Pre- and post partum mastitis in ewes caused by gram negative bacteria

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Abstract

The study conducted to compute the morbidity rate of clinical and subclinical ovine bacterial mastitis in pre- and postpartum periods; isolation and identification of these bacteria, estimation of specificity and sensitivity of California Mastitis Test and White Side Test for diagnosis of subclinical mastitis in 400 samples of mammary secretions collected from104 adult ewes; 200 milk samples obtained 2- 3 weeks before parturition and other 200 milk samples collected from the same ewes during the first week after parturition; in Al-Anbar province, in addition to study of efficiency of dry period therapy with slow releasing intramammary tubes contain benzathine penicillin and streptomycin sulfate for control of postpartum mastitis. The sensitivity and specificity of California Mastitis Test in pre-partum period reached to 69.70% and 49.44% respectively, whereas reached to 54.84% and 38.82% in postpartum period respectively, while, the sensitivity and specificity of White Side Test were 63.64% and 42.70 % in pre- partum period, and 40% and 52.25% in postpartum period respectively. In pre-partum period, The clinical mastitis due to gram negative bacteria appeared in 13 (6.5%) infected halves, where *pseudomonas aeruginosa, Mannheimia haemolytica, Pasteurella multocida, Escherichia coli, proteus vulgaris,* and *Serratia marcescens* isolated from 4 (30.77%), 3(23.8%), 2(15.38%), 2(15.38%), 1 (7.69%) and 1 (7.69%) halves respectively. The subclinical mastitis due to gram negative bacteria appeared 13 (6.5%) infected halves where *Mannheimia haemolytica, Proteus mirabilis, pseudomonas aeruginosa,Pasteurella multocida, Escherichia coli, Proteus vulgaris,* and *Histophillus ovis* isolated from 3 (23.8%), 3 (23.8%), 2(15.38%), 2 (15.38%), 1 (7.69%), 1 (7.69%) and 1(7.69%) halves respectively. From these 26 halves infected with gram negative bacteria, 8 halves were treated with intramammary tubes 2-3 weeks before parturition, thus in postpartum period 7 (87.5%) halves cured from mastitis after parturition while, one (12.5%) half remain infected. In non- treated group 10 (55.56%) out of 18 halves cured from mastitis in post partum period without treatment while, 8 (44.44%) halves remain infected, in addition to 13 newly infected halves in non- treated group. In post partum period, the clinical cases due to gram negative bacteria appeared in 10 halves where *Mannheimia haemolytica, pseudomonas aeruginosa, pasteurella multocida* and *E.coli* isolated from 5 (50%), 3 (30 %),1 (10%) and 1 (10%) halves respectively. Subclinical mastitis due to gram negative bacteria appeared in 22 (11%) halves, where *Mannheimia haemolytica, Pasteurella multocida, pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Yersinia enterocolytica* and *Histophillus ovis* isolated from 5 (22.72%), 5 (22.72%), 4 (18.18%), 3 (13.64%), 2 (9.09 %), 1 (4.55%), 1 (4.55%) and 1 (4.55%) halves respectively. It is concluded that the gram negative bacteria are important causative agents of mastitis even in dry period, and they have specific importance in postpartum period in ewes and the pre- partum therapy is an efficient practice for control of postpartum mastitis in ewes.

