

Study of fungal species in the uterus of cats referred to the clinic for ovariectomy during different estrus cycles

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Abstract

Reproductive system infections in cats, often caused by fungi, can negatively impact fertility and health, posing risks to humans as well. Fungi species exhibit variability in the uterus during different sexual cycles in animals due to the dynamic interactions between microbial communities and the hormonal changes associated with reproductive stages. However, studies on the frequency and prevalence of fungi in the uterine flora during different stages of the estrus cycle in cats are limited. This study aimed to identify fungal species that are present in the uteri of 24 cats referred for ovariectomy during their estrus cycle. Swab samples were cultured on Sabouraud dextrose agar with chloramphenicol, and yeasts and molds were examined using macroscopic, microscopic, and slide culture techniques. Definitive diagnoses were made using polymerase chain reaction (PCR), with amplified fragments sequenced and compared to the National Center for Biotechnology Information (NCBI) database. The abundance of different types of fungi at each estrus stage, across breeds, and age ranges were expressed as percentages. The Chi-Square test evaluated the relationship between age, breed, and fungal infection frequency. Results showed that 20 of the 24 cats (83.33%) had a fungal load, with *Aspergillus niger*, *Rhizopus*, *Penicillium*, and *Candida albicans* being the most common species. No significant relationships were found between age or breed and fungal load frequency. The findings of this study highlight a significant level of fungal species in the reproductive systems of cats. Although age and breed do not affect the presence of fungi, some differences may be seen in fungal species in various sexual stages of the cats. This underscores the urgent need for enhanced hygiene and management practices, effective treatment of genital diseases, and the maintenance of sanitary conditions for feline health.

Introduction

Fungal infections transmitted between cats and humans represent a significant public health concern, particularly due to the prevalence of zoonotic mycoses. This emerging transmission route underscores the need

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for heightened awareness and preventive measures to mitigate the risk of zoonotic fungal infections. Such infections can lead to various dermatological conditions, with certain fungi more commonly associated with feline hosts. Understanding the transmission dynamics and clinical implications of these infections is essential for effective prevention and management (1, 2). Despite the associated risks, awareness and preventive measures remain inadequate, emphasizing the urgent need for educational campaigns to mitigate the spread of these infections among pet owners and the general public (3, 4).

Knowledge of the microorganisms present in an animal's environment is crucial for understanding pathological processes. Vulvovaginitis can occur in both young and adult cats, although it is rare in older cats and more frequently observed in those under one year of age. This condition is primarily caused by bacterial infections, with fungal agents often identified as secondary infections. Important diseases affecting the feline reproductive system include vaginitis, cervicitis, and endometritis. Therefore, identifying persistent or occasional vaginal microbial populations is essential for understanding their potential role as endogenous sources of infection (5). Fungi are found in various environments, including soil, plants, water, fruits, trees, and animal secretions. Approximately 100 fungal species have been identified as pathogenic to humans and animals, particularly in conditions such as prolonged antibiotic therapy, intrauterine antibiotic therapy, and traumatic implantation (6). The genital tracts of various animals serve as major reservoirs for yeasts like *Candida albicans* and *Candida neoformans* (7). Additionally, fungi are part of the normal vaginal microbiota in some animal species, including camels and their presence varies depending on the stage of the estrous cycle, which is known to be an endogenous source of infection (8).

There is currently a lack of studies investigating the frequency and prevalence of fungi in the uterine flora during different stages of the estrus cycle in cats during the breeding season. Despite the significance of fungal infections in the female genital tracts of domestic cats, this area has not received adequate attention. With the increasing population of pet and stray cats in urban areas, the risk of fungal cross-contamination may pose a significant health threat. Many cats are referred to veterinary clinics for spaying, and being aware of potential fungal infections in the operated tissues could be beneficial for both the animals and the surgeons. Spaying, also known as ovariohysterectomy (OHE), involves the surgical removal of a female cat's reproductive organs. This procedure is the most common method of neutering pets, with a substantial number of animals undergoing it each year. The primary reason for performing ovariohysterectomy (spaying) in female cats is to prevent unwanted pregnancies and eliminate heat cycles. This procedure is widely recommended in veterinary clinics to manage reproductive health and control the population of pets effectively (9). Ideally, this surgery should be conducted before the cat reaches puberty (10).

