BOVINE MASTITIS CAUSED BY *Bacillus spp*. IN KHARTOUM STATE, SUDAN

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المستخلص

هذه الدراسة اجريت في ولاية الخرطوم- السودان- بين العاملين 2006- 2008. تم جمع مائة عينة لبن من أبقار مصابة بالتهاب ضرع اكلينيكي. النسب المئوية لالتهاب الضرع بلغت، 55% التهاب ضرع حاد، 44% التهاب ضرع مزمن و 1% التهاب ضرع مواتي. عينات اللبن تمت زراعتها في آجار الدم وآجار الماكونكي لمدة 24 ساعة في درجة حرارة 37 درجة مئوية. تم عزل الباكتريا العصوية من هذه العينات بنسبة 74%. وتشتمل على الأنواع الآتية بالنسب المئوية الآتية9% *B. subtilis*، 9% *B. cereus*، 11% *Bacillus coagulans*، 31% *B. mycoides*، 3% *B. circulans*، 4% *B. licheniformis*، 2% *B. lentus*، 2% *B. amyloliquefaciens* و *B. megaterium*.

Abstract

This study was conducted in Khartoum state, Sudan, between 2006- 2008. Hundered milk samples were collected from cows with clinical mastitis. The percentage of acute mastitis *55*% acute mastitis, 44% chronic mastitis and 1% gangrenous mastitis. Milk samples were cultured in Blood agar and MacConkey´s agar for 24 hours at 37º C. The isolation of *Bacillus* spp. amounted to 74% ; these constituted 31% *Bacillus coagulans*, 11% *B. cereus*, 9% *B. subtilis*, 9% *B. licheniformis*, 4% *B. circulans*, 3% *B. mycoides*, 3% *B. amyloliquefaciens*, 2% *B. lentus*, and 2% *B. megaterium*.

Keywords: Bovine, mastitis, bacillus

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Introduction

The term mastitis refers to the inflammation of the mammary glands regardless of the cause. It is characterized by physical, chemical and usually bacteriological changes in milk associated with pathological changes in the glandular tissue. The disease is responsible for decreased milk production, increased veterinary and treatment costs, increased labour costs and increased culling (Janzen, 1970; Asby *et al*., 1975; Dobbins, 1977; Blosser, 1979). Antibiotic therapy, without identifying the mastitis causing organisms, is frequently the veterinarian and dairy farmer's first choice of treatment for infected cows. Cases of mastitis that are refractory to any type of treatment occur frequently (Wager, 1981.; and Kirk, 1992).

Most strains of Gram positive spore forming bacteria can cause bovine mastitis like *Bacillus* spp (Reva *et al.*, 2004; Hong *et al.*, 2005). Some species of genus *Bacillus* are cause mastitis like *B. alvei, B. subtilis, B. megaterium* and *B. cereus* Elgadasi, (2003).

The objective of this research work was to detect the types of *Bacillus* spp. which can cause mastitis in dairy cattle in Khartoum state- Sudan.

Materials and Methods

The present study was carried out on selected dairy farms located in Khartoum state during the period from 2006 to 2008. The total numbers of examined animal was 500.The standard of milking hygiene in these farms was very poor and preventive measures, such as the use of udder disinfectants, post-milking teat dipping and dry cow therapy, were infrequent. Mastitis was diagnosed when there were visible or palpable signs of udder inflammation change in milk secretions, or through bacteriological examination of milk.

Milk samples were obtained from mastitic udders using sterilized sample bottles, with the usual aseptic precautions being taken. The sample were placed immediately on ice and brought to the Microbiology Laboratory at the department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum. The milk samples were examined

bacteriologically by inoculation onto 5% sheep blood and MacConkey´s agars. Plates were incubated aerobically at 37^{o}C and were examined for bacterial growth after 24-48 hours. Organisms were identified according to standard method of Barrow and Feltham (2003).

Results

The total number of infected udders was hundred. Percentage of different type of mastitis diagnosed during this study were: acute mastitis, 55% (55 mastitic quarters), chronic mastitis, 44% (44 mastitic quarters) and gangrenous mastitis, 1% (1 mastitic quarter).

Bacillus species and *Staphylococcus* species were isolated from 74% and 24% of cases. In one milk sample *Corynebacterium* species was identified and in another *Klebsiella* species was isolated. Nine *Bacillus* species were identified according to biochemical tests shown in Table 1, from cases with acute and chronic mastitis (Fig. 1 and 2). *Bacillus coagulans* constituted 31% of isolates, followed by *B. cereus* 11%, *B. subtilis* 9%, *B. licheniformis* 9%, *B. circulans* 4%, *B. mycoides* 3%, *B. amyloliquefaciens* 3%, *B. lentus* 2%, and *B. megaterium* 2%.

