

Study on Prevalence of Bovine Mastitis and Its Major Causative Agents in Salahadin City, Iraq.

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ABSTRACT

Key Words:

Prevalence of Bovine
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The Study have been done to know the most common causative agents which cause bovine mastitis in Salah Eldin City during the period from 1/1/2013 to 13/12/2013.

Diagnosis of the cases have been done for 200 Milk samples from Clinical and Sub Clinical Mastitis cattle .by using California mastitis test (CMT) ,enzyme–Linked Immune sorbent assay, culture media ,biochemical test and the antimicrobial susceptibility of isolated bacteria was done to determine its susceptibility.

A total of 200 cows were included in this study and out of these, 80 (40%) were found to be reactive by California mastitis test (CMT). Bacteriological analysis of milk samples collected simultaneously yielded positive result in 70(35%) of the samples, virological analysis of milk samples yielded positive result in 45 (22.5%), Fungi analysis of milk samples yielded positive result in 6(4.3) and finally Parasite detected in 4(2.9) .Bacteriological analysis carried out at the quarter level showed that obtain to 250 pathogens agents were positive for various bacteria , Viruses ,fungi and parasit. The predominant bacteria isolated were *Staphylococcus* species (47.11%), *Streptococcus* species (31.40%) and coli forms (9.92%). Other bacterial, fungi and parasite species were isolated at lower rates. The predominant virus were *Bovine herpesvirus1* and *Bovine herpesvirus 4* while Food and Mouth Disease virus was lower rate. Antimicrobial susceptibility test showed that most of the isolates in the study area were found to be highly sensitive to cloxacillin, gentamycin and amoxicillin, and moderately sensitive to oxytetracycline. Nevertheless, *Streptococcus* ,*Staphylococcus* and coli forms species isolated were resistant to penicillin.

دراسة حول انتشار التهاب الضرع البقري واهم مسبباته المرضية في محافظه صلاح الدين – تكريت، العراق.

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الخلاصة

اجريت هذه الدراسة لمعرفة المسببات المرضية الشائعة لحالات التهاب الضرع البقري في محافظه صلاح

الدين خلال الفترة من 1/1/2013 ولغاية 13/12/2013.

تم تشخيص المسببات المرضية ل 200 عينة حليب من ابقار مصابه بالتهاب الضرع السريري وتحت السريري. باستخدام اختبار كاليفورنيا للحليب الملتهب ، كتات الاليزا، الأوساط الزرعيه،الاختبارات الكيميوحيويه.كما تم اجراء اختبار فحص الحساسيه للجراثيم المعزوله لتأكد من حساسيتها لمضادات الحياتيه.

اشارت النتائج ل 200 بقرة مستخدمه بالدراسه ان 80(40%) اعطت تفاعلا موجبا لفحص كلفورنيا لحليب الملتهب و 70(35%) من عينات الحليب المجموعه اعطت نتيجة موجبه لتحاليل البكتريه، 50(25%) من عينات الحليب المجموعه اعطت نتيجة موجبه لتحاليل الفيروسيه، 6(4.3%) من عينات الحليب المجموعه اعطت نتيجة موجبه لتحاليل الفطريه واخيرا التحاليل الطفيليه كانت 4(2.9%). اشارت نتائج التحاليل البكتريه على مستوى الارباع الحصول على 250 مسبب مرضي متنوع (بكتيري والفيروسي والفطري والطفيلي). الانواع العقديه والانواع المسببيه وبكتريا القولون كانت من العزلات الشائعته العزل اما

الكلمات المفتاحية:

انتشار ، التهاب الضرع ، البقري ،
مسبباته المرضية ، صلاح الدين .
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بقية العزلات كانت اقل. BHV4 and BHV1 كانا اكثر انواع الفيروسات الشائعة التشخيص بينما كان فيروس الحمى القلاعية اقل نسبه. اشار اختبار فحص الحساسيه للجراثيم بان اغلب العزلات في منطقه الدراسه كانت عاليه الحساسيه لكلوكساسين،جنتاميسين والاموكسيلين ومتوسطه الحساسيه ل الاوكسي تتراسيكلين.نوعا ما العنقوديات ،المسبقيات وانواع من عزلات بكتريا القولون كانت مقاومه الامبسلين.

