Relationship between environmental microbial pollutants and mastitis in Egyptian buffaloes

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Abstract

The present work was conducted on 145 buffaloes, 4-8 years old and belonged to some governmental and private farms at Assiut governorate, Egypt. 91 milk samples were aseptically collected from mastitic animals. 494 apparently normal milk samples were collected from the normal healthy quarters of buffaloes and used for California mastitis test. 28 air samples and 40 soil samples were aseptically collected from the environment of animal enclosures. All the collected samples as well as those positive to California mastitis tests were bacteriologically examined. Affected animals with acute mastitis showed rise of body temperature (38.8-40.7ºC). Affected udders were red, swollen, hot and painful on palpation. Sometimes animals were inappetance. Cases with chronic mastitis showed variable degrees of tissue reactions including fibrosis and atrophy. Mastitic milk becomes watery, clotted, gelatinous and sometimes tinged with blood. Various bacterial isolates were found in milk samples of all cases with clinical mastitis. On the other hand, all positive milk samples to California mastitis test (19.2%) showed various bacterial isolates. The most prevalent bacterial isolates in mastitic buffaloes were *Strept. agalactiae* (76.5, 92, 9%), *Staph. aureus* (52.9, 71.4%), *E. coli* (30.2, 50.0%), *Enterobacter aerugenes* (35.7, 50.0%), and *Corynbact. S*pp.(27.9, 60.0%). Lower percentages were noticed with other isolated types. It seemed that, there is a great correlation between bacterial milk isolates and those of the environment (air and soil). Gentamycin and kanamycin were seen to be the drug of choice for control of mastitis in buffaloes.

Key words: mastitis , buffalo diseases , water pollution , environmental pollution, soil microbes .

Introduction

Buffaloes are regarded as the most important dairy animal in Egypt due to their milk with high fat content. Mastitis is considered one of the most important diseases affecting dairy herds and individual lactating animals. The disease causes great economic losses as it affects milk quality and quantity, high costs of drugs used for treatment and the possibility in loss of infected quarter **(Draz, A.A.** and **El. Gohary, 1992).**

From the public health point of view, consumption of mastitic milk causes many digestive troubles, in addition to many zoonotic diseases as brucellosis and tuberculosis **(Coles, 1980).** Air quality of animal houses is considered an important factor affecting the health and production of animals. Air may contain dust particles loaded with different types of microorganisms that may be disseminated in surrounding environment **(Blood, *et al*. 1983).** The soil and bedding materials provide excellent media for the maintenance and survival of numerous pathogens for considerable periods during which they may attack the healthy animals **(Samaha, 1983).**

The major method of transmission of mastitis pathogens from environment to the animal is by inadequate environmental management which includes wet bedding, dirty soil, milking wet udders, poor udder preparation, and dirty hands of milkers or milking machine problems. The aim of the present work is to study the bacteriological causes of mastitis and its incidence in buffalo breeding farms in Assiut governorate, Egypt and under the impact of some environmental factors. Also, the work will describe the suitable hygienic measurements that

can be taken in such conditions.

Material and Methods

The present work was conducted on 145 Egyptian native breed dairy buffaloes aged between 4 to 8 years. The animals belonged to some governmental and private farms in Assiut governorate, Egypt. The work was carried out during the period between November 2008 to June 2010.

Collected samples:

1-Milk samples: About 7 ml of milk samples were collected from quarters whether apparently healthy or show signs of mastitis in sterile McCartney bottles. Total of 43 mastitic milk samples (33 acute, 10 chronic), were collected from affected animals of the governmental and 48(34acute, 14 chronic), were collected from affected animals of private farms . Also, 95 apparently normal milk samples were collected (52) from some healthy governmental and (43) from private animal farms. The samples were kept in ice box (at 4°C) and transferred to the laboratory with a minimum delay for California test **(Crist and Harmon, 1991)**. The only positive samples to the test as well as those with clinical mastitis were bacteriologically examined **(Sobrial *et al.*, 1997).**

2-Air samples: About 20 ml sterile saline in sterile Petri-dishes was used for collection of air born bacteria from various animal houses. Total of 28 air samples were collected from the animal farms. The samples were collected at the mid-day and during the ordinary activities of animals and their attendants. The liquid was moved inside animal houses to trap the suspended dust particles. The collected samples were carried in ice box (at 4°C) with minimum delay for bacteriological examination **(Sobrial, *et al.*, 1997).**

3- Soil samples: Total of 40 soil samples were collected at various time periods from different places of animal houses. Each sample was removed at a depth of 5 cm, collected in sterile capped bottle, kept in ice box and delivered to laboratory for bacteriological examination **(Sobrial *et al.*, 1997)**.

