

CURRENT STATUS OF PREVALENCE OF YEAST-LIKE FUNGI IN THE ENVIRONMENT OF HORSES BRED IN POLAND*

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Abstract

This study presents an analysis of the yeast-like fungi population in the healthy horses' living environment. The study material was sampled from various elements and equipment of a stable. We compared the yeast-like fungi population from the breeding environment of five groups of horses (English Thoroughbred horses, Arabian horses, Polish Half-Bred horses, cold-blooded horses, and Hucul horses and Highland ponies). The samples were collected in the summer and winter period. The laboratory examination involved 260 samples and resulted in identification of yeast-like fungi belonging to 13 species. Over 50% of the samples collected from the horse environment in winter-time and fewer than 18% of the summer samples displayed fungal growth. The largest amounts of yeast fungi were isolated from samples collected from water and feed troughs. The laboratory analysis demonstrated qualitative diversity of the yeast-like fungi population depending on the breeding environment of the individual horse groups. Quantitative differences of isolates were additionally related to the season of the year.

Key words: horses, yeast-like fungi, qualitative analysis, winter/summer period

About 100 fungal species have been identified as pathogenic for humans and animals in such conditions as long-term antibiotic therapy, intrauterine antibiotic therapy and traumatic implantation (Tibary et al., 2005). Pathogenic fungi are characterised by low host specificity in comparison to other parasitic organisms. They display ability to adaptation and resistance to varied environmental conditions. Most yeast-like species are opportunistic microorganisms.

Current literature does not provide papers on the occurrence and composition of yeast-like fungi in the horse environment. Fungi may colonize mammalian skin

*This study was supported by University of Life Sciences in Lublin.

surface and mucous membranes, and they may play a role as a source of endogenous infection. The problem of fungal infections of the animal reproductive system has been addressed only in few papers. The massive use of antibiotics and hormonal therapies has resulted in prevalence of fungal infections in humans (Sobel et al., 1998). Verma et al. (1999) have demonstrated that various fungi may cause genital tract infections in cows and buffaloes. Fungal vaginitis, and uterine and cervical inflammation have been reported in horses (Blue, 1983; Pugh et al., 1986) and dogs (Cleff et al., 2005).

Knowledge of the microorganisms present in the animal environment is essential for better understanding of pathological processes. Few publications have reported the occurrence of opportunistic fungi in the animal reproductive system; however, some fungi are potentially pathogenic in cows (Garoussi et al., 2007), horses (Chengappa et al., 1984) and dogs (Cleff et al., 2005).

Yeast-like fungi are widespread in the environment, but literature provides no information about their particular species composition in the environment of animal husbandry. Due to their potential role as an endogenous infection source, it is crucial to identify the fungi species composition in the horse breeding environment.

The aim of the present work was to perform a qualitative analysis of the occurrence of yeast-like fungi in the environment of several groups of healthy horses bred in Poland.

Material and methods

The investigations were conducted using material from the breeding environment of five groups of horses kept at various equestrian centres in Poland. The breeding conditions and number of samples collected in the stable buildings and equipment are presented in Table 1. The maintenance conditions of the Thoroughbred horses, Arabian horses, Polish Half-Bred horses and the cold-blooded horses were similar, whereas the Hucul horses and Highland ponies were kept outdoors with the possibility to use shelters. The differences between the total number of isolated strains and the number of “no growth” samples and the total number of samples arise from isolation of more than one strain from one sample (see Tables 2–4).

The horses kept in the stables were fed in accordance with the current horse feeding standards (Horse Feeding Standards, 1997) providing mineral-vitamin supplements. The outdoor-bred horses were fed with hay supplemented with mineral licks between November and March; between April and October the horses grazed on pastures.

The experimental horses received permanent zootechnical and veterinary care. All the animals underwent a prophylaxis programme of parasitic invasion control and vaccinations. All the horses were healthy during the investigations. None of them received any treatment before the study (at least 1 month earlier) or afterwards (within maximum 1 month).

The temperature measurements were performed with an instant-read liquid thermometer. Relative humidity was assessed using the Assmann's aspiration psychrometer. The conditions of the horse environment were presented in Table 1.

The microbiological assay was performed after inoculation of 260 samples, 158 of which were collected in winter (object II, III, IV, and V) and 102 in the summer period (object I, III, IV, and V) (Table 1).