Table (1): The biochemical tests used for identification of the isolated Bacillus spp

Test	**Species and result of tests**								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Grams stain	+	+	+	+	+	+	+	+	+
Chains of cells	+	+	+	+	+	+	+	+	+
Motility	+	-	+	+	+	+	+	+	+
Spore position	*VX*	*VX*	*VX*	*VX*	*VX*	*VX*	*VX*	*VTX*	*VTX*
Swelling of the cell body by spores	-	-	+	-	-	-	-	+	+
Carbohydrate, acid from ASS:									
Glucose	+	+	+	+	+	+	+	+	+
Cellibiose	+	+	+	+	+	+	+	+	+
Galactose	-	-	+	+	-	+	+	+	+
Mannose	-	-	+	-	+	+	+	+	+
Raffinose	-	-	-	+	+	-	+	+	+
Salicin	+	+	-	+	+	+	+	+	+
Xylose	-	-	-	+	-	+	+	+	+
Citrate	+	+	-	+	+	+	+	-	-
Urease	+	+	+	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-
V.P	+	+	-	-	+	+	+	+	+
Nitrate	+	+	-	+	+	+	+	-	+
Casien hydrolysis	+	+	-	+	+	+	+	+	-
Oxidase	-	-	+	-	-	-	-	-	-

T: Spore terminal.
X: Spore oval
V: sub-terminal.

1- ***B coagulans.***
2- ***B. cereus.***
3- ***B. subtilis.***
4- ***B. licheniformis.***
5- ***B. circulans.***
6- ***B. lentus.***
7- ***B. mycoides.***
8- ***B. amyloliquefaciens.***
9- ***B. megaterium.***

