Isolation and identification of fungal microbiota from genital tract of ewes

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SUMMARY

The purpose of this study was to identify fungal microbiota in different parts of genital tract of ewes. The vagina, cervix, uterine body and uterine horns of 84 ewes was sampled using sterilized cotton swabs. Samples were cultured onto Sabouraud dextrose agar containing chloramphenicol (0.005%) and incubated at 30°C for 7-10 days. A total of 324 fungal colonies were determined as a combination of different filamentous fungi (47.2%) and yeasts (52.8%). Fungal isolates belonged to 15 genera including Candida spp. (27.2%; representing significant difference with other fungal species, p<0.05), Trichosporon spp. (16.7%), sterile hyphae (14.2%), Penicillium spp. (8.3%), Geotrichum spp. (7.1%), Alternaria spp. (7.1%), Cladosporium spp. (6.5%), Aspergillus spp. (3.7%), Fusarium spp. (3.4%), Rhodotorula spp. (1.9%), dematiaceous fungi (1.5%), Mucor spp. (1.5%), Rhizopus spp. (0.3%), Trichothecium spp. (0.3%) and Ulocladium spp. (0.3%). Among different Candida species, C. tropicalis and C. krusei were the most common yeast species. The total number of fungi found in the vagina (36.7%) was significantly higher than the results obtained from other parts (p<0.05). The most common fungi isolated from vagina, cervix and uterine horns were associated with Candida spp., while Trichosporon spp. were the most frequent fungi in uterine body. The results showed that Penicillium spp. and Candida spp., particularly C. tropicalis and C. krusei, were the most common components of the genital mycobiota of ewes and the number of fungi varied among different parts of genital system.

Keywords : ewe, genital tract, fungal microbiota, Penicillium, Candida, Candida tropicalis

RESUME

Isolement et identification de la microbiote fongique du tractus génital de brebis

L’application de cette étude a été d’identifier la microbiote fongique et de déterminer le nombre d’agents fongiques dans différentes parties de l’appareil génital de brebis. Les échantillons ont été pris dans différentes parties du tractus génital en incluant le vagin, le col, le corps utérin et les cornes utérines de 84 brebis en utilisant des tampons en coton stérilisés. Ils ont été cultivés sur la gélose de dextrose de Sabouraud contenant du chloramphenicol (0.005%) et ont été mis en incubation à 30°C pendant 7 à 10 jours. Un total de 324 colonies fongueuses a été obtenu sur l’ensemble du tractus génital. Les combinaisons de différents champignons filamentaux et levures ont été déterminées. Les isolats fongiques ont appartenu à 15 genres différents incluant Candida spp. (soit 27.2 %; différence significative avec les autres espèces fongiques, p<0.05), Trichosporon spp. (16.7 %), hyphes stériles (14.2 %), Penicillium spp. (8.3 %), Geotrichum spp. (7.1 %), Alternaria spp. (7.1 %), Cladosporium spp. (6.5 %), Aspergillus spp. (3.7 %), Fusarium spp. (3.4 %), Rhodotorula spp. (1.9 %), les champignons de la famille des démataciées (1.5 %), Mucor spp. (1.5 %), Rhizopus spp. (0.3 %), Trichothecium spp. (0.3 %) et Ulocladium spp. (0.3 %). Parmi de différentes espèces de Candida, C. tropicalis et C. krusei étaient les espèces de levure les plus communes. Le nombre total de champignons trouvés dans le vagin (36.7 %) était de façon significative plus élevé que dans les autres parties du tractus génital (p<0.05). Les champignons les plus communs isolés du vagin, le col et les cornes utérines ont été associés aux espèces de Candida spp., tandis que les espèces de Trichosporon spp. étaient les champignons les plus fréquents dans le corps utérin. Les résultats ont montré que les espèces de Penicillium spp. et Candida spp., particulièrement C. tropicalis et C. krusei, étaient les composants les plus communs de la microbiote génitale de brebis et le nombre de champignons a varié parmi de différentes parties de système génital.

Mots-clés : brebis, tractus génital, microbiote fongique, Penicillium, Candida, Candida tropicalis

Introduction

Sheep are important animal production resources in many parts of the world. Reproductive efficiency in the sheep is particularly important because of the seasonal nature of breeding and the relatively small number of lambs produced per sheep per year. Many different infectious agents can lead to reproductive problems in ewes [3] and knowledge of persistent or transient microbiota living in ewe’s cervicovaginal environment is relevant to a better understanding of these pathological processes [18]. Some of them cause infertility or reduce productivity with clinical signs which may include vaginal discharge or not. Little reports demonstrated normal fungal microbiota in genital tract of animals and some of these fungi were as the major fungal agents such as C. albicans in cows [8], C. zeylanoides (20.3%) in dromedaries [26], C. krusei (43.1%) in horses [4] and C. parapsilosis (21.7%) in dogs [6].