Table 1. Conditions of maintenance of various horse groups and the number of collected samples

Horse breeding environment						
Number of horses in the breeding facilities		Thoroughbred (I) 55	Arabian (II) 50	Polish Half-Bred (III) 33	Cold-blooded (IV) 19	Hucul horses and Highland ponies (V): 12 and 9 respectively
Breeding facilities		2 brick box stables	2 brick and 3 wooden box stables	a brick box stable	a brick box stable	Outdoor breeding with the use of a brick shelter
Mean outdoor temperature	winter	-	-15°C	-8°C	-4°C	-10°C
	summer	23°C	-	24°C	22°C	21°C
Mean indoor temperature	winter	-	from -7 to 8°C	3 °C	1 °C	-8 °C
	summer	21°C	-	19°C	20°C	20°C
Mean indoor humidity	winter	-	65%	76%	68%	56%
	summer	76%	-	73%	72%	78%
Number of samples						
Box walls		6	16	5/3	2/1	2/3
Box doors		6	9	5/3	2/1	1/2
Water troughs		6	12	5/3	2/1	2/3
Feed troughs		6	15	7/3	3/1	2/3
Vicinity of the window		6	12	4/3	2/1	-/1
Box bolt		6	9	4/3	2/1	1/2**
Wheelbarrow		-	2	2/3	1/1	2/3
Pitchfork		-	5	3/3	1/1	2/3
Bucket		-	5	5/3	1/1	2/3
Transport means		-	-	1/3	-/1	-
Manure shovel		-	-	2/3	-	-
Total number of samples		36	85	43/33*	16/10*	14/23*

*Number of samples collected in the winter/summer period.

**Applies to the shelter bolt.

All the samples were inoculated on the Sabouraud medium. In selective cultures macroscopically homogenous colonies were obtained, which, after growing, were assayed with the API 20C AUX (bioMérieux) biochemical tests.

Results

Representatives of 13 species of yeast-like fungi including eight species from the genus *Candida* (Table 2), were identified in the breeding environment of the five horse groups. No species were concurrently present in the environment of all the study groups. The largest number of species (9) was identified in the environment of the Polish Half-Bred horse group; a smaller number was found in the environment of the Arabian and Thoroughbred horses (5 and 4, respectively); the environments of the cold-blooded horses and the group of the Hucul horses and Highland ponies displayed three yeast species each.

Stephanoascus ciferrii was present only in the Thoroughbred horse group. *Cryptococcus laurentii* and *Cryptococcus sp.* were isolated from the environment of the Polish Half-Bred horses; two strains of *C. albicans* were obtained from the environment of the cold-blooded horses, and *C. krusei* was present in the environment of the outdoor-bred horses only.

The highest percentage of samples (60 samples) which demonstrated fungus growth was constituted by those collected from the breeding environment of the Arabian horses (Table 2). It was approximately two-fold higher than the percentage of positive samples obtained from the environment of the Thoroughbred, Polish Half-Bred and cold-blooded horses, and over three-fold higher than the samples from the shelter in the outdoor breeding (Hucul horses and Highland ponies).

C. tropicalis yeasts constituted the highest percentage of all the fungi identified in the environment of the Thoroughbred and cold-blooded horses. The most abundant yeast-like fungi in the environment of the Arabian horses comprised *C. glabrata* yeasts, while *C. guilliermondii* was predominant in the environment of the Polish Half-Bred horses. *C. krusei* and *C. rugosa* were identified to be the main yeasts in the environment of the Hucul horses and Highland ponies (Table 2).

Fungal growth was reported for almost 40% of the analysed samples, 84% of which were sampled in wintertime. In the breeding environment of the experimental horse groups, 11 yeast species were identified in the winter period and only five species in summer (Table 3). Besides the *C. sp.* group, only two (*C. guilliermondii* and *C. krusei*) out of all the identified species were found in the winter and summer periods. Over 50% of the winter samples collected from the horse-breeding environment and fewer than 18% of the summer samples were characterised by fungal growth.

More than 80% of the strains isolated from the winter samples consisted of three fungal species (*C. glabrata*, *C. rugosa* and *Trichosporon mucoides*). *C. tropicalis* constituted 50% of the strains isolated from the summer samples.

Among the 11 sampled sites of the breeding environment of the five horse groups, the highest percentage of fungal growth was recorded on water troughs and feed troughs (in more than half of the samples, each). The percentage of samples with fungal growth collected from the other sites was lower. Fifty-five percent of the isolated strains were found on the walls, water troughs and feed troughs. *C. glabrata*, *C. rugosa* and *Trichophyton mucoides* were predominant; each of them was isolated from nearly 73% of the sites examined.

