

Isolation and Identification of Bacterial Flora from Vagina in Normal Ewes (Slaughter and Living Ewes).

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Abstract: The present study was conducted to determine the normal bacterial flora of the vagina in slaughter and living ewes. For this purpose, a total of eighty of vaginal swabs were collected and examined to determine the normal bacterial flora of the vagina. Of all, 46(57.5%) of the samples were collected from the vaginas of living ewes and 34(42.5%) from the vaginas of slaughter ewes. *Escherichia coli* (56.25%) were the predominant isolated bacteria, in slaughter ewes were (52.94%) while living ewes (58.7%), were the predominant bacteria. The second dominant bacteria was *Proteus mirabilis* (13.75%) and *Klebsiella pneumonia* (12.5%). Other species were isolated at relatively lower rates *Staphylococcus aureus* (8.75%) and *Staphylococcus epidermidis* (coagulase negative) (3.75%), while *Streptococcus spp.* (5%) were the less frequent isolates. We concluded that *E. coli* were the most predominant bacteria of the vagina in slaughter and living ewes.

Keywords: living ewes, slaughter ewes, microflora, vagina.

I. Introduction

Knowledge about the vaginal normal bacterial flora is of paramount importance for the proper diagnosis and treatment of pathologic abnormalities of the reproductive tract in various species (1). Genital infections in domestic ruminants are often caused by opportunistic secondary invaders, especially *Escherichia coli* species, that have frequently been isolated in majority from ewes (2, 3), goats (4) and cows (5). Coliforms (of fecal origin) and other non-specific bacteria are also opportunistic pathogens in the reproductive tract. Under stressful conditions, these opportunist bacteria may cause genital infection that usually leads to reproductive failure in ruminants (6, 7, 8). Vaginitis, a common disease of the genital tract of domestic ruminants, is then often caused by secondary bacterial invaders, mainly the *Escherichia coli* species. It has then also been reported that intravaginal devices constitute a predisposing factor for the vaginitis caused by opportunistic micro-organisms (2, 3). There are many studies reported the microflora inhabited in genital system in ewes (3, 9, 10). Martins et al. (11) reported that microflora is usually harmless until presence of predisposing factors such as trauma or another infection which may be pathogenic and cause disease.

Therefore, the present study was conducted to isolate and identify the bacterial flora of the vagina in normal ewes (slaughter and living ewes) and estimate their prevalence.

II. Materials and Methods

1- Collection Of Samples

Eighty samples of vagina were taken from eighty ewes in Al-Hillah City, thirty four slaughter house and forty six ewes living at period from March to November 2014 and the swabs were rubbed gently on the mucosa from genital system after dissected by surgical blade for bacteriological studies (slaughter ewes). Each swab was cultured immediately or stored in a transport medium until cultured.

2- Bacteriological study

Culture media used for isolation and purification of bacteria included: Nutrient agar, Blood agar, MacConkey agar, Mannitol salt agar, SS agar medium and Eosin methylin blue, Media were prepared according to the manufacturer's instructions. Inoculated media were incubated aerobically at 37 °C for 24 hours. All the isolates were stored in brain heart infusion broth with 15% glycerol at deep freeze until further use.

3- Identification of bacterial isolates

Many different colony types were noted in the initial cultures and each colony type seen was recorded and subculture on sheep blood agar or other appropriate medium to provide a pure sub-culture. The initial examination of colonies was made by naked eye and using a dissecting microscope. The colonies seen were described in terms of their morphological characters such as size, elevation, outline, color and their effect on the medium and these were recorded. Colonies were presumptively identified by these characters and their identity confirmed by further tests. Smears were made from colonies of interest, fixed and stained by gram's method and the morphology and the staining reactions were recorded. The combination of colonial morphology, growth conditions, bacterial morphology and reaction to gram stain were used to reach a presumptive identification. The

biochemical tests were performed as catalase, oxidase. IMVC test (indol production, methyl red, vogas-proskauer and citrate utilization), TSI (triple sugar iron). The culture media prepare depended to routine methods (12).

III. Results and Dissociation

1- Bacteriological Results

The result of the bacteriological examination for the eighty ewes is positive result, as shown in fig (1). The number bacteria were isolated from 46 (57.5%) of the vaginas (living ewes) and 34 (42.5%) of the vaginas (slaughter ewes). This finding is in agreement with several researchers whom reported that 56.5-96.5% of ovine vagina showing positive isolates (13). Bacteria colonizing the vagina which cause reproductive failure in ewes and other domestic ruminants (6).



Figure (1): Percentage of bacterial isolates.

2- Laboratory identification of isolates

Cultural and staining characteristics of the bacteria isolated from vagina in ewes studied. *Escherichia coli* isolates are gram-negative, oxidase-negative, bacilli-shaped bacteria lactose fermenting. *Klebsiella pneumoniae* are gram-negative, pink colony in MacConkey agar and give sticky colonies. The isolates of *Proteus mirabilis* are gram-negative, non capsulated, motile rods by swarming motility. *Staphylococcus aureus* produce gray white or yellow colony on nutrient agar, white to golden colored colonies on blood agar. All isolate of *Staphylococcus aureus* were gram positive, arrange in clusters and coagulase positive. The presumptive streptococcus spp. produced small, circular and convex colonies on nutrient agar, pin-point colonies surround by clear zones of haemolysis on blood agar. The isolate of streptococcus spp. were gram positive and arrange in chains or pairs. Furthermore, these result agree with Quinn et al. (14) and Songer & Post (15).

