Int.J.Curr.Microbiol.App.Sci (2021) *10*(09): 1-7

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 09 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1009.001

Bovine Mastitis in Eldamazine Locality – Blue Nile State

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Keywords

Bovine mastitis – Edamazine locality – Sudan

Article Info

Accepted:
05 August 2021
Available Online:
10 September 2021

A B S T R A C T

This study conducted to examine the clinical bovine mastitis to isolate and identify the bacteria causing the disease and to determine the sensitivity pattern of isolates to commonly using antibiotic in Eldamazine locality, Blue Nile State. A total of 45 dairy cows clinically infected by different types of mastitis. Mastitis milk samples were collected from the animals and transported to microbiology laboratory for bacteriological examination. The samples were cultured in blood agar, MacConkey agar and purified in nutrient agar. Gram stain was used for morphological characteristics. Biochemical tests were done to all isolates. Sensitivity test was applied to gentamycin, ampicillin, vancomycin, ciprofloxacin, tetracycline and erythromycin. There were 3 types of mastitis were detected and these were acute mastitis with high prevalence (62.2%) followed by chronic mastitis (35.6%) and gangrenous mastitis (2.2%). The isolates were *Staphylococcus* spp (73.36%), *Streptococcus* spp (4.4%), *Bacillus* spp (8.9%) *Pseudomonas* spp, and *Escherichia* spp (8.9%). Also, the results revealed that high prevalence of *Staphylococcus epidermidis* (8.9%), followed by *Staphylococcus hyicus* (6.7%), *Streptococcus agalactia* (4.4%), and *Pseudomonas aeruginosa* (4.4%). Most effective antibiotics to isolated bacteria were ciprofloxacin, gentamycin, tetracycline, vancomycin and ofloxacin, while resistant to ampicillin and erythromycin. The species *Pseudomonas aeruginosa* were resistant to all antibiotics used. In conclusion, mastitis is associated with huge economic loss to farmers in the study area, and most effective control is prevention by using good management practices.

Introduction

Mastitis is a global problem as it adversely affects animal health, quality of milk and the economics of milk production, affecting every country. Including developed ones and causes huge financial losses (Biffa *et al.*, 2005). Mastitis is the most costly disease of dairy cattle due to economic losses from reduced milk production, treatment costs, increased labor, milk withheld following treatment, death, and premature culling (Kaneene and

Hurd, 1990; Miller *et al.,* 1993). Due to the heavy financial implications involved and inevitable existence of latent infection, it is obvious that mastitis is an important factor limiting dairy production. Disease is worth studying due to the financial loss involved as a result of reduced milk yield, discarded milk following antibiotic therapy, easily culling of cows, veterinary costs. Drug costs, increased labor, death of per acute cases and replacement costs. Additional economic incentives to control mastitis include consumers' acceptance and product shelf- life factors. Although all of these factors result in considerable economic loss, decreased milk production is the single most important economic consideration and this requires the development of methodologies of control program under prevailing husbandry system (Gera and Guha, 2011).

Mastitis is often the end result of interaction of several factors such as man, cow, environment, microorganisms and management (Blood *et al.,* 1989). Mastitis is a difficult problem to comprehend because, it is a disease caused by many factors. Both in large and in small-scale herds. Micro-organisms are responsible for the infection, but for them to enter the mammary gland and establish themselves to the point that they cause an infection, a multitude of factors may be involved. There are many factors acting simultaneously, and the disease generally involves interplay between management practice and infectious agents but with other factors, such as genetics, udder shape or climate (Bachaya *et al.,* 2011; Awale *et al.,* 2012). The occurrence of disease is an outcome of interplay between three major factors: infectious agents, host, and environmental factors (Berhanu, 1997).

Mastitis may be infectious caused by microbial organisms or non infectious resulting from physical injury to the gland (Campus. 2007). The infectious etiology is the most important and caused by one and / or the other pathogens, such as bacteria, virus, mycoplasma, yeast and algae (Welleberg *et al.,* 2002; Malinosuki *et al.,* 2006; Chanetonl *et al.,* 2008; Osumi *et al.,* 2008).

Mastitis is closely related to the production system and environment in which the dairy animals are kept (Mekibib *et al.,* 2010). That's why monitoring udder health performance is impossible without reliable and affordable diagnostic methods (Zadoks and Schukken, 2006).

Mastitis is the most common disease of dairy cows and the most common reason that cows are treated with antibiotics (Saini *et al.,* 2012). It represents one of most important diseases from economic standpoint.

