

UPDATE ON BOVINE MASTITIS ETIOLOGICAL, CLINICAL AND TREATMENT ASPECTS IN KHARTOUM STATE, SUDAN

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ABSTRACT: This study was conducted in certain area at Khartoum State determine the causative agent of bovine mastitis and the susceptibility of different isolates to different antibiotics use for treatment of bovine mastitis. The total number of dairy cows, which were examined in 34 investigated farms, equals 500. The result as follows: 55% acute mastitis, 44% chronic mastitis and 1% gangrenous mastitis. The isolated genera were as follows: 74% *Bacillus* spp., 24% *Staphylococcus* spp., 1% *Corynebacterium* spp. and 1% *Klebsiella* spp. The isolated species were as follows: 31% *Bacillus coagulans*, 11% *B. cereus*, 9% *B. subtilis*, 9% *B. licheniformis*, 4% *B. circulans*, 2% *B. lentus*, 3% *B. mycoides*, 3% *B. amyloliquefaciens*, 2% *B. megaterium*, 16% *Staphylococcus aureus*, 8% *Staphylococcus hyicus*, 1% *Corynebacterium* spp. and 1% *Klebsiella* spp. Lastly, the sensitivity test was applied using different antibiotics were as follows: Hundred percent of isolates were sensitive for Chloramphenicol and Ciprofloxacin, 91.6% for Gentamycin and Piperacillin/ Tazobactam, 83.3% for Pefloxacin and Tetracycline, 75% for Amikacin and Ofloxacin, 66.6% for Ceftizoxime, 33.3% for Co-Trimoxazole and Cefotaxime and 16.6% for Ampicillin/ Sulbactam. This study was depended at routine works at microbiological laboratory.

Keywords: Bovine Mastitis, Etiology, Clinical, Treatment, Khartoum, Sudan

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INTRODUCTION

Dairy industry has recently grown as a very important economic national source of income. In Sudan, many dairy owners introduced foreign blood. This might result in a progeny of mixed blood cows with lowered resistance to endogenous and locally prevailing diseases such as mastitis. Mastitis is considered the main disease in dairy herds (Kaneen and Bandhard, 1990). Mastitis is the inflammation of the mammary gland due to the injury of any type. However, the udder disease of major concern is that associated with microbial infection (Blood et al., 1983). The most common major pathogens include *Staphylococcus aureus*, *Streptococcus agalactiae*, Coliforms, Streptococci and Enterococci, while *Corynebacterium bovis* were considered to be minor pathogens. In the USA *Mycoplasma bovis* causes loss of \$ 32 per year as a result of the loss of the weight gain and diminished carcass value. (Rosengarten and Citti, 1999; Smith, 1990) reported that 62% of occurrences of clinical mastitis were due to environmental pathogens as coliform bacteria and most species of streptococci. Bovine mortality survey carried out in 1992, identified coliform mastitis as the single most important cause of death in dairy cows (Menziez et al., 1992). The most common coliforms are *Escherchia coli*, *Enterobacter aerogenes* and *Klebsiella* spp. The secretion of the clinically affected quarter is usually brownish and watery (Merk, 1998). Rowan et al. (2003) observed that all strains of *Bacillus* spp. implicated in mastitis and abortion in animals. Karmy (1990) isolated *Streptococcus agalactiae* from 5 cases, *E. coli* from 6 cases and *Corynebactrium* from 4 cases out of 32 camels suffering from mastitis.

The objectives of the study were: to study of the causative agent of mastitis based on clinical examination and apply the sensitivity to various antibiotics on bacterial isolates from mastitic milk samples.

