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Source: Journal of Wildlife Diseases, 16(1) : 141-149

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-16.1.141

OCCURRENCE OF HUMAN-ASSOCIATED YEASTS IN THE FECES AND POOL WATERS OF CAPTIVE BOTTLENOSED DOLPHINS *(Tursiops truncatus)*[1]

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Abstract: Total yeast counts at 20 and 37 C incubation from chlorinated salt water pools containing marine mammals averaged 40 per L and 12 per L, respectively. *Candida albicans,* the etiological agent of candidiasis in mammals, was found in 32% of 123 water samples although numbers were low (average of 1.2 cells per L). The yeast was isolated only once from feces from one Atlantic bottlenosed dolphin *(Tursiops truncatus)* but was recovered from three fecal samples from an asymptomatic beluga whale *(Delphinapteras leucas)* which suggested that this animal may be a carrier. Three yeasts *(Candida tropicalis, C. parapsilosis,* and *Torulopsis glabrata)* associated with human disease accounted for 73% and 88%, respectively, of the 37 C isolates from water and animals. The data indicate the routine presence of potentially pathogenic yeasts in water and various marine mammals. Captive environments characterized by antimicrobial treatment (e.g., chlorine) may provide appropriate conditions for resistant microorganisms, including yeasts, to become opportunistic pathogens in susceptible marine mammals or to become established in others which act as healthy carriers.

INTRODUCTION

Clear documentation of the yeast *Candida albicans* as a human pathogen appeared more than a century ago. In addition to the classical oral, vaginal, and cutaneous pathology in humans[10,30] more recent studies have focused on a wider variety of clinical symptoms and causes.[1,15]

Species of *Candida* have been implicated in respiratory tract, dermatological, and systemic mycoses in several types of marine mammals.[6,7,8] More specifically, *C. albicans* involvement in mycoses have been reported;[18,19,21,28] in fact, Sweeney and Ridgway[27] indicated that after *Nocardia, Candida* is the second most frequently isolated genus from systemic mycoses in marine mammals.

During the course of the development of a selective medium for the detection and enumeration of *C. albicans* in recreational waters,[6] the organism was isolated from water systems containing captive marine mammals at two large aquariums in Connecticut and Florida. While there are some data on specific bacteria as pathogens in captive marine animals,[9,11,19,27] little is known about the normal bacterial flora,[13] and no reports are available on commensal yeasts associated with cetaceans. Consequently, the following work was initiated to establish some preliminary baseline data on prevalence of yeasts in captive bottlenosed dolphins and waters in which they live.

MATERIALS AND METHODS

All sampling was conducted at the Mystic Marinelife Aquarium in Mystic, Connecticut. The water supply is local

[1] Contribution No. 134 from the University of Connecticut, Marine Research Laboratory, Noank, Connecticut 06340, USA.

tap water; commercial grade NaCl is added to maintain a salinity of 26 ppt. Chlorine, as liquid NaOCl (15% solution), is added twice a day to maintain a residual of 0.3 - 0.5 mg/L free chlorine. The water is circulated through a series of rapid-sand filters. Water temperature varies between 16 and 20 C. Total organic carbon levels are generally between 5-13 mg/L.[25] After the study had begun, a commercial ultraviolet (UV) light water treatment unit[2] was installed in the system and was located just past the filters. Approximately 760 L of water per min was exposed to continuous UV irradiation and then distributed to three pools with eventual circulation back through the filters.

