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Isolation and Identification of Bacterial Flora from Reproductive Tracts of Normal Ewes in Glasgow

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Abstract

The present study was conducted to determine the normal bacterial flora of the uterus, cervix and vagina. For this purpose, a total of forty-five reproductive tracts were examined. Of all, thirty-five reproductive tracts were collected from cull ewes at Paisley abattoir and examined to determine the normal bacterial flora of the uterus and vagina. In addition, nine ewes which had been culled from the Cochno flock for non reproductive reasons were also examined for residual infection in the reproductive tract. For the 35 ewes, the species isolated were similar to those present in the vaginal discharge, but no bacteria were isolated from 4 (11%) of the vaginas and 12 (34%) of the uteri. E. coli and Streptococcus spp. were the predominant bacteria, but Bacillus and Proteus spp. were the less frequent isolates. For the nine ewes, bacteria were recovered from all the vaginal samples and the uteri and cervix of all but three animals (67%) had no bacteria. In two animals, bacteria were not recorded from the cervix but were isolated from the uterus and in three no bacteria were isolated from either cervix or uterus. This isolation may reflect residual infection or could be due to contamination at sampling or even a low level of commensal colonisation.

Keywords: Cull ewes, bacteria, microflora, vagina, uterus, cervix.
Introduction

Reproductive efficiency in the sheep is particularly important because of the seasonal nature of breeding and the relatively small number of lambs produced per ewe per year. Many diseases are classified as specific and non-specific, cause problems in the reproductive system in the sheep. Some of them cause infertility or reduce productivity with clinical signs which may include vaginal discharge or not. Abortion and infertility can result from infection or from non-infectious causes. The most common infections to cause abortion and infertility in the UK are Enzootic Abortion in ewes (*Chlamydophila*) and Toxoplasmosis which infect the placenta and cause abortion. Animals infected with these agents may not be detectable clinically but can usually be identified by laboratory means either in the individual or the flock. *E.coli* and *A. pyogenes* are the most common non-specific infections in sheep (Farid et al., 1986, Ramadan et al., 1997, Mavrogianni et al., 2007, Atwa and Rady 2007). Opportunist infections with a variety of bacteria are more important causes of endometritis worldwide and significantly affect fertility (Henderson 1990). The importance of studying such microorganisms is related to diseases caused by them due to stress and reduction of the immunity of the reproductive system (Al-Dahash and Fathalla 2000).

Mavrogianni et al., (2007), monitored bacteria in the uterus in ewes which had undergone lambing and found *E.coli*, *Apyogenes*, staphylococci and streptococci were present after interference. There is little published information about the bacterial normal flora of reproductive tract in the sheep in UK. Therefore, the present study was conducted to isolate and identify the bacterial flora of the uterus, cervix and vagina in normal sheep.

Materials and Methods

Sampling

In present study, a total of fort-five reproductive tract were examined. Of all, thirty five reproductive tracts were collected from cull ewes at Paisley abattoir and examined to determine the normal bacterial flora of the uterus and vagina. The reasons for culling the ewes in this study were general and not linked to fertility. In addition, 9 ewes which had been culled from the Cochno flock for non-reproductive reasons were also examined for residual infection in the reproductive tract. Rectal temperatures were taken prior to slaughter. They were euthanized at the Faculty of Veterinary Medicine using a captive bolt pistol and exsanguinated to ensure death and the reproductive tract was then examined. The gross appearance was recorded and bacteriological samples were taken from the uterus. Samples were also taken for histology and put in 10% formaldehyde to be processed to be examined if time permitted. Any visible abnormalities were recorded. The reproductive tracts were longitudinally dissected and samples were taken from vagina, cervix and uterus using sterile swabs.

Bacteriological Materials, Methods and Reagents

Media used in this study consisted of 7% sheep blood agar, 7% horse blood agar, MacConkey agar, Chocolate agar and Campylobacter medium. Media were prepared according to the manufacturer’s instructions.