MATERIAL AND METHODS

Sampling

A total of 500 suspected mastitic cows were examined clinically for presence of mastitis. Hundred milk samples from mastitic cows were collected. This was done during period extending from December 2006 to April 2007. Mastitis was diagnosed when there were visible or palpable signs of udder, inflammatory changes in milk secretions, or through bacteriological examination of milk. During the study 100 milk samples were encountered

from 41 cows suffering from clinical mastitis. Examination of each cow was carried out according to the enclosed questionnaire of bovine mastitis, this questionnaire included data about area, cow, udder and milk. The enclosed questionnaire was to determine the roles played by environment, farm hygiene, ventilation, drainage system, floor type and building of farm as a contribution causing factor for mastitis. Milk samples were taken under critical aseptic condition for bacteriological studies by collection in sterile Bijou bottles after cleaning the outer surface of the udder and teat with potassium permanganate and with cotton wool soaked in 70% alcohol. The fore milk was stripped off and about 5 ml of milk were drawn in sterile Bijou bottle. All samples collected were immediately placed on ice in a thermo flask after collection.

PH examination

This test was done to determine milk pH by using of a special paper (manufactured by Kruse Company in Denmark). The test was applied by adding one drop of milk on yellow spot, where in a few seconds the colour was change in positive cases.

Culture

The two media used in culture were Blood agar and MacConkey's agar. Plates were examined for cultural characteristics and biochemical reactions according to standard keys (Barrow and Feltham, 2003).

RESULTS AND DISCUSSION

Questionnaire

The farmers responded highly to the questionnaire regarding general farm data. The main system applied was semi-intensive system. The ventilation was satisfactory in all farms while in the University of Khartoum and Hilat kuku farms were excellent. Drainage system was satisfactory in all farms except Shambat farms which were poor. Farm housing materials were a mixture between modern and traditional. Type of floor was found ranged between earth and concrete (Table 1).

PH examination

A total of 100 milk samples gave a positive reaction to pH paper. Positive sample revealed change in colour from yellow to green or bluish green .because the pH of milk will increase to alkaline in infected cows with mastitis., while negative sample revealed no change of the yellow spots or change to light green.

Clinical status of mammary glands

The greatest number of type of mastitis was acute mastitis (55%), followed by chronic cases (44%) and gangrenous cases (1%) (Figure 1).

Bacterio logical examinations

Four genera of bacteria were isolated from the milk samples. The isolated bacteria were as follows: *Bacillus* spp. (74%), *Staphylococcus* spp. (24%), *Corynebacterium* spp. (1%) and *Klebsiella* spp. (1%) (Figure 2). Figure (3) showed the percentage of isolated *Bacillus* spp. from 100 mastitic cows. Figure (4) showed the percentage of isolated *Staphylococcus* spp., *Corynebacterium* spp. and *Klebsiella* spp.

Sensitivity test

Figure (5) showed the percentage of effectiveness of different antibiotics against different isolated bacteria (Table 2) showed the name and concentration of antibiotics and degree of effectiveness. Drug susceptibility profiles of aerobic bacterial isolates, including *Bacillus* spp., *Staphylococcus aureus*, *Staphylococcus hyicus* and *Klebsiella* spp. were subjected to various drugs. Susceptibility of Gentamycin (GM) and Piperacillin/Tazobactam (TZP) were about (91.6%), (83.3%) were susceptible to Pefloxacin (PF) and Tetracycline (TE), (75%) were susceptible to Amikacin (AK) and Ofloxacin (OF), (66.6%) were susceptible to Ceftizoxime (CI), (33.3%) were susceptible to Co-Trimoxazole (BA) and Cefotaxime (CF), (16.6%) were susceptible to Ampicillin/sulbactam (AS).but the best antibiotics in all this are Chloramphenicol (CH) and Ciprofloxacin (CP) because the percentage of the effectiveness was 100% that mean all isolated organisms are susceptible to these two drugs.

This study was conducted in Omdurman and Khartoum North because these towns are considered as the largest milk producing and marketing. Mastitis is a complex disease caused by several microorganisms.

