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Research paper

Caprine Subclinical Mastitis in Atbara Area, River Nile State

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ABSTRACT

The study investigates Caprine Subclinical Mastitis (CSM) using local breed (Nubian), Saanen and Cross breed (Nubian+Saanen) goats in Atbara Area, Sudan. The study was carried out by using two field tests; California mastitis test (CMT) and White side test (WST). Results revealed that the prevalence of CSM out of 100 half/udder milk samples was 56% and 44% according to CMT and WST, respectively. Bacterial isolation and identification was done according to Cowan and Steel. The predominant isolated bacteria were: Staphylococcus, Pasteurella, Aeromonas, Micrococcus, Escherichia and Klebsiella. Streptococcus, Corynebacterim and Enterobacteria were isolated with lesser frequencies. Results revealed that Nubian breed was more resistant to causative bacteria (33.3% susceptibility), followed by Saanen (66.6%) and Cross breed (88.8%). Antimicrobial susceptibility tests were carried out by disc diffusion method on the Mueller Hinton agar. Out of six studied antibiotics Ciprofloxacin and Gentamicin showed 100% activity against both G+ve and G-ve isolates, while Chloramphenicol showed 100 and 60% activity against G+ve and G-ve, respectively. However 77.7% of isolates were resistant to Amoxicillin. CMT is more precise than WST in screening for CSM.

Key words: goats, subclinical mastitis, CMT, WST, antibiotics

التهاب الضرع تحت السريري في الماعز بمنطقة عطبرة- ولاية نهر النيل

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هذه الدراسة تستكشف التهاب الضرع تحت السريرى مستخدمة الماعز المحلى النوبى والسعانين وهجين من النوبى والسعانين بمنطقة عطبرة- السودان. اجريت الدراسة باستخدام اثنين من الاختبارات الحقلية، اختبار كاليفورنيا لالتهاب الضرع (CMT) واختبار الوايت سايد (WST). اظهرت الدراسة ان انتشار المرض المذكور فى 100 عينة (عينات نصف الضرع) حليب هو 56 و44% لنوعى الاختبارين المذكورين CMT و WST على التوالى. عزل ومعرفة البكتريا تم حسب طريقة كاون وستيل. النوع السائد من البكتريا المعزولة هى المكورات العنقودية(ستافيلوكوكس)، الباستوريلا، الايروموناس، المايكروكوكس، الاشريشية، الكلبسيلا، اما ستريبتوكوكس، الكوراينباكترم، الانتيروباكتر فقد تم عزلهم بصورة اقل. اثبتت النتائج ان الماعز النوبى اكثر مقاومة للبكتريا المسببة للمرض (بمستوى اصابة 33%) ثم السعانين (بمستوى اصابة 66.6%) والهجين (بمستوى اصابة 66.8%). تم اجراء اختبار فعالية المضادات الحيوية بطريقة الانتشار القرصى فى اجار مولر هنتون. بين المضادات الست التى تم اختبارها اظهر كل من سبروفلاكسين و جنتاميسين فعالية بنسبة 100% ضد البكتريا الموجبة والسالبة لصبغة جرام، بينما اظهر كلورامفينوكول فعالية بنسبة 100% و 60% ضد البكتريا الموجبة لصبغة جرام والبكتريا السالبة لصبغة جرام، على التوالى. اظهرت 77.7% من البكتريا المعزولة مقاومة للاموكسسلين. اختبار CMT كان اكثر دقة من اختبار WAS فى الكشف عن التهاب الضرع.

كلمات مفتاحية: الماعز، التهاب الضرع السريرى، اختبار كاليفورنيا، اختبار وايت سايد

Introduction

Subclinical mastitis is an invisible abnormality of milk and udder in goats which is characterized by an increase in somatic cell and/or leukocyte counts (Radostitis *et al*., 1994). The importance of goats as a source of meat and dairy products has been well discussed and documented in a recent review by Haenlein (2004). This importance is also reflected by increase in goat population during the last two decades.

Subclinical and chronic mastitis are most persistent and widespread forms of intermammary infection (IMI) affecting high milk producing breeds of goats (Bergonier *et al.*, 2003). The proportion of udder halves with subclinical IMI in goats in deferent countries ranges from 35% to 70% (Menzies and Ramanoon, 2001 and Leitner *et al*., 2004). Subclinical mastitis in goats is by far, the most expensive disease as it reduces milk and cheese yields because of deterioration of milk quality in infected glands, reflected by elevated Somatic Cell Count (SCC) and the presence of microorganisms (Countreras *et al*., 2007). In goats IMI, the coagulase-negative S*taphylococci* (CNS) are the most prevalent microorganisms isolated from chronic and subclinical infections (Bergonier *et al*., 2003).

