

PREVALENCE OF BOVINE SUBCLINICAL MASTITIS IN DAIRY COWS AND BUFFALOES IN SOME VILLAGES IN ISMAILIA GOVERNORATE

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Abstract

A total of 173 quarter milk samples collected from 297 dairy cows and 178 buffaloes belonging to housed bovine animals of the farmers in some villages of Ismailia Governorate, was subjected to C.M.T. to determine the subclinical mastitic cases. The incidence of subclinical mastitis was 22.2% in cows and 14.6% in buffaloes.

The occurrence of subclinical mastitis, as related to the age of animals, showed that the incidence was increased with advancing age.

Most of subclinical mastitic cases were recorded in one or two quarters.

Bacterial examination of the milk samples revealed various pathogens. *Staph. aureus* was the prevalent microorganism in dairy cows, while, in dairy buffaloes, *Escherichia coli* was the predominant organism.

INTRODUCTION

Bovine mastitis is the most costly disease of dairy cattle. It causes great economic losses due to reduction of milk yield and milk quality, in addition to the public health problems from consuming infected milk. Also, using antibiotic in treatment of mastitic cases without following the medical rules, consumption of milk of treated udder may cause allergy to consumers in addition to the possibility of acquiring resistance to the applied antibiotics.

The incidence of subclinical mastitis in Egypt was recorded by Abd El-Moniem (1979) in cows and buffaloes in Sharkia, Akl (1988), in buffaloes in Behira, Radwan (1988) in cows and buffaloes at Kafr El-Sheikh, El-Rashidy *et al.* (1990) of lactating cows and buffaloes in Giza Governorate and Morcos *et al.* (1991) in dairy cows in

Nubaria. The difference in results of subclinical mastitis in different governorates may be due to the hygienic measurements, animal case and management.

Therefore, the aim of the present work was to study the incidence of subclinical mastitis among cows and buffaloes in some villages of Ismailia Governorate in relation to many factors. This work included the isolation and identification of the causative agents, application of sensitivity test and finally suggesting the methods of control.

MATERIALS AND METHODS

Collection of milk samples :

173 quarter milk samples were collected aseptically from 297 apparently healthy dairy cows and 178 buffaloes, aged between 3-13 years old. They were subjected to California Mastitis Test (C.M.T.) according to the method recommended by Schalm *et al.* (1971). Positive milk samples were immediately kept in an ice box and examined bacteriologically.

Bacteriological examination :

Bacterial cultures were made from milk after pre-incubation at 37°C, for 18-20 hours (Schalm *et al.* 1971 and Aziz *et al.* 1975). Culture media used were sheep blood agar for the most of the bacteria causing mastitis, MacConkey agar plate to any Gram-negative bacteria that can be able to grow on the medium, Edward's medium which is highly selective for streptococci, Mannitol salt agar for staphylococci. The main characteristics and tests for identification of isolated organisms were colonial appearance, Gram stained smear, catalase test, oxidase test and haemolysis on blood agar.

The staphylococci were further identified by means of coagulase production using staphylase test.

The streptococci were identified by catalase test, CAMP reaction, and fermentation of sugars, viz, asculin, raffinose, salicin, inulin, trehalose, sorbitol, mannitol, maltose, sucrose and lactose.

Coliform bacteria were identified, using indole production, urea hydrolysis, growth in citrate, triple sugar Iron reaction, methyl red, Voges Proscour and

motility tests. Identification of the isolated microorganism was done according to Koneman *et al.*, (1988) and Quinn *et al.* (1994).

Sensitivity test :

Susceptibility of the most predominant isolates to different antibiotics was done by disc diffusion method according to Finegold and Martin (1982). The antibiotic discs used were penicillin (10 i.u.), ampicillin (10 mg), streptomycin (10 mg), neomycin (30 mg), tetracycline (30 mg), gentamicin (10 mg), polymyxin B (300 i.u.) chloramphenicol (30 mg) and nitrofurantion (300 mg) (oxiod).

RESULTS

Of the 475 dairy cows and buffaloes examined by California Mastitis test, 92 animals (19.4%) were found to be suffering from subclinical mastitis.

Bacteriological examination of 173 quarter milk samples showed that all of them were positive.

Regarding the occurrence of subclinical mastitis as related to the age of animal, Table 1 showed that the incidence was increased with advancing age.