Microbial infections of the genital tract may lead to temporary or permanent infertility in ewes as well as abortion and prenatal and neonatal loss in pregnant subjects [1]. Fungal infections of the genital tract of animals have not received much attention in the past. However, with indiscriminate use of antibiotics and hormonal therapy, fungal infections are becoming more common in humans [30, 31]. Verma et al. [35] showed that different fungi including Candida spp., Aspergillus spp., Penicillium spp., Acremonium spp., Cladosporium spp., Mortierella spp., Aureobasidium pullulans and zygomycetes could cause genital infections in cows and buffalos. Vaginitis, endometritis, and cervicitis due to fungal agents have been described in horses [5, 22], cows
Materials and Methods

SAMPLING PROCEDURE

A total of 84 non-pregnant ewes (Zell breed), aged > 2 years old, were included in this study from March 2016 to August 2016. These animals belonged to different farms in humid regions of northern Iran, Mazandaran province, Joybar city (one of the most important locations of sheep breeding). With regard to high consumption of sheep meat in most regions of Iran, ewes were purchased by Iranian Veterinary Medicine Organization (IVMO) from their farms and selected for this study. The animals were chosen based on no previous genital infections noted in their files as well as physical examinations. Animals with a recent history of disease, or that had been administered local or systemic drugs or antimicrobial agents in the last six months were excluded from this study. All the animals had a history of sexual interaction.

In slaughter houses, the carcasses were immediately opened. Subsequently, the complete genital tract was aseptically removed, ligated with a nylon suture and immediately transferred under cool condition to Mycology Center, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran. Samples were taken from the internal surfaces of vagina, cervix, uterine body and uterine horns using sterile cotton swabs without transport media (LP Italiana S.p.A., Milano, Italy). One swab was taken from each site. Special care was taken to ensure that the swab did not come into contact with the other body sites. A total of 336 samples from different parts of genital tract were submitted for mycological examination. The protocol for this experiment was approved by the Institute Animal Care and Use Committee of Amol University of Special Modern Technologies, Amol, Iran.

SAMPLE CULTURE AND FUNGAL IDENTIFICATION

Samples were inoculated onto Sabouraud dextrose agar (Merck Co., Darmstadt, Germany) supplemented with antibiotic (chloramphenicol; 0.005%) and kept at 30°C for 7-10 days before being considered negative. Visual examinations of the fungal colonies were made and their characteristics including texture, pigment, and rate of growth on medium were recorded [27]. Colonies were examined under a light microscope to determine the number of fungal isolates in different parts of genital tract of ewes.

STATISTICS

Data analysis was performed via SPSS (version 20; SPSS Inc., Chicago, IL, USA) using one way analysis of variance (ANOVA) and Tukey post-hoc tests. The comparisons were performed among different species of fungi as well as among different sites of reproductive tracts. A P value less than 0.05 was considered statistically significant.

Results

Fungal agents were isolated from 73.8% (62/84) of the sample processed. The organisms isolated and the total number of fungal species are illustrated in Table 1. A total of 324 fungal isolates were obtained from the genital samples. After investigation, 153 mixed filamentous fungi (47.2%) and 171 yeasts (52.8%) were detected. Six different fungi were found in 1 ewe, 5 in 2, 4 in 6, 3 in 15, 2 in 17 and 1 in 21. The number of fungal agents was ranged from 27 in a subject to 1 in 21 animals.

Fungal isolates belonged to 15 genera including Candida spp. (27.2%), Trichosporon spp. (16.7%), sterile hyphae (14.2%), Penicillium spp. (8.3%), Geotrichum spp. (7.1%), Alternaria spp. (7.1%), Cladosporium spp. (6.5%), Aspergillus spp. (3.7%), Fusarium spp. (3.4%), Rhodotorula spp. (1.9%), dematiaceous fungi (1.5%), Mucor spp. (1.5%), Rhizopus spp. (0.3%), Trichothecium spp. (0.3%) and Ulocladium spp. (0.3%). There was significant difference between the incidence rate of Candida spp. and other fungal genera (p<0.05). As shown in Figures 1 and 2, C. tropicalis and C. krusei (29.5%) and A. flavus (50%) were the most common yeasts and moulds isolated from the genital tract, respectively.
**Table I**: Frequency of fungal species isolated from different parts of genital tract of ewes.

