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Isolation of Bacteria Associated with Mastitis and Measuring Level of Lactoferrin in Cow's Milk

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Abstract:
The study was conducted in Salah al-Din Governorate in areas adjacent to the city of Tikrit to diagnose mastitis in cows using various physical, chemical and bacterial tests, study the factors that accompany the disease, identify the types of bacteria causing the disease, record the clinical signs of the disease, compare the accuracy of the tests among them, and determine sensitivity and resistance. Microbiology of antibiotics and knowledge of the relationship of lactoferrin concentration to clinical and subclinical mastitis

This study included (50) cows, 40 of which suffered from clinical and subclinical mastitis, and 10 of which were healthy and showed no signs of infection. For the period from April 2022 to January 2023, vital signs and physical signs in the milk were recorded using special forms that were prepared in advance. For all animals. 50 ml of milk was collected from all quarters of the udder and placed in sterile tubes. Bacterial screening operations were performed on the samples and the concentration of lactoferrin was measured through the separation process and the whey was stored in the freezer at a temperature of -20°C until ELISA tests were performed on them to measure the concentration of lactoferrin in the milk serum. .

a. The results of aerobic bacterial tests in cows showed that the total incidence of clinical and subclinical mastitis was (27.5%) and (72.5%), respectively. Bacteriological examinations were performed on all samples, and the gram-positive bacterial species were isolated from the milk. They were different in percentages, such as Coagulase negative Staphylococci, the most prevalent bacteria with a percentage of (42.23%): Staphylococcus intermedius 9.85%, Staphylococcus hyicus 23.94, Staphylococcus saprophyticus 1.40%, Staphyl epidermidids coccu 7.04 %, as for Staphylococcus aureus, the percentage was the highest (25.35%). Followed by Streptococcus spp, 9.85%. The prevalence of Gram-negative bacteria varied in percentages, such as Escherechia coli 12.67%, Proteus mirabilis 11.26%, Manheimia hemolytica 1.40%, Pseudomonas aeroginosa 4.22%, Pasteurella spp. 2.81%. The results of lactoferrin showed significant differences at the level (P>0.05) in the milk of cows suffering from clinical and subclinical mastitis, which had the highest concentration from healthy cows (control).

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عزل الجرثومي المصاحبة لالتهاب الضرع وقياس مستوى اللاكتوفرين في حليب الابقار

الخلاصة

اجريت الدراسة في محافظة صلاح الدين في مناطق مجاورة لمدينة (تكريت) لتشخيص التهاب الضرع في الأبقار بمختلف الفحوصات الفيزيائية ,الكيميائية والبكتيرية ,ودراسة العوامل التي ترافق المرض, وتحديد أنواع البكتريا المسببة للمرض ,وتسجيل العلامات السريرية للمرض ,ومقارنة دقة الفحوصات فيما بينها وتحديد حساسية ومقاومة الجراثيم للمضادات الحيوية ومعرفة علاقة تركيز اللاكتوفرين بالتهاب الضرع السريري وتحت السريري

شملت هذه الدراسة (50) حيوأنا من الأبقار (40) منها تعأني من التهاب الضرع السريري وتحت السريري و10 منها سليمة لا توجد علامات التهاب عليها ,للفترة من نيسأن 2022 الى كأنون الثأني 2023 تم تسجيل العلامات الحيوية والعلامات الفيزيائية على الحليب باستمارات خاصة تم إعدادها مسبقاً لجميع الحيوانات. تم جمع 50 مل من الحليب من جميع ارباع الضرع ووضعة في أنابيب معقمة ,اجريت على العينات عمليات الورع الجرثومي وقياس تركيز اللاكتوفرين من خلال عملية الفصل وتخزين مصل الحليب في التجميد بدرجة حرارة -20 م لحين اجراء فحوصات الاليزا عليها لقياس تركيز اللاكتوفرين في مصل الحليب.

أظهرت نتائج الاختبارات الجرثومية الهوائية في الأبقار أن النسبة الإجمالية للأصابه بمرض التهاب الضرع السريري و تحت السريري وكأنت (27.5%) و (72.5%) على التوالي. اجريت على جميع العينات عمليات الفحص البكتريولوجي وتم عزل الأنواع الجرثومية موجبة الكرام من الحليب وكأنت مختلفة في النسب الجرثومة الأكثر أنتشارا بنسبة 42% Coagulase negative *staphylococci* حيث، كأنت *Staphylococcus intermedius* 9.85 % , *Staphylococcus hyicus* 23.94 , *Staphylococcus saprophyticus* 1.40 %, *epidermidids Staphylococcu* 7.04 % , اما *Staphylococcus aureus* فكأن أنتشار 9.85 %. *Streptococcus spp* كأنت النسبة المئوية لها هي الاعلى 25.35%)) . وتليها الـ *Escherechia coli* % , *Proteus mirabilis* الجراثيم سالبة الجرام متفاوت بنسب حيث كأن كتالي 12.67 11.26 % , *Manheimia hemolytica* 1.40 % , *pseudomonas aeroginosa* 4.22 %, *Pasteurella spp*. 2.81 % أظهرت نتائج اللاكتوفرين فروقات معنوية عند مستوى (P>0.05) في حليب الأبقار المصابة بالتهاب الضرع السريري وتحت السريري هي الاعلى تركيزاً من الأبقار السليمة السيطرة