Table 2. Frequency of isolation of yeast species from different parts of the breeding environment

Strains	Number of strains				
	Thoroughbred	Arabian	Polish Half-Bred	Cold-blooded	Hucul horses and Highland ponies
<i>C. albicans 1</i>				1 (12.50)	
<i>C. albicans 2</i>				1 (12.50)	
<i>C. famata</i>		10 (13.70)	2 (9.52)		
<i>C. glabrata</i>		35 (47.95)	3 (14.29)		
<i>C. guilliermondii</i>	3 (27.27)		7 (33.33)	1 (12.50)	
<i>C. krusei</i>					3 (42.86)
<i>C. rugosa</i>		12 (16.44)	2 (9.52)		3 (42.86)
<i>C. tropicalis</i>	5 (45.45)			5 (62.50)	
<i>C. sp.</i>	1 (9.09)		1 (4.76)		1 (14.29)
<i>Cryptococcus humicola</i>		3 (4.11)	1 (4.76)		
<i>Cryptococcus laurentii</i>			1 (4.76)		
<i>Cryptococcus sp.</i>			1 (4.76)		
<i>Stephanoascus ciferrii</i>	2 (18.18)				
<i>Trichosporon mucoides</i>		13 (17.81)	3 (14.29)		
Total N (%)	11 (100)	73 (100)	21 (100)	8 (100)	7 (100)
No-growth samples N (%)	25 (69.44)	33(38.82)	55 (72.37)	18 (69.23)	30 (81.08)
Number of examined samples	36	85	76	26	37

Table 3. Frequency of yeast species isolated from different parts of the breeding environment of the five groups of healthy horses in relation to the season

Strains	Winter period No. (%)	Summer period No. (%)	Total No. (%)
<i>C. albicans 1</i>	1 (0.83)		1 (0.83)
<i>C. albicans 2</i>	1 (0.83)		1 (0.83)
<i>C. famata</i>	12 (10.00)		12 (10.00)
<i>C. glabrata</i>	38 (31.67)		38 (31.67)
<i>C. guilliermondii</i>	8 (6.67)	3 (2.50)	11 (9.17)
<i>C. krusei</i>	1 (0.83)	2 (1.67)	3 (2.50)
<i>C. rugosa</i>	17 (14.17)		17 (14.17)
<i>C. tropicalis</i>		10 (8.33)	10 (8.33)
<i>C. sp.</i>	1 (0.83)	2 (1.67)	3 (2.50)
<i>Cryptococcus humicola</i>	4 (3.33)		4 (3.33)
<i>Cryptococcus laurentii</i>	1 (0.83)		1 (0.83)
<i>Cryptococcus sp.</i>	1 (0.83)		1 (0.83)
<i>Trichosporon mucoides</i>	16 (13.33)		16 (13.33)
<i>Stephanoascus ciferrii</i>		2 (1.67)	2 (1.67)
Total N (% sample)	101 (84.17)	19 (15.83)	120 (100)
No-growth samples	78	84	162
Number of samples	158	102	260

Table 4. Frequency of yeast species isolated from different parts of the breeding environment of five groups of healthy horses

Infrastructure Isolated strains	Box wall	Box door	Water trough	Feed trough	Vicinity of the window	Box bolt	Wheelbar- row	Pitchfork	Bucket	Transport means	Manure shovel
<i>C. albicans</i> 1				1							
<i>C. albicans</i> 2				1							
<i>C. famata</i>		1	5	2	1	2	1				
<i>C. glabrata</i>	9	3	4	7	6	4	3	2			
<i>C. guilliermondii</i>	2	1	4				1	1	1		1
<i>C. krusei</i>				2			1				
<i>C. rugosa</i>	3	2	3	1	2	3			2		1
<i>C. tropicalis</i>	2	1	4	1		1			1		
<i>C. sp.</i>				2				1			
<i>Cryptococcus humicola</i>			1					3			
<i>Cryptococcus laurentii</i>				1							
<i>Cryptococcus</i> sp.									1		
<i>Stephanodascus ciferrii</i>				1		1					
<i>Trichosporon mucoides</i>	2		1	6	1	2	1	1	2		
Total N											
(% samples)	18 (15.00)	8 (6.67)	22 (18.33)	25 (20.83)	10 (8.33)	13 (10.83)	7 (5.83)	8 (6.67)	7 (5.83)	0 (0.00)	2 (1.67)
No-growth samples	24	22	15	17	22	20	9	11	13	5	3
Number of samples	38	29	34	40	29	28	14	18	20	5	5

Discussion

Current literature does not provide reports about occurrence and composition of yeast-like fungi in the animal breeding environment, including horse husbandry. Such information could contribute to establishing the possible relation between horse diseases and prevalence of yeast-like fungi in their environment. Since there are no such reports in the literature, the present results may serve as a point of reference for further environmental husbandry studies.