INTRODUCTION:

Diary production is a biologically efficient system that converts feed and rough ages to milk. Milk which is a very nutritional food i.e. rich in carbohydrate, proteins, fats, vitamins and minerals, provides an important dietary source for the majority of rural as well as a considerable number of the urban and peri-urban population .However, its reduced production quality deterioration is a great concern in association n with health risk to consumers due to the presence zoonotic pathogens and antimicrobial drug residues FAO(2003b),Sisay *et al.*,(2012)

Mastitis is a multi factorial disease and very difficult to control. It results from injury, chemical irritation and infection caused by different bacterial species. Mastitis is most expensive disease of dairy animals resulting in the reduction of milk production and quality. The estimated annual losses due to mastitis are about \$ 184 per animal. These expenses in terms of reduction of production, discarding milk, drug therapy, veterinarian charges, premature culling, and extra use of labour Baloch *et al.*, (2011).

Mastitis is caused by a group of infective and potentially pathogenic bacteria , viruses , fungi and parasite. The incidence of bacterial species in mastitis milk samples of different animals was studied worldwide. The incidence of coagulase negative *Staphylococci* (1.50%), coagulase-positive *Staphylococci* (1.59%), *Streptococcus* sp. (*Streptococcus dysgalactiae*, 3.7%; *Streptococcusuberis*, 0.83%), *Enterococcus* sp. (1.04%), *Escherichia coli* (1.56%), *Pseudomonas* sp. (1.59%), and yeast (1.5%) was recorded in Korea Park *et al.*,(2007).

The bacterial agents responsible to cause inflammation of udder are classified as either contagious or environmental, based upon their primary reservoir and mode of transmission. *Staphylococcus aureus* and *Streptococcus dysagalactiae* are recognized as contagious bacterial species, commonly transmitted among dairy animals through contact with infected milk. The pathogens reside in environment are of 2 types, one is Coliforms (*Escherichia coli*, *Klebsiella*) and other is *Streptococcal* species other than *Streptococcus dysgalactiae* entering into the udder between milkings , when teats are exposed to mud, manure, and dirty bedding materials, the epidemiology of bacterial mastitis in dairy animals has been studied using various molecular typing methods. Several studies reveled that only a few specialized clones are responsible for a broad geographic distribution Radostit *et al.*,(2010).

Economic losses due to mastitis are recognized world wide as a major problem on dairy farms. Financial loss involved as a result of permanent loss of production in individual cows, is carded milk following antibiotic therapy, early culling of cows, veterinary costs, drug costs, increased labor, death of per acute cases and replacement costs Bishi(1998).

Some studies have been conducted so far on the prevalence and major causes of bovine mastitis mostly in cross bred dairy cattle in different parts of the country Workineh *et al.*,(2002),Biffa *et al.*, (2005), Sori ., *et al* (2005).

Materials and Methods:

study include physical examination of the udder and teats, California mastitis test (CMT), enzyme–Linked Immune sorbent assay, bacteriological culture and antimicrobial sensitivity tests.

1-Physical examination of udder:

The udder was first examined visually and then thoroughly palpated to detect possible fibrosis, signs of inflammation, visible injury, atrophy of the tissue and swelling of the supra mammary lymph nodes. Rectal temperature of those cows with clinical mastitis was taken to check systemic involvement. Information related to the previous health history of the mammary quarters was obtained from the owners of the cows. Viscosity and appearance of milk secreted from each mammary quarter were examined for the presence of clots, flakes, blood and watery secretions.

Antimicrobial sensitivity test:

In vitro antibiotic sensitivity test (Kirby-Baur disc diffusion) method was carried out in order to identify the most effective drugs for mastitis treatment in the study area. A loop full of colony from the growth of isolates was transferred to the nutrient broth in tubes and incubated at 37°C for 5 h. Mueller-Hinton agar which was used as plating medium was inoculated with broth (bacterial suspension) by using cotton swab. Then antibiotic impregnated paper disc (Oxoid, UK) were applied and pressed onto the plate with forceps. Plates were incubated at 37°C for 18 h. The diameters of zones of growth inhibition were measured in millimeter and interpreted as sensitive, intermediate and resistant to different antibiotics Quinn., *et al* (2010). The drugs used were oxytetracycline, penicillin, cloxacillin, amoxacillin and gentamycin.