Table 2 showed that most of subclinical mastitic cases had one or more quarters infected.

Relative frequency of different types of organisms in cows, Table 3 revealed that, *Staph aureus* was the predominant organism (32.78%), while, in buffaloes the predominant organism was *Escherichia coli* (43.14%). From 144 organisms isolated from cows and 60 organisms from buffaloes, there were double infections illustrated in Table 3 and Fig.3.

In vitro, antibiotic susceptibility of 204 isolates from cows and buffaloes, showed that 99.5% of isolates were sensitive to gentamicin followed by a descending order nitrofurantion, streptomycin neomycin, ampicillin, tetracycline, and chloramphenicol, while, penicillin and polymixin B showed restricted efficiency (Table 4).

DISCUSSION

Subclinical mastitis is prevalent among dairy cows and buffaloes in Egypt.

This present survey has estimated the incidence in the housed bovine animals of the farmers in some villages of Ismailia Governorate.

The incidence recorded in this study was 22.2% and 14.6% among cows and buffaloes, respectively. Similar data were recorded in Egypt by Aranaout (1961) among cows (22.5%) and El-Rashidy *et al.* (1990) among buffaloes. The present percentages are considered to be lower than those reported by many investigations on large herds level recorded by El-Bayomi and Mahmoud (1987), 51.79% in cows and El-Rashidy *et al.* (1988), 62.08%, Radwan (1988), 51.11%, 39.71% of cows and buffaloes, respectively.

On the other hand, El-Guindy *et al.* (1964), Nada and Khalil (1966), Nada and Goda (1975) reported a lower incidence of bovine subclinical mastitis.

According to the relationship between age and the incidence of subclinical mastitis, the obtained results showed that the incidence was increased with advancing age (Table 1). These results are in agreement with the findings of Abd El-Moniem (1979), Wilesmith *et al.* (1986) and Amany (1993).

Table 1. The occurrence of subclinical mastitis related to age of animal.

Age/years	Cow			Buffalo		
	Exam.	Infect.	%	Exam.	Infect.	%
3-5	70	13	18.6	39	2	5.13
5-7	128	25	19.5	72	10	13.88
7-9	73	21	28.77	63	11	18.03
9-11	16	7	43.75	6	3	50
11-13	10	0	0	0	0	0
Total	297	66	22.2	178	26	14.6

It was evident from the present study, that, most infected cases involved one or two quarters (Table 2). Also, El-Rashidy *et al.* (1990) reported that, cases of one or two quarters infected were detected in an incidence of 54.14% and 27.61%, respectively, in cows.

Table 2. Distribution of intramammary infection in quarters (66 cows and 26 buffaloes).

Type of animal	One quarter		Two quarters		Three quarters		Four quarters	
	No	%	No	%	No	%	No	%
Cow	22	33.3%	28	42.2	8	12.1	5	7.6
Buffalo	10	38.5%	10	38.5	3	11.5	3	11.5

The results showed that *Staph aureus* was the most frequent organism in cows representing 32,78% of examined milk samples (Table 3). These results agreed with those of Said and Amira (1968), Aziz *et al.* (1975), Nada and Goda (1975), Tawfik *et al.* (1984) in Egyptian Buffaloes, Morcos *et al.* (1991) in cows.

Table 3. Frequency of different types of organisms isolated from milk samples.

Organism	Cow (122 milk samples)		Buffalo (122 milk samples)	
	No.	%	No.	%
Single infection :				
staph. aureus	40	32.78	11	21.57
Staph. epidermidis	18	14.75	6	11.76
E.coli	30	24.59	22	43.14
Str. agalactiae	7	5.38	2	3.92
K.pneumoniae	5	4.10	1	1.96
Mixed infection :				
Staph.aureus	10	8.20	2	3.92
+Staph. epidermidis				
Staph. aureus	3	2.46	0	0
+ K.pneumoniae				
Staph. epidermidis	1	0.82	0	0
+ E.coli				
Staph. epidermidis	3	2.46	3	5.88
+ E.coli				
Staph. epidermidis	2	1.64	0	0
+ E.coli				
Staph. epidermidis	3	2.46	2	3.92
+ Str.agalacitae				
E.coli	0	0	1	1.96
+ K.pneumoniae				
Str. agalactiae	0	0	1	1.96
+ K.pneumoniae				

Staphylococci are normal inhabitants of the skin, present in greatest numbers on the surface of the udder, and easily transmitted during milking. The types of mastitis produced by *Staph. aureus* range from subclinical to the peracute life-threatening form, one of which is gangrenous mastitis (Quinn *et al.* (1994).