The estrous cycle in female cats comprises five distinct phases, during which significant hormonal changes occur, particularly in proestrus and estrus. These changes can increase the risk of infections (11). Similar to other species, female cats become more susceptible to *Candida* infections due to these hormonal fluctuations (12). The physiological alterations during both the estrous cycle and pregnancy play a crucial role in the proliferation of *Candida* species. Specifically, increased levels of estrogen during the estrous cycle modify the vaginal environment, promoting conditions that favor *Candida* colonization (13, 14). This is further exacerbated during pregnancy, when elevated estrogen and progesterone levels continue to alter the vaginal ecosystem, leading to increased *Candida* growth (15). Based on this, we hypothesized that the uterine fungal flora could differ during the various phases of the estrous cycle. Some of the normal fungal flora, such as *Candida* species, can become infectious agents under certain conditions. Therefore, the objective of the present study was to investigate the fungal flora and assess the frequency of different types of fungi present in the uteruses and ovaries of healthy cats.

Materials and Methods

The study was conducted on 24 female Domestic Short-Haired (DSH), Scottish Fold (SF), and Persian cats (*Felis catus domesticus*), averaging 2.8 ± 0.5 kg in weight from February 2023 to September 2023. These cats were referred by their owners to the Specialized Hospital of the Faculty of Veterinary Medicine at the University of Tabriz for ovariectomy surgery. Throughout the study, treatment protocols followed the World Medical Association's Statement on the Use of Animals in Biomedical Research, ensuring that unnecessary stimulation of the animals was avoided at all stages. All surgeries were performed under appropriate anesthesia by an expert surgeon. Informed consent forms for surgery were obtained from animal owners.

Surgery

Ovariectomy was routinely performed on all subjects in this study. The procedure commenced with premedication utilizing acepromazine maleate (1%, 0.1 mg kg⁻¹, IM, Alfasan, Woerden, The Netherlands), followed by the induction of general anesthesia through a combination of diazepam (0.5%, 0.2 mg kg⁻¹ IV, Chemi Darou, Tehran, Iran) and ketamine HCl (10%, 10 mg kg⁻¹ IV, Bremer, Germany). Anesthesia was maintained with 1% halothane (Halothane BP, Nicholas Piramal India Limited, India) in oxygen. The ventral abdomen was clipped and surgically prepared from the xiphoid to the pubis. A 4-cm incision was made in the middle third of the caudal abdomen to expose the *linea alba*, which was subsequently incised. The uterine horns were grasped with an OHE hook and exteriorized, while the suspensory ligament was broken near the kidney. The ovarian pedicle was transected using the two-forceps method and absorbable suture material for ligatures (polyglycolic acid, 3-0, SUPA, Tehran, Iran). A ligature was applied around the broad ligament if the patient was in estrus or if the broad ligament exhibited significant vascular or fat infiltration. The uterine body cranial to the cervix was double ligated using the figure-eight suture method and a circumferential ligature prior to transection. The abdominal wall was closed in three layers (*linea alba*, subcutaneous tissue, and skin) utilizing a simple continuous suture pattern with both absorbable (polyglycolic acid, 3-0, SUPA, Iran) and nonabsorbable (polyamide, 3-0, SUPA, Tehran, Iran) suture materials (16).

Sampling

The estrus cycle of the animals was determined by evaluating ovaries using the previously reported method (17). We did not recognize any cat in the interestrus period. Sampling was conducted using intrauterine and ovarian swabs during surgery. The swab samples were cultured on Sabouraud dextrose agar containing chloramphenicol and incubated for two weeks at temperatures ranging from 25 to 28 °C. During the incubation period, the resulting yeasts and molds were examined based on their colony characteristics, as well as macroscopic and microscopic features. Possible yeasts were identified by observing germ tube formation and colony color on *Candida* chrome agar medium. If necessary, for definitive identification, the ITS region of the fungal ribosomal DNA was amplified using PCR, and the resulting product was sequenced and compared to the NCBI database. If the fungal species could not be confirmed through sequencing, it was reported at the genus level.