Mastitis is a disease of considerable economic importance worldwide for both dairy goats and cattle (Radostitis *et al*., 1994). Poor management of sanitary conditions, lack of therapeutics and control measures play vital role in the development of this disease in goats.

In developed countries proper strategies of disease monitoring and control have been developed, however in the River Nile State, little information is available regarding prevalence of subclinical mastitis in goats. This study aims to investigate the prevalence of Caprine Subclinical Mastitis in the mentioned area as well as its etiological agents and their susceptibility to different antibiotics to support effective measure treatment.

Materials and methods

Source and collection of samples

A total of one hundred half-udder milk samples from different locations (twenty samples each) were collected from apparently healthy 50 goats (with no evidence of acute mastitis) of varying age (1-10 years). Samples represented three breeds of goats: Local breed (Nubian), Sannen and Cross breed (Nubian+Sannen). Milk samples were drawn after morning milking. After discarding

the first few mls of milk, the teat end of each half-udder was thoroughly cleaned with cotton soaked in 70% ethanol. Two mls of milk samples were squeezed from each half into the cup of the test plate for CMT and WST at the field (location of samples) and 10ml milk samples were collected into sterile bottles immersed in ice. Samples were analyzed in Atbara Veterinary Research Laboratory.

Field screening tests

The CMT and WST were performed as primary filed screening tests on milk samples to detect subclinical mastitis and determine the prevalence rate of disease according to both methods by taking positive samples as a numerator and the total tested samples as a denominator.

Bacteriological examination

For primary isolation of bacteria blood base agar (Columbia, scharlau, 01.034) and Nutrient agar [PLASMATEC, M8] were prepared according to instructions of the manufacturer. Primary isolated bacteria were inoculated on blood agar slants, incubated overnight at 37°C to allow growth and then preserved at 4°C. For bacterial identification all inoculated plates incubated aerobically at 37°C daily for up to 7 days and evaluated by growth and changes in the medium. Further identification was done according to Cowan and Steel (1993) which included; Gram's stain, aerobic growth, motility test, spore formation and biochemical tests including catalase activity and oxidase test.

Antibiotic susceptibility test

The test was carried out by the disc diffusion method using commercial separated discs of the following antibiotics:

1. Tetracycline (T)30 (HIMEDIA, SD 037-5CT) 30 mcg /disc
2. Amoxicillin (AM)10 (MIMEDIA, SD 001-5CT) 10 mcg/disc.
3. Streptomycin (S)10 (HIMEDIA, SD 031-5CT) 10 mcg/disc
4. Ciprofloxacin (CF)5 (HIMEDIA. SD 060-5CT) 5 mcg/disc.
5. Gentamicin (G)10 (HIMEDIA. SD 016 – 5 CT) 10 mcg/disc.
6. Chloramphenicol (C)30 (HIMEDM, SD 006-5CT) 30mcg/disc.

The test was done according to the guidelines of Clinical Laboratory Standard Institute (CLSI, 2000). The Mulleler Hinton agar was used to perform the antibiotic susceptibility test. Results were interpreted according to the zone size interpretative chart as "susceptible", "intermediate" or " resistant".

Results

Table (1) shows results of field screening tests. The total number of milk samples with positive CMT were 56% while samples with positive WST were 44%. The percentage of positive samples in different locations ranged from 35-70% and 20-70% with CMT and WST, respectively. The results of the screening tests for the 50 goats revealed that the overall prevalence rate was 28 (56%) and 22 (44%) on the basic of CMT and WST, respectively.

Table (2) shows the influence and prevalence of caprine subclinical mastitis on the basis of age, breed and parity. The results revealed that goats above four years of age showed higher prevalence rate of mastitis, 57.1% and 59.1% for CMT and WST, respectively. Goats in category of 2-4 years of age showed prevalence rate of mastitis with 35.7 and 36.3% by CMT and WST, respectively. In category of 1 – 2 years of age goats showed further decrease to 7.1 and 4.5% by CMT and WST, respectively. Regarding breeds results, prevalence rate of mastitis for Nubian goats was 10.7 and 18.1%, for Saanen was 39.2 and 27.2% and for cross breed was 50 and 54.5% on the basis of CMT and WST, respectively. On the basis of parity, the highest prevalence rate was 46.4 and 50% recorded with CMT and WST, respectively in goats having three or more parities.

Table (3) shows the overall bacterial isolation confirmed in 76 samples out of 100 half-udder examined milk samples, which represent 76%. Out of 76 positive samples, there was 61 (80.1%) were single isolates and 15 (19.9%) were mixed infection.