التهاب الضرع قبل وبعد الولادة في النعاج المتسبب عن الجراثيم السالبة لصبغة غرام

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الخلاصة

صممت الدراسة لحساب نسبة الإصابة بالتهاب الضرع السريري وتحت السريري في الأغنام والمتسبب عن الجراثيم السالبة لصبغة غرام في فترة ما قبل الولادة وفترة ما بعد الولادة، عزل وتوصيف هذه الجراثيم وتقدير حساسية وخصوصية اختبار كاليفورنيا واختبار وايت ساید في تشخيص التهاب الضرع تحت السريري في 400 عينة من 104 نعجة، حيث جمعت 200 عينة حليب خلال 2 – 3 أسابيع قبل الولادة و200 عينة حليب اخرى جمعت من نفس النعاج خلال الأسبوع الأول بعد الولادة في محافظة الأنبار، كما واستهدفت الدراسة اختبار كفاءة العلاج خلال فترة الجفاف بعصارات الضرع البطيئة التحرر الحاوية على المضادات الحيوية penicillin benzathine و streptomycin sulfate في السيطرة على حالات التهاب الضرع بعد الولادة. أظهرت النتائج ان حساسية وخصوصية اختبار كاليفورنيا في تشخيص التهاب الضرع تحت السريري قبل الولادة بلغت 69.70% و49.44% على التوالي بينما بلغت 84%. 54 و% 38.82 على التوالي بعد الولادة. أما حساسية وخصوصية اختبار وايت ساید قبل الولادة فبلغت 63.64% و42.70% على التوالي، في حين بلغت 40% و52.25% على التوالي بعد الولادة. في فترة ما قبل الولادة، ظهرت حالات التهاب الضرع السريري المتسببة عن الجراثيم السالبة لصبغة غرام في 13 (6.5%) نصف ضرع، حيث عزلت جراثيم *pseudomonas aeruginosa , Mannheimia haemolytica, Pasteurella multocida, Escherichia coli, proteus vulgaris Serratia marcescens* من 4 (30.77%)، 3 (23.8%)، 2 (15.38%)، 2 (15.38%)، 1 (7.69%) و1 (7.69%) نصف ضرع على التوالي. ظهر التهاب الضرع تحت السريري المتسبب عن الجراثيم السالبة لصبغة غرام في 13 (6.5%) نصف، حيث عزلت جراثيم *Mannheimia haemolytica, Proteus mirabilis, pseudomonas aeruginosa, Pasteurella multocida, Escherichia coli, Proteus vulgaris, and Histophillus ovis* 3 (23.8%)، 3 (23.8%)، 2 (15.38%)، 2 (15.38%)، 1 (7.69%)، 1 (7.69%) و1 (7.69%) نصف ضرع على التوالي. من بين 26 نصفا مصابا بالتهاب الضرع المتسبب عن الجراثيم السالبة لصبغة غرام تم علاج 8 غدد لبنية بعصارات الضرع خلال 2– 3 أسابيع قبل الولادة فشفيت 7 (87.5%) أنصاف منها بعد الولادة بينما بقي نصف واحد (12.5%) مصابا بعد الولادة. أما في المجموعة التي لم تعالج قبل الولادة، فإن 10 (55.56%) أنصاف من مجموع 18 نصفا شفيت من التهاب الضرع بعد الولادة مقارنة بثمانية (44.44%) أنصاف بقيت مصابة، إضافة إلى 13 نصفا أصيب بالتهاب الضرع بعد الولادة بعد ان كان سليما قبل الولادة في المجموعة غير المعالجة. ظهرت حالات التهاب الضرع السريري المتسببة عن الجراثيم السالبة لصبغة غرام في فترة ما بعد الولادة في 10 أنصاف، حيث عزلت جراثيم *Mannheimia haemolytica , pseudomonas aeruginosa, pasteurella multocida, E.coli* من 5 (50%)، 3

(30%)، 1 (10%) و1 (10%) نصف على التوالي. ظهر التهاب الضرع تحت السريري المتسبب عن الجراثيم السالبة لصبغة غرام في 22 نصفا، حيث عزلت جراثيم *Mannheimia haemolytica, Pasteurella multocida, pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Yersinia enterocolytica and Histophilus ovis,* من 5 (22.72%)، 5 (22.72%)، 4 (18.18%)، 3 (13.64%)، 2 (9.09%)، 1 (4.55%)، 1 (4.55%) و1 (4.55%) نصف على التوالي. يستنتج من هذه الدراسة أن الجراثيم السالبة لصبغة غرام تعتبر من المسببات المهمة لالتهاب الضرع حتى في فترة الجفاف وتمتلك أهمية خاصة في فترة ما بعد الولادة وان علاج الضرع قبل الولادة من الإجراءات الكفؤة للسيطرة على التهاب الضرع بعد الولادة في النعاج.

Introduction

Mastitis is inflammation of the parenchyma of the mammary gland Regardless of the cause. Mastitis is therefore characterized by a range of physical and chemical changes in the milk and pathological changes In the glandular tissue. The most important change in the milk include discoloration, the presence of clots and the presence of large number of leukocytes(1). Mastitis is a global problem as it adversely affects animal health, quality of milk and economics of milk production and every country including developed ones suffer huge financial losses (2) due to reduced milk yield (up to 70%), milk discard after treatment (9%), cost of veterinary services (7%) and premature culling (14%) (3). Although clinical cases of mastitis are a source of loss, more important economically is subclinical mastitis due its higher prevalence and associated decrease in milk production. Although, subclinical mastitis occurs worldwide, its economical importance is especially significant in the Mediterranean countries (4). Bacterial infections account for the highest percentage of mastitis in ruminants, and the proportion of particular etiological factors is determined by the climatic zone, region, flock size and study period, among others (5). Several pathogens may cause mastitis but *Staphyl*ococcus spp. are the most frequently diagnosed causal microorganisms of Mastitis in sheep. Other pathogens such as *Streptoc*occus spp., *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Mannheimia haemolytica*, *Corynebacterium* spp can produce Mastitis in small ruminants, but there percentage are lower. In addition, severe cases of Mastitis related to incorrect preventative strategies have been attributed to the *Serratia marcescens*, *P.aeruginosa* or *Burkholderia cepacia* (4, 6, 7, 8). In Iraq the morbidity rate of clinical mastitis ranged between 3.55% (9) and 13.17% (10), whereas the morbidity rate of subclinical mastitis ranged between 15.24% (11) and 36.08% (10). The annual reports of the state company of veterinary medicine revealed that 35.45%, 39.69% and 20.79% of all cases of mastitis in Iraq have been reported in Al-Anbar province during the years of 1994, 1995 and 1998 respectively (10). The references demonstrate that gram-negative infections are not common in the dry period because of the high concentration of lactoferrin in the dry secretions (1) while it increase soon after parturition, therefore, this study was conducted to investigate this phenomenon; the sensitivity and specificity of CMT and WST; the role of self- cure phenomenon in relief of mastitis in postpartum period and the efficacy of dry- off therapy against gram negative bacteria.