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<tbody>
<tr>
<td><strong>Vagina</strong></td>
<td>12(3.7)</td>
<td>8(2.5)</td>
<td>4(1.2)</td>
<td>10(3.1)</td>
<td>4(1.2)</td>
<td>2(0.6)</td>
<td>1(0.3)</td>
<td>1(0.3)</td>
<td>1(0.3)</td>
<td>0</td>
<td>0</td>
<td>119(36.7)</td>
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<tr>
<td><strong>Cervix</strong></td>
<td>8(2.5)</td>
<td>8(2.5)</td>
<td>7(2.2)</td>
<td>5(1.5)</td>
<td>1(0.3)</td>
<td>4(1.2)</td>
<td>1(0.3)</td>
<td>1(0.3)</td>
<td>0</td>
<td>1(0.3)</td>
<td>1(0.3)</td>
<td>67(20.7)</td>
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<tr>
<td><strong>Uterine body</strong></td>
<td>7(2.2)</td>
<td>8(2.5)</td>
<td>5(1.5)</td>
<td>3(0.9)</td>
<td>3(0.9)</td>
<td>0</td>
<td>1(0.3)</td>
<td>1(0.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>67(20.7)</td>
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<tr>
<td><strong>Uterine horns</strong></td>
<td>19(5.9)</td>
<td>3(0.9)</td>
<td>7(2.2)</td>
<td>3(0.9)</td>
<td>4(1.2)</td>
<td>5(1.5)</td>
<td>2(0.6)</td>
<td>2(0.6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>71(21.9)</td>
</tr>
<tr>
<td><strong>Sum</strong></td>
<td>46(14.2)</td>
<td>27(8.3)</td>
<td>23(7.1)</td>
<td>21(6.5)</td>
<td>12(3.7)</td>
<td>11(3.4)</td>
<td>5(1.5)</td>
<td>5(1.5)</td>
<td>1(0.3)</td>
<td>1(0.3)</td>
<td>1(0.3)</td>
<td>324(100)</td>
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<tr>
<td><strong>Total</strong></td>
<td>153(47.2)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td>324(100)</td>
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</table>

* Significant difference was observed between the incidence rate of *Candida* species and other fungi isolated ($p<0.05$).
Fungal isolates were recovered from different parts of genital tract including vagina, cervix, uterine body and uterine horns. The total number of fungi detected in the vagina (36.7%) was significantly higher than the results obtained from other parts (p<0.05).

Discussion

The fungal microbiota 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 fungal species, with its array of virulence factors, is introduced into the host, disease does not always occur. Conversely, under selected conditions, commensal endogenous fungi can participate in disease processes [14]. About 100 species of fungi are identified as pathogens of animals under predisposing conditions, such as abnormal susceptibility, prolonged antibiotic treatment, intrauterine therapy and traumatic implantation [33]. However, there are limited data on the incidence of fungal vaginitis, cervicitis, endometritis and metritis, as well as the pathogenesis and treatment of reproductive infections in ewes. The resident fungal microbiota of the genital tract is still unknown in ewes and this knowledge would be very useful in assessing the accuracy of treatments.

The present study indicated a large range of fungal microbiota isolated from ewes’ genitalia living in humid regions of northern Iran. Fungal isolates were obtained from 62 (73.8%) of 84 ewes examined. This value is higher than the results obtained by other investigators in relation to other animals [4, 8, 19]. The higher prevalence of mycobiota agents in the present study may be attributed to the high density of animals and topographic and climate variations, particularly high relative humidity of Joybar city. In this study, some sheep were colonized by one kind of fungi isolate. However, some ewes were colonized by 2, 3, 4, 5 and/or 6 kinds of fungi isolates. Literature reviews showed that the synergism of the multiple microorganisms and the type of synergistic microorganism have severe effect on reproductive tract and resulted in pathological changes which lowered pregnancy rate in animals [16]. Different filamentous fungi along with yeasts were isolated from ewes in this study. From 47.2% filamentous fungi detected, the most predominant fungi were classified as sterile hyphae, *Penicillium* spp. and *Aspergillus* spp. Current literature does not provide information about prevalence and composition of filamentous fungi in the genital tract of ewes. In general, our findings are in accordance with several observations related to the presence of different filamentous fungi in other female animals such as cow [8], buffalo [35], horse [24] and dog [25]. filamentous fungi are ubiquitous in nature and are usually saprophytic. These fungi can produce reproductive failure in animals either as a direct result of establishing infection in the genital system or by producing toxigenic metabolites, particularly by *Penicillium* spp. and *Aspergillus* spp. Mycotic abortion caused by *Aspergillus* spp. and *Penicillium* spp. is the most important consequence of fungal infection of the genital tract, although fungi have been implicated occasionally in other syndromes such as vulvovaginitis or endometritis. The genera of *Aspergillus* and *Penicillium* can grow in a suitable substrate under appropriate conditions. They can produce toxins which are accounted the majority of abortion cases and can cause reproductive system infections in animals [13, 34]. In the present study, *A. flavus*, *A. terreus*, *A. nidulans*, *A. niger* and *A. glaucus* were detected in the genital tracts of ewes. There have been no previous reports of the isolation of the above-mentioned *Aspergillus* species in sheep. In studies conducted by Pal et al. [20] and Giri et al. [9], *A. terreus* was isolated from cases of endometritis in cows.