Table (4) illustrates the correlation between screening test and bacterial isolation. All +ve bacterial isolates showed false-negative reactions in the field screening test. This reveals that false negative reactions of bacterial isolates were 20 (26.3%) and 32 (42.1%) in CMT and WST, respectively.

Table (5) represent the identification of the bacterial isolates to the genus level. It shows that all the isolates belonged to nine different genera. Four genera were Gram positive bacteria

isolated bacteria namely, *Staphylococcus, Streptococcus, Micrococcus* and Corynebecteria. The rest were Gram negative bacteria genera (*Pasteurella, Klebisella, Escherichia, Entrobacter* and *Aeromonas*). The most common bacterial genera recovered from positive studied samples were *Staphylococcus* (25%), *Pasteurella* (19.7%), *Aeromonas* (13.1%), *Micrococcus* (8%), *Escherichia* (6.5%) and *Klebsiella* (4%). *Streptococcus*, *Corynebateriim* and *Enterobacter* were isolated with lesser frequencies.

Table (6) shows the results of goat breeds susceptibility test to the different genera of bacterial isolates which revealed that the Nubian breed (local breed) was more resistant to subclinical mastitis and susceptible only to 33.3% of causative bacteria, which were, *Staphylocaccus, Corynebcaterium* and *Aeromonas*. The Saanen breed was susceptible to 66.6% and cross breed showed minimum resistant and had been susceptible to 88.8% of isolated bacteria.

Table (7) illustrate the effectiveness of six antibiotics, Ciprofloxacin (CF), Streptomycin (S), Amoxicillin (Am), Chloramphenicol (C), Gentamicin (G) and Tetracycline (T) against each of the nine isolated genera of bacteria. The results showed that Ciprofloxacin and Gentamicin were very effective against both G+ve and G-ve bacteria (100%). Further, Chloramphenicol showed 100% and 60% effectiveness against G+ve and G-ve organisms, respectively.

Discussion

In this study, the test of CMT was effective in field screening for the presence of subclinical mastitis, because it was positively correlated with SCC (Mcdougall *et al*., 2001). Previous studies reported that CMT might be useful for detection of healthy udders (Karzis *et al*., 2007 and Petzer *et al*., 2008). Somatic cell count is the most widely used indicator of udder health in cow, sheep and goat milk, but unfortunately SCC is difficult to interpret in goats. Compared with sheep and cows, SCC in goat milk and healthy udder is relatively high and it increases throughout the lactation and also with parity (Paape and Capuco, 1997). The highest prevalence rate was recorded in goats having three or more parities. Age and parity are the most significant factors in determining the prevalence of mastitis in goats. Milk somatic cell count increases with age and lactation as reported by Zeng *et al*. (2008) and Zamin *et al*. (2010). Further, an increase in prevalence related to parity was reported in goats by Leitner *et al*. (2001) and Bergonier *et al*. (2003).

Schaeren and Maurer (2006) reported a great variation in SCC among farms and among individual animals. Similarly, in this study, the percentage of false negative reaction of CMT regarding to the bacterial isolates proved that no correlation between the field screening test (CMT) and SCC for individual animals. However, elevated SCC is mainly a response to infection (Poutrel *et al*., 1997). Therefore, measurement of SCC seems likely to be a reliable way to detect goats with intermammary infection (IMI).

Regarding overall bacterial isolation, false- negative reaction of CMT was less about 15.8% than WST. Accordingly, CMT is more sensitive and more accurate than WST in screening subclinical mastitis in tested goats. These results agreed with that obtained by Iqbal *et al*. (2003), who approved that WST is not very responsive in detecting mastitis positive cases. The negative result of CMT and positive bacterial isolation for the same samples, may be attributed to the fact that some of the bacteria could not be detect by CMT due to their lower pathogenicity or otherwise their mere presence as udder microflora (Elliot *et al.*, 1976 and Motie *et al*., 1985).

The most dominant bacteria in milk samples was *Staphylococcus* (25%). However, Zamin *et al*. (2010) found that the presence of *Staphylococcus* as the causative agent of subclinical mastitis in goats was (45.3%). Similar results were also reported in other countries such as, USA (38.2%), Spain (70.0%) and Kenya (60.3%) (White and Hinckley, 1999; Sanchez *et al*., 1999 and Ndegwa *et al*., 2001).

The highest prevalence percentage of subclinical mastitis was detected in the Cross breed, followed by Saanen and Nubian breeds, respectively. This may be due to the fact that Nubian goat (local breed) had more adaptation to the regional environment compared to others. Breed differences in susceptibility to SCM could be attributed to differences in intramammary infection, milk yield or genetic factors (Gonzalo *et al*., 2005).

