# Vaginal flora of Iraqi sheep and goats during different reproductive stages

ISSN: 1999- 6527

## N. W. Zaid Dep. of Surgery and Obstetric/ College of Veterinary Medicine/ University of Baghdad

#### **Abstract**

The recent study was carried on 26 ewes and 21 does in the Animal farm of Veterinary Medicine College in Baghdad University during 2007. Bacterial swabs were taken from the female vagina in different reproductive status. The results revealed that there were significant differences in the multiparturient and postparturient animals comparing with the ewe lamb and pregnant ewes, while in the does there were no significant differences between these stages. The highest bacterial isolation in the sheep was the *Enterobacter* species 29.6% and *Escherichia coli* 18.5%. In the goat, the *Pseudomonas aeruginosa* and *Streptococcus faecalis* was 21.8%. The investigation showed that there was a possibility of isolation of 12 different types of bacteria from the reproductive tract and the probability of presence of more than one type of bacteria in the same swabs. In this study the bacteria isolated appears to be affected by the reproductive status in the ewes. This finding not observed in the does which might be more resistant to infection.

# الجراثيم التعايشية لمهابل الأغنام والماعز العراقية خلال المراحل المختلفة للتناسل

نزيه ويس زيد فرع الجراحة والتوليد/ كلية الطب البيطري/ جامعة بغداد

#### الخلاصة

أجريت الدراسة الحالية على 26 نعجة و 21 ماعز محلي في الحقل الحيواني التابع لكلية الطب البيطري، جامعة بغداد خلال عام 2007. أخذت المسحات الجرثومية من مهابل الإناث خلال حالات تناسلية مختلفة. أكدت النتائج الحالية وجود فرق معنوي في النعاج الغير حوامل المتعددة الولادة وبعد الولادة مقارنة مع الفطائم الغير حوامل والنعاج الحوامل، بينما في الماعز لم يسجل مثل هذا الفرق الإحصائي ما بين هذه المراحل المختلفة. بينت الدراسة أن أعلى نسبة على ناللجراثيم في الأغنام العراقية كانست ودامل والنعاج العراقية والمحافظة والمحافظة العراقية عزل 12 كانست عزليت المشاهدات إمكانية عزل 12 كانست عوامل والنعام الجراثيم من تجويف الأجهزة التناسلية الأنثوية وإحتمالية وجود أكثر من نوع لنفس المسحة الجرثومية. أظهرت العزلات الجرثومية تأثراً واضحاً بالتغيرات التناسلية في النعاج، مثل هذه الملاحظة لم تلاحظ في إناث الماعز والتي ربما تبدو أكثر مقاومة للتغيرات التناسلية.

#### Introduction

The vaginal flora are made of many species of microorganisms that varies throughout the life cycle (1). The importance of studying such microorganisms is related to diseases caused by them due to reduction of the immunity of the reproductive system (2). Several studies mentioned that there were several types of bacteria found in the reproductive tract (3-6). In Iraq the workers isolated similar microorganisms (7-10). In the same direction many of researchers indicated that the reproductive system contains normal flora (11-14). The Iraqi ewes were studied by the researches (15-19) whom found that most of ewe's reproductive systems contain normal flora, while in the goat mentioned by (17, 20 and 21) were reported that the non specific infections appear to play a minor role in causing infertility in does. Because of the little information's about the effect of the reproductive stages on the normal flora in the Iraqi ewes and does, the present study was performed.

#### **Materials and Methods**

26 Iraqi ewes and 21 Iraqi does were examined and vaginal swabs were taken for the bacteriological studies. These females were presented in animal farm of the Veterinary Medicine College of Baghdad University during 2007, their ages ranged from (1.5-2.5) years and weight average (35-40) kilograms. All the females were kept under a good healthy conditions. After clinical examination and recording data, the females classified into (10, 5) ewe lamb non pregnant, (4, 8) multiparturient non pregnant, (9, 5) pregnant in the last two months and (3, 3) after parturition for ewes and does respectively. The swabs were cultured and the media were incubated at 37°C under aerobic and anaerobic conditions for 24-48 hours, the isolation and the identification of bacteria was done according to the methods of Sneath *et al.* (22) and Quinn *et al.* (23).