In this study, *Candida* spp. were the most predominant yeast isolates, followed by *Trichosporon* spp. and *Geotrichum* spp. These yeasts were hypothesized to be transitory contaminants of the genital area or transient colonizers. In fact, due to the reduced number of isolations and the lack of a relationship among ewes, the real role of them in the normal genital microbiota of ewes remains to be determined in further studies. Our results showed a high prevalence of *Candida* species (27.2%) in genital tract of examined ewes. The predominance of *Candida* yeasts was expected, since their role as members of female genital microbiota has been reported as a natural condition of women [21] and animals such as cow [8], dog [6], horse [4], cat [7] and monkey [32]. Yeasts of genus *Candida* are widely distributed in the environment and frequently colonize skin and mucous membranes such as oral cavity, genital and gastrointestinal tracts of mammalians [2].

Among different *Candida* species, *C. tropicalis* and *C. krusei* (29.5%) were the most frequent species isolated from different parts of ewes genital canal, particularly in vagina. The isolation of *C. krusei* and *C. tropicalis* appears not to have been recorded earlier, although their involvement in different forms of candidiasis in human was well documented [10, 12]. The observation in this study differed from those of the other researchers in human and cow that presented *C. albicans* as major yeast microbiota with frequencies of 85-90% and 39.1%, respectively [8, 29]. The explanation for this difference in our population is not clear. However a study with a larger sample in different regions could conclusively establish the possible relationship between these *Candida* species and ewes. The high predominance of *C. tropicalis* and *C. krusei*
in most samples suggests that this microorganism may be a resident member of the normal genital microbiota of ewes. It is necessary to mention that C. krusei has been recognized as a potentially multidrug-resistant (MDR) fungal pathogen, due to its intrinsic fluconazole resistance combined with reports of decreased susceptibility to both fluconazole and amphotericin B [17]. The MDR phenotype exhibited by C. krusei poses a therapeutic dilemma when one is considering treatment choices for animals with genital candidiasis, especially for those with prior exposure to fluconazole.

Among the non-Candida yeasts, T. beigelii (16.7%) and G. candidum (7.1%) were found as the predominant yeasts of the genital tract in ewes. The close similar results were reported by other investigators in some animals such as camel (T. beigelii; 10.1% and G. candidum; 7.5%) [27], lion tamarin (T. beigelii; 11.8%) [19], horse (G. candidum; 8.0% and T. beigelii; 2.6%) [4] and cow (G. candidum; 8.7%) [28].

Fungal isolates were recovered from different parts of genital tract including vagina (36.7%), cervix (20.7%), uterine body (20.7%) and uterine horns (21.9%) of ewes. The value found in the vagina was significantly higher than results obtained from other parts. Candida spp. were the most frequent fungi isolated from vagina, cervix and uterine horns, while Trichosporon spp. were the most frequent fungi in uterine body. The primary reservoir for infectious agents that colonize into the genital canal is the caudal genital tract including external genitalia such as vulvovaginal, although contamination from fecal matter (pneumovagina, poor perineal conformation, etc.) or due to iatrogenic means (after perineal conformation, etc.) or due to iatrogenic means (after uterine culture/cytology/ biopsy or artificial insemination) is also possible. Therefore, it is suggested that vagina and cervix should be cultured when fungal infections are suspected or identified. From this study, mycoflora of cervix may play an important role in uterine body and horns infection and could stay dormant in the genital tract tissues, passed to next breeding season or could be localized after coitus due to contamination from livestock.

In conclusion, there were a variety of fungi that have been identified from genital tract cultures with Penicillium spp. and Candida spp. (particularly C. tropicalis and C. krusei) as the most common filamentous fungi and yeasts, respectively. The data acquired in this study provide helpful insights into the occurrence of fungal microbiota in ewes’ genitalia and confirm the importance of both clinical examinations and fungal cultures for the diagnosis of reproductive infections. Further investigations should be done to understand the role of opportunistic fungal agents in various theriogenological processes.

Conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

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References