Table 1 - General farm hygiene, ventilation, drainage system, housing type, building material and floor type

Farm's name	Ventilation	Housing type	General farm hygiene	Drainage system	Building material	Floor type
Falasteen	Satisfactory	Free stall and Pen	Satisfactory	Satisfactory	Traditional	Earth
Eltebna	Excellent	Stanchion	Satisfactory	Satisfactory	Mixture	Concrete
Hilat Kuku	Satisfactory	Free stall and Pen	Poor	Satisfactory	Traditional	Earth
Elhalfaia	Satisfactory	Free stall and Pen	Satisfactory	Satisfactory	Mixture	Earth
Elsamrab	Satisfactory	Free stall and Pen	Satisfactory	Satisfactory	Mixture	Earth
The University	Excellent	Stanchion and pen	Satisfactory	Satisfactory	Mixture	Earth
Shambat	Poor	Free stall	Poor	Poor	Traditional	Earth

Excellent: International Required Standard; Satisfactory: Modered International Required Standard; Poor: Absent of International Required Standard.

Table 2 - Name and concentration of antibiotics and degree of effectiveness

Isolated bacteria	Name, concentration and effectiveness of antibiotics											
	AS 20mcg	BA 25mcg	CF 30mcg	TZP 100/10mcg	CH 30mcg	CP 5mcg	CI 30mcg	TE 30mcg	OF 5mcg	GM 10mcg	AK 30mcg	PF 5mcg
<i>B. coagulans</i>	-	+++	-	-	+++	+++	+++	-	-	+++	-	-
<i>B. cereus</i>	-	-	-	+++	+++	+++	-	+++	+++	+++	+++	+++
<i>B. circulans</i>	-	-	-	+++	+++	+++	-	+++	+++	++	++	++
<i>B. mycoides</i>	-	-	-	+	+++	+++	-	++	-	-	-	-
<i>B. licheniformis</i>	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	+++
<i>B. megaterium</i>	-	-	+++	+++	+++	+++	+	++	-	+++	+++	+++
<i>B. lentus</i>	-	++	+++	+++	+++	+++	-	-	++	++	+++	+++
<i>B. subtilis</i>	-	-	-	+++	+++	+++	+	+++	+++	+++	+++	+++
<i>B. amyloliquefaciens</i>	-	-	-	+++	+++	+++	+	+++	+++	+++	+++	+++
<i>Staph .aureus</i>	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Staph. Hyicus</i>	-	-	-	++	++	+++	+++	+++	+++	+++	+++	+++
<i>Klebsiella spp.</i>	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

(+++): Highly sensitive; (++) : Moderate sensitive; (+): Poor sensitive; (-): Non sensitive

Sandholm et al. (1995) and still it's the most important problem in dairy industry (Dodd 1985; Fetrow and Mann, 1991). Three forms of mastitis were classified in this study acute, chronic and gangrenous mastitis; this classification is different from Philpott (1967) who classified mastitis according to the severity of inflammatory response into four forms peracute, acute, chronic and mild. Most of the surveyed farms were small, so problems of ventilation and drainage have been clearly observed. Most dairy farms building materials were traditional made of mud, wood with old iron sheets for the door. These traditional building may cause an injuries on the udder and teat and hence predispose for mastitis occurrence. The floor surfaces were aclear hazards to the animals. Mud and excessive moisture increase coliform organisms contaminating the udder (William, 1995).

In this study the types of *Staph spp.* isolated from acute and chronic mastitis were *Staph aureus* and *Staph hyicus*. This agrees with the finding of DaRong et al. (2010); Jan et al. (1998). Radostits et al. (1994) mentioned that *Staph aureus* is the first microorganism incriminated in bovine mastitis. A predominance of *Staph aureus* mastitis in cows has been reported by Watts (1988); Falade et al. (1989) and Carlos (1990). Elsayed (2000) isolated *Staph aureus* and *Staph hyicus* from 499 milk samples from different domestic animals: cows, sheep, goat and camels. AlAyies (2004) isolated *Staph aureus* (73.7%) and *Staph hyicus* (6%) from 100 bovine mastitic milk samples. Isolation of *Klebsiella spp.* in this study is in accord with (Cullor, 1992), who found that 20% of bovine mastitic cases, in Nordic countries caused by coliform of which about 85% were *E. coli* and the rest were *Klebsiella spp.*, and other *Enterobacteria* were isolated. This is in agreement with Mc Donald et al. (1970); Ibrahim and Habiballa (1978). To the best of knowledge gangrenous mastitis caused by *Klebsiella spp.* is considered as the first report in Sudan. In this study nine species of *Bacillus* were isolated from acute and chronic mastitis, this in agreement with Jan et al. (1998). The percentage of incidence of *Bacillus coagulans* was high and this confirms the findings of Nail et al. (2003). Also *Bacillus cereus* was also isolated by Nail et al. (2003). Other species of *Bacillus* were isolated like, *B. licheniformes*, and this in accord with results of findings of Logan (1988); Nail et al. (2003); Parvanta (2000). The isolation of *Bacillus alvei*, *B. subtilis*, *B. megaterium* and *B. cereus* during this study in agreement with Elgadasi (2003). *B. licheniformis*, *B. amyloliquefaciens*, *B. circulans*, *B. lentus* and *B. mycooides*, to the best of our knowledge for the first time to be recorded for mastitis in cow in Sudan.