Streptococcus agalactiae was present in 5.38% and 3.92% in subclinical mastitic cows and buffaloes, respectively. The relative importance in this organism has also been shown by its high incidence in other surveys. Zakarya (1969), Khalil

1995/96 are presented in Table (2).

Analysis of variance showed that flax genotypes significantly differed in all the six seed characters i.e., no. of capsules /plant, no. of seeds/capsule, no., of seeds/plant, seed index and seed yield/plant as well as per faddah.

Concerning number of capsules per plant, its mean value ranged from 25.18 for Giza 8 cv. to 10.95 for Gawhar with superiority ratio of 129.95%. The other flax genotypes which recorded higher number of capsules per plant (over 20 capsules) are namely S.2465/1, S.421/3, Giza 6, S.420/140 and Giza 7. While the another flax genotypes yielded less than 20 capsules/plant and more than Gawhar capsules number, where the ascending regularity of these genotypes was as follow : Viking (16.22), Iriana (16.55), S.162/12 (18.17), S.282/98 (18.69), S.329/2 (19.69), S.402/2 (19.71) and Giza 5 cv. (19.78). The grand mean for this trait was 19.84 ± 1.001 with c.v. % value of 18.86%.

For number of seeds per capsule, results obtained illustrated that Giza 8 variety had the highest mean value (8.42) and the descending regularity was as follows, S.2465/1 (8.26), S.421/3 (7.38), Giza 6 (7.32), S.420/140 (7.04), Giza 7 (7.00), Giza 5 (6.77), S.402/2 (6.74), S.329/2 (6.69), S.282/98 (6.52), S.162/12 (6.37), Iriana cv. (6.30), Viking cv. (6.28) and the lowest number of seeds/capsule was Gawhar (5.67).

Regarding number of seeds per plant, results indicated that this character ranged from 81.70 to 190.25 seed/plant for Gawhar and Giza 8, respectively. The remainder flax genotypes recorded mid estimates between the lowest and highest seeds/plant and the arrangement was in the following manner; S.2465/1 > S.421/3 > Giza 8 > S.420/140 > Giza 7 > Giza 5 > S.402/2 > S.329/2 > S.282/98 > S.162/12 > Iriana > Viking.

In respect with seed index trait, results showed that S.2465/1 was superior and ranked first in its seed index (11.797 g.) followed by Giza 8 cv. (9.537 g.), (S.421 g.), Giza 6 (9.014 g.), 420/140 (8.818 g.), Giza 7 (8.713 g.), Giza 5 (8.411 g.), S.421/3 (8.404 g.), S.329/2 (8.371g.) and S.282/98 (8.09g.) and the lowest four values of this trait obtained by Iriana (4.879 g.), Viking (5.003 g.), Gawhar (7.726 g.) and S.162/12 (8.020 g.).

In relation to seed yield per plant as well as per faddan, similar trend was observed in this concern among the flax genotypes. Data revealed that Giza 8 variety

Table 4. Sensitivity of the isolated strains from subclinical mastitic cows and buffaloes to the various antibiotics..

Organism	No. of tested strains	Antibiotics									
		Gentamicin	Nitrofurantoin	Streptomycin	Neomycin	Ampicillin	Tetracycline	Chloramphenicol	Penicillin	Polymyxin B	
<i>Staph. aureus</i>	67	(67) 100%	(62) 92.5%	(51) 76.1%	(45) 67.2%	(64) 95.5%	(41) 61.2%	(31) 46.3%	(0) 0%	(0) 0%	
<i>Staph. epidermidis</i>	44	(44) 100%	(40) 90.9%	(37) 84.1%	(36) 81.8%	(42) 95.4%	(21) 47.7%	(16) 36.4%	(39) 88.6%	(0) 0%	
<i>E.coli</i>	67	(66) 98.5%	(61) 91.0%	(56) 83.6%	(45) 67.2%	(23) 34.3%	(43) 64.2%	(60) 89.5%	(14) 20.9%	(12) 17.9%	
<i>Str. agalactiae</i>	17	(17) 100%	(16) 94.1%	(11) 64.7%	(15) 88.2%	(11) 64.7%	(13) 76.5%	(6) 35.3%	(4) 23.5%	(2) 11.7%	
<i>Klebsiella</i>	9	(9) 100%	(7) 77.8%	(6) 66.7%	(6) 66.7%	(2) 22.2%	(7) 77.8%	(9) 100%	(1) 11.1%	(0) 0%	
Total (No.) %	204	(203) 99.5%	(186) 91.18%	(161) 78.92%	(147) 72.06%	(142) 69.6%	(125) 61.27%	(122) 59.8%	(58) 28.43%	(14) 6.86%	