Inoculation of Cultures

Swabs were used to inoculate the isolation plates and the material was streaked with a bacteriological loop to give 4 dilutions of the inoculums. Inoculated plates were incubated in aerobic conditions with 10% per cent CO2 microaerobic and anaerobic conditions. Aerobic conditions were used for sheep blood and MacConkey agars. After inoculation the plates were placed into an incubator (Swallow Incubator) at atmospheric pressure at 370C, then examined for any growth at 24 and 48 hours after incubation and the findings were recorded. Chocolate agar cultures were incubated under 10% CO2 incubation achieved by placing the plates in the CO2 incubator (LEEC, Incubator). Microaerobic conditions were used for Campylobacter plates which were placed in a Don Whitley micro aerobic incubator (MACS-VA500-Microaerophiic work station) in an atmosphere consisting of 0.85% nitrogen 0.5% oxygen 0.5% CO2 and 5% hydrogen. Inoculated Horse blood
agar plates were incubated in an anaerobic incubator in an atmosphere consisting of balance % nitrogen 20% oxygen 20 % CO2 and 0 to 5.0% hydrogen (Don Whitley MK3Anaerobic Work Station Incubator).

**Identification of Bacterial Isolates**

**Presumptive Identification**

Many different colony types were noted in the initial cultures and each colony type seen was recorded and subcultured on sheep blood agar or other appropriate medium to provide a pure subculture. 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, 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 to reach a presumptive identification.

**Confirmation of Identity**

Throughout this study, confirmation of bacterial identity was carried out using the Analytical Profile Index (API) (bioMérieux) system which uses enzymatic tests in conjunction with a special database. The API system comprises a range of panels of biochemical tests used to identify species in family or generic groups. API 20E is used to identity Enterobacteraceae and API 20 Staphylo identify species of staphylococci whereas, API Coryne and API STRIPS were used to identify species of Corynebacterium and streptococci, respectively. The choice of an appropriate panel or strip was based on the presumptive identification of the organism.

**Results**

**Cull Ewe Reproductive Tracts**

No gross abnormalities were recorded in the vagina, cervix or uterus of the 35 cull ewe reproductive tracts.

**Bacteriological Results**

The result of the bacteriological examination for the thirty five ewes is shown in table (1). The species isolated were similar to those present in the vaginal discharge, but no bacteria were isolated from 4(11%) of the vaginas and 12 (34%) of the uteri.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Uterus</th>
<th>Vagina</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>1</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Corynebacteriumspp</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Enterococcus spp</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Bacillus spp</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Proteus spp</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No bacteria isolated</td>
<td>12</td>
<td>4</td>
<td>16</td>
</tr>
</tbody>
</table>

The result of the bacteriological examination for the nine ewes is shown in Table (2) and Figure (1). Bacteria were recovered from all the vaginal samples and the uteri and cervix of all but three animals (67%). In two animals, bacteria were not recorded from the cervix but were isolated from the uterus. In three ewes; no bacteria were isolated from either cervix or uterus.
Table 2: Bacteriological founding in the reproductive tracts of 9 cull ewes.

<table>
<thead>
<tr>
<th>Sheep number</th>
<th>Type of discharge assisted uterus</th>
<th>Cervix</th>
<th>Vagina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. propinquum spp</td>
</tr>
<tr>
<td>143</td>
<td>1 -</td>
<td>C. auris</td>
<td>C. jejicium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brevibacterium spp</td>
<td>Enterococci</td>
</tr>
<tr>
<td>149</td>
<td>1 -</td>
<td>E. coli A. heamolyticum spp</td>
<td>Staphylococcus C. jejicium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A.</td>
</tr>
<tr>
<td>349</td>
<td>1 -</td>
<td>no growth</td>
<td>no growth</td>
</tr>
<tr>
<td>4036</td>
<td>1 -</td>
<td>no growth</td>
<td>no growth</td>
</tr>
<tr>
<td>4146</td>
<td>1 -</td>
<td>E. coli</td>
<td>no growth</td>
</tr>
<tr>
<td>4958</td>
<td>n/a</td>
<td>C. striaum</td>
<td>c. urealyticum</td>
</tr>
<tr>
<td>25427</td>
<td>n/a</td>
<td>C. striaum</td>
<td>no growth</td>
</tr>
<tr>
<td>25780</td>
<td>2 +</td>
<td>no growth</td>
<td>no growth</td>
</tr>
<tr>
<td>26763</td>
<td>1 +</td>
<td>no growth</td>
<td>no growth</td>
</tr>
<tr>
<td>total isolated</td>
<td></td>
<td>3/9</td>
<td>5/9</td>
</tr>
</tbody>
</table>

N/A: Not available.