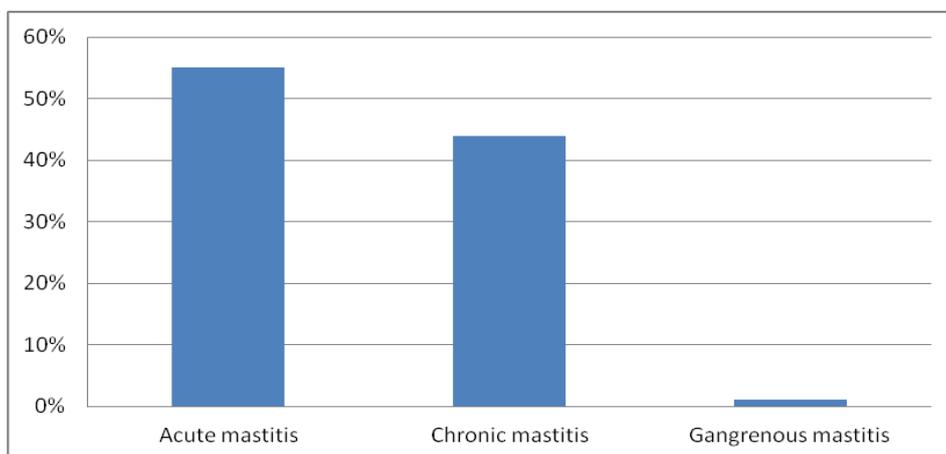


Figure 1. Classification of mastitis according to the clinical state of the mammary gland

