

## ISOLATION AND IDENTIFICATION OF BACTERIAL SPECIES FROM VAGINA OF APPARENTLY HEALTHY EWES

R. Manickam<sup>1</sup>, T. Lurthu Reetha<sup>2</sup> and B. Puvarajan<sup>3</sup>

<sup>1</sup>Asst.Prof., <sup>2</sup>Prof. & Head, <sup>3</sup>Assoc. Prof.

Department of Veterinary Microbiology, Veterinary College and  
Research Institute, Orathanadu, Tamil Nadu, India

E-mail: manickam75vet@gmail.com (<sup>1</sup>Corresponding Author)

**Abstract:** The present study was conducted to determine the normal bacterial flora of the vagina in healthy ewes. For this purpose, a total of thirty eight of vaginal swabs were collected and examined to determine the normal bacterial flora of the vagina. Of the total 38 samples, the dominant isolates from vaginal swabs of ewes were gram-negative bacilli, the frequently isolated species being *Escherichia coli* (55.26 %). The second dominant bacterium was *Staphylococcus epidermidis* (52.63), *Staphylococcus aureus* (39.47%). Other species were isolated at relatively lower rates, *Klebsiella pneumonia* (23.68%), *Proteus mirabillis* (18.42%) and *Streptococcus spp.* (10.52%). We concluded that *E. coli* were the most predominant bacteria of the vagina in healthy ewes. The bacterial flora constitutes a predisposing factor for the vaginitis leads to reproductive failure. Therefore, the present study was conducted to isolate and identify the bacterial flora of the vagina in normal ewes and estimate their prevalence.

**Keywords:** Healthy ewes, vagina, microflora.

### 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 <sup>[1]</sup>. 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 <sup>[2,3]</sup>, goats <sup>[4]</sup> and cows <sup>[5]</sup>. 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 <sup>[6]</sup>. Vaginitis, a common disease of the genital tract of domestic ruminants, is then often caused by secondary bacterial invaders, mainly the *Escherichia coli* species <sup>[7,8]</sup>. There are many studies reported the microflora inhabited in genital system in ewes <sup>[9,10]</sup>. The microflora is usually harmless until presence of predisposing factors such as trauma or another infection which may be pathogenic and cause disease <sup>[11]</sup>.

## **Materials and Methods**

### **Collection of Samples**

Thirty eight swabs were collected from the mucosa of genital tract of apparently healthy ewes and each swab was stored in a transport medium until cultured. The swabs were transported to the laboratory for further bacteriological analysis.

### **Bacteriological study**

The collected swabs were inoculated into brain heart infusion broth and incubated at 37°C for 24 hours. After that the culture was inoculated onto different selective and differential agar plates. Culture media used for isolation and purification of bacteria include 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.

### **Identification of bacterial isolates**

The colonies seen were described in terms of their morphological characters such as size, elevation, outline, colour 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 for presumptive identification. The biochemical tests were performed as catalase, oxidase, IMVC test (indol production, methyl red, voges-proskauer and citrate utilization), TSI (triple sugar iron). The culture media was prepared according to the routine methods <sup>[12]</sup>.