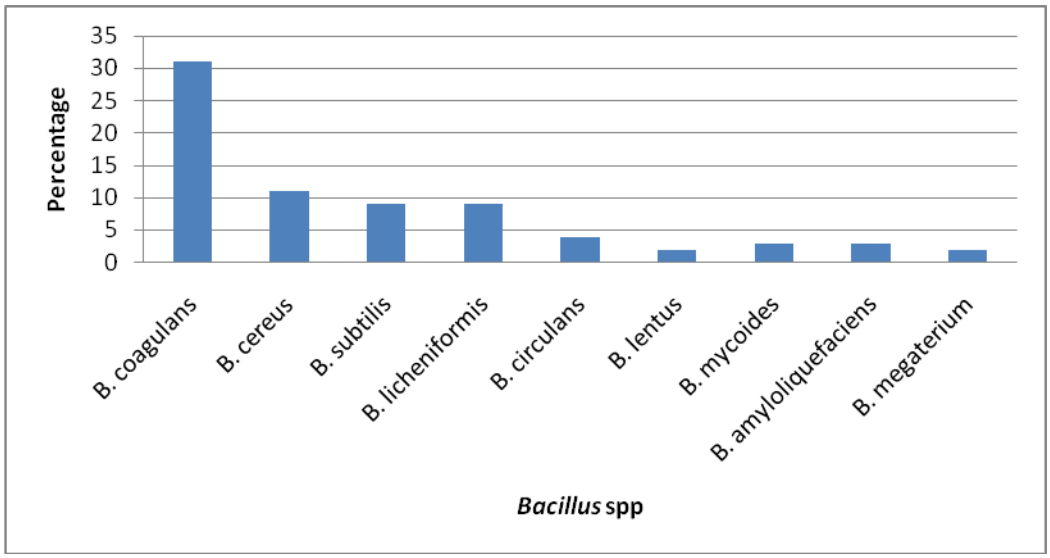

Fig. 1: The percentage of isolated Bacillus spp. from 100 mastitic cows

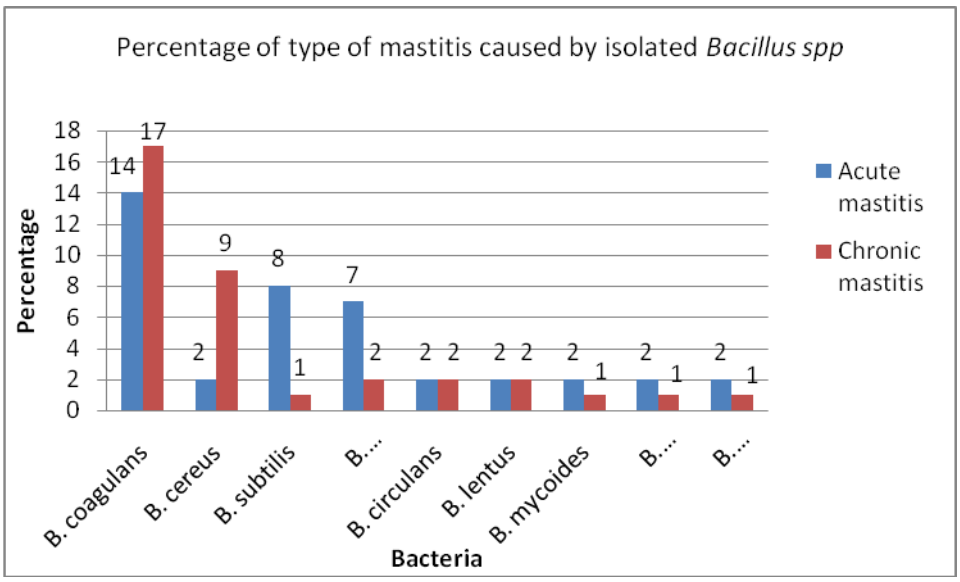

Fig. 2: Percentage of acute and chronic mastitis caused by isolated Bacillus spp. from 100 mastitic cows

Discussion

Bacillus species are widely distributed in nature and most species exist in soil, water, dust, air, feces and on vegetation. The first case reported by Brown and Scherer (1957) was attributed to the introduction of the organism during treatment of chronic intra-mammary infections when a single plastic syringe was used by a dairy farmer to infuse the quarters with an antibiotic solution. In our 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 similar to the findings of Nail *et al*., (2003). *Bacillus cereus* was also isolated by Nail *et al*., (2003).

Other species of *Bacillus* were isolated like, *B. licheniformes,* and this is in accord with results of Jones and Turnbull (1981) Logan, (1988), Nail *et al.*, (2003) and Parvanta, (2000). The isolation of *Bacillus alvei*, *B. subtilis*, *B. megaterium* and *B. cereus* during this study is in agreement with Elgadasi, (2003). *B. licheniformis*, *B. amyloliquefaciens*, *B. circulans*, *B. lentus* and *B. mycoides*, to the best of our knowledge are reported here for the first time in bovine mastitis in Sudan.

On conclusion *Bacillus* spp. should be considered as one of the causes of mastitis in dairy farms and further studies are needed in this aspect.

References

Asby, C. B.; Ellis, P. R.; Griffin, T. K. and Kingwill, R. G. (1975). The benefits and costs of a system of mastitis control in individual herds. Study No. 17, Natl. Inst. Res. Dairying, Shinfield, Reading, UK.

Barrow, G. I. and Feltham, R. K. A. (2003). Cowan and Steel's manual for identification of medical bacteria. 3rd Cambridge press.

Blosser, T. H. (1979). Economic losses from the national research program on mastitis in the United States. J. Dairy. Sci. **62**: pp. 119-127.

Brown, R.W.; and Scherer, R. K. (1957). A report of two cases of acute mastitis caused by *Bacillus cereus* .Cornell Vet.**47**: pp: 266.

Dobbins, C. N. (1977). Mastitis losses. J. A. V. M. A. **170**: pp. 1129-1132.

Elgadasi, S. D. G. (2003). Identification of bacteria isolated from mastitic milk of cattle, sheep and goats in Khartoum State and study of antimicrobial sensitivity. (M.V.Sc). Thesis, University of Khartoum.

Hong, H. A.; Duc, L. H. and Cutting, S. M. (2005). The use of bacterial spore formers as probiotics. FEMS Microbiology Reviews **29**: pp. 813-835.

Jan, M.; Sargeant, H.; Morgan, S; Ken, E.; Leslie, M. J. I. and Anna, B. (1998). Clinical mastitis in dairy cattle in Ontario: Frequency of occurrence and bacteriological isolates. Can Vet. **39**: pp. 33-38.

Janzen, J. J. (1970). Economic losses resulting from mastitis: a review. J. Dairy Sci. **53**: pp. 1151-1155.

Jones, T. O.; and Turnbull, P. C. B. (1981). Bovine mastitis caused by *Bacillus cereus*. Vet. Rec. **108**: pp: 272.

Kirk, J. H. (1992). Diagnosis and treatment of difficult mastitis cases. Proc. 31st Annual Meeting of the National Mastitis Council. pp: 26-33.

Logan, N. A. (1988). Bacillus species of medical and veterinary importance. J. Med. Microbiol. **25**: pp. 157-165.

Nail, J. R.; George C. C.; Gemmell, G. and Lain, S. H. (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): pp. 2372-2376.

Parvanta, M. F. (2000). Abortion in a dairy herd associated with *Bacillus licheniformis*. Tierarztliche Umschau. **55**. pp. 126.

Reva, O. N.; Dixelius, C.; Meijer, J. and Priest, F. G. (2004). Taxonomic characterization and plant colonizing abilities of some bacteria related to *Bacillus amyloliquefaciens* and *Bacillus subtilis*. FEMS Microbiology Ecology. **48**: pp. 249-259.

Wager, L. A. (1981). *Mycoplasma* and *Prototheca* infections. Refractory mastitis on the increase. Proc. 20th Annual Meeting of the National Mastitis Council. pp: 156-160.