() = No. of sensitive.

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REFERENCES

1. Abd El-All, B.E. 1989. Studies on bacteria causing mastitis in cattle in Assiut . Thesis, Ph. D., Assiut Univ.
2. Abd El-Monim M.M. 1979. Incidence of mastitis in a village of Sharkie province and its treatment. Thesis, M.V.Sc. Fac., Vet Med., Zagazig University .
3. Akl, K.M. 1988. Studies on subclinical mastitis in cattle and buffaloes in Behera governorate. Thesis, M.V.Sc. Alex. Univ.
4. Amany, M.R.K. 1993. An investigation on the etiology of subclinical mastitis on bovines. Thesis, M.V.Sc. Fac. Vet. Med., Cairo Univ.
5. Arnaout, A.H. 1961. Incidence of mastitis among buffaloes and cows at the Bahtim breeding station and the economical value obtained after treatment. Egypt. Vet. Med., Assoc, Cairo, 21 : 126 .
6. Aziz, M.A.; Y. Zein El-Abdin and Y.A. Farag. 1975. Control of subclinical mastitis in a buffalo dairy herd. Egypt. J.Vet. Sci., 12 (2) : 135 .
7. Davidson, J.N. 1980. Antibiotic resistance patterns of bvine mastitis pathogens. Proc. NMC 19 th Anim. Mtg. 181.
8. El-Bayomi, Kh. M. and A.A. Mahmoud. 1987. Some genetic and environmental factors affecting the incidence of subclinical mastitis in dairy cows. Vet. Med. J., 35 : 269 .
9. El-Guindy, H., H.F. Farrag, and L. Abou El-Azm. 1964. A study of the treatment of mastitis in buffalo and cow in Egypt. Vet. Med. and Small. Clin., 59 : 380 .

10. El-Rashidy, A.A., L., Bedier, A.Amin, A.S. Mostafa and M.S. Tawfik. 1988. Etiology and diagnosis of subclinical mastitis among herds in Egypt. *J. Egypt. Vet. Med. Assoc.* 48 (2) : 189 .
11. El-Rashidy, Amal A., M.M. El-Garhy, K.N. Metias, A.A. Ahmed and M.S. Tawfik. 1990. Incidence of bovine subclinical mastitis in individual cases belonging to Egyptian Farmers in Giza Province. 4th Sci. Cong., Fac. Vet. Med., Assiut Univ., 3 : 789
12. Finegold, S.M. and W.J. Martin. 1982. *Diagnostic Microbiology*. 6th Ed., The C.V. Mosby Co., St. Louis Toronto, London .
13. Khalil, A.D., A.A. Barakat, E.M. Sayour, M.E. Samira, Gibaly and Y.S. Ismail. 1972. Studies on bovine mastitis. *Proc. 10th Arab. Vet. Cong.*
14. Koneman, E.W., S.D. Allen, V.R. Dowell, H.M. Sommers, M.M. Ianda and W.C. Winn. 1988. *Colour Atlas and Text book of Diagenostic Microbiology*. 3rd Ed., J.B. Lippincott Co., New York, London .
15. Mahmoud, A.A. 1988. Some studies on subclinical mastitis in dairy cattle. *Assiut Vet. Med. J.*, 20 (39) : 149 .
16. Morcos, M.B., Soheir Shoukry, E.R. Zaki E.M. Reyad and H. El-Sawah. 1991. Studies on subclinical mastitis in a commerical dairy herd in Western Nubaria. *J. Egypt. Vet. Med. Assoc.*, 51 (1 and 2) : 261.
17. Nada, S. and F.M. Goda. 1975. Incidence of micro-organisms isolated from mastitis in dairy cattle at El-Haram localities and their sensitivity to antibiotics with special reference to kanamycine in vitro. *J. Egypt. Vet. Med. Assoc.*, 35 (3) : 113 .
18. Nada, S. and M.G. Khalil. 1966. Incidence and antibiotic sensitivity of some organisms isolated from apparent healthy milk. *J. Arab. Vet. Med. Assoc.*, 26 : 241.
19. Quinn, P.J., M.E. Carter, B. Markey and G.R. Carter. 1994. *Clinical Veterinary Microbiology*. Mosby-London. WCTH 9LB, England.

