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Isolation and Identification of Aerobic vaginal Bacteria and Fungi from Adult Cows in AL- Kufa district/ Al-Najaf province-Iraq

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Abstract

Fifty-six cows were examined in this study. Clinical vaginitis was appeared on 19 out of 56 cows with incidence rate 33.92% . Vaginal swabs were collected from the posterior area of the vagina from all animals for microbiological isolation (bacteria and fungus). Totally, 95 and 42 bacterial isolates were isolated from intact vagina and cows with vaginitis respectively. The isolates from the normal vagina were *E. coli* 22.1% followed by *Staph. Spp. Proteus Spp*., *Pseudomonas Spp* and *Neisseria* with incidence rate 18.94%, 9.47%, 9.47% and 3.15% respectively. However, in cows suffered from clinical vaginitis, *Staph. Spp* . recorded the highest percentage 26.19% followed by *Staph aureus*, *E. coli*, *Proteus* and *Pseudomonus Spp*. 21.42 %, 11.9%, 9.52% and 9.52 % respectively while Neisseria was a lowest percentage 2.38%. The mycoflora that isolates from intact vagina were *Aspergillus spp*., *Mucor spp*. and *Penicillium spp.* with incidence rate 16.21%, 2.7% and 13.51% respectively. While, *Penicillium* with incidence rate 36.84% was the only fungal isolate, which were isolated from the cow with vaginitis.

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Introduction

The normal microbial flora of the bovine urogenital tract is made up of a dynamic mixture of aerobic, facultative anaerobic and strict anaerobic micro-organisms (Songer and Post, 2005). The genital system becomes contaminated with micro flora by different ways usually by ascending these flora from the environment during the breeding, parturition or genital manipulation, in most females. The infectious organisms are eliminated by natural genital defense mechanisms, however in some females, these mechanism fails to protect the genital system from the contamination and lead to cause

the reason of infertility (Torres *et al.*, 1994) .Vaginitis is an inflammation of mucus membrane of the vagina. It is often caused by microorganism such as *Corynebecterium spp*., *Arcanobecterium pyogens* , *E. coli* and *Staphylococcus spp*., and cause many reproductive problems such as early embryonic death ,abortion, premature delivery , stillbirth and death of newborn calves (Amine *et al* .,1996). This study was designed to isolate and identify the vaginal microorganism (bacteria and fungi) from natural cow and cow with vaginitis.

Materials and Methods

This study was carried out in Al- Najaf governorate /Kufa district from March to July 2012. During this period, 56 local adult cows were examined, and according to case history and vaginal examination using vaginal speculum, the animals were divided into two categories cows with intact vagina or with vaginitis.

Isolation and identification of aerobic bacteria

Firstly, the vulvar area was washed with water and disinfected solution to avoid contamination. Then, the vaginal swab was taken from the posterior area of the vagina from each animal by introducing cotton tipped swab. The swab was placed in a sterilized labeled test tube containing transport media (nutrient broth 5ml), then, betimes, these samples were sent to the laboratory. All samples were incubated at 37 C^o for 24 hrs. Then, 1-2 drops of these samples were streaked on different media such as MacConcky, blood agar, SS agar and manitol agar and incubated at 37^{o}C for 24 hrs. The isolates were identified according to colonies morphology, Gram's stain reaction and biochemical tests as mentioned by Cheesbrough, (2000).

Isolation and identification of fungi

The fungal swabs were taken by the same manner of bacterial swabs. Then, the samples were inoculated onto Sabouraud glucose agar (Merck Co., Darmstadt, Germany) supplemented with antibiotic (chloramphenicol; 0.005%) and kept at 38 ^{o}C for 7–10 days before being considered negative. Visual characteristics of the fungal colonies are including texture, pigment, and rate of growth on medium, as well as the microscopical examination under a light microscope to determine the morphological structures of the fungus species on slide mounted in lactophenol-cotton blue (Carter and Chengappa, 1991). The identification of fungus was performed according to (Dwight and Zee, 1999).