Molecular detection by polymerase chain reaction (PCR)

DNA extraction was performed using a physical-chemical method using 0.5 mm diameter glass beads in the presence of denaturing agents. The process began by inoculating one ounce of fresh *Aspergillus* cultures (one week old) into 250 ml flasks containing Sabouraud broth medium, which were then incubated at 28 °C for 72 hours on a shaker. Following the incubation period, the fungal mycelia were collected and washed with Tris-EDTA buffer. DNA extraction was performed using a combination of glass beads, phenol, and chloroform (18). The extraction buffer consisted of 1 mM EDTA (pH = 8), 1% SDS, 10 mM Tris-HCl (pH = 8), 100 mM NaCl, and 2% Triton X-100. A portion of the mycelium was vortexed in a microtube with glass beads, the extraction buffer, and proteinase K. After adding saturated phenol solution, the mixture was centrifuged for 5 minutes. The

supernatant was transferred to a new microtube, and 500 µL of chloroform was added before centrifugation. The supernatant was then extracted with 3 M sodium acetate buffer (pH = 5.5) and isobutanol. The DNA precipitate was washed with 70% alcohol, dissolved in 30 to 50 µL of TE buffer, and stored at -20 °C (18). The extracted DNA was electrophoresed on a 1.5% agarose gel (Figure 1A).

Polymerase chain reaction (PCR) was performed to amplify a fragment of the rDNA gene located in the ITS region, utilizing primers selected based on the research of Glass & Donaldson (Table 1). A 50 µL PCR mixture was prepared, which included 20 ng of genomic DNA, PCR buffer containing 10 mM Tris HCl, 50 mM potassium chloride, 2 mM MgCl₂, 0.2 mM dNTP, 0.4 µM of each primer, and 2U of Taq polymerase. This mixture was then placed in a Primus 96 plus thermocycler, where it underwent an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles consisting of denaturation at 95 °C for 45 seconds, primer annealing at 56 °C for 45 seconds, extension at 72 °C for 1 minute, and concluding with a final extension at 72 °C for 5 minutes (19).

Table1. ITS primer sequences, which were used in this study (19).

Primer	Sequence	Product size (bp)
ITS1	TCC GTA GGT GAA CCT GC GG	600
ITS4	TCC TCC GCT TAT TGA TAT GC	600

To assess the initial results of the PCR reaction, electrophoresis of the products was conducted on a 1.5% agarose gel in TBE buffer for 30 minutes at a voltage of 100 V (Figure 1A). The beta-2 tubulin primer yielded one band (Figure 1B). The PCR product was subsequently sequenced to identify the species based on nucleotide differences. Initially, the PCR products were purified using a specialized kit, and nucleotide sequences were determined from both ends of the DNA using forward and reverse primers specific to each gene region. Sequencing was performed using an ABI 3730 DNA analyzer (ABI series, Applied Biosystems, USA), employing the Sanger method (20). The sequencing results were presented in the form of chromatograms. For definitive identification, the chromatograms were analyzed using bioinformatics methods, comparing the obtained sequences with information available in databases such as the National Center for Biotechnology Information (NCBI) to determine the species of *Aspergillus* and *Penicillium*. The nucleotide sequences were analyzed using MEGA 7.0.2, BioEdit ver. 7.0.9, and Chromas software.

