, 2008 and Singh *et al.*, 2005**). The organism is ubiquitous and can colonize in the skin as well as in the udder .The intramammary infusion (IMI) with *S. aureus* predominantly cause subclinical mastitis resulting in a chronic infection lingering lifelong **(Bannerman *et al*., 2008; Riollet *et al*., 2000; Yang *et al*., 2008).** *S. aureus* infections; in its peracute mastitis presentation generates gangrene and severe tissue damage. In comparison with *Streptococcus*, *S. aureus* is more difficult to be eradicated. *S. aureus* infections cause a 45% decrease on milk production per quarter, reflected as a 15% per infected animal **(NMC, 1999).**The chronic, subclinical infections account for approximately 80% of mastitis related costs, due to reduced milk yield and product quality **(Shim *et al*., 2004**). In practice, an elevated somatic cell count (SCC), over 300,000 to500, 000 cells / ml, indicates high prevalence of infected glands with *S. aureus* in a herd **(NMC, 1999).** The major reservoirs of *S. aureus* are infected udders, teat canals, and teat lesions, but these bacteria also have been found on teat skin, muzzles, and nostrils. The bacteria are spread to uninfected quarters by teat cup liners, milkers' hands, washcloths, and flies. Staphylococci do not persist on healthy teat skin but readily colonize damaged skin and teat lesions. The organisms multiply in infected lesions and result in increased chance of teat canal colonization and subsequent udder infection **(Petersson -Wolfe 2010).*Escherichia coli*** do not normally live on the skin or in the udder but enter the teat canal when the cow comes in contact with a contaminated environment. The pathogens normally found in feces bedding materials, and feed. Cases of environmental mastitis rarely exceed 10% of the total mastitis cases in the herd **(Awale *et al*., 2012)**. This imagined why *E.coli is* one of the most frequently isolated pathogens from both clinical and chronic infection, it was more severe than the other bacterial causes and it tended to be more severe in early lactation and during the housing period, resulting in inflammation that ranges from subacute to per-acute. Recurrent clinical mastitis caused by *E. coli* in a cow that express persistent intramammary infection (IMI) is known to exist in the same quarter can be caused by a persistent IMI or may be associated with recurrent IMI. **(Lipman *et al.,* 1995) Thomson *et. al.,* (1988) and Salwa *et. al.,* 2011.**

Forty four *Staph.aureus* isolates (60%) was sensitive to Ciprofloxacin followed by gentamycine (58. %) and enrofloxacin (50%) Similar antibiogram patterns were reported by **Sumathi *et al.,* (2008)** and **Choudhuri (2000)**, workers found highest sensitivity of mastitic agents to enrofloxacin, gentamycine **(Kumar and Sharma, 2002**) and Chloramphenecol (**Rao *et al.,* 1989**) and least sensitivity to Ampicillin (**Dhakal et al., 2007)** and Cloxacillin (**Rao *et al.,* 1989)**. More number of isolates showed resistance to Amoxicillin, Ampicillin, Cloxacillin, Doxycyclilin, ox tetracycline and streptomycin. Indiscriminate and frequent use of these antibiotics in animals could be the reason for their ineffectiveness against staph. aureus isolates. Similar antibiogram pattern were reported by **Goswami et al (2002), and Chhabra and Arora (2006).**Treatment failure in mastitis is due to indiscriminate use of antibiotics without testing in vitro sensitivity. This practice increases economic losses due to costly treatment and also results in development of resistance to antimicrobials. Antibiogram studies are essential to avoid further complications. Identification of mastitis causing pathogens and the results of the antibiotic resistance of the isolated bacteria are important prerequisites for implementation of effective control of mastitis **(Dhakal *et al*., 2007).** The poor response of *S. aureus* mastitis to antibiotic and low success rate include poor penetration of antibiotics into areas of scarring and inflammation, inactivation of antibiotics by milk and serum components, intracellular or metabolically inactive organisms, bacterial forms, resistance to antibiotics and improper treatment procedures. *Staphylococcus aureus* as a result of toxin produce the cell membrane was destroyed and directly damage milk producing tissue. White blood cells (leukocytes) are attracted to the ar-