Five Atlantic bottlenosed dolphins *(Tursiops truncatus)* and two beluga whales *(Delphinapteras leucas)* regularly inhabit the major water system that consists of a large (main) pool and two smaller side pools (north and south); total volume is 1.5×10^6 L. These three tanks were sampled most frequently (a total of 116 times). On seven occasions, a holding facility connected to the whale-dolphin system was sampled. This housed several California sea lions *(Zalophus californianus)* and harbor seals *(Phoca vitulina).*

Water Sampling

Samples were collected in sterile 4L wide-mouth plastic bottles that contained 3.2 ml of a 10% $Na_2S_2O_7$ solution to neutralize chlorine effects during transit,[3] a period that never exceeded one hr. For yeast counts, 500 ml volumes of water were filtered through black 0.8 μm porosity membrane filters[3] and cultured on Sabouraud dextrose agar[4] containing 150 mg/L chloramphenicol[5] (SD^+). Duplicate plates were incubated at 20 (74 samples) and 37 C (106 samples) and counted after 2-5 days incubation. For specific detection of *C. albicans,* 1 L volumes of water were filtered through white 1.2 μm porosity membrane filters[6] and incubated at 37 C for 3 days on mCA agar.[6]

Dolphin Sampling

Five bottlenosed dolphins (four females and one male) designated K, Sa, C, S, and D were included in this study. They ranged in weight from about 160 to 200 kg and from 3 to 18 years in age. Their period of residence in the aquarium was between 8 mo and 5 yr.

Anal swabs were obtained during routine examination of the animals. Several dolphins were isolated in one of the smaller pools and the water was completely drained. Sterile cotton-tipped swabs were inserted to a depth of 3 to 4 cm in the anus and rotated several times. Plates of SD^+ and mCA were swabbed immediately and the cotton tip of the swab was placed in a flask of SD^+ broth. All cultures were incubated at 37 C. Flask contents were examined periodically for the presence of yeasts using phase microscopy. Positive cultures were streaked on SD^+ and mCA media for isolation.

On several occasions, fecal samples were cultured. These were obtained immediately after an animal had defecated, while a pool was dry, or after the dolphins had playfully "beached" themselves on the edge of the full main pool. In some cases, swabs were taken from the feces and treated as above; in other instances, portions of the wet feces were diluted in sterile artificial seawater,

[2] Model AL-CSL-24, Aquafine, Burbank, California 91504, USA.

[3] AABG047S0, Millipore Corporation, Bedford, Massachusetts 01730, USA.

[4] Difco Laboratories, Detroit, Michigan 48232, USA.

[5] Sigma Chemical Company, St. Louis, Missouri 63178, USA.

[6] RAWG047S0, Millipore Corporation, Bedford, Massachusetts 10730, USA.

and various volumes filtered as for water samples. Cultures were isolated as above.

Yeast Recovery and Identification

Yeast colonies were selected randomly from membrane filter platings of water samples. Selection was based on differences in color, shape, size, consistency, and surface texture of colonies. Isolations from anal swab and fecal samples consisted either of all developing colonies or representatives of all colonial types based on the characteristics above.

All colonies were streaked to ensure purity and maintained on SD agar slants. Identification was based on accepted procedures[29] and standard descriptions.[5,16]

RESULTS

Culture of the 74 samples of water collected from the main, north, south, and sea lion-harbor seal pools showed an average yeast count of 40 per L when incubated at 20 C. Incubation at 37 C (106 samples) yielded an average count of 12 yeasts per L. Although the absolute numbers are not significant by themselves, the relative similarity in counts between the four areas indicated a general homogeneity of the system. At 20 C incubation, counts ranged from 28 - 53 per L; at 37 C the range was 6 - 14 per L.

One factor considered was the initiation of the UV treatment during the study. Approximately 20% of the water samples were taken prior to UV installation. Yeast counts per L at 20 C and 37 C averaged 33 and 10, respectively, before UV treatment was begun. These compared favorably with the total averages above (40 and 12). While there was no obvious UV effect on either group of yeasts in the tank areas sampled, studies have been made on yeast and bacteria populations in the water in the system just before and after UV irradiation and in more remote areas of the pools. These results will be reported elsewhere.