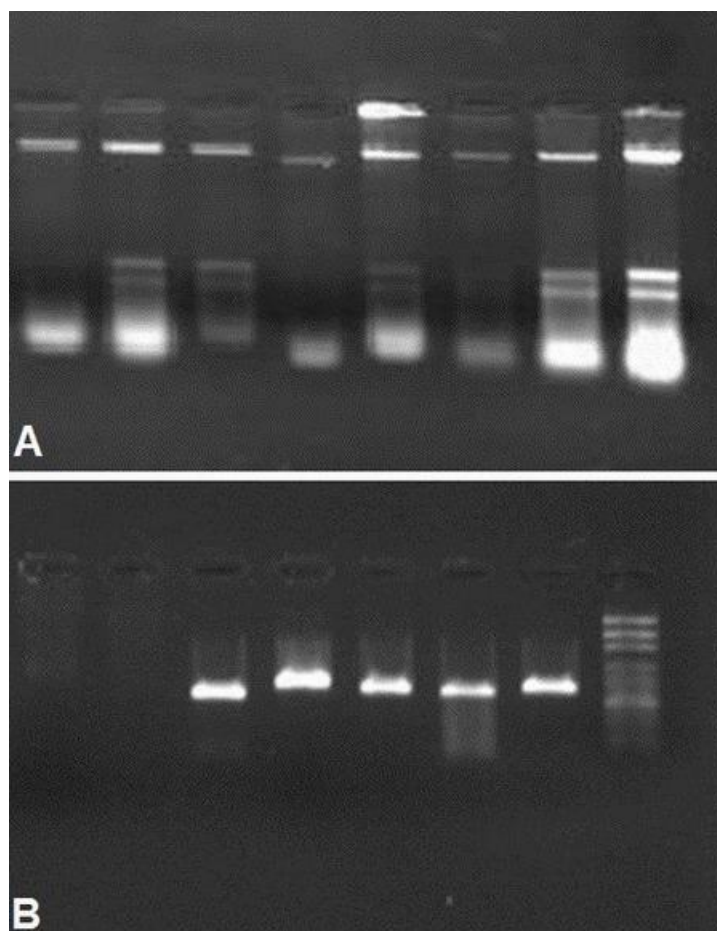


Fig. 1. A: DNA extracted on a 1.5% agarose gel. **B:** Band created from amplification of the ITS region of the ribosomal DNA gene.

Statistical analyses

Minitab software (version 16.2.0, Minitab Inc., State College, PA, USA) was used for statistical analysis. The abundance of different types of fungi at each estrus stage, different breeds, and different age ranges were expressed as percentages. To examine the results of the effect of age range and different races on the isolated fungi, the Chi-Square test was performed to evaluate the relationship between categorical variables and the frequency of fungal infections, and a $P < 0.05$ was considered a statistically significant difference.

Results

In this study, 20 out of 24 cats (83.33%) had various fungal species. One fungal species was isolated from 6 cats (25%), two fungal species from 12 cats (50%), and three fungal species from 2 cats (8.33%). No significant association was found between the fungal species isolated and the cats. *Aspergillus niger* (*A. niger*), *Rhizopus*, *Penicillium*, and *Candida albicans* (*C. albicans*) were the most common fungi isolated.

The results from various heat cycles are summarized in Table 2. In the proestrous cycle, *A. niger* exhibited the highest frequency with 3 occurrences, accounting for 33.3% of the total. *Rhizopus* followed with 2 occurrences (22.2%), while both *C. albicans* and no fungal contamination also had 2 occurrences each (22.2%). During the estrous cycle, both *A. niger* and *C. albicans* had 3 occurrences each (27.3%), while *Aspergillus flavus* (*A. flavus*) had 2 occurrences (18.2%). The fungi *Rhizopus*, *Candida* species, and *Cladosporium* had the lowest frequency,

each with 1 occurrence (9.1%). In the diestrus cycle, *A. niger* dominated with 6 occurrences (54.5%), followed by *Penicillium* with 2 occurrences (18.2%). Again, *Rhizopus*, *Candida* species, and *Cladosporium* had the lowest frequency with 1 occurrence each (9.1%). In the anestrus cycle, *Rhizopus* had the highest frequency with 4 occurrences (44.4%), followed by *Penicillium* with 2 occurrences (22.2%). *A. niger*, *Geotrichum*, and no fungal contamination had the lowest frequency with 1 occurrence each (11.1%).

Table 2. Fungal species isolated in different sexual cycles of cats that were evaluated in this study.

