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ISOLATION OF *Klebsiella* Spp. FROM GANGRENOUS MASTITIS IN CATTLE IN KHARTOUM STATE, SUDAN

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ABSTRACT: This study was conducted in Khartoum State to determine the causative agent of gangrenous mastitis in bovine. Hundred dairy cows were examined after collected aseptically from 41 cows suffering from mastitis. All these cows were examined by visual inspection and palpation of mammary gland and supra-mammary lymph nodes. The milk samples were examined bacteriologically. The result was 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 isolation of *Klebsiella* spp. from gangrenous mastitis in Frisian cow is considered as the first of its type in Sudan.

Keywords: Bovine, Gangrenous, Mastitis, Klebsiella, Khartoum, Sudan.

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INTRODUCTION

Bovine mastitis, defined as "inflammation of the mammary gland" is the most economically important disease in dairy milk production worldwide (Bradley, 2002; Gruet et al., 2001). This disease can have an infectious or noninfectious etiology, and the infectious pathogen is the most important ones that frequently due to infection by one and/or the other pathogens, such as bacteria, viruses, mycoplasma, yeasts and algae (Chaneton et al., 2008; Malinowski et al., 2006; Osumi et al., 2008; Watts 1988; Wellenberg et al., 2002). Fortunately the vast majority of mastitis is of bacterial origin and just a few species of bacteria account for most cases, such as *E. coli*, *S. aureus*, *Str. uberis*, *Str. dysgalactiae* and *Str. agalactiae* (Aarestrup et al., 1995; Annemüller et al., 1999; Aouay et al., 2008). The main causative agent of gangrenous mastitis in cattle is *Staphylococcus aureus, this was reported by* dairy farmers of Ontario 1995 (2011).

Rate of coliform infection is higher during the dry period than during lactation; and dry cow therapy (treating cows with antibiotics at the end of lactating period) has been shown to have no effect on reducing the coliform infection rate (Smith et al., 1985). An outbreak of coliform mastitis is described in a dairy herd from the State of Rio de Janeiro, Brazil. During a four-month period 14 fatal cases of *Klebsiella pneumoniae*-related mastitis were observed in a herd of 104 lactating cows (Arq, 2001). Contaminated cow´s surrounding is the cause of *Klebsiella* spp. mastitis. Unlike contagious forms of mastitis which spread from cow-to-cow during milking, *Klebsiella* come from environmental sources, such as manure and organic material/bedding (recycled manure, wood shavings, etc.) (Arq, 2001).

Klebsiella bacteria can enter the teat canal both during and between milking. Dirty udders, especially when wet, have enormous bacterial populations. High rainfall, hot and humid weather, and moist environments can trigger heavy bacterial growth and increase incidence of mastitis caused by Klebsiella. The clinical signs of infected cow with mastitis caused by Klebsiella are: Fever, swollen/warm quarter (usually only 1 quarter affected), abnormal milk, decreased appetite, depression, diarrhea, and standing away from other herd-mates are common clinical signs of Coli Mastitis (Christina et al., 2011). *Klebsiella* spp. are most numerous in sawdust bedding as reported by Bramley and Neave (1975), Fairchild et al. (1982) who both reported *Klebsiella* spp. Outbreaks when cows were bedded using fresh sawdust.

The main objective of this study was to investigate the causative agents of bovine mastitis with special reference of mastitis caused by *Klebsiella* spp.

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MATERIAL AND METHODS

The samples were subjected to pH detection by using an indicator paper (manufactured by Kruse Company in Denmark). This test was applied by adding one drop of milk on yellow spot, in a few seconds the colour should change. The milk samples were collected aseptically from individual cows from infected quarters and then were subjected to bacteriological analysis by culturing on Blood agar and MacConkey´s agar. Isolation and identification was carried out according to the method of Barrow and Feltham (2003). The disc of different antibiotics was used for sensitivity test on the isolates according to Barrow and Feltham (2003).