4- Antibiogram pattern: In-vitro susceptibility of the organisms to various commercial antibiotic agents was tested by the disc diffusion technique on Muller-Hinton agar. The plates were incubated at 37°C. Zones of growth inhibition were evaluated according to **CLSI (2002)**.

Results

A- Buffaloes with clinical mastitis: Cases with acute mastitis showed rise of body temperature (range 38.8- 40.7ºC). Affected udders were red, swollen, hot, and painful on palpation. Sometimes the animals refused the food. The milk became watery, clotted, gelatinous, and sometimes tinged with blood. Cases with chronic mastitis showed a variable amount of tissue reactions including fibrosis and atrophy. Prevalence of mastitis as well as the isolated bacterial causes was shown in tables 1 and 2.

B- Buffaloes with subclinical mastitis: The condition is considered a serious form as it happens with no obvious symptoms and the animals produced apparently normal milk for long time. Out of 277 and 217 tested milk samples, 52(16.3%) and (16.9%) were positive for California mastitis test (CMT). Tables 1 and 2 showed the frequency of isolated bacteria in both clinical and sub clinical mastitis of farm animals.

C- Bacterial isolates of air and soil: The numbered and frequency percentages of bacterial isolates in air and soil samples in the environment of the farms were shown in (table, 3).

D- Antibiogram: The obtained results revealed variations in the in vitro- sensitivity tests to the various chemotherapeutic and antibiotics used. The results showed that Gentamycin and Kanamycin were the most effective antibiotics for the control of mastitis in buffaloes (table, 4).

Table (1). Prevalence of bacterial mastitis among buffaloes

Animals	No. of exam. animals	No. of exam. quarters	Total no. of +ve quarters	%	Types of mastitis				Negative quarters	
					clinical		Subclinical			
					Total		Total			
					No.	%	No.	%	No.	%
Governmental Farms	80	320	95	29.7	43	13.4	52	16.3	225	70.3
Private farms	65	260	91	35	48	18.5	43	16.9	169	65

Table (2). Prevalence of isolated bacteria among dairy buffaloes with mastitis in governmental and private farms.

Isolated bacteria	Clinical mastitis								Subclinical mastitis			
	Gov. farm				Private farm				Gov.farm (52)		Priv.farm (43)	
	Acute(33)		Chronic (10)		Acute (34)		Chronic (14)					
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Strept. Agalactiae	20	60.6	5	50	26	76.5	13	92.9	13	25	25	58.1
Strept.Dysagalactiae	0	0	1	10	3	8.8	0	0	0	0	8	18.6
Staph..Epidermidis	8	24.2	6	60	0	0	2	14.3	6	11.5	11	25.6
Staph. Aureus	12	36.4	7	70	18	52.9	10	71.4	7	13.5	10	23.3
E.coli	5	15.2	5	50	5	14.7	3	21.4	5	9.6	13	30.2
Klebsiella.Pneumoniae	8	24.2	4	40	2	5.9	0	0	0	0	3	7.0
Pasteurella.Aeruginosa	3	9.1	3	30	1	2.9	3	21.4	0	0	4	9.3
Entero.aerugenes	4	12.1	5	50	11	32.4	5	35.7	1	1.9	5	11.6
Coryn. spp.	9	27.3	6	60	8	23.5	4	28.6	3	5.8	12	27.9

Table (3). Occurrence of bacteria in air and soil samples in houses of dairy buffaloes

Isolated bacteria	Governmental farms				Private farms			
	Air (no.14)		Soil (no.20)		Air (no.14)		Soil (no.20)	
	No.	%	No.	%	No.	%	No.	%
Staph.Aureus	3	21.4	5	25	4	28.6	6	30
Strept.Epidermidis	1	7.1	3	15	1	7.1	3	15
Strept.Faecalis	0	0	2	10	1	7.1	3	15
Staph.bovis	1	7.1	2	10	2	14.3	2	10
Strept.Faecium	1	7.1	3	15	2	14.3	4	20
Staph.durans	0	0	3	15	1	7.1	2	10
Coryn.spp.	3	21.4	5	25	5	35.7	7	35
E.coli	2	14.3	13	65	3	21.4	16	80
K.pneumoniae	0	0	1	5	1	7.1	3	15
Pasteurella aeroginosa	1	7.1	2	10	1	7.1	4	20
Proteus spp.	0	0	3	15	1	7.1	2	10

Table (4). Antibiogram pattern of most bacterial isolates from mastitic buffaloes.