Table 1 includes data for the occurrence and numbers of *C. albicans* cells in pool waters. The overall percent of positive samples was 32%, with samples from the main pool and the sea lion-harbor seal holding area showing approximately twice the frequency of occurrence noted in the north and south pools. It may be that the fewer number of samples from these two locations accounted for these observations. However, the numbers of *C. albicans* recovered was also higher in these two areas. One possibility is that dolphin K shed large numbers of *C. albicans* cells into the main pool water when there. But equally large numbers were not found in the smaller pools (north or south). One would expect to find larger numbers per L there because of the considerably smaller volume and a greater residence period. As seen in Table 1, the reverse was true.

A more plausible explanation was prompted by sampling the feces from one of the two beluga whales which was confined exclusively to the main pool.

TABLE 1. Frequency of occurrence and average density of *Candida albicans* in various pool waters containing marine mammals.

		Pos. for *C. albicans*		Avg. no *C. albicans*/L	
Pool	No. samples	No.	% of total	All samples	Only pos. samples
Main	13	8	62	2.9	4.8
North	62	14	23	0.6	2.7
South	41	13	32	1.2	3.9
Sea lion-harbor seal	7	4	57	3.1	5.5
Total	123	39	Avg. 32	Avg. 1.2	Avg. 3.8

Only three fecal samples were obtained from this animal but all were positive for *C. albicans*. One sample was diluted, counts made, and a density of 75 *C. albicans* cells/g wet weight of feces was calculated. From data provided by Ridgway,[20] an average figure of approximately 9 g of feces were produced per kg of dolphin body weight over a 24 h period under experimental conditions. The whale in question weighs about 1,000 kg. If it can be assumed that a whale yields feces in the same proportion to body weight as a dolphin, then the whale is responsible for about 9,000 g/day of feces. If the figure above of 75 *C. albicans* cells/g is nearly correct, the whale is shedding about 675,000 cells/day into the water. If one assumes further that these cells are uniformly distributed throughout the whale-dolphin water system, a "standing crop" of *C. albicans* due to the one whale alone would be about 0.45 cells/L, or slightly over one-third of that shown in Table 1 for all samples (avg. of 1.2 cells/L). There are, of course, other factors to be considered. The filters probably remove some yeast cells. Moreover, while there is some information on the effects of UV[22] and chlorine[14] on *C. albicans in vitro*, the system studied here requires additional evaluation in terms of reduction efficiency. We have examined anal swabs from only two harbor seals and no yeasts were recovered. Other pinnipeds as carriers (see below) may be responsible for introducing *C. albicans* into the water. Human attendants enter the water daily for routine pool scrubbing but probably were not a contributing factor in introducing *C. albicans*.

Table 2 shows the occurrence of yeast species isolated from all the various pool waters sampled and from the several dolphins examined. Three yeasts *(C. tropicalis, C. parapsilosis,* and *Torulopsis glabrata)* accounted for 73% and 88%, respectively, of the isolates from water and animals. All other species were much less frequent and all have been previously reported from seawater, soil, and/or warm-blooded animals. *T. haemulonii* was of interest

TABLE 2. Frequency of occurrence of yeast species isolated at 37 C from dolphins and pool water.

	Pool water		Dolphins	
	No. of isolates	% of Total	No. of isolates	% of Total
Candida tropicalis	46	55	20	63
C. parapsilosis	8	10	4	13
Torulopsis glabrata	7	8	4	13
C. albicans	—[1]		1	3
C. pelliculosa var. *cylindrica*	1	1	1	3
C. humicola	1	1	1	3
T. bovina	0	0	1	3
T. haemulonii	10	12	0	0
T. candida	3	4	0	0
T. inconspicua	4	5	0	0
C. guilliermondii var. *guilliermondii*	2	2	0	0
C. lusitaniae	1	1	0	0
T. maris	1	1	0	0
Total	84		32	

[1]See Table 1 for pool water data

because it was encountered with a greater frequency than several other species and also since original descriptions[16] were made on isolates from seawater and fish. The main diet of the marine mammals studied was thawed fish which may have been the source of *T. haemulonii.*

No pink yeasts were observed on any 37 C platings; however, they were seen occasionally on plates from tank water and incubated at 20 C. A total of 20 isolations were made with four species identified *(Rhodotorula rubra, R. glutinis* var. *glutinis, R. graminis,* and *R. minuta* var. *minuta).* The first two accounted for 18 of the 20 isolates.