1-Introduction

Over the past century, great progress has been made in controlling mastitis. But it is due to changing population dynamics, herd structure and more stringent processing standards that make mastitis a complex disease and it remains a major problem in the dairy industry. Hence, there is a need for more intensive research in all areas where milk production, especially milk, is among the most important sources of food for most of the world's population (1). The increasing global demand for dairy products is driven by the need to increase average milk production per cow. Increases in milk production have resulted from genetic selection as well as improved cow nutrition and management. One of the biggest problems affecting high milk yield is poor udder health, especially due to mastitis (2).

Mastitis is defined as an inflammation of the udder that affects many dairy cows (3). Mastitis can be classified into clinical and subclinical forms. Clinical mastitis (CM) is an infection characterized by sudden onset, change in the composition and appearance of milk, decreased milk production, and signs of local inflammation in the affected

udder areas. On the other hand, subclinical mastitis (SCM) is an infection that does not show obvious symptoms of local inflammation. There are no clear indications of SCM in the milk or on the udder, however it reduces milk production. This type of disease is more common than clinical mastitis (4). SCM is responsible for 70% of economic losses and is one of the major factors limiting milk production (5). Different screening methods are used to diagnose SCM based on the physical and chemical changes of milk (6). Field tests such as the California mastitis test (CMT) and the white side test (WST) are preferred as screening tests for subclinical mastitis due to their ease of use and ability to produce rapid and satisfactory results (7). However, bacteriological cultivation of samples has been Milk serves as an essential gold standard method for definitive diagnosis of subclinical mastitis and evaluation of intra-udder infection (8). More than a hundred different microorganisms have been isolated from bovine mastitis, Staphylococcus, Streptococci and Gram-negative bacteria are the most frequently isolated pathogens (9). Some types of Staphylococcus aureus bacteria are characterized by being a common cause of this disease, as recent studies have agreed that they are the cause of mastitis in cows in all cities of the world, as the bacteria that cause the disease is able to infect the host with virulence factors possessed by the main bacteria that cause the disease (10). On the other hand, environmental mastitis can be widely known as an infection that affects the udder due to causes in which the environment in which these cows live can be the primary source of those causes (4).

2-Materials and Methods

Collect milk samples

Milk samples were taken in the morning. According to (11), milk samples were collected from all quarters of the udder separately in sterile tubes. The nipples are cleaned with water and then left to dry, then the nipples are sterilized with a 70% diluted alcohol solution. The first three nipples are discarded and 50 ml is taken. From the milk in sterilized tubes and immediately transfer it to a refrigerated box to be transported to the laboratory.

3-Result

Percentage of bacterial isolates from cases of mastitis:

The percentage of each bacterial species isolated and recognized from udder milk samples is shown in (Figure1 and Table 1). The species identified from milk samples were staph. Aureus25%, Staph. Intermedius9.85%,Staph. Hyicus23.94%,Staph. Epidermidis7.04%,Staph. Saprophyticus 1.40%, Pseudomonas aeruginosa 4.22%, Proteus mirabilis 11.26%, Streptococcus Spp9.85%, E. coli 12.67%, Pasteurella spp2.81%, M. hemolytica1.40%, the most common pathogens detected in staph mastitis. Aureus, Staph. Hyicus, E. coli.

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Table No. (1) shows bacteria isolated from mastitis

ت	نوع البكتريا المعزولة	عدد العزلات	النسب المئوية للعزلات
1	*staph. aureus*	18	%25.35
2	*Staph. intermedius*	7	%9.85
3	*Staph. hyicus*	10	%23.94
4	*Staph. epidermidis*	5	%7.04
5	*Staph. saprophyticus*	1	%1.40
6	*Pseudomonas*	3	%4.22
7	*Proteus mirabilis*	8	%11.26
8	*Streptococcus Spp*	7	%9.85
9	*E. coli*	9	%12.67
10	*Pasteurella spp*	2	%2.81
11	*M. hemolytica*	1	%1.40