3- Distribution of the bacterial isolates

Of the total 80 samples, the dominant isolates from vaginal swabs of ewes were gram-negative bacilli, the frequently isolated species being *Escherichia coli* (56.25%), in slaughter ewes were (52.94%), while in living ewes (58.7%). The second dominant bacterium was *Proteus mirabilis* (13.75%) and *Klebsiella pneumoniae* (12.5%). Other species were isolated at relatively lower rates *Staphylococcus aureus* (8.75%), *Staphylococcus epidermidis* (3.75%) and *Streptococcus* spp. (5%). The bacterial isolates and their absolute/relative abundance are presented on Table (1) and Fig. (2). This is in accordance to several observations (8, 9, 13, 16, 17, 18, 19). Changes in the environment of the vagina may lead to alterations on the microflora, e.g. due to pH modifications during estrus or pregnancy. Members of the microflora may therefore act as opportunistic and play an important role the development of vaginitis and infections in reproductive superior tract, impairing the reproductive performance and therefore determine important economic hazards (11).

Table (1): Bacterial species isolated from the vaginal swabs of ewes.

Bacterial isolates	No.(%) from vaginal swabs of slaughter ewes	No.(%) from vaginal swabs of living ewes	Total No. (%)
<i>Escherichia coli</i>	18(52.94)	27(58.7)	45(56.25)
<i>Staphylococcus aureus</i>	3(8.82)	4(8.7)	7(8.75)
<i>Staphylococcus epidermidis</i>	2(5.88)	1(2.17)	3(3.75)
<i>Streptococcus</i> spp.	1(2.94)	3(6.52)	4(5)

Proteus mirabilis	4(11.76)	7(15.22)	11(13.75)
Klebsiella pneumonia	6(17.65)	4(8.7)	10(12.5)
No. (%) of isolated bacteria	34(42.5)	46(57.5)	80(100)

On the other hand, in our study we found that the higher percentage of isolated bacteria were *Escherichia coli* and *Klebsiella pneumonia* from the anatomical site of vagina of slaughter ewes, while the higher percentage of isolated bacteria from the living ewes were *Escherichia coli* and *Proteus mirabilis* as shown in fig.2 . This result agree (7, 20, 21, 22).

In present study these bacteria may be present as saprophytes or (opportunists) which under conditions of stress may result in disease, the role played by these (non-specific) bacteria is not known but may result in disease under unfavorable conditions of stress (23).

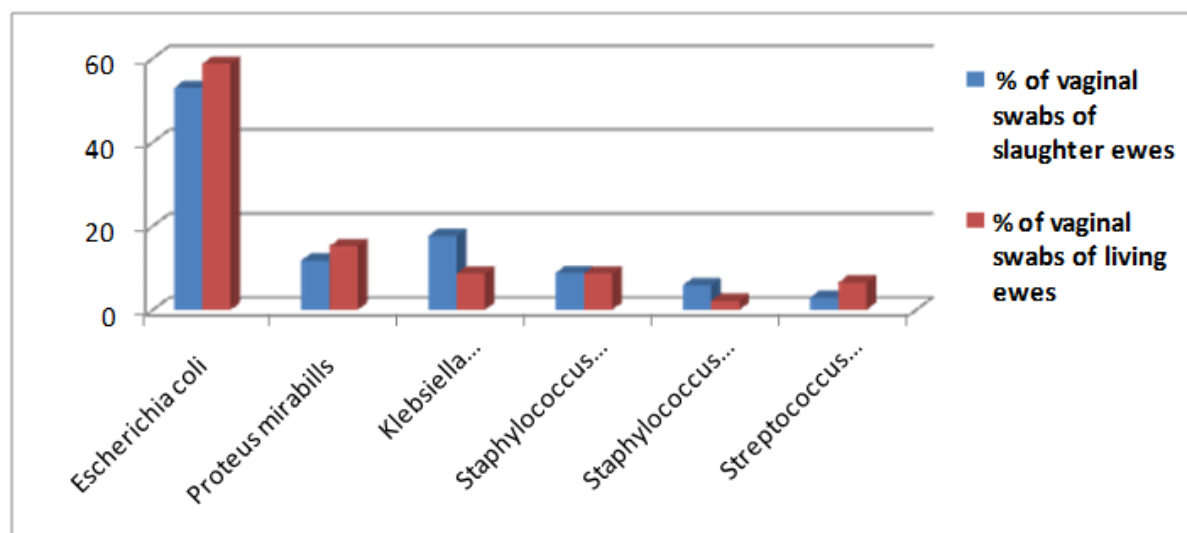


Figure (2): Relative proportion of different bacteria isolated from vaginal swabs of slaughter and living ewes.

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