Pathogens(No.) / Chemotherapeutic antibiotic discs ↓	*S.Agalactiae(102)*	*S.Dysagalactiae(12*	*S.Epidermidis(33)*	*S. Aureus(64)*	*E.coli(36)*	*k.pneumoniae(17)*	*P.aeruginosa(14)*	*E.Aerogene(31)*	*Coryn. Spp.(42)*
Ampicillin	36	0	12	13	0	0	0	0	4
(25 ug)	35.2	0	36.4	29.7	0	0	0	0	9.5
Cefacetrile	48	1	17	10	3	9	0	2	8
(30ug)	47.1	8.3	51.5	15.6	8.3	52.9	0	6.5	19
Choramphenicol	62	0	24	41	0	2	0	0	11
(30ug)	60.8	0	72.7	64.1	0	11.8	0	0	26.2
Erythromycin	10	3	5	7	2	3	0	12	15
(5ug)	9.8	25	15.2	10.9	5.6	17.6	0	38.8	35.7
Gentamycin	100	10	29	56	21	51	11	8	28
(10ug)	98	83.3	87.9	87.5	58.3	88.2	78.6	25.8	66.7
Kanamycin	98	10	28	58	29	16	14	26	21
(30ug)	96	83.3	84.8	90.6	80.6	94.1	100	83.9	50
Oxolinic acid	0	0	0	0	0	0	0	0	0
(2ug)	0	0	0	0	0	0	0	0	0
Penicillin	78	5	18	15	8	8	0	0	3
(10ug)	76.5	41.6	54.5	23.4	22.2	47.1	0	0	7.1
Streptomycin	76	7	12	45	36	7	9	7	0
(10ug)	74.5	58.3	36.4	70.3	100	41.1	64.3	22.6	0

Discussion

Buffaloes have some characteristics that may contribute to greater risk of mastitis. For example, the udder is more pendulous and teats are longer in comparison with cattle. Conversely, buffaloes have a long narrow teat canal, which may be expected to prevent the invasion of microorganisms **(Moroni *et al*., 2006)**.

The prevalence of mastitis (table, 1) among the governmental buffalo farms (13.4) was lower than that of the private farms (18.5%). The same trend was observed in the subclinical cases. The negative quarters for MCT were 225 milk samples (70.3%) in governmental farms that were higher than that of private farm animals (169 milk samples, 65%).

Our data in this respect were greatly lower than other investigators that may attributed to the variations in the hygienic management. (Table2), revealed isolation of different types of bacteria from mastitic buffaloes with various frequencies according to type of mastitis. The incidence of *Strept. agalactiae, Staph. aureus* and *Enterobacter aerugenes* in private farms with acute were 76.5%, 52.9% and 32.4% respectivily and that was higher than those of governmental buffalo farms. Also, the isolates of these microorganisms were higher in private farm animals with chronic mastitis than cases of other farms. Lower data were previously reported **(Mosharaf, 2004)**.

Higher incidence of *Strept. agalactiae* and *Staph. aureus* (58.1% and 23.3%) were reported in private farm animals with subclinical mastitis as compared with the governmental farm animals (25% and 13.5%) respectively. Mean while, lower data of bacterial incidence in german dairy cows with subclinical mastitis were previously reported **(Mosharaf, 2004)**.

The high prevalence of clinical and subclinical

mastitis in dairy cows of southern Ethiopia was mainly caused by *Staph. aureus, Strept. agalactiae* and *E. coli* **(Ahmed, 1993)**. *E. coli* and *Corynbacterium spp*. constituted prominent incidence in buffaloes with subclinical mastitis were high (30.2% and 27.9%) in the governmental and private farms respectively.

The incidence of isolated bacteria from mastitic Egyptian buffaloes were *E. coli* (41.02%), *Staph. aureus* (21.09%), *Strep. dysagalactiae* (10.16%), *Strept. epidermidis* (9.38%) and *Strept. agalactiae* (8.59%), **(Bramley *et al*., 1996)** that slightly varied with our results. *Klebsiella. pneumoniae* and *Pasteurella. aeruginosa* were isolated from many cases of clinical and subclinical mastitis of animals in both groups (Table, 2).

In general, the difference in microbial incidences may be explained on the basis of environmental conditions, endemic state of mastitis pathogens, animal management, resistance of animals, technique of milk sampling and the subsequent handling of collected samples. Also, the effect of season of the year may have a great influence on microbial incidence. (Table 3), showed high values of incidences of *Staph. aureus* (28.6%), *S. bovis* (14.3%), *S. faecium* (14.3%), *corynbacterium spp*. (35.7%), *E. coli* (21.4%), *K. pneumoniae* (7.1%) and *proteus spp*. (7.1%) in air samples of the enclosures of the private buffalo farms as compared to the governmental ones. Also, other types of bacteria as *Strept. epidermidis*, *Strept.faecalis, Staph. durans* and *Pasteurella. aeruginosa* were present in small percentages as air, impurities in animal dwellings.