Interestingly, the breeding environment of the Polish Half-Bred horses, where the largest number of strains was isolated, mostly contained yeast-like fungi that were characteristic of the environment of the Arabian and Thoroughbred horses (Table 2). Furthermore, the highest percentage of various yeast-like fungi identified in the environment of the experimental horse groups (Table 2) may indicate that the prevalence of fungal species is specific for the breeding environment of a particular animal group.

The lowest strain diversity was reported from the breeding environments of the cold-blooded and primitive horses (Table 2). The lowest percentage (less than 19%) of the samples with fungal growth was characteristic of the samples collected from the shelter of the outdoor-bred horses (Table 2). This may indicate the effect of horses' presence on the quantitative and qualitative composition of yeast-like fungi population in the breeding environment. This is confirmed by abundant growth of yeast-like fungi in the samples collected in the winter period and by the great diversity of strains (Table 3). These observations can be explained by the changed horse maintenance conditions, as the animals use paddocks for a limited time.

A reverse phenomenon was observed in the summer period, when the yeast growth was found in 19 of 102 analysed samples (Table 3).

It is interesting that the highest growth of yeast-like microorganisms was observed in the water troughs and feed troughs (Table 4). This may indicate two sources of yeast-like fungi: the horses themselves or their feed. In order to corroborate this theory, further research of the composition of microbiota on horses' mucous membranes and in the feed samples is indispensable.

Fungal diseases pose a serious problem in human and animal medicine. Reports of investigations of candidiasis-causing microorganisms conducted in 1992–2006 demonstrate that yeast species from the genus *Candida* (*C. albicans*, *C. parapsilosis*, *C. glabrata* and *C. tropicalis*) were the predominant etiological factors of invasive diseases worldwide (Trofa et al., 2008). Similar results were presented by Pfaller and Diekema (2010), who found that approximately 95–97% of all severe *Candida*-associated invasive fungal infections (blood, normally sterile body fluids) of human tissues and organs were caused by five species: *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*. The remaining 3–5% of the severe invasive *Candida*-associated fungal infections were caused by 15–18 various species, e.g. *C. guilliermondii*, *C. lusitaniae* and *C. rugosa* (Pfaller et al., 2008).

Chengappa et al. (1984) found that the most prevalent fungal species isolated from a variety of clinical specimens from cows, pigs, dogs, horses, cats and birds

were *C. albicans*, *C. krusei*, *C. tropicalis* and *C. parapsilosis*, which constitute 71.4% of all species from the genus *Candida*.

The results of the prevalence analysis of the yeast-like fungi obtained in the present study seem striking, bearing in mind the fact that the several fungal species causing severe fungal infection were present in the environment of healthy horses, which did not exhibit any disease symptoms.

The present study (Table 2) showed occurrence of *Candida albicans*, *C. glabrata*, *C. tropicalis* and *C. krusei* in the breeding environment of various groups of horses, which did not exhibit any signs of fungal diseases; however, these species are responsible for candidiasis – an invasive disease in immunocompromised human (Trofa et al., 2008; Pfaller et al., 2008). Among these species, only *C. glabrata* and *C. krusei* may be regarded as “emerging” causes of severe invasive fungal infections due to their resistance to the commonly applied antifungal drugs (Trofa et al., 2008).

The biggest problem is posed by occurrence of *Stephanoascus ciferrii* in the horse breeding environment, which in literature is exclusively referred to as the cause of invasive mycosis in humans (Soki et al., 2010) and in the Persian cat suffering from outer ear infections (Kano et al., 2000). The *Stephanoascus ciferrii* species tends to acquire drug resistance. It was isolated, among others, from a patient with refractory otitis media, acute myeloid leukemia and from an immunocompromised patient. According to the literature, it may also be the causative agent of surface and deep mycoses (Soki et al., 2010). Chengappa et al. (1984) have found the yeast species in question to be the cause of vaginal infection in mares. In our study, however, the presence of *Stephanoascus ciferrii* on the feeding trough and box bolt in the stable of the Thoroughbred horses (Tables 2 and 4) was not accompanied by any discernible health effects in the animals. It can be concluded, therefore, that *Stephanoascus ciferrii* occurring in the breeding environment of Thoroughbred horses is an opportunistic fungus.