Materials and Methods

- **Flocks and survey design:** 104 pregnant ewes from local breeds belonging to 4 flocks in Al- Anbar province were subjected to the study from July 2010 to January 2011. Age of these ewes ranged from 2- 5 years, 200 mammary secretion samples were collected during the last 3 weeks of gestation period, in addition to 200 milk samples during the first week after lambing were collected from the same ewes.
- **Sampling method:** Milk samples were collected according to (1) where the udder and teats were cleaned with water and left to dry and then were swabbed with cotton soaked in 70% ethyl alcohol. The first three streams were discarded before 5- 10 ml of milk were collected in sterile tubes. The samples were transported to the laboratory on ice and tested by California Mastitis Test and White side test within 6 hours of collection and refrigerated if the culture delay until the next day.
- **Clinical examination and epidemiological data:** In pre- partum period, In addition to the clinical examination, the information regarding the identity, age, period of gestation, number of previous parturitions had been recorded. After parturition, the ewes reexamined clinically before collection of milk samples, and the information included date and nature of parturition, number of born lambs and their healthy status had been reported.
- **Case definition:**

1. **Subclinical mastitis:** Halves and their milk samples which had no grossly detectable abnormalities, but positive for California Mastitis Test, White side test and\ or were bacteriologically positive considered as subclinically infected halves.
2. **Clinical mastitis:** Halves and their milk samples which had detectable abnormalities, with or without positive results in bacteriological examination were considered as clinically infected halves.

- Field tests:

1. California Mastitis Test (CMT): was applied to the apparently healthy samples according to (12).

2. White side test: was applied to the samples according to (13). Sensitivity and specificity of both tests have been calculated according to (14) as the following:

Sensitivity = A/ (A + C)

Specificity = D/ (B + D).

	Disease present	Disease absent
Test positive	A	B
Test negative	C	D

Fifth: Culture media and biochemicals.

Media	Manufacturer
Blood Agar	HIMEDIA
Brain Heart infusion Agar	Central Drug House (p) LTD
Brian Heart infusion Broth	Central Drug House (p) LTD
Briliant Green Agar Base Modified	HIMEDIA
H2O2	SIGMA
MacConkey Agar Base	HIMEDIA
Maltose	Fluka
Mueller – Hinton Agar	HIMEDIA
Nacl Sodium chloride	SIGMA
Nutrient Agar	HIMEDIA
Nutrient Broth	HIMEDIA
Pepton water	HIMEDA
Potassium hydroxide 99%	SIGMA
Sodium hydroxide pellets	(BDH) limited pool England
Sucrose	Fluka
Urea Agar Base	HIMEDIA

- **Laboratory tests:**

1. Catalase test according to (15)
2. Oxidase test according to (15)
3. Oxidation\ fermentation test according to (16)
4. Motility test according to (16)
5. Biochemical tests according to (15)
6. API 20 E according to (16)

- **Stains:** Gram stain according to (16)

Results and Discussion

As appeared in Table (1), 26 halves were showed clinical mastitis in pre- partum period in compare to 11 halves infected with clinical mastitis in post partum period either due to gram negative or positive bacteria. 66 halves were showed subclinical mastitis in pre- partum period while, 45 halves infected with subclinical mastitis in post partum period either due to gram negative or positive bacteria. This decline in number of infected halves may be due to the dry- off therapy in the last period of pregnancy.

Table (1) Number of infected and non- infected halves

	Number of samples	**Number of infected halves**		**Sum of mastitic halves**	**non – infected halves**
		Clinical	Subclinical		
Pre-partum	200	26	66	92	108
Postpartum	200	11	45	56	144
Total	400	37	111	148	252

As shown in tables 2,3,4 and 5, the results of CMT and WST reveal that CMT is more accurate than WST in both pre- and postpartum periods but, the sensitivity and specificity of both tests in this study were less than that revealed in other studies (9,10,11) because this study carried out on ewes in pre- and postpartum period in which the mammary secretions differ in constituents from the milk in lactation period leading to high rate of false results (12).