The statistical analysis was done by using analysis of variance and least significant differences to determine the variance between the groups at (P<0.01) according to the methods of Al-Mohammed *et al.* (24).

#### **Results**

The results of the study showed that there were 12 different types of bacteria isolated from the vagina of 47 animals (26 ewes and 21 does) in different status of reproduction. The positive single isolated bacteria was 21 (44.7%) swabs, while the positive mixed isolated swabs were reached 12 (25.5%) which classified into double isolation that appear to be 8 (17%) swabs, the triple isolation found in 3 (6.4%) swabs, finally quadruple isolation showed in 1 swab (2.1%). The clean swabs were 14 (29.8%) from the total females (table 1).

The swabs isolated from the sheep females showed that there were 11 single isolates (42.3%), 5 double isolates (19.2%), 2 triple isolates (7.7%) and clean swabs was 8 (30.8%), on the other hand, the isolated bacteria from the goats appeared to be 10 (47.6%) as a single isolates, 3 (14.3%) as a double isolation, 1 (4.8%) as a triple isolation, 1 of the swabs (4.8%) showed a quadruple isolation and 6 (28.6%) free from bacteria.

The species of the bacteria present in table (2) was as the following: 8 (29.6%) *Enterobacter* spices present in swabs taken from the sheep vagina, 5 (18.5%) *Escherichia coli*, 4 (14.8%) *Lactobacillus* spices, 3 (11.1%) *Proteus mirabilis*, 2 (7.4%) for *Klebsilla pneumoniae*, *Streptococcus faecalis*, *Salmonella dublin* and 1 (3.8%) *Staphylococcus aureus*.

The highest bacteria isolated from the goat vagina was 5 (21.8%) *Pseudomonas* aeruginosa and *Streptococcus faecalis*, 2 (8.7%) *Staphylococcus epidermidis*,

Staphylococcus aureus, Lactobacillus species, Enterobacter spices, Proteus mirabilis, 1 (4.3%) Brucella melitensis, Escherichia coli and Listeria monocytogenes (table 2).

Table (3) showed that the percentage of isolation in non pregnant ewe lamb was (60%, 80%), the non pregnant multipara was (100%, 62.5%), while the pregnant was (55.6%, 80%), and in the puerperium periods was (100%, 66.7%) for the ewes and does respectively. There were significant differences (P<0.01) between each of the non pregnant multipara and post parturient periods of the ewes and between each of the non pregnant ewe lamb and the pregnant females (table 3). The does showing no significant differences among these stages (table 3).

Table (1) The percentage of isolated numbers in the different stages of reproduction

| reproduction         |                             |                           |          |            |                             |                              |          |            |  |
|----------------------|-----------------------------|---------------------------|----------|------------|-----------------------------|------------------------------|----------|------------|--|
| Isolation<br>numbers | Ewes percentage<br>(number) |                           |          |            | Does percentage<br>(number) |                              |          |            |  |
|                      | Non<br>pregnant<br>heifers  | Non pregnant<br>multipara | pregnant | pureperium | Non<br>pregnant<br>heifers  | Non<br>pregnant<br>multipara | pregnant | pureperium |  |
| Single               | 40%                         | 75%                       | 33.3%    | 33.3%      | 40%                         | 50%                          | 60%      | 33.35%     |  |
|                      | (4)                         | (3)                       | (3)      | (1)        | (2)                         | (4)                          | (3)      | (1)        |  |
| Double               | 10%                         | 25%                       | 11.1%    | 66.7%      | 20%                         | 12.5%                        | 20%      | -          |  |
|                      | (1)                         | (1)                       | (1)      | (2)        | (1)                         | (1)                          | (1)      | (0)        |  |
| Triple               | 10%                         | -                         | 11.1%    | -          | 20%                         | -                            | -        | -          |  |
|                      | (1)                         | (0)                       | (1)      | (0)        | (1)                         | (0)                          | (0)      | (0)        |  |
| Quadruple            | -                           | -                         | -        | -          | -                           | -                            | -        | 33.35%     |  |
|                      | (0)                         | (0)                       | (0)      | (0)        | (0)                         | (0)                          | (0)      | (1)        |  |
| Positive             | 60%                         | 100%                      | 55.5%    | 100%       | 80%                         | 62.5%                        | 80%      | 66.7%      |  |
|                      | (6)                         | (4)                       | (5)      | (3)        | (4)                         | (5)                          | (4)      | (2)        |  |
| Negative             | 40%                         | -                         | 44.5%    | -          | 20%                         | 37.5%                        | 20%      | 33.3%      |  |
|                      | (4)                         | (0)                       | (4)      | (0)        | (1)                         | (3)                          | (1)      | (1)        |  |

