# Mycotic mastitis in sheep

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## Abstract

The study was aimed to investigate the mycotic mastitis in sheep during the period from October 2011 to May 2012 in different areas of Al-Diwaniya province. 253 ewes were examined, and from which 500 milk samples were collected (495 samples from apparently healthy ewes that examined by California Mastitis Test (CMT ) in addition to five samples from sheep infected with clinical mastitis) for isolation and identification of yeasts and molds adopted the method of culturing on Saboaurad Dextrose and Corn meal agar in addition to Chrome agar and biochemical tests as well as specific yeast kits (Integral system yeast plus) for diagnosing the mycotic agents. Results were indicate that the incidence of mastitis in ewes was 17.8%, while the percentage of mycotic mastitis was 9.4%, (0.4% and 9% of clinical and subclinical forms respectively). Yeasts were isolated and identified grossly by colony shape, size, and color, and by biochemical testing which represents 9.61% of the fungal causes, and the yeasts isolates were *Candida famata* and *Rhodotorula rubra* from the clinical cases of mastitis only. Molds 90.38% also were isolated, as a high isolates of *Asperigllus niger 28.84%*, *Asperigllus flavus* 23%, *Asperigllus fumigauts* 17.30%, *Pencillium spp* 13.33%, *Asperigllus terrus* 5.76%, and the least percentage of isolation 3.84% was of the *fusarium spp*.
**Key words: Mycotic mastitis, sheep, yeasts, molds.**

# التهاب الضرع الفطري في الأغنام

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## الخلاصة

هدفت الدراسة للتحري عن حدوث التهاب الضرع الفطري في الأغنام للفترة من تشرين الأول لسنة 2011 ولغاية أيار 2012 في العديد من مناطق محافظة الديوانية. شملت الدراسة فحص 253 نعجة وتم جمع 500 عينة حليب منها 495 عينة من نعاج سليمة ظاهريا فحصت باستخدام اختبار كاليفورنيا بالإضافة إلى خمسة عينات من نعاج مصابة سريريا بالتهاب الضرع ولغرض عزل وتشخيص الخمائر والاعفان و اعتمدت طريقة الزرع على الأوساط الخاصة (وسط سابرود ووسط دقيق الذرة إضافة إلى وسط الكروم) والاختبارات الكيمياوية اضافة الى استخدام عدة integral system yeast plus لغرض تشخيص الخمائر . أظهرت النتائج إن نسبة الإصابة بالتهاب الضرع قد بلغت 17.8 % في حين كانت نسبة التهاب الضرع الفطري 9.4 % بشكليه السريري وتحت السريري (0.4 % ، 9%) على التوالي. تم عزل وتشخيص الخمائر من خلال شكل المستعمرات النامية و طرق التشخيص المختلفة حيث شكلت ما نسبته 9.61% من المسببات الفطرية وكانت الخمائر المعزولة *candida famata* و *Rhodotorula rubra* والتي عزلت من الحالات السريرية لالتهاب الضرع فقط ، إما الفطريات الأخرى المعزولة كانت هي الاعفان وبنسبة 90.38 % وكانت أعلى نسبة عزل للعفن *Asperigllus niger* بنسبة 28.84 % بينما كانت نسبة *Asperigllus flavus* هي 23% في حين بلغت نسبة *Asperigllus fumigauts* 17.30 % وكانت *Pencillium spp* 13.33 % ونسبة *Asperigllus terrus* 5.76 % وكانت اقل نسبة عزل للعفن *fusarium spp* هي 3.84 % .
**الكلمات المفتاحية: التهاب الضرع الفطري ، الاغنام ، الاعفان ، الخمائر.**

## Introduction

Mastitis is a worldwide important disease of productive ewes and it is a common health problems in ewes characterized by inflammation of mammary glands, usually is caused by noninfectious and or infectious agents which more serious in mostly bacteria, yeast, filamentous fungi, mycoplasma, viruses, algae (1). The economic importance of mastitis is coming principality from premature culling of ewes with udder abnormalities, mortality of ewes and lamb, low weight gain of the lamb, reduction in