RESULTS:

Physical examination of udder:-

Out of 200 cows examined physically for udder condition health were showed sign of fibrosis, cardinal signs of inflammation, visible injury and tick infestation, atrophy of the tissue of udder and swelling of the supra mammary lymph nodes.

Prevalence of mastitis

A total of 800 quarters from 200 milking cows were investigated. Among these, 700(87.5%) quarters were found to be patent while the remaining 100 (12.5%) were blind. other indications of mastitis were also observed during this cross-sectional study. The results of CMT carried out on milk samples collected from those patent teats showed that 80 (40%) of the cows gave positive reaction. The bacteriological culture result showed that 70 (35%) of the cows yielded bacterial agents. The Virological analysis result showed that 45 (22.5%) of the cows yielded viral agents. The fungiological culture result showed that 3 (1.5%) of the cows yielded fungi agents and finally parasitology analysis result showed that 2 (1%) of the cows yielded parasite agents (Table 1).

At the quarter level, 250 (35.7%) of them were positive for CMT, 160 (22.9%) of them were positive for bacterial growth and 100(14.3) of them were positive for Viral analysis (Table 2).

Table 1. prevalence of mastitis according to pathogen type in Salah Edin cows

	No of Cows	Porportion	
		Number	%
1-	Positive for CMT	80	40
2-	Positive for bacterial growth	70	35
3-	Positive for Viral examination	45	22.5
4-	Positive for Fungi examination	3	1.5
5-	Positive for Parasite examination	2	1
	Total Number examined	200	

Table 2. Prevalence of clinical and subclinical mastitis in cow and quarter level on the basis of CMT culture result viral and parasite analysis.

	Mastitis type	CMT		Culture		viral analysis	
		No. of animals	No. of quarters	No.of animals	No.of quarters	No.of animals	No. of quarters
1	Clinical Mastitis	30(37.5)	100(40)	30(42.9)	80(50)	45(100)	100(100)
2	SubClinical Mastitis	50(62.5)	150(60)	40(57.1)	120(75)	-----	----
Total		80 (40)	250(35.7)	70(35)	160(22.9)	45(22.5)	100(14.3)

Bacterial, fungi culture parasite and viral analysis:

The results of Bacterial, fungi culture on milk samples collected from mastitis cattle showed the involvement of 150 bacteria belonging to 7 species and 6 yeast belonging to one species. Both contagious and environmental bacteria were isolated from milk samples collected from mastitis cows. The predominant bacterial species isolated was *Staphylococcus aureus*(37.5%), followed by *Streptococcus agalactiae* (20.6%) , followed by *Escherichia coli* (16.9) and *corynbacterium pyogens* (9.4 %). From both the clinical and subclinical mastitis cases, *Staphylococcus aureus* *Streptococcus agalactie* ,*Escherichia coli* and *corynbacterium pyogens* were the predominant bacteria isolated during this study .The results of parasite and viral anylysis on milk samples collected from mastitis cattle showed the involvement of 45 virus belonging to 3 species and 4 parasite t belonging to one species (Table3).

Table3. Types of Bacterial, fungi, parasites and viruses isolated of milk samples from clinical and sub clinical mastitis

	Bacterial isolated	Number of isolates (%)		
		Clinical mastitis	Subclinical mastitis	Total
1	<i>Staphylococcus aureus</i>	25(35.7)	35(35%)	60(37.5)
2	<i>Streptococcus agalactiae</i>	13(18.6)	20(22.2)	33(20.6)
3	<i>Escherichia coli</i>	12(17.1)	15(16.7)	27(16.9)
4	<i>corynbacterium pyogens</i>	10(14.3)	5(7.1)	15(9.4)
5	<i>Klebsiella spp</i>	3(4.3)	5 (7.1)	8(5)
6	<i>Proteus spp</i>	1(1.4)	2(2.2)	3(1.9)
7	<i>Bacillus cereus</i>	1(1.4)	2(2.2)	4(2.5)
8	<i>Candidia spp</i>	3(4.3)	3(3.3)	6(3.8)
9	<i>Toxoplasmosis</i>	2(2.9)	2(2.2)	4(2.5)
Total isolated		70	90	160
10	<i>Bovine herpesvirus 1</i>	25(55.6)	-----	25(55.6)
11	<i>Bovine herpesvirus 4</i>	15(33.3)	-----	15(33.3)
12	<i>Foot-and-mouth disease virus</i>	5(11.1)	-----	5(11.1)
Total Viral		45		45