The most important changes in the milk are discoloration and presence of clot (Blood *et al.,* 1983), In the Sudan mastitis become one of the major problems in recent years and causes heavy economic losses to milk producers. Bovine mastitis isthe major problem for milk producers throughout the world and responsible for substantial losses of revenue annually (Suzan *et al.,* 2016).

The objectives of this study were to identify the types of mastitis among dairy cows in the study area, isolation and identification of the positive agents and determination of sensitive antibiotics to the isolates.

Materials and Methods

Study area

This study was conducted in Eldamazine locality, Blue Nile State, Sudan. This area lies between longitudes 33-35 and latitudes 10-12 and 492 meters above sea (G. D. of Animal Resources, 2013).

Collection of milk samples

A total of 45 mastitic cows were examined clinically from August to November 2020. Milk samples (45 samples) were taken under a septic condition for bacteriological examination and transported to microbiology laboratory in Eldamzine town.

Bacteriology

All steps of sterilization were used according to Barrow and Feltham (2003). Preparation of culture media also were done (Oxoid, 2006). Films were made from purified culture and biochemicals tests were done for identification of the isolates (Barrow and Feltham, 2003). Also, sensitivity test was done by using of ampicillin (Sulbactam) 20mg, ofloxacin (OF) 5mg, gentamycin (GN) 10mg, vancomycin (VA) 30 mg, tetracycline (TE) 30 mg, ciprofloxacin (CIP) 5 mg and erythromycin € 15mg to all isolated bacteria.

Statistical analysis

Bacterial count in the data was analyzed by using descriptive method. The data of antibiotics was analyzed by one way ANOVA and the statistical significance was set at p-value of ≤ 0.05.

Results and Discussion

Clinical status of mastitis

The 45 milk samples were collected from dairy cows infected with mastitis. The clinical signs for acute mastitis were severe inflammation with swelling, heat and pain of the quarter. The milk samples containing blood, clots and discoloration of normal color. While the chronic mastitis was detected by duration and atrophy of the mammary gland, fibroid udder with the watery secretion. But gangrenous type of the udder characterized with bluish coloration and bloody secretion (Table 1). Acute mastitis revealed high incidence (62.2%) followed by chronic mastitis (35.6%), but gangrenous mastitis revealed low incidence (2.2%).

Bacteriology

Gram positive bacterial were identified as etiological agents of mastitis to these animals were (Table 2) *Staphylococcus aureus* (57.8%), *Staphylococcus epidermidis* (8.7%), *Staphylococcus hyicus*(6.7%, *Streptococcus agalactiae* (4.4%), and *Bacillus cereus* (8.9%), while Gram negative bacteria were *Escherichia coli* (8.9%) and *Pseudomonas aeruginosa* (4.4%) was identified (Table 3).

Antibiotic sensitivity test

The isolated bacteria *Bacillus cereus* showed sensitivity to erythromycin and *E. coli* showed resistant to vancomycin and ofloxacin, but *Pseudomonas aeruginosa* was resistant to all antibiotics used (Table 4). Generally, most of the isolates were sensitive to antibiotics used with high significant difference between them ($p \leq 0.05$).

Mastitis among bovine is common disease and several surveys conducted in Sudan showed that the prevalence is high (Mustafa, *et al.*, 1977; Ibrahim and Habiballa, 1978).

In this study, *Staphylococcus aureus* was high (57.8%). This finding in agreement with Radostits *et al.*, (2007) who mentioned that these species is the incremented in bovine mastitis. This organism has been reported as udder pathogen (Bagadi, 1970; Adlan *et al.*, 1980; Watts, 1988; Mamoun and Bakheit, 1992; Mwahib, 2010).

Table.1 Classification of different types of mastitis in mastitic cows (n=45) in Eldamazine locality, Blue Nile State

Type of mastitis	Number of cases
Acute mastitis	62.2%
Chronic mastitis	35.6%
Gangrenous	2.2%
Total	**100%**

Table.2 The species of Gram-positive bacteria isolated from mastitic cows (n=45) in Eldamazine locality, Blue Nile State

Name of bacteria	Number of bacteria	Percentage
Staphylococcus aureus	26	57.8%
Staphylococcus epidermidis	4	8.9%
Staphylococcus hyicus	3	6.7%
Streptococcus agalactia	2	4.4%
Bacillus cereus	4	8.9%
Total	**39**	**86.7%**

Table.3 The species of Gram-negative bacteria isolated from mastitic cows (n=45) in Eldamazine locality, Blue Nile State

Name of bacteria	Number of bacteria	Percentage
Escherichia coli	4	8.9%
Pseudomonas aeruginosa	2	4.4%
Total	**6**	**13.3**