Table (2) The percentage of bacterial types in the different stages of reproduction

| Types of bacteria          | Ewes percentage (number) | Does percentage (number) |
|----------------------------|--------------------------|--------------------------|
| Enterobacter spices        | 29.6% (8)                | 8.7% (2)                 |
| Escherichia coli           | 18.5% (5)                | 4.3% (1)                 |
| Lactobacillus spices       | 14.8% (4)                | 8.7% (2)                 |
| Proteus mirabilis          | 11.1% (3)                | 8.7% (2)                 |
| Klebsilla pneumoniae       | 7.4% (2)                 | - (0)                    |
| Streptococcus faecalis     | 7.4% (2)                 | 21.8% (5)                |
| Salmonella dublin          | 7.4% (2)                 | - (0)                    |
| Staphylococcus aureus      | 3.8% (1)                 | 8.7% (2)                 |
| Staphylococcus epidermidis | - (0)                    | 8.7% (2)                 |
| Brucella melitensis        | - (0)                    | 4.3% (1)                 |
| Pseudomonas aeruginosa     | - (0)                    | 21.8% (5)                |
| Listeria monocytogenes     | - (0)                    | 4.3% (1)                 |
| Total bacterial isolates   | (27)                     | (23)                     |

Table (3) The percentage of bacterial isolation in the different stages of reproduction

| Reproductive stages Animals | Non pregnant<br>heifers | Non pregnant<br>multipara | pregnant | pureperium |
|-----------------------------|-------------------------|---------------------------|----------|------------|
| Ewes positive               | 60%                     | 100%                      | 55.5%    | 100%       |
| •                           | bc                      | a                         | bcd      | a          |
| Ewes negative               | 40%                     | 0%                        | 44.5%    | 0%         |
| E wes negative              | cd                      | e                         | bcd      | e          |
| Does positive               | 80%                     | 62.5%                     | 80%      | 66.7%      |
| Does positive               | ab                      | abc                       | ab       | abc        |
| Doog pogetive               | 20%                     | 37.5%                     | 20%      | 33.3%      |
| Does negative               | de                      | cde                       | de       | cde        |

<sup>-</sup> The small letters represented significant differences at level (P<0.01)

#### **Discussion**

The study indicated that there were several bacterial types presented in the female genital system which has no effect on the reproductive function. This finding agreed with several workers (7, 8, 9, 15 and 17). In the present work (69.2%) of ovine vagina contained bacterial microflora, this finding was inagreement with several researchers whom reported that 56.5-96.5% of ovine vagina showing positive isolates (15-17), while in the does (71.4%) has been reported, this was approximately similar to the finding of Al-Delemi (17) in diestrus phase.

The probability of presence of more than one bacterial isolates in one swabs, this is similar to the finding of Al-Delemi (17).

The pregnant ewes showed (55.5%) of the bacterial isolates, this is less than the percentage reported by Aziz *et al.* (15) in Al-Mosul, while in the does the result was (80%). This result was might be firstly reported in Iraqi goats.

In the post parturient animals, the sheep showed (100%) positive culture. This is similar to the results of Abdullah *et al.* (18) and Zaid *et al.* (19) in the same periods, in the does, the percentage lower than that of the ewes which was (66.7%) and this was the first done in caprine in pureperium periods.

In non pregnant females, the ewe lamb and multiparturient ewes showed a positive culture of 60% and 100% respectively. This percentage was inagreement with Aziz *et al.* (15), Al-Hamedawi *et al.* (16) and Al-Delemi (17), the does kids and multipara reported an isolates of 80% and 62.5%, this was in accordance to the finding of Al-Delemi (17).