Table 3 lists yeast species recovered from anal swabs and/or fecal samples from individual dolphins. Since the number of samplings of each animal and the total number of yeast isolations were relatively small, it is inappropriate to overgeneralize. Nonetheless, some comments can be made. *C. albicans* was not found routinely in all animals; it was isolated only once from one dolphin (K). Two animals (K and Sa) had been receiving antifungal medication (Nystatin, [7] approximately 6,000,000 units per day oral and intramuscular) before and during the sampling period. While the effectiveness of this treatment has been questioned in cetaceans with established candidiasis,[18] it may account for the rare occurrence of *C. albicans* in uninfected animals. This assumes the yeast is a normal commensal inhabitant of the intestinal tract of marine mammals as it is in terrestrial mammals. From the data above, it appears that *C. albicans* can occur in large numbers in the feces of unmedicated beluga whales. Table 3 indicates that other human-associated yeasts, particularly *C. parapsilosis, C. tropicalis,* and *T. glabrata,* occur in dolphin feces and are found in pool waters containing these animals (Table 2). Dolphins K and S were of particular interest because only three yeasts *(C. albicans, C. parapsilosis,* and *C. tropicalis)* were recovered, but all are significant in occurrence in human infections.[1]

DISCUSSION

There is little information in the extant literature on microorganisms associated with marine mammals other than known pathogens. Johnston and Fung[13] concluded that several species of *Streptococcus* recovered from captive porpoises (no genus given) probably were

TABLE 3. Occurrence of yeast species in individual dolphins.

Yeast	K (4[1]) Freq. of isolation	Sa (4) Freq. of isolation	C (4) Freq. of isolation	S (4) Freq. of isolation	D (2) Freq. of isolation
Candida albicans	1/1	0/0	0/0	0/0	0/0
C. humicola	0/0	1/1	0/0	0/0	0/0
C. parapsilosis	2/1	0/0	0/0	2/2	0/0
C. pelliculosa var. *cylindrica*	0/0	0/0	0/0	0/0	1/1
C. tropicalis	5/2	2/2	0/0	12/3	1/1
Torulopsis bovina	0/0	1/1	0/0	0/0	0/0
T. glabrata	0/0	4/3	0/0	0/0	0/0

[1]Number of times sampled
[2]Number of isolations/number of times isolated

[7] E. R. Squibb & Sons, New York, New York 10022, USA.

introduced by human attendants. They also indicated that, while many bacteria appeared to be common to man and porpoises and can cause infections in both, no inordinate hazard to the health of man occurs from association with these animals. However, upon capture, several species of Enterobacteriaceae were cultured as well as six species of *Pseudomonas* and two species of *Staphylococcus*. Schroeder *et al.*[23] concluded that leptospirosis and salmonellosis of marine animals may be transmissible to man although data are not available.

Our concern herein was more in the complementary direction; i.e., is there a threat to the health of captive dolphins posed by pathogenic yeasts in their environment? The data above indicate the presence of several species of pathogenic yeasts that are common to man and marine mammals but it is not yet known if captive animals are threatened by these organisms. No background material exists on the occurrence of these organisms in wild or captive dolphins in the nondiseased state. The purpose here was to examine the "normal" yeast flora of several captive dolphins with special regard to the human-associated yeast species. *C. albicans*, the primary cause of candidiasis in marine mammals, was found infrequently in the feces of one of two antibiotic-treated dolphins and not at all in unmedicated animals, although it could be routinely recovered in small numbers from pool waters. The source may have been an apparently healthy beluga whale which, as a carrier, could have represented a significant reservoir of *C. albicans* in the captive environment. The possibility that an animal in a captive colony may serve as a carrier has not been reported previously.