Results

According to the clinical examination, the vaginitis was diagnosed in 19 cows out of 56 with incidence rate 33.92%. The main clinical signs were secretion of mucopurulent discharge from vulva with obvious congestion of mucous membrane. The main microorganism that isolated from the normal vagina were, *E. coli* (22.1%) followed by *Staphylococcus Spp., Proteus*, *Pseudomonas* spp. and *Pseudomonus Spp.* with incidence

rate 18.94%, 9.47%, 9.47% and 9.47% respectively, while Neisseria was a lowest percentage 3.15 % table (1).

Table (1) Reveals the bacterial isolates of intact vagina

Isolates	Nunmber	Percentage%
E.coli	21	22.1
Staphylococcus Spp.	18	18.94
Protius Spp.	9	9.47
Pseudomonus Spp.	9	9.47
Staphylococcus aureus	13	13.68
Corynebacterium Spp.	8	8.42
Enterococcus Spp.	7	7.37
Campylobacter Spp.	7	7.37
Neisseria Spp.	3	3.15

In the cow with clinically vaginitis, *Staphylococcus Spp*. revealed the highest incidence rate 26.19% followed by *Staphylococcus aureus*, *E. coli*, *Proteus Spp*. and *Neisseria Spp*. with incidence rate 21.42. %, 11.9%, 9.52% and *Pseudomonus Spp*. 21.42 %, 11.9%, 9.52% and 9.52 % respectively while Neisseria was a lowest percentage 2.38%, table (2).

Table (2) Reveals the isolates from cow with clinical vaginitis

Isolates	Number	Percentage%
Staphylococcus *spp.*	11	26.19
Staphylococcus aurous	9	21.42
E. coli	5	11.9
Protius Spp.	4	9.52
Pseudomonus Spp.	4	9.52
Corynebacterium Spp.	2	4.72
Enterococcus Spp.	3	7.14
Campylobacter Spp.	3	7.14
Neisseria	1	2.38

The main fungal isolates from intact vagina were *Penicillium Spp.*, *Mucor Spp.*, and *Aspergillus Spp*., with incidence rate 16.21% (6 out of 37), 13.51% (5 out 37) and 2.7% (1 out of 37) respectively, while only *Penicillium Spp* was isolated from cows with vaginitis 36.84% (7 out of 19).

Discussion

The present study demonstrated the variation between commensal microflora in intact and inflamed bovine vaginas. Previously, it was mentioned that the composition of bovine vaginal flora was highly variable (Gani *et al.*, 2008; Dohmen *et al.*, 1995). In addition, the main source of vaginal bacteria is variable according to species including contamination from environment, skin or fecal materials (Torres *et al* 1994). The results

revealed that the *E. coli* and *Staphylococcus* were the predominant isolates from intact vagina. These results are in agreement with others (Nooralden *et al* 2012; Ayhan *et al.,* 2010). However, this results are in disagreement with other previous studies (Gani *et al.,* 2008), where *Staphylococcus* was predominant 37.8% .The variation in predominant species was not unexpected due to obvious variation in sanitary procedures ,management ,individual body condition and breeding system adopted by private small rural herds, which were examined in the present study.

Infectious vaginitis characterized mainly by mucopurullent discharge and it might advance to cervicitis and endometritis (Dohmen *et al* 1995). The possibilities of the occurrence of the predominant isolates in the present study (*Staphylococcus spp.*, and *E. coli),* might be originated from environment, fecal materials or contaminated semen however, most contamination occurs in post calving period (Studer and Morrow,1978). Noorldine *et al* (2012) were examined 90 cows submitted to artificial insemination. They found that the predominant isolate in heifer was *Staphylococcus 36.7*% followed by *E. coli* 30% , while in multiparous cows the predominant isolate was *E. coli* 38.3% followed by Staphylococcus 20% .In other hand, Gani *et al.,* (2008) found that *Staphylococcus spp* isolated mainly from repeat breeder cows in percentage of 37.8% followed by *bacillus*, *E coli* and *pseudomonas* in percentage rate 35.15%, 29.7 % and 18.9 % respectively.

In conclusion, this study revealed the high prevalence rate of vaginitis among cows reared in private small farms distributed around Kufa district, in addition to the differences in the bacterial isolates, which were isolated from healthy and inflamed vaginas.

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