ea of inflammation where they attempt to fight off the infection. Initially, the bacteria damage the tissues lining the teat and gland cisterns within the quarter. Then they move up into the duct system and establish deep-seated pockets of infection in the milk secreting cells (alveoli) **fig(1).**This is followed by walling-off of bacteria by scar tissue and the formation of abscesses, which is responsible for the poor response to antibiotic treatment. Alveolar and duct cells may be destroyed and milk yield is reduced. These degenerated cells may combine with leukocytes and clog the milk ducts that drain the alveolar areas, contributing to further scar tissue formation. The ducts may reopen and release ***S. aureus*** organisms to other areas of the mammary gland. Further abscess formation occurs resulting in spread the infection to other animals and become as a source of infection also antibiotic sensitivity test reveled resistance and it becomes difficult to successfully treat because the drugs are not able to penetrate to all infection sites and because the bacteria live inside the white blood cells, ***Staph.aureus*** produces an enzyme that inactivates most penicillin-based treatments, resulting in ineffective antibiotics as shown in antibiotic sensitivity test. Hematological evaluation of diseased cows showed significant decrease in erythrocytic count, hemoglobin concentration and packed cell volume. These changes may be due to the harmful effect of bacterial toxin on the haemopoietic system and also the effect of toxin on the endothelial cell of blood vessels The finding was in agreement with **Lovell (2001**) who reported that infected cow developed anemia, (**jain 2000**) stated that bacteria exotoxin cause massive removal and The finding was in agreement with **Lovell (2001**) who reported that infected cow developed anemia, (**jain 2000**) stated that bacteria exotoxin cause massive removal and degradation of red cell membranes by the reticuloendothelial system, hemolytic anemia was resulted. Infected cow showed significant leucocytosis with absolute neutrophilia, lymphocylosis on the endothelial cell of blood vessels; this result was agreement with that reported by (**Coles 1986**) these changes may be attributed to bacterial infection **Lovell (2001)**.

In our study the obtained results could be attributed to the tissue destruction and stimulation of leucopoiesis by the nucleic products) The serum level of total protein were scientifically decrease due to liver damage loss of appetite and malnutrition in diseased cow significant elevation was observed in the percent of gamma globulin , may be due to production of antibodies and may be due to increase lymphocyte **(Schalm 1986))**. serum biochemical analysis diseased cow revealed an impairment of liver function, significant increase in ALT , AST and AP activities this elevation is may be due to bacterial exotoxin on the liver reported that ALT is more specific than AST in early hepatocellular damage while AST has both cytoplasmic and mitochondrial significant elevation was observed in the percent of gamma globulin , may be due to production of antibodies and may be due to increase lymphocyte (**Schalm 1986)) .**serum biochemical analysis of diseased cow revealed an impairment of liver function, significant increase in ALT, AST and AP activities this elevation is may be due to bacterial exotoxin on the liver (**Withby *et al.*,1988**) reported that ALT is more specific than AST in early hepatocellular damage while AST has both cytoplasmic and mitochondrial isoenzymes and tend to released more than ALT in chronic hepatocellular disease .The authors added that alkaline phosphatase is anchored to cell membrane and particular in billiary canaliculi our results agree with **(Schalm 1986** Non significant changes were noticed in urea and creatinine. The bacteria not effect on kidney, our results agree with **(Schalm 1986 and Elsangart 2011)** who reported that the blood picture of buffloes suffering from sub clinical masitis showed anemia associated with significance increase in total leucocitic count with nutrophilia and eosinopeni **Fabiny and Eringhausen (1971).** The most pronounced pathological changes in under tissue consisted of focal damage to alveolar epithelium, degenera-

tive and necrotic change the picture is in close resemblance to those described by **Corleton and Megaviv, 1995**, **Fatma *et al* .,2003**. Since milk accumulation encourages bacterial proliferation **Alawa *et al.*, 2000** where the udder distention will predispose it to insult and trauma and the udder become more vulnerable for the acute necrotizing action of bacteria toxins which play a great role in tissue damage this observation support our result.

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Sastry G.A. (1983). Veterinary Pathology 6th edition.CBS Publishers and Distributors. Delhi **Secondruforum RUFORUM Biennial Meeting 20 -24 September 2010, Entebbe, Uganda** Milking procedures and prevalence of mastitis and mastitis specific-pathogens in selected small holder dairy herds in Zimbabwe Simbarashe Katsande1, Matope, G.2, Pfukenyi, D.M.3 & Ndengu, M.3 Department of Animal Science, Faculty of Agriculture and Veterinary Sciences, P. O. Box MP 167

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