The survival of *C. albicans* under conditions of seawater[7] and chlorine[14] contact has been documented. It is possible that the yeast does pose a potential health hazard in aquariums to animals that may be physiologically stressed. Chlorine or other antimicrobial compounds added to water, in addition to antibiotic chemotherapy, could affect the normal commensal microflora to the extent that chemical-resistant pathogens have a greater competitive advantage in establishing infections in stressed animals.

In addition to the infrequent occurrence of *C. albicans*, other human pathogens (*C. tropicalis, C. parapsilosis, T. glabrata, R. rubra,* and *R. glutinis*),[1,10,24] were commonly isolated from feces and were encountered commonly in the captive environment. The prevalence of infections in marine mammals of *Candida* and other yeasts[17] are becoming more frequent in the literature but no quantitative or epidemiological data are available. In the captive state, aquatic mammals may be subject to primary or secondary disease caused by normally nonpathogenic but still opportunistic microorganisms, including yeasts.[2] The isolation of the bacterium *Edwardsiella tarda* from three species of marine mammals has indicated that this organism is a common opportunistic invader in sick or injured animals.[8] Numbers of *C. albicans* have been shown to increase both in humans and dogs during prolonged confinement.[4,12] When the host is stressed, *C. albicans* and, undoubtedly, others may become opportunistic pathogens.

The question remains: Do water-borne yeasts pose a health hazard to captive marine mammals? The observations noted here do indicate a *potential* threat, particularly if certain animals function as carriers. This situation is unlikely to occur in open water where the distance between potentially pathogenic microorganisms may be considerable. One cannot dismiss the possibility of the exposure of natural marine mammal populations to human pathogens in coastal waters receiving treated or untreated domestic wastewater. Captive situations characterized by antimicrobial treatment (e.g., chlorine), however,

may present the appropriate conditions for *normally* nonpathogenic microorganisms, including yeasts, to become opportunistic pathogens if they are unaffected by conventional treatment methods. Conceivably, they may become established in the intestinal tract of healthy animals (the carrier state) to be subsequently shed into the environment and thus represent a possible threat to other, susceptible animals.

Ahearn[1] listed 10 opportunistic yeast pathogens in probable frequency of infection in man. With the exception of *Cryptococcus neoformans* (ranked second), which has been reported only recently in dolphins,[17] six of the first seven were noted in the present study from dolphins and/or pool waters *(C. albicans, C. tropicalis, C. parapsilosis, T. glabrata, C. guilliermondii,* and *R. rubra).* Perhaps careful examination and culture before and after necropsy will reveal a greater frequency of yeast involvement in marine mammal pathology than had been thought.

While the animal and water system studied here may be unique in some respects in the combination of physical-chemical factors producing the captive environment, the human influence is minimal because trainers and attendants do not spend extended periods of time in the water. As such, the observations presented here are probably an accurate reflection of the microbiological conditions without significant human impact.

It is suggested that all mammals newly-introduced into a captive environment be subject to microbiological examination including culture for potentially pathogenic yeasts. Furthermore, all animals and pool waters should be monitored periodically for the presence of these organisms.

Aquaria provide a unique combination of economic, scientific, recreational, conservational, and educational functions. Clearly, these values can only be maintained by proper understanding of adequate mammal husbandry techniques including disease prevention.

Acknowledgements

Gratitude is extended to Stephen Spotte, Vice President and Director, Mystic Marinelife Aquarium, for cooperation and expertise during various phases of this research and for manuscript review. I am particularly indebted to Dr. J. Lawrence Dunn, Staff Veterinarian, for obtaining anal swabs from (usually) uncooperative dolphins. Thanks are due Frank Heard, Curt Horton, and other members of the Aquarium training department for help in collecting samples. Mrs. Kathleen M. Feldman provided invaluable assistance in collection and laboratory anaylses.

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Received for publication 14 April 1979