The non pregnant multipara and pureperium ewes showed significant increase in bacterial isolates in a comparison with the non pregnant ewe lamb and pregnant. This is inagreement with the findings of several workers (4, 7, 8, 10, 15, 16 and 17). The higher bacterial isolates in non pregnant ewes than those of pregnant may be due to the contamination of the vagina with bacteria as the non pregnant ewes having opened cervix in comparison with the completed closed cervix of pregnant (15). In the non pregnant animals the delay of uterine bacteria clearance may be due to many factors that include: decrease the myometrial activity (frequency, intensity and duration), vascular changes in the endometrium, altered hormonal response and finally the altered mucus production (25-28). The significant differences in post parturient periods were related to the anatomical and histological characteristics of the cervix which remained relaxed for several days after parturition and provided entrance for bacteria into the uterine cavity, where sloughed tissue and fluid and blood maintained a liquid medium favorable for bacterial growth (11, 12 and 29).

Does had no significant differences between the pregnant and non pregnant or even post parturient females in bacterial isolates. These agreed with the finding of (17 and 21) that indicate that the non specific bacteria may play a minor role in infertility of the does.

The large percentage of bacteria which has been isolated in the ewes was *Enterobacter* spices and *Escherichia coli* with other bacteria. This is in accordance to several observations (15, 16 and 17). While in the does the *Streptococcus faecalis* and *Pseudomonas aeruginosa* had the highest percentage isolates. All isolated bacteria in the does were similar to the studies of Al-Delemi (17).

From the results we conclude that there were several species of normal bacterial flora found in the genital tract of the sheep and goat during different status of reproduction, and the status of reproduction affected the bacterial content in the ewes while this not observed in the doe.

### References

- 1. Hafez, B & Hafez, E.S.E. (2000). Reproduction in Farm Animals. 7<sup>th</sup> ed. Lippincott Williams and Wilkins, USA. PP: 13-29.
- 2. Al-Dahash, S.Y. & Fathall, a M.A. (2000). Veterinary Obstetrics. 2<sup>nd</sup> ed. Book House for Printing and Publishing. Al-Mousl University. PP: 29-31.
- 3. Elliott, L.; McMahon, K.J.; Gier, H.T. & Marion, G.B. (1968). Uterus of the cow after parturition: bacterial content. Am. J. Vet. Res., 29 (1): 77-81.
- 4. Studer, E. & Morrow, D.A. (1978). Postpartum evaluation of reproductive potential: comparison of findings from genital tract examination per rectum, uterine culture and endometrial biopsy. J. Am. Vet. Med. Ass.,172 (4): 489-494.
- 5. Messier, S.; Higgins, R.; Couture, Y. & Morin, M. (1984). Comparison of swabbing and biopsy for studying the flora of the bovine uterus. Canadian Vet. J., 25: 283-288.
- 6. Noakes, D. E.; Wallacce, L. & Smith, G. R. (1991). Bacterial flora of the uterus of cows after calving on two hygienically contrasting farms. Vet. Record, 11 (128): 440-442.
- 7. Azawi, O. I.; Ali, J. B. & Ali, D. S. (1995). Non-specific genital tract microflora of Iraqi local breed cows. Iraqi J. of Vet. Sci., 8 (2): 261-265.
- 8. Al-Hilali, H.A. & Al-Delemi, D.H. (2001). The uterine bacterial flora of normal reproductive tract, non-pregnant Iraqi cows. The Veterinarian, 11(1):112-120.
- 9. Al-Delimi, D. H. J. (2002). The uterine bacterial flora of reproductive tract of the Iraqi she-camels. Al-Qadisiya J. Vet. Med. Sci., 1 (2): 55-59.
- 10. Griffin, J. F. T.; Hartigan, P. J. & Nunn, W. R. (1974). Non-specific uterine infection and bovine fertility I. Infection patterns and endometritis during the first seven weeks post-partum. Theriogenology, 1 (3): 91-106.
- 11. William, B. L.; Senger, L. R. & Ward, A. C. S. (1988). Relationships between days post partum, observed estrus and uterine microflora in commercial dairy cows. Theriogenology, 30 (3): 555-561.
- 12. Hussain, A. M.; Daniel, R. C. W. & Boyle, D. O. (1990). Postpartum uterine flora following normal and abnormal puerperium in cows. Theriogenology, 34 (2): 291-302.
- 13. Bekana, M.; Jonsson, P.; Ekman, J. & Kindahi, H. (1994). Intra uterine bacterial findings in post-partum cows retained fetal membranes. Zentralbl Veterinarmed, 41 (9): 663-670.
- 14. Torres, E. B.; Nakao, T.; Hiramune, T.; Moriyoshi, M.; Kawata, K. & Nakada, K. (1997). Streptococcus and uterine bacterial flora in dairy cows following clinical normal and abnormal puerperium. J. Reprod. Dev., 43: 157-163.
- 15. Aziz, D. M.; Al-Sultan, M. A. H. & Al-Jawally, E.A.K. (2000). Uterine microflora in Awassi ewes. Iraqi J. Vet. Sci., 13 (1): 201-205.
- 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. 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.
- 18. Abdullah, A. H.; Zaid, N. W.; Ibrahim, A. H. & Khadim, A. A. (2006). Bacterial elimination during post parturient period in Awassi ewes. Diyala J. Appl. Res., 2 (2): 13-17.

