Pathogenicity of Certain Species of Monilia*

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VIEWED in the light of recent work, it appears that the conflict of opinion as to the pathogenicity of the monilia group of fungi depends upon the reported multiplicity of species, and the failure to recognize a variability in the virulence of different strains of the same species.

We have shown that many of the cultures classified as different species are identical organisms,[1] and that cultures isolated by us [2] from sputum of bronchitic patients, cases of thrush, and of vaginitis, all belong to 1 of 3 species, typified by either *M. parapsilosis*, *M. albicans*, or *M. candida*. The relative frequency with which organisms belonging to these species are found in association with inflammatory lesions of the human body and the result of pathogenicity studies also seems important in relation to the results reported in this paper. In our study of 150 cultures isolated from sputum we encountered only 5 strains of *M. parapsilosis* and 5 strains of *M. candida;* all others were *M. albicans*.

Plass, Hesseltine and Bouts [3] in a study of monilia vulvo-vaginitis report 28 cases due to *M. pinoyi*, 1 *M. metalondinensis*, 4 *M. krusei*, and 4 unclassified. We have shown that *M. pinoyi*, *M. metalondinensis* and *M. albicans* are identical organisms.

Redaelli [4] reports his results in the production of experimental moniliasis by intravenous, subcutaneous and intra-peritoneal inoculation of rabbits. He used 5 cultures: *M. pinoyi*, *M. metalondinensis*, *M. tropicalis*, *M. krusei*, *M. macedoniensis*. He was able to kill rabbits by intravenous inoculation in 5 to 6 days with all the cultures except *M. krusei* and *M. macedoniensis*. In previous reports we have shown that these 2 organisms do not belong to the monilia group of yeast-like fungi, but that *M. krusei* is a mycoderma and *M. macedoniensis* produces asci and is therefore an endomycete. He has, then, in this group only 3 monilias: *M. pinoyi* and *M. metalondinensis*, which are identical organisms, both being typified by *M. albicans*, and *M. tropicalis*, which is typified by *M. candida*. In his paper he neither discussed the variation in dosage required to kill nor the pathology of the lesion encountered sufficiently to indicate whether his cultures of *M. albicans* and *M. candida* differed in any particular in their pathogenicity or virulence.

Dowling [5] in a paper in which he reports experimental lesion produced by the intradermal injections of *M. albicans* also records the result of his animal experimentation with 3 cultures. He was able to kill rabbits by intravenous inoculation of 1 of these cultures. This culture was *M. pinoyi* Castellani and we have shown it to have all of the cultural characteristics of

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M. albicans. He does not name the other 2 cultures used but says that neither was pathogenic for rabbits.

Nye, Zerfas, and Cornwell [6] have studied a number of yeast-like fungi isolated from the gastrointestinal tract of well people and others afflicted with various diseases. They did not give a species name to any of their organisms but classified them as para-saccharomyces A, B, C, and so forth. All of their cultures classified as para-saccharomyces A were fatal for rabbits when given intravenous in doses of 2 to 100 million cells per 1,800 gm. of body weight. All others produced no lesions, macroscopic or microscopic, except para-saccharomyces B, which produed no gross lesions but microscopic lesions when given in large doses, 100 million cells per 1,800 gm. of body weight. A study of this paper reveals that the cultures classified as para-saccharomyces A are identical with our Type II organism, *M. albicans,* and the para-saccharomyces B correspond to our Type III, *M. candida,* while the para-saccharomyces C probably is the same as our Type I, *M. parapsilosis.*

In work just completed we have shown that all cultures of monilia which we have recognized as *M. albicans* will produce either death or extensive lesions when administered intravenously in doses varying from 0.5 to 1.5 million cells per 100 gm. of body weight; that cultures of *M. candida* have much less virulence, requiring doses varying from 5 to 35 million per 100 gm. of body weight to produce lesions or death and that *M. parapsilosis* has no pathogenicity; *i.e.,* even tremendous doses caused neither clinical manifestation in the animal nor macroscopic or microscopic lesion in any organ. The variability of doses required to produce death and to establish clinical and postmortem evidences for the various cultures is shown in Table I.