Estrus cycle	Types of fungi	Frequency (No.)	Frequency (%)
Proestrus	<i>Aspergillus niger</i>	3	33.3
	<i>Rhizopus</i> spp.	2	22.2
	<i>Candida albicans</i>	2	22.2
	No infection	2	22.2
Estrus	<i>Aspergillus niger</i>	3	27.3
	<i>Rhizopus</i> spp.	1	9.1
	<i>Candida albicans</i>	3	27.3
	<i>Candida</i> spp.	1	9.1
	<i>Aspergillus flavus</i>	2	18.2
	No infection	1	9.1
Diestrus	<i>Aspergillus niger</i>	6	54.5
	<i>Rhizopus</i> spp.	1	9.1
	<i>Penicillium</i> spp.	2	18.2
	<i>Candida</i> spp.	1	9.1
	<i>Cladosporium</i> spp.	1	9.1
Anestrus	<i>Aspergillus niger</i>	1	11.1
	<i>Rhizopus</i> spp.	4	44.4
	<i>Penicillium</i> spp.	2	22.2
	<i>Geotrichum</i> spp.	1	11.1
	No infection	1	11.1

The results from various breeds are summarized in Table 3. In the DSH cats, *A. niger* exhibited the highest frequency with 6 occurrences, accounting for 27.3% of the total. *Rhizopus* and *C. albicans* followed with 4 occurrences each (18.2%). Additionally, *Penicillium*, *Candida* species, *A. flavus*, and no fungal contamination each had 2 occurrences, contributing 9.1% of the total. In the Scottish Fold breed, *A. niger* was the most frequent with 4 occurrences, representing 57.1% of the total. *Rhizopus*, *Penicillium*, and *C. albicans* each had 1 occurrence (14.3%), the lowest frequency in this breed. In the Persian cats, both *A. niger* and *Rhizopus* showed the highest frequency with 3 occurrences each (27.3%). *Penicillium*, *Cladosporium*, *Geotrichum*, and no fungal contamination each had 1 occurrence (9.1%).

Table 3. Fungal species isolated from various cat breeds that were evaluated in this study.

Breed	Types of fungi	Frequency (No.)	Frequency (%)
DSH	<i>Aspergillus niger</i>	6	27.3
	<i>Rhizopus</i> spp.	4	18.2
	<i>Penicillium</i> spp.	2	9.1
	<i>Candida albicans</i>	4	18.2
	<i>Candida</i> spp.	2	9.1
	<i>Aspergillus flavus</i>	2	9.1

SF	No infection	2	9.1
	<i>Aspergillus niger</i>	4	57.1
	<i>Rhizopus</i> spp.	1	14.3
	<i>Penicillium</i> spp.	1	14.3
	<i>Candida albicans</i>	1	14.3
Persian	<i>Aspergillus niger</i>	3	27.3
	<i>Rhizopus</i> spp.	3	27.3
	<i>Penicillium</i> spp.	1	9.1
	<i>Cladosporium</i> spp.	1	9.1
	<i>Geotrichum</i> spp.	1	9.1
	No infection	2	18.2

DSH: Domestic Short-Haired; SF: Scottish Fold.

The results obtained at different ages are presented in Table 4. In the age group under 1 year, *A. niger*, *Rhizopus*, *Penicillium*, and no fungal contamination each had 2 occurrences, accounting for 20.0% of the total. *C. albicans* and *A. flavus* each had 1 occurrence, contributing 10.0% of the total. In the 1 to 2 years age range, *A. niger* exhibited the highest frequency with 8 occurrences (36.4%), followed by *Rhizopus* with 6 occurrences (27.3%), and *C. albicans* with 3 occurrences (13.6%). The fungi *Candida*, *Cladosporium*, and no fungal contamination had the lowest frequency, each with 1 occurrence (4.5%). In the age group over 2 years, *A. niger* again had the highest frequency with 3 occurrences (37.5%), followed by *Penicillium* with 2 occurrences (25.0%). In this age range, *C. albicans*, *Candida* species, and *A. flavus* had the lowest frequency, each with 1 occurrence (12.5%).

Table 4. Fungal species isolated from cats that were evaluated in this study based on age.