## **Results**

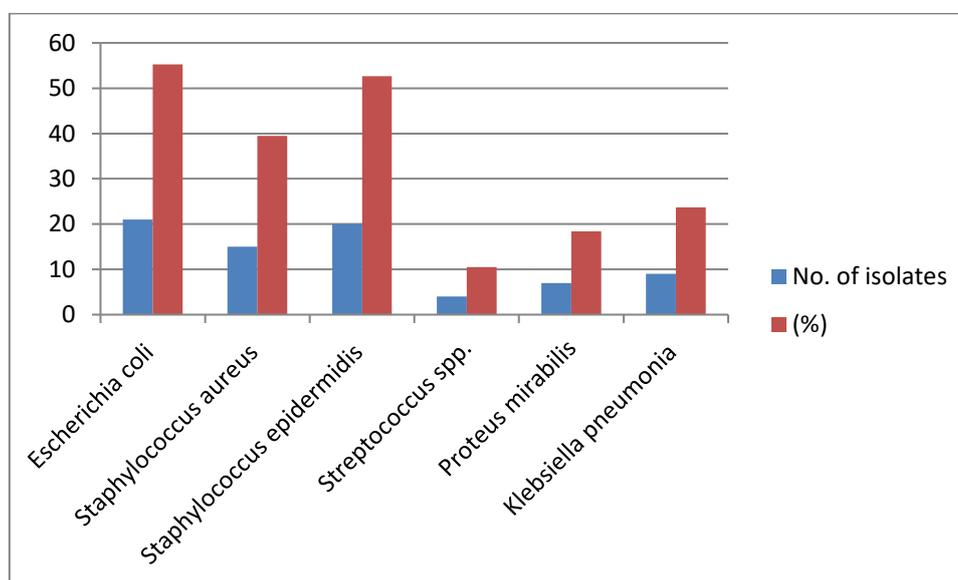
### **Bacteriological Results**

The result of the bacteriological examination for the thirty eight ewes is as shown in table (1). The bacteria were isolated from all the vaginal swabs (100%). This finding is in agreement with several researchers whom reported that 56.5-96.5% of ovine vagina showing positive isolates <sup>[13]</sup>.

Of the total 38 samples, the dominant isolates from vaginal swabs of ewes were gram-negative bacilli, the frequently isolated species being *Escherichia coli* (55.26 %). The second dominant bacterium was *Staphylococcus epidermidis* (52.63), *Staphylococcus aureus* (39.47%). Other species were isolated at relatively lower rates, *Klebsiella pneumonia* (23.68%), *Proteus mirabills* (18.42%) and *Streptococcus* spp. (10.52%).

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

Bacterial isolates	No. of isolates	(%)
<i>Escherichia coli</i>	21	55.26
<i>Staphylococcus aureus</i>	15	39.47
<i>Staphylococcus epidermidis</i>	20	52.63
<i>Streptococcus spp.</i>	4	10.52
<i>Proteus mirabilis</i>	7	18.42
<i>Klebsiella pneumonia</i>	9	23.68

**Figure 1: Bacterial species isolated from the vaginal swabs of ewes**

## Dissociation

### 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 pneumonia* is gram-negative, pink colony in MacConkey agar and gives 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. These results are in accordance with some researcher<sup>[14, 15]</sup>.

### **Distribution of the bacterial isolates**

The bacterial isolates and their absolute/relative abundance are presented on Table (1) and Fig. (1). These are in accordance to several observations<sup>[16,17]</sup>. 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<sup>[11]</sup>.

In our study we found that the higher percentage of isolated bacteria were *Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus* followed by *Klebsiella pneumonia* from the anatomical site of vagina of healthy ewes. These results agree with some researcher<sup>[7, 18]</sup>.

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<sup>[19]</sup>.

Finally, we conclude that it is necessary to check the mild bacterial infection which are occult, should be screened at the earliest if there is any problem in the reproduction apart from nutritional and hormonal imbalances and to prevent the reproductive failure in due course.

### **References**

- [1] Martins, G.; Igueira, L.; Penna, B.; Brandão, F.; Vargas, R.; Vasconcelos, C. & Lilenbaum, W. (2009). Prevalence and antimicrobial susceptibility of vaginal bacteria from ewes treated with progestin-impregnated intravaginal sponges. *Small Ruminant Research*. 81(2): 182-184.
- [2] Sargison, N.D.; Howie, F.; Mearns, R.; Penny, C.D.; & Foster, G. (2007). Shiga toxin-producing *Escherichia coli* as a perennial cause of abortion in a closed flock of Suffolk ewes. *Veterinary Record*. 160(25): 875–876.
- [3] Manes, J.; Fiorentino, M.A.; Kaiser, G.; Hozbor, F.; Alberio, R.; Sanchez, E. & Paolicchi, F. (2010). Changes in the aerobic vaginal flora after treatment with different intravaginal devices in ewes. *Small Ruminant Research*. 94 (1-3): 201-204.
- [4] Ababneh, M.M. & Degefa, T. (2006). Bacteriological findings and hormonal profile in the postpartum Balady goats. *Reproduction in Domestic Animals*. 41(1): 12 -16.