Fig. 1: Isolation of bacterial species from cull ewe vaginas and uteri.
Discussion

The microbiological flora of the lower female genital tract provides a dynamic, complex example of microbial colonization, the regulation of which is not fully understood. When an exogenous bacterial species, with its array of virulence factors, is introduced into the host, disease does not always occur. Conversely, under selected conditions, commensal endogenous bacteriaca can participate in disease processes (Larsen and Monif 2001).

The study indicated that there were several bacterial types presented in the female genital system which has no effect on the reproductive function. Similar findings were reported in sheep (Aziz et al., 2000; Al-Delemi 2005), Cows (Al-Hilali et al., 2001) and camels (Al-Delimi et al., 2002). The predominant bacteria in the ewes were Streptococcus spp and E. coli. This is in accordance to several observations (Delimi et al., 2002, Zaid 2009). In does, the Streptococcus faecalis and Pseudomonas aeruginosa had the highest percentage isolates (Zaid 2009). In buffalo, E coli, C bovis and Micrococcus spp have been found the predominant (El-Jakee et al., 2008).

In present study, isolation may reflect residual infection or could be due to contamination at sampling or even a low level of commensal colonization. It is possible that E. coli isolated from ewe no. 4146 represents contamination, but it does not seem likely that the C. urealyticum and C. striatum isolated from ewe no. 25427 could be due to contamination. A. pyogenes, a known cause of chronic infection, was present in the vagina of ewe no. 149, but was not recovered from other parts of the tract of this animal.

Ahlers and Gruent (1993) found that lochial secretion taken as early as one day after birth contain mostly E coli and streptococci, suggesting that streptococci is normal bacterial flora (Huszenicz et al., 1999). Corynebacteria were isolated from grossly normal reproductive tracts at post-mortem and were unlikely to be faecal contaminants. These findings suggest that corynebacteria are common in the ewe reproductive tract but provide little clear evidence for their role in vaginal discharge or infertility. C. jeikeium was present in 5 of the 9 animals, C. striatum was present in 3 animals and these species would seem most likely to be common inhabitants of the reproductive tract.

In present study, sheep showed no abnormalities in the reproductive tract. However, the decision of culling was based on udder and feet faults. Decisions regarding culling often depend on the severity and nature of the weakness. Ewes showing an abnormality of the udder that will impair milk production or the sucking behaviour of lambs are culled, whereas those showing minor faults of the feet or mouth may be kept for further breeding. The market price for cull ewes and the availability and cost of replacements have also to be considered. All the nine ewes had lambed in April 2007 and 7 of the 9 had had discharge in 2007. Thus the finding that all 9 reproductive tracts were grossly normal was not totally expected.

The bacteriological results from the cull ewe uteri provide additional information about bacteria present in the reproductive tract of the ewe. A limited range of organisms was found with more bacteria being present in the vagina than in the uterus. Once more the E.coli and Streptococcus spp were the main isolates and as with some of the streptococci, they could have been derived from faecal contamination of the vagina at slaughter or handling of the isolated viscera. However, since considerable precautions were taken, it is considered more likely that these are ‘normal’ bacterial flora. Thus by implication, the sampling technique used in the field was perhaps less critical than at first thought. In effect therefore they were representative of unbred animals during the mating season, and their flora and its distribution provided a baseline for the bacteriology of discharge. For disease to occur, exogenous or endogenous bacteria that possess pathogenic prerequisites must attain replicative dominance. Their ability to do so is potentially governed by inhibitory or synergistic interrelationships with other microbes (Larsen and Monif, 2001)

Given that the tracts were grossly normal, the bacteriological findings should reflect the “normal” flora of the reproductive tract of the ewe at mating. Bacteria were present in all three parts of the reproductive tract, the vagina, cervix and uterus but
the highest numbers isolated were from the vagina. Keeping of the ewes under non stressful condition is warranted to avoid clinical infection of reproductive tract with opportunistic bacteria.

References