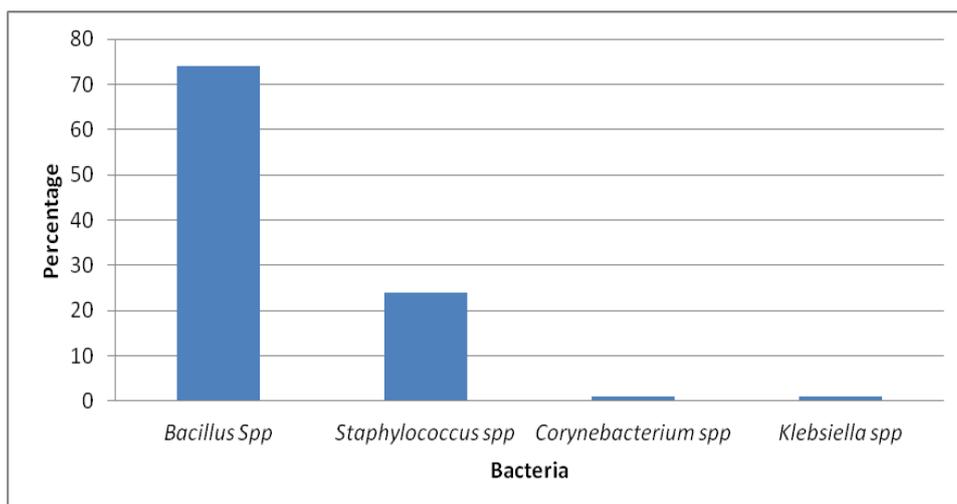


Figure 2. Percentage of isolated bacteria from 100 mastitic cows

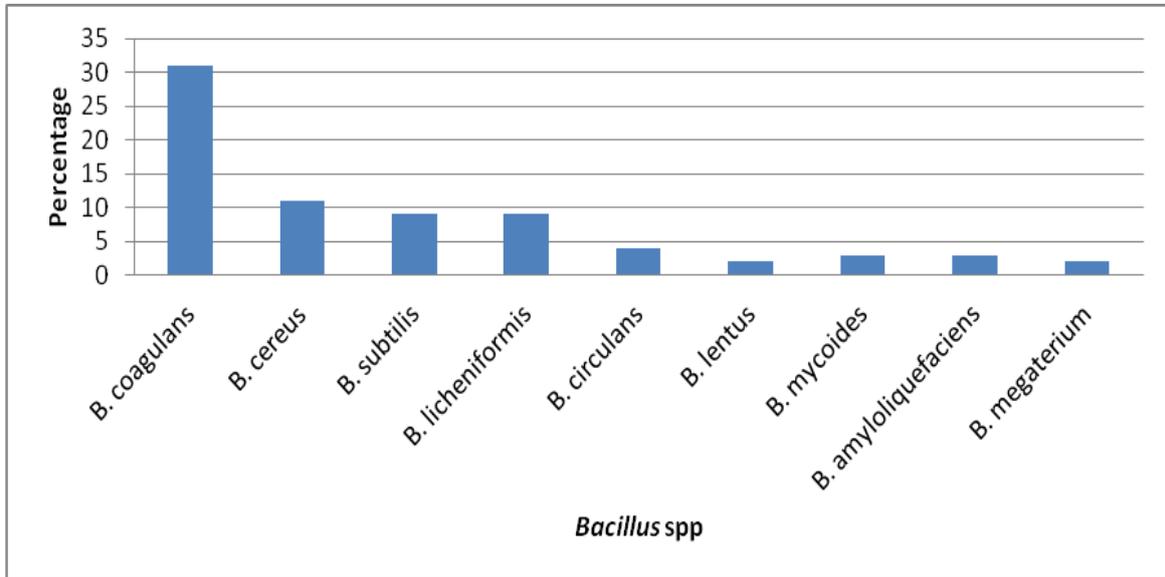


Figure 3. The percentage of isolated *Bacillus* spp. from 100 mastitic cows

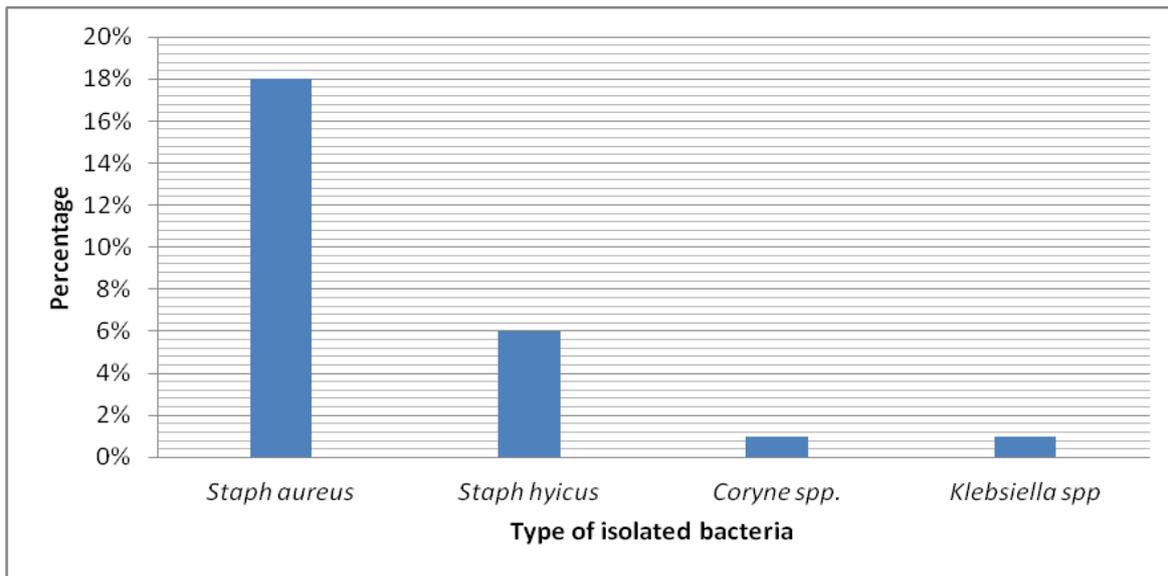


Figure 4. The percentage of other isolated bacteria from 100 mastitic cows

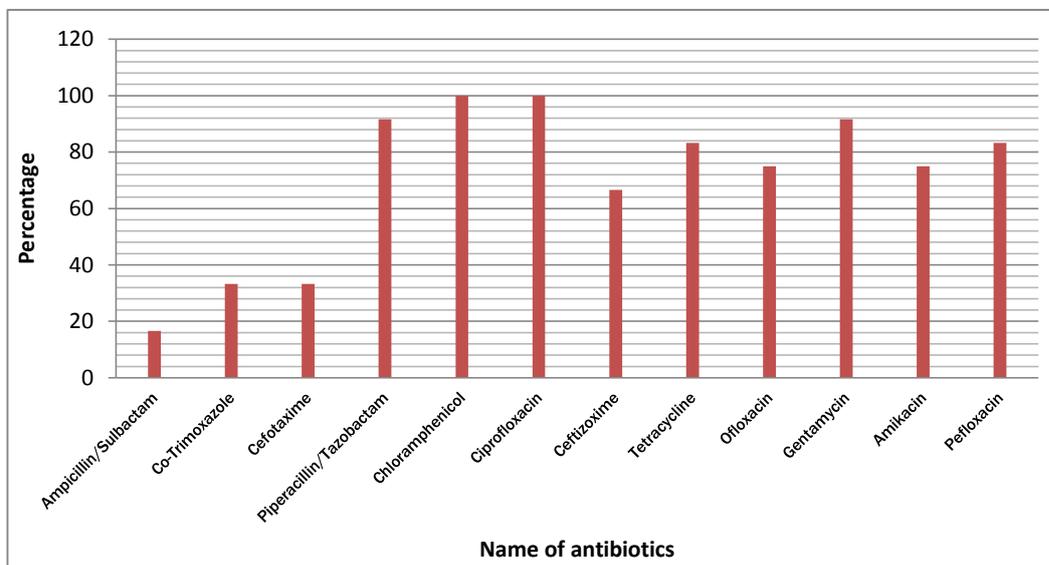


Figure 5. The percentage of effectiveness of different antibiotics against different isolated bacteria

CONCLUSION

All cases of bovine mastitis should be handled carefully and reported comprehensively with respect to microscopic examination of milk with special emphasis on milk pH, bacterial examination and the effectiveness of different antibiotics against mastitic cases and it is need more an efficient work specially for detect sub-clinical mastitis in the future.