the milk yield, alteration of the qualitative characteristics of milk and others related losses include labor, feed cost for orphan lambs as well as veterinarian costs as drugs , fees and careful management (2). Ovine mycotic mastitis is usually caused by yeasts but mastitis due to filamentous fungi mostly Aspergillus spp. has been reported higher than yeast . It occurs as sporadic cases or sometimes as outbreak, however, the seriousness of infection depends on the number of organisms present in the glands and the species involved (3). Generally, the studies have suggested that mycotic mastitis incidence is increased (4)3% and (5) 9.9% .The increasing incidence of mycotic infection will be lead to increase resistance of mycotic agents to antifungal drugs that will make public health problem in medication responsibility (6). In Iraq, a poorly particular mycotic mastitis ewes studying was performed, there was only one research corresponding by (7) but another as (8) could be isolated some mycotic agents in their studies which non-restricted on mycotic mastitis in ewes as specific.

## Materials and methods

### Sample collection

Five hundred milk samples were collected randomly from (253) lactating Awassi sheep distributed in different areas in Al-Diwaniyia province during November 2012 to May 2013 as 495 milk samples from (sub clinical mastitic ewes) as well as 5 milk samples (clinical mastitic ewes) according to (9) after accurate exam of udder and about 10 ml of milk was collected in sterile test tube after discarding the first three milking streams, then samples were placed in racks for eases of handling and transported in cold box to the laboratory for California mastitis test examining. From each half of udder, a squirt of milk sample was placed in each of the cups on the CMT paddle and an equal amount of CMT reagent was added to each cup and mixed well, Reactions were graded as Negative and Trace, and +1,+2,+3 for positive reactions (10).

### Isolation and identification of mycotic mastitis agents:

All milk samples of clinical mastitis and positive CMT samples were centrifuged 3000 rpm/15 min, and precipitate was cultured on Sabourauds dextrose agar (SDA)and potatoes dextrose agar (PDA) which containing chloramphincol (0.05gm/l) and incubated separately at 25ºC and 37ºC to reveal dimorphism, examined grossly (include size, shape, color and smell of colony on PDA and SDA media) and microscopically , they were not considered negative for growth until after one weeks of incubation (11).

### Identification of molds

Diagnosis of isolated molds depended upon macroscopic examination (morphological features: colors, consistency of growth on agar as well as external general color of their petri dishes) (12) and microscopic examination by making microslide culture technique to observe the shape and structure of hyphae and spores(13).

### Identification of yeasts

Yeasts identified according to(12) and(14) to observe morphological features, germ tub and capsule as well as we use the integral system yeast plus kit (Liofilchem (Italy)) and Chromagar (Himedia (India).

### Statistical analysis

Data were analyzed using SPSS program (15). Chi-square test ($p \leq 0.05$), used to detect the significant variance.

## Results

During the study period, 500 milk samples were collected from (253) lactating ewes examined for mastitis. Table (1) was showed that clinical mastitis as 1% which recorded in only 5 milk samples and 84 (16.8%) milk samples were positive for CMT and the total mastitic milk samples 89 (17.8%). Mycotic mastitis in ewes 47( 9.4%) was recorded as 2(0.4%) clinical form and 45(90%) as subclinical mastitis (Table 2) There were fifty two mycotic isolates were isolated 2 (3.84%) and 50 (96.15%) from clinical and subclinical mycotic mastitis respectively in Table (3), which revealed molds were highly significant in percentage of mycotic isolation 47 (90.38%) as 100% and 90% from subclinical and clinical mycotic mastitis than yeast 5 (9.61%) which isolated as 10% from

**Table (1) : Clinical and subclinical mastitis in examined ewes.**

| examined ewes (253) | Total mastitis | | Clinical mastitis | | Subclinical mastitis | |
|---|---|---|---|---|---|---|
| Milk samples (500) | No | % | No | % | No | % |
| | 89 | 17.8 | 5 | 1 a | 84 | 16.8 b |

**Table (2) : Clinical and subclinical mycotic mastitis.**

| No. of examined Milk samples | Mycotic mastitis Milk samples | | Mycotic clinical mastitis | | Mycotic subclinical mastitis | |
|---|---|---|---|---|---|---|
| | No | % | No | % | No | % |
| 500 | 47 | 9.8 | 2 | 0.4a | 45 | 9b |