Air samples collected from cattle byres in Egyptian environment revealed the presence of many types of microorganisms with various percentages **(Dego and Tareke, 2003)**. It was clear from table 3 that, the frequency of various isolated bacteria from the soil samples were higher than those recorded in air samples of enclosures of farm animals.

Our results revealed that*, Staph. aureus, Strept. epidermidis, Strept. faecium, Corynbacterium spp., E. coli, k. pneumoniae* and *P. aeruginosa* were the highly predominant types of bacteria isolated from the soil of animal enclosures. Our results were nearly similar to some investigators **(Quinn *et al*. 1994** and **Quinn, *et al*. 2002)**. It was clear that dirty soil might act as a potential reservoir of several Pathogens as causative agents for diseases. Table 4, indicated that there was a marked difference regarding the sensitivity to antibiotics between the different bacterial isolates.

Although **(Alacam *et al*., 1989; Ozenc, 2008)** found that the most effective antibiotics determined against IMI agents isolated from buffaloes infected quarters under in vitro conditions were Cephtiofur and trimetoprim+ sulfametoxazol. Penicillin, cloxacilin and bacitracin had the lowest sensitivity for all isolates. It seems that cephalosporins and enrofloxacin are the best antibiotic agents against S. aureus. Others have reported that in buffaloes, amoxicillin + clavulanic acid were most effective. Gentamycin and kanamycin were found to be the antimicrobial agents of choice that can be used for treatment and control of mastitis in buffaloes in Egypt.

Variable degrees of sensitivity were observed with the other used antibiotic discs. Oxolinic acid appeared to be ineffective chemotherapy. Variation in sensitivity may be due to the nature distribution of bacteria, regional differences, wrong dose, duration of the drugs as well as the plasmid that help in formation of resistant natural strains.

Conclusion

The animal environment constitutes a dangerous vehicle for certain pathogens of veterinary and human importance.

It has been proved that the air and soil of animal enclosures are one of the sources of pathogenic and potentially pathogenic microorganisms.

There was a great prominent correlation between mastitis and the environmental pathogens. Air inside animal enclosures should be improved. Soil of animal enclosure should be always kept clean with hygienic disposal of secretions and excretions as well as animal wastes.

Teat disinfect must be carried out either immediately prior to milking or more commonly after milking. Gentamycin and kanamycin were proved to be highly effective antibiotics that can be used for treatment of mastitis in buffaloes.

Slaughtering of all buffaloes with recurrent or chronic mastitis was among the most important preventive measures of control.

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العلاقة بين التلوث الميكروبي البيئي والتهابات الضرع في الجاموس المصري

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الملخص العربى

اشتملت الدراسة على 145 من سلالات الجاموس المحلي من عمر 4-8 سنوات في بعض مراكز ومزارع محافظة اسيوط العينات الماخوذة من لبن الحيوانات ظاهريا سليمة او تظهر عليها اعراض التهاب الضرع

العدد الكلي من 43 عينة لبن (33 حاد و10 مزمن) وايضا 95 عينة من البان طبيعية ظاهريا من مزارع المحافظة نقلوا الى المعمل لعمل اختبار كاليفورنيا على عينات اللبن. العينات الموجبة للاختبار تم عمل فحص بكتريولوجي لها. تم ايضا اخذ عينات من الهواء والتربة المحيطة بالحيوان وعمل فحص بكتريولوجي لهم . ثم لمعرفة المضاد الحيوي المناسب للعلاج تم عمل اختبار حساسية الميكروب للمضاد الحيوي حيث اثبت ان المضاد الاكثر فاعلية للعلاج هو جنتاميسين اوكاناميسين . وبفحص الضرع المصاب اكلينيكيا وجد انه متورم احمر اللون وملتهب اما اللبن من الضرع المصاب فكان مائي به تجلطات ومواد جيلاتينية احيانا ممزوجة بهم .

من الفحص والتحليل اتضح ان 217 عينة لبن موجبة لاختبار كاليفورنيا .تم عزل عدد من البكتريا من الهواء والتربة المحيطة بالحيوان . اوضحت الدراسة ايضا ان مزارع المحافظة للجاموس اقل في التعرض للاصابة من مزارع الاهالي