Similarly, *Candida krusei*, one of the causes of invasive fungal infections in humans (Trofa et al., 2008), has been found in patients with hematologic malignancies and in transplant recipients (Pfaller et al., 2008). It has also been reported as a causative agent of infection in horses (Chengappa et al., 1984). The *C. krusei* yeasts were identified in the environment of primitive horses (Hucul horses and Highland ponies) (Table 2) but they did not produce disease symptoms in the animals. As in the case of *Stephanoascus ciferrii*, this may suggest their opportunistic rather than invasive nature.

The other isolated microorganisms included *Candida tropicalis* strains, whose presence was demonstrated in the summer period in the Thoroughbred and cold-blooded horses (Tables 2 and 3). *C. tropicalis* in humans are considered to have increasing importance, which is confirmed by its prevalence in patients with hematological malignancies (Pfaller et al., 2008). In mares, this yeast has been reported as a causative agent of genital tract infections (Chengappa et al., 1984). Other causes of fungal infection of the reproductive system in mares, i.e. *Aspergillus fumigatus* and *Candida albicans*, have been reported by Blue (1983). The presence of *C. albicans* in horses has also been found by Chengappa et al. (1984), who isolated the fungus

from an aborted foetus. Two *Candida albicans* strains have been found in our own studies. Both were isolated from the environment of the cold-blooded horses in the winter period (Tables 2 and 3). They did not cause mycosis, likewise the other identified fungi in the environment of the horses (Table 1).

According to the literature, one of the most virulent yeasts is *Candida guilliermondii* and *Candida rugosa*. They have been found by Chengappa et al. (1984) as causative agents of cervical, uterine and vaginal infection in mares. These yeast fungi have also been identified in the breeding environment of the horse groups examined in this study (Table 2). As in the case of the other species, their presence in the environment did not induce disease symptoms in healthy, immunocompetent horses. These observations suggest that the isolated yeasts may be a part of the natural microbiota of horses' organisms or a part of contamination of their environment.

In conclusion, the current results of the study of the yeast-like fungi prevalence in the horse breeding environment have shown occurrence of representatives of yeast commonly regarded as opportunistic microorganisms. It is noteworthy that their presence in the test material was not associated with any health risk. No reproduction disturbances were observed either during the pre-study period, the study period or during the one-year post-study period. This suggests that the fungi, which according to e.g. Chengappa et al. (1984) may lead to infection manifested by disease symptoms, are the natural component of the environmental biota. Given the characteristics of fungal infections, it should be emphasized that they are often a secondary symptom of decreased immunity caused by another infectious or environmental agent that is difficult to detect.

The presented results of investigations of the breeding environment as well as its qualitative and quantitative characteristics appear to be related to specific groups of horses and the breeding environment. The variability of the occurrence of the yeasts, their diversity and dependencies between them render further research in this area advisable. Elucidation of these relationships will allow identification of the real threat posed by yeast microorganisms and clarification of the specific role of mycoses in the pathology of horse breeding.

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Accepted for printing 27 VI 2012

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Aktualny status występowania grzybów drożdżopodobnych w środowisku hodowlanym koni w Polsce

STRESZCZENIE

Niniejsza praca przedstawia analizę występowania grzybów drożdżopodobnych w środowisku życia zdrowych koni. Materiał do badań pobrano z różnych elementów stajni i wyposażenia. W pracy porównano częstość izolacji grzybów z próbek pochodzących ze środowiska hodowlanego 5 grup koni (pełnej krwi angielskiej, czystej krwi arabskiej, małopolskich i zimnokrwistych oraz koni rasy huculskiej i Highland ponies). Próby pobierano w okresach letnim i zimowym. Łącznie przebadano laboratoryjnie 260 prób, z których wyhodowano i zidentyfikowano grzyby drożdżopodobne należące do 13 gatunków. Wykazano zróżnicowanie częstości izolacji różnych gatunków w zależności od środowiska hodowlanego poszczególnych grup koni. Różnice te związane były ponadto z porą roku. Z prób pobranych ze środowiska koni w okresie zimowym w ponad połowie przypadków wystąpił wzrost grzybów, natomiast w okresie letnim – w niespełna 18%. Najwięcej drożdżaków wyizolowano z prób pobranych z poidel i żłobów.