RESULTS

Among the Hundred examined cows we find one was infected with gangrenous mastitis. Examination of the mammary gland produced a yellowish secretion in the left hind quarter, and the consistency of the secretion in the other quarters was normal in the infected cattle with gangrenous mastitis. The clinical signs observed in the cow affected by gangrenous mastitis were increase in body temperature, sloughed the mammary gland from the body and the oozing of purulent secretions. The pH indicator paper revealed change in colour from yellow to green. Table 1 shows the percentage of *Klebsiella* spp. and other isolates from various type of mastitis, there were *Bacillus* spp. (74%), *Staphylococcus* spp. (24%), *Klebsiella* spp 1% and *corynebacterium* spp. (1%). The various type of mastitis diagnosed were acute mastitis 55%, chronic mastitis 44% and 1% for gangrenous mastitis showed Figure 1. Table 2 shows the results of the antibiotic sensitivity test. *Klebsiella* was found equally sensitive to all antibiotics tested.

Table 1 - The percentage of isolated bacteria from milk samples

Bacteria	Percentage %
Bacillus spp.	74
Staphylococcus spp.	24
Klebsiella spp.	1
Corynebacterium spp.	1

Table 2 - The effectiveness of different antibiotics against Klebsiella spp

Klebsiella spp.	The effectiveness of different antibiotics against *Klebsiella* spp. and name of antibiotic											
	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
Results	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

AS: Ampicillin/Sulbactam; CI: Ceftizoxime; BA: Co-Trimoxazole; TE: Tetracycline; CF: Cefotaxime; OF: Ofloxacin; TZP: Piperacillin/ Tazobactam; GM: Gentamycin; CH: Chloramphenicol; AK: Amikacin; CP: Ciprofloxacin; PF: Pefloxacin; (+++): Higly sensitive.

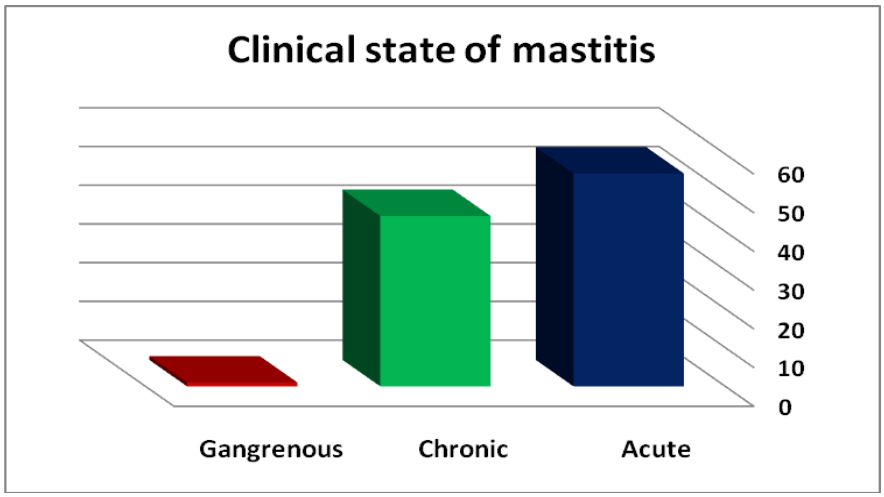

Figure 1 - The percentage of types of mastitis

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DISCUSSION

In the literature dealing with gangrenous mastitis, there is considerable discussion concerning the relative importance of various bacteria in the pathogenesis of the condition (Minett 1937; Schalm, 1944). The environmental mastitis becomes more prevalent in a herd, the probability increases in early-lactation. The greatest number of cases in the first 21 days after calving, before breeding starts. Coliforms are found in soil, water and manure and they also inhabit the intestinal tract of cows (Eberhart et al., 1979; Fairchild et al., 1982). *Klebsiella pneumoniae* and *Escherichia coli* are the most common species associated with mastitis (International Dairy Federation, 1999) in dairy cattle. However, a significant number of cases were recorded until 70 days after calving. Then, as lactation progressed, the numbers became much more sporadic. With increased environmental mastitis in the summer, the occurrence of such cases in some cows close to breeding is likely (Perrin et al., 2007).

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.

CONCLOUSION

Regardless of this result should be make an experimental infection with *Klebsiella* spp. to confirm that bacteria can cause gangrenous mastitis and use PCR to detect what strain of these bacteria or the toxin can cause gangrenous mastitis.

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