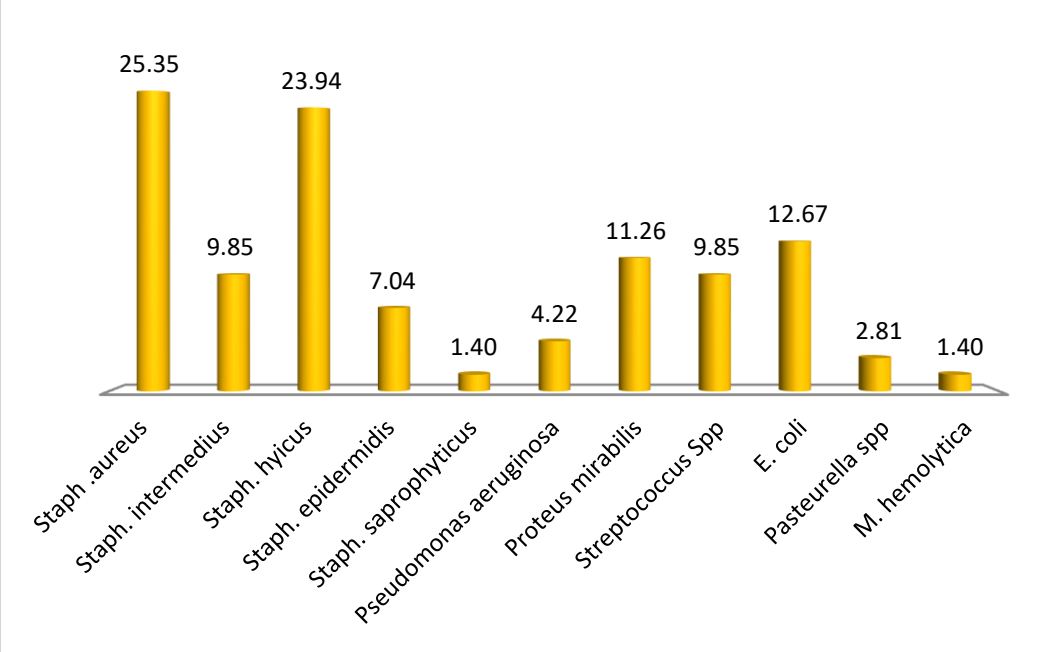

Figure No. (1) shows bacteria isolated from mastitis

The study included 200 milk samples from all quarters of infected and healthy cows used in the study at different periods to determine lactoferrin concentrations. No bacterial growth was detected in 58.2% (93/160) quarter of the milk samples, while in the other 41.8% (93/160) samples bacteria were found. The test results showed

The concentration of lactoferrin in the milk of uninfected animals (10 cows) ranged from 2.73±0.6 ng/ml (n = 67). The results were divided into animals infected with clinical and subclinical mastitis, as shown in Table No. (2) .

Table No. 2 shows lactoferrin concentrations in cows without mastitis and cows with clinical mastitis and under the bed.

Lactoferrin concentration range)ng/mL(	Lactoferrin concentration rate)ng/mL(	Number of positive samples	Number (milk samples(	Number (cows)	Cows
3.2 - 2.2	c0.6 ± 2.73	0	40	10	Uninfected cows
10.4 - 7	1.65 ± 8.71 a	28 %27.5	44	11	cows with clinical mastitis
6.5 - 4	b1.2 ± 5.23	39 %72.5	126	29	cows with subclinical mastitis

4- Discussion

The majority of isolates in milk samples during the study were Staphylococcus spp. has been registered

Similar results regarding the higher percentage by (12) and (13) who found that Staphylococcus spp was the most frequently isolated bacterial group. In addition to (14), Coagulase-negative staphylococci are the main causative agents of subclinical mastitis (30-95%), especially in dairy cows. Several studies showed that CNS species were one of the important causes of mastitis in ruminants (15).

staph is registered. aureus in this study in clinical and subclinical cases, and this is consistent with (16).

(17). 28.5%. All CMT-positive specimens were cultured to determine the causative agent. Of the 170 cultured samples, 153 samples were positive for the causes of subclinical mastitis. The predominant bacteria isolated were Staphylococcus species. Of these, Staphylococcus aureus (44.9%) was the predominant type of Staphylococcus. The Gram-negative enterobacteria Escherichia coli (8.8%) (18).

In this study, S. saprophyticus was isolated at a low percentage (1.40%). This is consistent with that of (19).

(20) who recorded that Staph. saprophyticus (1.2%), but our percentage does not agree with their results. Staph was isolated. intermedius of cows with mastitis (9.85%). This result is consistent with the result of (21) and (22). During the current study, E.coli was isolated in a percentage of 12.67%, and this is consistent with the results of (23), who reported that the percentage of E.coli bacteria was (0.0%-7.8%.(

It is also consistent with the result of (24), which found that the percentage of E. coli was (10.34%). Proteus mirabilis was isolated in the study at a rate of (11.94%), and this result is consistent with The results of the study showed significant differences at ($P \leq 0.05$), and the highest percentage in clinical mastitis was 1.65 ± 8.71 (ng/mL). This result is consistent with (25), whose results appeared somewhat similar to the results of the current study.. .

(26) Lactoferrin has an important role in udder protection. Many factors affect lactoferrin in cow's milk, such as the stage of lactation, milk production, and infections that occur within the udder. This is consistent with the results of the study, which showed significant differences in animals with clinical and subclinical mastitis.

(27) indicated that cows suffering from mastitis affect the level of lactoferrin in milk. The concentration of lactoferrin obtained from cows with mastitis was higher than that of healthy cows. The study is consistent with the current study.

The study is consistent with (28), whose results showed that the concentration of lactoferrin in healthy cows is the same as the study found, and this is consistent with our results.

5-Conclusion

Staphylococcus aureus (CNS) was the most common intra-udder pathogen followed by Staphylococcus aureus**.**

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