20. Radwan, S. 1988. Relationship between bacteriological examination and enzymatic changes in subclinical mastitis in buffaloes and cattle. Thesis, M.V.Sc., Fac. Vet. Med., Cairo Univ.
21. Said, A.H. and S.A. Amira. 1968. Diagnosis, incidence and treatment of subclinical mastitis in dairy buffaloes. J. Vet. Sci., U.A.R. (5) : 151.
22. Said, A.H. and A.S. Abdel Malek. 1974. Diagnostic tests of mastitis. J. Vet. Sci., U.A.R., 5 (2) : 171.
23. Schalm, O.W., E.J. Carroll and N.C. Jain. 1971. Bovine mastitis. School Vet. Med. California, Univ, Davis, California .
24. Tawfik, M.S., S.M. Nashed, G.F. Goda, S.M. El-Kholy, A.F. Farid and S. Nada. 1984. Preliminary studies on bacteria recovered from milk of apparently healthy udders in dairy cattle. Ass. Vet. Med. J., 12 (23) : 91.
25. Wilesmith, J.W., P.G. Francis and C.D. Wilson. 1986. Incidence of clinical mastitis in a cohort of British dairy herds. Vet. Rec., 118,199 .
26. Zakarya, A.H.F. 1969. A comparative study on different methods used for detection of subclinical mastitis. Thesis, Ph.D., Fac. Vet., Med., Cairo Univ.

مدى انتشار التهاب الضرع الغير ظاهرى فى الابقار والجاموس الحلابه فى محافظة الاسماعيليه

سهير شكرى و ماجدة شبانا

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شملت هذه الدراسه عمل مسح شامل بين التجمعات الحيوانية الصغيره فى قرى محافظة الاسماعيليه باجراء اختبار حقلى سريع (اختبار كاليفورنيا) على عدد ٢٩٧ بقرة حلابه و ١٧٨ جاموسه حلابه من مختلف الاعمار للكشف عن حالات التهاب الضرع الغير ظاهرى.

وقد تم جمع عدد ١٧٣ عينه لبن إيجابيه للاختبار السريع من عدد (٦٦ بقرة و ٢٢ جاموسه) وفحصها بكتريولوجيا، بفحص العينات على أوساط بيئية مختلفة خاصة بالميكروبات المسببه لالتهاب الضرع وإجراء الاختبارات البيوكيميائية والسيرولوجية على الميكروبات المعزوله أمكن عزل الميكروبات المرضيه بنسبه ٢٢,٢% فى الابقار الحلابه و ١٤,٦% فى الجاموس الحلاب، وكانت أكثر الميكروبات المعزوله الميكروب العنقودى الذهبى فى الابقار والميكروب القولونى فى الجاموس.

وبدراسه تأثير العمر على نسبه التهاب الضرع الغير ظاهرى وجد أن الاصابه تزداد بتقدم العمر.

وقد أوضحت الدراسه العلاقة بين وجود المرض ومدى اصابه اجزاء الضرع كما أجريت اختبارات الحساسيه بالمضادات الحيويه المختلفه للميكروبات التى تم عزلها وقد اعطى الجنتاميسين أحسن النتائج.

هذا ونوصى بضرورة الاهتمام بأرشاد الفلاح والمربى باتباع الطرق السليمه بعملية الحلب وتربيته الحيوانات ورعايتها والفحص الدورى للحيوانات وفحص عينات اللبن بالاختبارات الحقلية السريعة لاكتشاف المرض مبكرا.