Age (year)	Types of fungi	Frequency (No.)	Frequency (%)
<1	<i>Aspergillus niger</i>	2	20
	<i>Rhizopus</i> spp.	2	20
	<i>Penicillium</i> spp.	2	20
	<i>Candida albicans</i>	1	10
	<i>Aspergillus flavus</i>	1	10
	No infection	2	20
1-2	<i>Aspergillus niger</i>	8	36.4
	<i>Rhizopus</i> spp.	6	27.3
	<i>Candida albicans</i>	3	13.6
	<i>Candida</i> spp.	1	4.5
	<i>Cladosporium</i> spp.	1	4.5
	<i>Geotrichum</i> spp.	1	4.5
	No infection	2	9.1
>2	<i>Aspergillus niger</i>	3	37.5
	<i>Penicillium</i> spp.	2	25.0
	<i>Candida albicans</i>	1	12.5
	<i>Candida</i> spp.	1	12.5
	<i>Aspergillus flavus</i>	1	12.5

There was no statistically significant difference between age range and the frequency of fungal infections ($\chi^2 = 15.263$, $p = 0.506$). Similarly, there was no statistically significant difference between race and the frequency of fungal infections ($\chi^2 = 14.144$, $p = 0.588$).

Discussion

The study of uterine fungi in cats across different estrous cycles offers significant insights into the microbial environment and the influence of hormones. While the presence of bacteria has been documented, the specific role of fungi remains less understood. Research indicates that the microbial population, including fungi, fluctuates during the estrous cycle. Notably, studies have shown that no bacteria were isolated from the uteri of clinically healthy cats, suggesting a potential absence of significant microbial presence, including fungi (21). In contrast, studies in dogs have demonstrated that the uterine environment can support fungal growth, indicating species-specific differences in uterine microflora dynamics during the estrous cycle (22). Our study revealed that 83.33% of the cats examined had fungal flora caused by various species. Supporting this, Garoussi et al. (23) reported that 69% of the feline population had vaginal fungi. Specifically, fungal isolates were found in 25% of household cats, 22% of stray cats, and 22% of cats from industrial dairy herds, with the highest prevalence of infections occurring in cats aged 1-2 years. A total of 22 different fungal isolates were identified, with *Penicillium* species (11%) and *Aspergillus* (4%) being the most common. Interestingly, healthy cats can be infected by 2-4 different fungal agents (23). Both studies align in highlighting the significant presence of fungi in the genital tracts of female cats. Importantly, no significant relationships were found between the isolated fungal species and the cats' age or breed.

Fungi species exhibit variability in the uterus during different sexual cycles in animals, driven by the dynamic interactions between microbial communities and hormonal changes associated with reproductive stages. This variability can significantly influence reproductive health and outcomes. The estrous cycle, characterized by distinct hormonal phases, alters the uterine environment, leading to changes in microbial populations, including fungi (24, 25). Throughout the estrous cycle, the expression of estrogen and progesterone receptors fluctuates, impacting uterine conditions. For instance, lower receptor expression during diestrus may increase the uterus's susceptibility to microbial colonization (26). Additionally, structural changes in the myometrium during estrus and pregnancy suggest that hormonal fluctuations could affect the uterine microflora, potentially fostering conditions conducive to fungal growth (27). Research on mice indicates that different reproductive stages correlate with specific microbial profiles, suggesting that microbial diversity may play a role in reproductive success (28). In rabbits, the widest variety of fungi was observed during the diestrus stage, indicating a potential link between microbial diversity and reproductive phases (25). Fungal species, particularly those in the Basidiomycota, demonstrate a relationship between sexual reproduction and pathogenicity, influenced by the host's reproductive cycle (29, 30). Mogheiseh et al. (24) found *Cladosporium* and *Penicillium* isolated during estrus, while yeast appeared in diestrus. Our observations revealed differences in fungal species isolated across various sexual stages, with *Cladosporium* and *Penicillium* being more prevalent in the diestrus phase. However, while the presence of fungi is often linked to reproductive stages, some studies suggest that certain fungi, like *Klebsiella oxytoca*, do not show a clear relationship with the estrous cycle, indicating that not all microbial interactions are directly influenced by reproductive phases (25). This was also true for *Aspergillus niger* in our study, as this fungus was detected in all sexual stages of the cats.