- 19. Zaid, N. W.; Abdalla, A. H.; Ibrahim, A. H. & Khadim, A. A. (2007). Effect of prostoglandine PGF2α on bacterial elimination during puerperium in Awassi ewes. Al-Anbar J. Agric. Sci., 5 (1): 211-217.
- 20. Miller, S. J. & Raja, C. K. S. V. (1982). Infertility in sheep and goats. In: Reproduction in Farm Animals (Theriogenology). Sane, C.R.; Luktuke, S.N.; Deshpande, B.R.; Kaikini, A.S.; Velhankar, D.P.; Hukeri, V.B. and Kodagali, S.B. Varghese publishing house, Bombay. PP: 371-382.
- 21. Arthur, G. H.; Noakes, D. E.; Pearson, H. & Parkinson, T. J. (1996). Veterinary Reproduction and Obstetrics. 7<sup>th</sup> ed. W.B. Saunders Company.
- 22. Sneath, P. A.; Mair, N. S.; Sharp, M. E. & Hott, J. G. (1986). Bergey's Manual of Systematic Bacteriology. William and Wilkinis, USA. PP: 1013-1035.
- 23. Quinn, P. J.; Markey, B. K.; Carter, M. E.; Donnelly, W. J. C.; Leonard, F. C. & Maghire, D. (2006). Veterinary microbiology and microbial diseases. 6th ed. Black well Publishing company, Great Britain.
- 24. Al-Mohammed, N. T.; Al-Rawi, K. M.; Younis, M. A. & Al-Morani, W. K. (1986). Principles of Statistics. Book House for Printing and Publishing. Al-Mousl University.
- 25. Troedsson, M. H. (1999). Uterine clearance and resistance to persistent endometritis in the mare. Theriogenology, 52: 461-471.
- 26. Nikolakopoulos, E. & Watson, E. D. (1999). Uterine contractility is necessary for the clearance of intrauterine fluid but not bacteria after bacterial infusion in the mare. Theriogenology, 52: 413-423.
- Rigby, S. L.; Barhoumi, R.; Burghardt, R. C.; Colleran, P.; Thompson, J. A.;
   Varner, D. D.; Blanchard, T. L.; Brinsk, S. P.; Taylor, T.; Wilkerson, M. K.
   & Delp, M. D. (2001). Mares with delayed uterine clearance have an intrinsic defect in myometrial function. Biol. Reprod., 65: 740-747.
- 28. Card, C. (2005). Post-breeding inflammation and endometrial cytology in mares. Theriogenology, 64: 580-588.
- 29. Hussain, A. M. & Daniel, R. C. W. (1992). Phagocytosis by uterine fluid and blood neutrophils and hematological changes in postpartum cows following normal and abnormal parturition. Theriogenology, 37 (6): 1253-1267.