**Table ( 3) : Percentage of mycotic agents isolated from mycotic mastitis.**

| Mycotic isolates | | | Mycotic mastitis | | | |
|---|---|---|---|---|---|---|
| | No | % | Clinical form | | Subclinical form | |
| | | | No | % | No | % |
| Yeast | 5 | 9.61 A | —— | ——a | 5 | 10 A b |
| molds | 47 | 90.38 B | 2 | 100a | 45 | 90 Bb |
| Yeast + molds | 52 | | 2 | 3.84 a | 50 | 96.15 b |

**Small letters represent statistics horizontally**

**Table (4 ): Percentage of mycotic species isolates from mastitic ewes.**

| Mycotic pathogen | | No of isolates | % |
|---|---|---|---|
| yeast | *Candida famata* | 4 | 7.69 C |
| | *Rhodotorularubra* | 1 | 1.92 C |
| mold | *Asp .niger* | 15 | 28.84 B |
| | *Asp. flavus* | 12 | 23 B |
| | *Asp. fumigatus* | 9 | 17.30 A |
| | *Asp.terrus* | 3 | 5.76 C |
| | *Pencilliumspp* | 6 | 13.33 A |
| | *Fusariumspp* | 2 | 3.84 C |
| Total mycotic isolates | | 52 | 100 |

**Different letters refers to significant differences at ($p < 0.05$)**

Subclinical form, while it was not isolated from clinical mastitic form. The mycotic species that isolated from mastitic ewes included *Asperigllus niger* and *A.flavus* were isolated from mastitis milk with highly significant percentage 28.84%, and 23% when compare with *A.fumigatus* and *Pencillium spp* as 17.3% and 13.33 respectively, whereas the lowest percentage of *Rhdotorula rubra* , *A.terrus* and *Fusarium spp* 1.92%, 5.76%, 3.84% respectively (Table4). Percentage of *Rhdotorula rubra*, *A.terrus* and *Fusarium spp* 1.92%, 5.76% , 3.84% respectively (Table 4).

## Discussion

These results of clinical and subclinical mastitis in ewes were in agreement with results by (16) and (17) who recorded 19%, 17% as percentage of mastitis in ewes in Newzealand and Pakistan respectively as well as showed the clinical and subclinical mastitis ranged between (1-4%), (80-86%) respectively. In contrast, other results by (18)which revealed high percentage of mastitis 28% and clinical mastitis 11%, but with low subclinical mastitis percentage 40% when compared with our results. This variation between our results and others was attributed to many causes like numbers of samples, study season, ewes breed, rearing system, type of samples testing, prophylactic programs that applied in their flock or not, flock size and managements routines. The present results of mycotic mastitis percentage was closely to that repots by (19) and (5) which appeared (9.1%,9.9%), (0.4% ,0.8%), (43%,46%) of mycotic, subclinical and clinical mastitis of ewes. But our results were regarded highly percentage when compared with mycotic mastitis 3% that including 0.0% subclinical and 3% of clinical mycotic mastitis by (20) in Mongolia , and this wide variety in percentage of mycotic mastitis occurrence in ewes that depending in firstly on climatic status, sanitary measurement also relating to using antibiotic in continuous ,sometimes high doses as intramammary infusion therapy as well as environmental bacterial mastitis that lead to necrotic tissues in udder induce mycotic infections. (21). About results of molds and yeasts isolation that showed in our study were also recorded by (5) who isolated molds with high percentage 60% than yeasts 11%, as well as (8) found subclinical mastitis caused by yeast and molds than clinical from caused by molds only. The present study was showed that *Asperigllus spp* isolates (39) out of 52 mycotic isolates which in agreement with resultant of (22) by isolate *Aspergillus*

*spp* as 86% of molds and represent as 64% from mycotic agents as causative agents of mycotic mastitis in ewes in Newgersy state, while (23) in Iran found *Pecillium spp* isolates as high significant 66% in isolation as well as *Asperigllus spp* 42% than yeast , which thought to be these molds have availability to grow at different macro and micro-environmental condition as well as can cause different disease of both animals and human which act as sources of these molds and spread from one site to another. In contrast (24) reported that ovine mastitic aspergillosis was very rare ,but *A.fumigatus* can cause bovine, goat mastits as well as in ewes,whereas ,*Candida spp* and *Fusarium spp* could be isolated with variant percentage especially from lactating ewes shortly after intramammary antibiotic treatment. Yeasts isolation with low significant percentage than in similarity with that summarized explanation by (25) most yeasts infection with unapparent sings as well as spontaneously recover.

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