Antimicrobial sensitivity test:

A total of 160 isolates from clinical and subclinical mastitis were tested for their *in vitro* antimicrobial sensitivity. The results showed that the majority of the isolates were highly sensitive to cloxacillin, gentamicin and amoxicillin and moderately sensitive to oxytetracycline. Nevertheless, *Streptococcus*, *Staphylococcus* and *coli* forms species isolated were resistant to penicillin table(4).

Table4. antimicrobial sensitivity tests on the bacterial isolates.

	Pathogen	Total isolate	cloxacillin	Gentamicin	amoxicillin	penicillin	oxytetracycline
1	<i>Staphylococcus aureus</i>	60	59/1	58/2	59/1	10/40	30/30
2	<i>Streptococcus agalactiae</i>	33	31/2	32/1	30/33	7/26	15/15
3	<i>Escherichia coli</i>	27	26/1	26/1	25/2	8/19	20/7
4	<i>Corynebacterium pyogenes</i>	15	13/2	14/1	14/1	5/10	6/9
5	<i>Klebsiella ssp</i>	8	7/1	6/2	7/1	3/5	2/6
6	<i>Proteus spp</i>	3	2/1	2/1	3/3	1/2	1/2
7	<i>Bacillus cereus</i>	4	3/1	3/1	3/1	1/3	1/3

The numerators show the number of sensitive isolates, while the denominator show the number of resistant isolates among tested. OXY, Oxytetracycline (30 µg); GEN, gentamicin (5 µg); AMO, amoxicillin (10 µg); PEN, penicillin (10 IU); CLO, cloxacillin (5 µg); S/R, sensitive/resistance.

DISCUSSION:

In the present study the overall prevalence of mastitis in cow was 90%, which can significant value regarding the economic and public health importance of the disease to the study area. The overall mastitis prevalence reported in the present study is higher than previous findings of other authors in different regions of Ethiopia like: 61.1% by Tolla (1996) in south Wello, 63.0% by Geressu (1989) in Addis Ababa, 68.1% by Zerihun (1996) in Addis Ababa, 85.6% by Nesru (1986) in Dire-Dawa and 36.9% by Darsema (1991) in Dire-Dawa Eastern Ethiopia. The differences in prevalence of mastitis in the present study and other reports could be attributable to difference in breeds of targeted cows, farm, level of production and differences in study methods and materials employed by the investigators, also mastitis is a complex disease, involving interactions of various factors which include management, environmental, animal risk factors and causative agents, its prevalence will vary Radostit et al., (2010).

the prevalence of clinical mastitis(52.5%) is higher than subclinical mastitis(47.5%) which is supported by reports of several investigators 44.0% and 21.0% by Lemma (2005), 47.2% and 6.3% by Tolossa (1987) 43.1% and 41.2% by Takele (1987) for subclinical and clinical in mastitis, respectively.

The prevalence of subclinical mastitis in this study is higher than prevalence reported by other research Geressu(1989), Mengistu,(1986), Robertson,(1985)

, respectively. In the present prevalence report of clinical mastitis is also higher than prevalence reports of several other investigators; 21.1% by Takele [1987] and 21.0% by Lemma (2005) in Arsi region and 20.0% prevalence reported by Bagadi (1970) in Sudan. In general, subclinical mastitis has been reported to be lower than clinical mastitis owing to the defense mechanism of the udder, which reduces the severity of the disease Hussein et al., (1997).

The quarter level overall mastitis prevalence of mastitis in both agro-ecological settings is in close agreement with the works of other authors Matios et al.,(2009) and Abera et al.,(2010). However, it is higher than previous reports Lemma,(2005) and Takele, (1987) It has been suggested

that variation in the quarter prevalence of mastitis in different reports is directly related with the variation in the overall prevalence of the disease in the areas. From the 250 CMT positive quarter milk samples, 160 (64%) were bacteriologically positive up on culturing, while 90 (36%) were bacteriologically negative, which is in line with the results of Aregaw, (1992), who reported 18% bacteriologically negative samples. Our finding is higher than that of Biffa, et al.,(2005) and Sori, et al., (2005).