Although several fungi have been identified in the uteri of apparently healthy cats in our study, the presence of fungal flora without clinical symptoms is a plausible scenario, supported by various studies. While specific research on uterine fungal flora is limited, related findings suggest that cats can harbor fungi asymptomatically. For example, studies have shown that cats can carry various fungal species, such as *Aspergillus* and *Penicillium*, without exhibiting clinical signs of infection (31). Additionally, research on bacterial flora in the reproductive tracts of healthy cats indicates that the presence of microorganisms, including anaerobes, is typical (32). This suggests a similar situation for fungi, where non-pathogenic species might reside without causing disease. The ability of fungi to exist without causing symptoms may reflect the immune system's capacity to manage these

organisms effectively. However, environmental stress or immunocompromised states could lead to opportunistic infections, as seen in cases involving *Aspergillus candidus* (33). Conversely, while asymptomatic fungal flora may be common, it is essential to monitor for potential shifts in health status, as latent infections can become problematic under certain conditions.

The majority of fungi isolated in this study can be classified as zoonotic fungal pathogens. *Candida* spp. are part of the normal flora in both humans and animals, but they can become pathogenic under certain conditions, such as immune dysfunction or antibiotic use, leading to infections like candidiasis. As zoonotic agents, these fungi suggest that animals, including cats, can act as reservoirs for these pathogens (34, 35). Specifically, *C. albicans* is commonly found in the mucous surfaces of the oral cavity, gastrointestinal tract, and vagina of humans and domestic animals. When immune dysfunction occurs, *C. albicans* can switch from a commensal organism to a pathogenic one, capable of infecting various tissues and potentially causing a fatal systemic disease (36).

Aspergillus species, particularly *A. niger*, are prevalent in various environments and can lead to serious health issues in both animals and humans. Understanding the transmission dynamics and clinical implications of these infections is crucial for raising awareness and implementing preventive measures. Although *A. niger* can be transmitted from cats to humans, direct zoonotic transmission is less common compared to other fungal pathogens. Environmental exposure is a significant factor, as *A. niger* is ubiquitous in soil and decaying organic matter, which can be inhaled or come into contact with the skin (37, 38). In cats, sinonasal aspergillosis caused by *A. niger* is a common infection, presenting symptoms such as nasal discharge and facial swelling (39). In humans, exposure to *A. niger* can lead to allergic reactions or invasive infections, particularly in immunocompromised individuals (37). Additionally, zoonotic fungal infections caused by *Penicillium* and *Aspergillus* species can be transmitted from cats to humans through direct contact or environmental shedding, making these infections increasingly recognized as significant health concerns due to changing environmental factors (40).

Rhizopus infections in cats are relatively uncommon but can lead to significant health issues. Notably, *Rhizopus* microsporus has been identified in cases of feline gastrointestinal eosinophilic sclerosing fibroplasia, where it was associated with severe gastrointestinal symptoms (41). While these infections are rare, they can result in severe complications, emphasizing the need for awareness and prompt intervention in affected cats (42). Similarly, fungal infections caused by *Cladosporium* species can lead to serious health problems, including chromoblastomycosis and cerebral phaeohyphomycosis. These infections are often linked to underlying conditions such as immunosuppression, making accurate diagnosis and treatment critical. For instance, Zambelli & Griffiths (43) reported a case of a cat with feline immunodeficiency virus (FIV) that developed chromoblastomycosis due to *Cladosporium carrionii*, which proved resistant to itraconazole treatment. Earlier, Mariani et al. (44) documented two domestic shorthair cats that exhibited neurological symptoms due to *Cladosporium* spp., confirmed through postmortem histopathology.

Conclusion

The findings of this study highlight a significant level of fungal species in the reproductive systems of cats. This underscores the urgent need for enhanced hygiene and management practices, effective treatment of genital diseases, and the maintenance of sanitary conditions for feline health.

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Ethical approval

The study was approved by the Research Ethics Committee of the University of Tabriz (approval code: IR.TABRIZU.REC.1404.052).

Conflict of Interests

Authors declare that they have no conflict of interest.

Artificial Intelligence Statement

The authors used PopAi tool to check the grammar of the manuscript. After using this tool/service, the authors review and edit the content if needed and maintain responsibility for the content of their work.

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