Table (2) Sensitivity and specificity of CMT IN pre- partum period

Results of CMT	**Results of culture**		**Total**
	+	-	
+	46	45	91
-	20	44	64
Total	66	89	155

Sensitivity = 46/ 66 = 69.70%
Specificity = 44/ 89 = 49.44%

Table (3) Sensitivity and specificity of WST in pre partum period

results of WST	**Results of culture**		**Total**
	+	-	
+	42	38	87
-	24	51	68
Total	66	89	155

Sensitivity = 42/66 = 63.64%
Specificity = 38/89 = 42.70%

Table (4) Sensitivity and specificity of CMT in post partum period

Results of CMT	Results of culture		Total
	+	-	
+	17	52	69
-	14	33	47
Total	31	85	116

Sensitivity = 17/31 = 54 .84%
Specificity = 33/85 = 38.82%

Table (5) Sensitivity and specificity of WST in post partum period

Results of WST	Results of culture		Total
	+	-	
+	18	53	71
-	27	58	85
Total	45	111	156

Sensitivity = 18/ 45 = 40%
Specificity = 58/111 = 52.25%

The results of bacterial culture, isolation and identification revealed that 26 (13%) halves infected with gram negative bacteria in the pre- partum period, in compare to 32 (16%) halves in the postpartum period, as shown in Table 6, this elevation in the number of infected halves may contribute to the effect of vigorous suckling of the lambs that leads to wounds in the udder thus predisposing to mastitis (17), the effect of stress during parturition (18) which suppress the immunity and facilitate the chance of microorganisms to infect the udder and exposure of the udder to the contaminated ground during parturition and soon after.

Table (6) Number of halves infected with gram negative bacteria

Period	Type of mastitis		Total
	Clinical	Subclinical	
Pre- partum	13	13	26
Postpartum	10	22	32
Total	23	35	58

From 26 halves infected with gram negative bacteria, 8 halves were treated with slow-releasing intramammary tubes 2- 3 weeks before parturition while 26 halves allowed without treatment to evaluate the efficacy of treatment in pre- partum period, thus in postpartum period 7 (87.5 %) halves cured from mastitis after parturition in compare with one (12.5%) half remain infected, while in non- treated group, cases of mastitis was increased from 18 in pre- partum period to 31 cases in postpartum period as shown in Table 6. This result agree with (8) who demonstrated that antibiotic dry- off therapy significantly reduce the incidence of intramammary infections in dairy ewes. This decline in number of infected halves in postpartum period in treated group prove the efficacy of treatment of mastitis during the dry period where in this period the bacteria are more susceptible to the antimicrobial drugs (1), in addition to the role of the self- cure phenomenon where (6, 7, 19, 20) demonstrated that the spontaneous cure rate at parturition, which can be especially high for small ruminants, is 20–60%. This phenomenon may be contributed to many defense mechanisms of the udder, including lysozyme, immunoglobulins, leukocytes, the high concentration of lactoferrin in the dry secretions and elimination of the pathogens through the milk (1, 7, 12).

Table (7) Efficacy of Pre- partum dry period therapy

	Treated halves		Non – treated halves	
	Pre-partum	**Postpartum**	**Pre-partum**	**Postpartum**
Halves infected with gram negative bacteria	8	1	18	31
halves Not infected with gram negative bacteria	79	86	95	82
Total	87	**87**	**113**	**113**

The gram negative bacteria that isolated in this study summarized in Table (8) where shows that *M.haemolytica* and *P. aeruginosa* were the most prevalent gram negative bacteria. This result agree with (5, 7, 8) whose demonstrated the importance of these bacteria as causative agents of mastitis in addition to other bacteria. Other gram negative bacteria also isolated in this study; *E.coli, H. ovis, K.pneumoniae, P. multocida, P.mirabilis, P. vulgaris, S. marsescence, Y. enterocolytica.* most of these bacteria was also isolated in other studies in Iraq (9, 10, 21).

Table (8) The gram negative bacteria that recovered from the infected halves

Period	**Type of mastitis**	***E.coli***	***H. ovis***	***K.pneumoniae***	***M.haemolytica***	***P. aeruginosa***	***P. multocida***	***P.mirabilis***	***P. vulgaris***	***S. marsescence***	***Y. enterocolytica***	**Total**
Pre- partum	Clinical	1	0	0	2	5	3	0	1	1	0	13
	subclinical	2	1	0	4	1	1	3	1	0	0	13
Postpartum	Clinical	2	0	0	5	2	1	0	0	0	0	10
	subclinical	2	1	1	5	5	5	2	0	0	1	22
Total		7	2	1	16	13	10	5	2	1	1	58

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