Table.4 Degree of antibiotics effectiveness on isolated bacteria from mastitic cows (n-45) in Eldamazine locality Blue Nile State

Isolated bacteria	AS	OF	CN	VA	TE	CIP	E
Staph aureus	5.73±3.77	16.62±2.21	18.62±1.50	15.04±2.63	13.81±2.17	18.85±1.35	7.00±2.67
Staph epidermidis	4.00±3.37	16.25±2.22	19.50±1.29	15.00±0.82	16.25±2.06	18.00±1.63	2.50±3.32
Staph hyicus	4.00±3.61	15.33±1.53	19.33±1.15	13.67±1.53	16.33±2.08	18.67±2.08	8.67±2.52
Strepto agalactiae	5.50±4.95	20.00±1.41	19.50±0.71	16.50±2.12	15.00±1.41	19.50±2.12	11.00±2.83
Pseudomonas aeruginosa	0.00±0.00	1.50±2.12	60.00±1.41	0.00±0.00	2.00±2.83	6.00±1.41	6.50±2.12
Escherichia coli	7.00±2.16	7.25±2.63	17.75±2.22	3.25±3.95	14.00±3.16	21.00±1.83	11.75±2.50
Bacillus cereus	0.50±1.00	16.00±1.83	19.25±0.96	11.25±3.30	17.50±1.91	15.50±1.91	13.00±1.63
Sig	*	**	**	**	**	**	**

Sig = significance * = $p \leq 0.05$ ** $p \leq 0.01$ ns = not significant

Reem (2008) revealed that the high incidence might be attributed to the increasing in number of animals per farm and spreading of strain resistance. Also, in this study, *Staphylococcus hyicus* (6.7%) and *Staphylococcus epidermidis* (8.0%) were isolated (Elsayed, 2000; Mashaer, 2017). In this results *E. coli* (Table 3) was isolated as coliform bacteria that caused the disease with rapid multiplication (Radositits *et al.,* 2007).

Isolation of *Bacillus cereus* (8.9%) from mastitic milk samples could be attributed to failure of sanitary programs which help in the elimination of the causative agents. This supported by Quinn *et al.,* (1994) who mentioned that the organisms were isolated from mastitic milk of bovine (Nail *et al.,* 2003; Reem, 2008).

The species *Streptococcus agalaciae* (4.4%) was isolated in this study and considered the cause of the disease and has ability to adhere to the mammary gland tissues (Radostits *et al.,* 2007).

Also, the isolation of *Pseudomonas aeruginosa* (4.4%) may be due to existing of the organism commonly in the water environment during udders washing (Radositis *et al.,* 2007; Madut *et al.,* 2009).

The results revealed that most of isolates were sensitive to antibiotic used (Table 4). Tetracycline possesses antimicrobial activity by binding to the ribosomal subunit (30 S) of the susceptible organism that interfering with bacterial protein synthesis in growing or multiplying organisms (Gale and Folkes, 1953; Suzuka *et al.,* 1966). Because of this tetracycline group is inhibiting the growth of a wide variety of bacteria. The result of resistant of *Pseudomonas* spp to all antibiotics including gentamicin in contrast to finding of Adams (2001) who stated that this organism is sensitive to the therapy.

The tricyclic glycopeptide vancomycin is active against gram positive cocci, enterococci and aerobic gram negative bacteria, but N-alkyl vancomycin is 5 times more active than vancomycin (Nagarajan *et al.,* 1989). However, aminopenicillins generally active against some *Enterobacteriaceae* and gram positive (Adams, 2001). Also, ofloxacin is bactericidal that inhibiting bacterial DNA replication and transcription (Ferrero *et al.,* 1995; Drlica and Zhao, 1997). This drug activity against most of gram-negative and gram-positive bacteria. Generally, the resistant to the antibiotics is depending on the type of pathogen and its severity, duration of infection and stage of lactation (Radostits *et al.,* 2007).

The study concluded that continuous monitoring of the disease in animals is important in Eldamazine locality due to common spreading among herd and this is indicating that the disease is serious problem across the herds in this area. The management measures are most essential for the well-being of dairy herds which can be achieved through the detection of mastitis in early stages and treatment of the disease.

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How to cite this article:

Myson Ali Babiker, Mohamed Ismail Mohamed Fangama, Siham Elias Suliman and Mohamed Abdelsalam Abdalla. 2021. Bovine Mastitis in Eldamazine Locality – Blue Nile State. *Int.J.Curr.Microbiol.App.Sci.* 10(09): 1-7. doi: https://doi.org/10.20546/ijcmas.2021.1009.001