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REFERENCES

- AlAyi AAM (2004). Studies on Staphylococci associated with bovine mastitis in Khartoum State. (M.V.SC). Thesis, University of Khartoum.
- Barrow GI and Feltham RKA. (2003). Cowan and Steel's manual for identification of medical bacteria. 3rd (ed) Cambridge press.
- Blood DC, Radostits OM and Henderson JB (1983). Veterinary Medicine, 6th ed. Bailliere Tindal, London, Pp. 451.
- Carlos, ADM (1990). Characteristic of *Staphylococcus aureus* from subclinical bovine mastitis in Brazil. Br. Vet. J, 146: 443-448.
- Cullor J (1992). Tests for identifying antibiotic residues in milk; How well do they work? Vet. Med, 87: 1235-1241.
- DaRong C, Shan YZ, Zhao H Y, Wen WD, Zhi XM, Zhi RS and Huai CS (2010). Prevalence of bacterial infection responsible for bovine Mastitis. African Journal of Microbiology Research, 4 (11): 1110-1116.
- Dodd FH (1985). Progress in mastitis control. Kiel. Milchwirtsch. Forschungsber, 37: 160-223.
- Elsayed NI (2000). Staphylococcal species in normal and mastitic milk of some domestic animals. (M.V.Sc). Thesis, University of Khartoum.
- Falade S, Nwanaza L and Wulaya A (1989). The incidence of bovine mastitis in Kenya. Bull. Anim. Hlth. Prod. Africa, 26: 55-61.
- Fetrow J and Mann D (1991). Production losses from mastitis: Carry over from the previous lactation. J. Dairy. Sci, 74: 833-839.
- Ibrahim, A. E. and Habiballa, N. (1978). Persistent mastitis in dairy herd of a breeding center. Sud. J. Vet. Sci. Anim. Husb, 19: 96.
- Jan M, Sargeant, Morgan S, Ken EL, Mary JI and Anna B (1998). Clinical mastitis in dairy cattle in Ontario: Frequency of occurrence and bacteriological isolates. Can Vet J, 39.
- Kaneen J and Bandhard HS (1990). The national animal health monitoring system in Michigan cost estimates of selected dairy cattle diseases. J. Vet. Med, 8: 127-140.
- Karmy SA (1990). Bacteriological studies on mastitis in small ruminants and she-camel in Upper Egypt. J. Egypt. Vet. Med. Assoc, 50: 69-70.
- Logan NA (1988). Bacillus species of medical and veterinary importance. J. Med. Microbiol. 25: 157-165.
- McDonald JS and Anderson J A (1981). Antibiotic sensitivity of Staphylococci isolated from infected bovine mammary glands. Cornell. Vet, 71(4): 391-396.
- Menzies FD, Bryson DG, McCallion T and Matthews DA (1992). A study of mortality among suckler and dairy cows in Northern Ireland in 1992. Vet. Rec, 137: 531-536.

- Merk Veterinary Manual (1998). 8th (ed). Printed in the USA by National publishing, Inc. Philadelphia, Pennsylvania. Pp. 1009-1012, Mercy.
- Nail JR, George CC, Gemmell G and Lain SH (2003). Production of Diarrhoeal Enterotoxins and other Potential Virulence factors by Veterinary of Bacillus species Associated with Non Gastrointestinal infections. Appl. Environ. Microbiol 69 (4): 2372-2376.
- Parvanta MF (2000). Abortion in a dairy herd associated with *Bacillus licheniformis*. Tierarztliche Umschau. 55: 126.
- Philpott WN (1967). Influence of subclinical mastitis on milk production and milk composition. J. Dairy. Sci. 50: 978.
- Radostits OM, Blood DC and Gat (1994). Veterinary Medicine. 12th ed. Balliere. Tindall. London, Pp. 510-560.
- Rosengarten R and Citti C (1999). Variant colony surface antigenic phenotypes within *Mycoplasma* strain population: Implication for species identification and strain standardization. J. Clin. Microbial, 34: pp. 149-158.
- Rowan N J, Caldow G, Gemmell CG and Hunter IS (2003). Production of Diarrheal Enterotoxins and Other Potential Virulence Factors by Veterinary Isolates of Bacillus Species Associated with Nongastrointestinal Infections. Appl. Environ. Microbiol, 69: 2372-2376.
- Sandholm M, Tuula HB, Liisa K and Sata P (1995). The bovine udder and mastitis. The University of Helsinki Press. OY, Jyveskyla, Finland.
- Smith MC (1990). Exclusion of infectious diseases from sheep and goat farms. The veterinary clinics of North America: food animal practice (USA), 6: 705-720.
- Watts JL (1988). Etiologic agents of bovine mastitis. Vet. Microbiol, 16: 41-66.
- William GR (1995). Diseases of Dairy Cattle 1st ed. Philadelphia: Alea and Febiger book. Williams and Wilkins. Pp: 287.