The result obtained from bacteriological analysis of the sample revealed that the predominant organism isolated from mastitis milk were *S. aureus* followed by *S. agalactia*. Geressu,(1989), Zerihun,(1996), Nesru,(1986) and Mekbib, *et al.*, (2009) from Ethiopia and that of Madut, *et al.*,(2009) in cows from Sudan. The predominance of these two bacterial species is due to frequent colonization of teats as they are commensals of the skin. Then they can easily get access to the teat canal during milking or suckling and can be transmitted from quarter to quarter and from cows to cows during milking practices. Their ability to exist intra cellular and localize within micro-abscessation in the udder and hence, resistant to antibiotic treatment MacDonald,(1997). could also be important factor contributing to the predominance of these organisms. However, the incidence of the *S. agalactia* in this study is greater than that of Mekbib,*et al.*,(2009) and lowers than that reported by Tolossa,(1987) and Mekuria, (1986) who found *Streptococcus* species to be 53.55% and 45.50%, respectively. The third predominant bacterial species isolated were the coliforms. This finding is comparable with the results of Kerro and Tareke (2003), in which the coliform accounts for 14.10% and were the third predominant pathogens from dairy cows in Southern Ethiopia. *E. coli* were the predominant bacteria among the coliforms with an isolation rate of 5.60% in this study which is in consent with the observations of MacDonald,(1997), Mekuria, (1986) and Biffa.*et al.*,(2005) who reported 4.60, 3.64 and 3.14%, respectively from different parts of Ethiopia. In this study, *Klebsiella* spp accounted for 5% among coliforms which is higher than Sisay, *et al.*, (2012). who reported 2.5%. other coliform, fungi and parasite exist in the mammary gland of the cow and the major root of transmission for these organisms occur during milking through milkers' hands, teat cup liners and udder clothes Paape, *et al.*,(1991) . The results of this study are in line with this fact as all the herds visited use hand milking and washing hands, udder and teats before milking is not practiced, poor housing (drainage, barn cleaning and often muddy bedding) conditions in the present study. Contamination of teat ends is a major predisposing risk factor in the development of environmental mastitis, due to the fact that environmental pathogens can survive and multiply in organic bedding materials and housing conditions that can influence teat contamination rates.

The result obtained from virological analysis of the sample revealed that the predominant viruses isolated from mastitis milk were Bovine herpesvirus 1 and Bovine herpesvirus 4, The above mentioned studies demonstrate that BHV1 and BHV4 have been isolated from natural cases of mastitis due to their immunosuppressive properties, More research is warranted to establish a possible indirect role of BHV4 infections in bovine mastitis, e.g. as a result of immunosuppression. The virus can infect cells involved in the immune system, e.g. mononuclear blood cells (macrophages), and recently, a possible role of BHV4 has been postulated by playing a role in damaging vascular tissues Lin, *et al.*, (2000). while the third predominant virus isolated from clinical mastitis was FMD virus which causes clinical mastitis can induce teat lesions, for instance in the ductus papillaris, which result in a reduction of the natural defence mechanisms of the udder and indirectly in bovine mastitis due to bacterial pathogens. Wellenberg, *et al.*, (2002) .

The *in vitro* disc sensitivity test showed that cloxacillin is the most effective drug followed by gentamycin and amoxicillin in the study area. Similar results have been published by Sumathi, *et al.*,(2008) and Sisay, *et al.*,(2012) in support of our findings who reported gentamycin to be the drug of choice in Bangalore. However, *Staphylococcus* and *Streptococcus* species showed the existence of resistance to streptomycin and penicillin. These two antibiotics are most widely used in many parts of Iraq. They are the most commonly available and affordable antibiotics to farmers

under Ethiopia condition. They are sometimes the only available antibiotics in many veterinary clinics. It might be this wide use of these drugs and inappropriate administration which have contributed to the development of resistance by the predominant bacterial agents in then area. The prevailing subclinical mastitis in the area also supports this hypothesis. The effectiveness of cloxacillin detected in this study agrees with findings of Quinn,*et al.*, (2010) who asserted that cloxacillin is an effective drug against *S. aureus*, *S. agalactae*, *E. coli* and environmental streptococci.

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