Susceptibility test of the bacterial isolates in vitro showed that Ciprofloxacin and Gentamicin were the most effective antibiotics in controlling Caprine Subclinical Mastitis, followed by Chloramphenicol. However, the isolated bacteria are more resistant to Amoxicillin. In relation to antibiotic susceptibility Castro *et al*. (2001) reported that Gentamicin shows high susceptibility to bacteria in vitro. On the other hand, Da Silva *et al*. (2004) found that bacteria in Caprine mastitis are more resistant to Penicillin G. This may be due to the frequently use of the

same antibiotics which may lead to antibiotic resistant (Tras *et al*., 2007). Further, different results obtained from antibiotics susceptibility may be mainly due to misuse of antibiotics and difference of bacterial strains. Similarly, Gentamycin, Ampicillin and Tetracycline were reported to be the most effective drugs in treating CSM in Camels (Hawari and Hassawi, 2008).

Table (1): Overall prevalence rate (%) of subclinical mastitis according to the field screening tests

Locations	Examined samples	+ve samples (%)	
		CMT	WST
1	20	14 (70%)	14 (70%)
2	20	8 (40%)	8 (40%)
3	20	7 (35%)	4 (20%)
4	20	14 (70%)	9 (45%)
5	20	13 (65%)	9 (45%)
Total	**100**	**56 (56%)**	**44 (44%)**

CMT: California mastitis test; WST: White Side test

Table (2): Prevalence of Caprine subclinical mastitis related to age, breed and parity

Test	Age/years			Breed			Parity		
	1-2	2-4	>4	Nubian	Sannen	Cross	First	Second	≥ third
CMT	2 (7.1%)	10 (35.7%)	16 (57.1%)	3 (10.7%)	11 (39.2%)	14 (50.0%)	6 (21.4%)	9 (32.1%)	13 (46.4%)
WST	1 (4.5%)	8 (36.3%)	13 (59.1%)	4 (18.1%)	6 (27.2%)	12 (54.5%)	4 (18.1%)	7 (31.8%)	11 (50%)

Table (3): The percentage of overall bacterial isolation

Total of examined samples (%)	Overall +ve sample (%)	+ ve with single isolates (%)	+ ve with mixed isolated (%)
100	76	61	15
%	76%	80.1%	19.9%

Table (4): Percentage of false-negative reaction of both CMT and WST regarding bacterial isolation results

+Ve B.Iso*	Test	+ve Results	False – ve reaction (%)
76	CMT	56	20 (26.3%)
	WST	44	32 (42.1%)

* Bacterial isolation

Table (5): Percentage of different genera of both G+ve and G-ve bacterial isolates

Infection	Gram positive isolates	No. & % of +ve samples
Single	*Staphylococcus*	19 (25%)
	Streptococcus	01 (1.3%)
	Micrococcus	06 (8%)
	Corynebacterium	01 (1.3%)
	Pastcurella	15 (19.7%)
	Klebisella	03 (04.0%)
	Escherichia	05 (06.5%)
	Enterobacter	01 (01.3%)
	Acromonas	10 (13.1%)
Mixed	*Micrococcus* + *Streptococcus*	01 (1.3%)
	Staphylococcus + *Aeromonas*	03 (4.0%)
	Escherchia + *Pasteurella*	03 (4.0%)
	Staphylococcus + *Streptococcus*	03 (4.0%)
	Pasteurella + *Aeromonas*	02 (2.6%)
	Streptococcus + *Aeromanas*	03 (4.0%)

Table (6): Susceptibility of tested goat breeds to different genera of bacterial isolates

Bacterial Genera	Nubian breed	Sannen breed	Cross breed
Staphylococcus	+	+	+
Streptococcus	-	+	-
Micrococcus	-	+	+
Corynebcaterium	-	-	+
Pasteurella	+	+	+
Klebisella	-	-	+
Enterobactor	-	-	+
Escherichia	-	+	+
Aeromonas	+	+	+

Table (7): Antibiotic susceptibility of different genera of bacterial isolates

Type of Isolate	Genera	The antibiotics susceptibility					
		CF	**S**	**Am**	**C**	**G**	**T**
Gram positive	*Staphylococcus*	S	M	R	S	S	M
	Streptococcus	S	S	S	S	S	M
	Micrococcus	S	M	R	S	S	M
	Corynebacterium	S	M	R	S	S	S
Gram negative	*Pasteurella*	S	M	R	S	S	R
	Klebsiella	S	S	M	M	S	S
	Enterobacter	S	S	R	S	S	R
	Escherichia	S	M	R	M	S	R
	Aeromonas	S	R	R	S	S	S

S= Susceptible M= Moderate R= Resistant

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