- [5] Sheldon, I.M.; Williams, E.J.; Miller, A.N.; Nash, D.M. & Herath, S. (2008). Uterine diseases in cattle after parturition. *Veterinary Journal*. 176(1): 115 - 121.
- [6] Levinson, W.E. & Jawetz, E. (1994). *Medical Microbiology and Immunology*. Third edition. Prentice- Hall Int. Inc., Englewood Cliffs, New Jersey-USA. 20-23.
- [7] Shallali, A.A.; Hussein, A.M.; Salih, M.M.; & Dafalla, E.A. (2001). A preliminary report on bacteria isolated from the female genital tract of sudanese sheep and goats. *The Sudan Journal of Veterinary Research*. 17(1): 55-63.
- [8] Mshelia, V.T.; Maina, V.A.; Okon, K.; Mamza1, S.A.; Peter1, I.D. & Egwu, G.O. (2014). Microbiological studies on genital infections in slaughtered ewes from tropical arid zone of Nigeria. *Sokoto Journal of Veterinary Sciences. J. Veterinary Sciences*. 12(1): 18-22.
- [9] Zaid, N.W. (2009). Vaginal flora of Iraqi sheep and goats during different reproductive stages. *Al- Anbar J. Vet. Sci*. 2 (1): 25-30.
- [10] AL-Saffar, A. (2010). Effect of cervical flora during breeding season on future fertility in Iraqi Awassi ewes. *Kufa Journal for Veterinary Medical Sciences*. 1(1):1-8.
- [11] Martins, G.; Brandão, F.Z.; Figueira, L.; Penna, B.; Renato., V.; Vasconcelos, C. & Lilenbaum, W. (2009). Prevalence and antimicrobial susceptibility of Staphylococci isolated from the vagina of healthy ewes. *R. bras. Ci. Vet*. 16(1): 37-40.
- [12] Forbes, B.A.; Sahn, D.F. & Weissfeld, A.S. (2007). *Bailey and Scotts' Diagnostic microbiology* 12th ed. Elsevier.
- [13] Al- Delemi, D.H.J. (2005). The normal bacterial flora in the vaginal cavity of Iraqi cows, sheeps, goats and camels during the luteal phase. *Al-Qadisiya J. Vet.Med. Sci*. 4 (1): 23-29.
- [14] Quinn, P.J.; Carter, M.E.; Markey, B.; & Carter, G.R. (2004). *Clinical veterinary microbiology*. An imprint of Elsevier limited. 284- 286.
- [15] Songer, J.G. & Post, K.W. (2005). *Veterinary microbiology. Bacterial and fungal agents of animal diseases*. 1st ed. Elsevier Saunders.
- [16] Al-Hamedawi, T.M.; Khammas, D.J. & Ai-Ubaidi, A.S. (2002). Effect of estrus synchronization on vaginal flora and subsequent fertility in ewes. *Iraqi J. Vet. Sci*. 16 (1): 73-79.
- [17] Petit, T.; Spergser, J.; Rosengarten, J.; & Arich, J. (2009). Prevalence of potentially pathogenic bacteria as genital pathogens in dairy cattle. reproduction in Domestic Animals.44, 88-91.

- [18] Emikpe, B.O.; Oyero, O.G.; & Akpavie, S.O. (2009). Isolation and antibiogram of aerobic nasal bacterial flora of apparently healthy West African dwarf goats. *Revue d'elevage et de Medecine Veterinaire des Pays Tropicaux*. 62(1): 17-21.
- [19] Bukar, k. Y.M.; Amin, J.D.; Zaria, L.T. (2007). Bacteria flora of the anterior genitalia of the sahelian doe in Maiduguri-borno state, Nigeria. *Nigerian Veterinary Journal*. 28(2): 60-62.