The animals receiving the larger doses died within 24 to 48 hours while those receiving the smaller doses died on the 6th to 8th day with symptoms of uremia. The doses described as morbidity doses did not prove fatal but caused an acute illness for several days, followed by recovery.

Just as the size of the dose required to cause death or produce acute and chronic illness had marked out 3 species of monilia, parapsilosis, albicans and candida, so the clinical course following the injections also sharply differentiated the 3 species. We were not

TABLE I

DOSE DIFFERENCES OF MONILIA SPECIES

(Dose in Millions per 100 Grams Body Weight)

Species	*Lethal Dose*	*Morbidity Dose*
	Millions	Millions
Monilia parapsilosis, Type I.....	None	200 *
Monilia albicans, Type II.....	1.5	0.5
Monilia candida, Type III.....	35.0	6.0

Dose Variability of Type II

Strain	*Lethal Dose*	*Morbidity Dose*
	millions	millions
4135	1.5	0.5
2112	1.5	0.5
22353	1.5	0.5
2117	3.0	1.0
33691	6.0	2.5

Dose Constancy of Type III

Strain	*Lethal Dose*	*Morbidity Dose*
	millions	millions
2113	30.0	5.5
1369	25.0	5.0
750	30.0	5.5
23669	35.0	6.0
14999	35.0	6.0

* No lesions

CHART I

TYPE II RABBIT—BRIEF PROTOCOL OF RABBIT NO. 19 INOCULATED INTRAVENOUSLY WITH 2 C.C. OF CULTURE NO. 41351 * (54,000,000 FUNGUS CELLS)

Clinical Course Before Inoculation

Date	Activity	Weight Grams	Temperature	W.B.C.	N.P.N.
6- 8-32	Well nourished and active	1,820	102.0	10,300	42.8
	Clinical Course After Inoculation				
6-10-32	Ill, hunched, does not eat		105.8	2,000	47.0
6-11-32	Ate carrots, appeared better		103.8	10,650	
6-13-32	Ate very little		103.8	36,050	
6-14-32	Ill, did not eat		105.6	17,300	133.0
6-15-32	Ill, hunched, slow respiration		104.8		
6-16-32	Died at noon	1,580	104.6	22,000	260.0

* *M. albicans*

able to cause death or even any symptoms in animals inoculated with *M. parapsilosis,* even though the doses were extremely large, 200 million per 100 grams of body weight. The charts, I and II, show the contrast in clinical courses run by animals inoculated with *M. albicans* and *M. candida* respectively.

With such a wide difference in the number of cells required to establish a moniliasis, and such a distinct difference in the acuteness of the manifestation, it is to be expected that the gross and microscopic lesions found at autopsy would reveal differences in the intensity and distribution of lesions.

Animals dying within 24 to 48 hours following intravenous injections of *M. albicans* revealed at post-mortem examination a distribution and intensity of lesions which clearly differentiated the reaction of rabbits to this species. The *Monilia albicans* rabbits showed multiple lesions throughout the body involving the kidney (Figure I,d) and

CHART II

TYPE III RABBIT—BRIEF PROTOCOL OF RABBIT NO. 23669 II INOCULATED INTRAVENOUSLY WITH 5 C.C. OF CULTURE NO. 23669 * (300,000,000 CELLS)

Clinical Course Before Inoculation

Date	Activity	Weight Grams	Temperature	W.B.C.	N.P.N.
12-30-31	Well nourished and active		102.4	11,000	
12-31-31			104.0	13,000	39.5
1- 1-32			102.4	9,300	
1- 2-32			102.4	9,500	
1- 4-32		2,275	102.4	11,000	
	Clinical Course After Inoculation				
1- 5-32	Hunched, did not eat		105.6	12,600	
1- 6-32	Did not eat; appears ill	2,270	104.6	12,600	
1- 7-32	Ate; more active	2,300	104.6	12,200	44.5
1- 9-32		2,225	104.0	15,000	
1-10-32	Appeared well		104.0	17,100	
1-11-32		2,325	103.4	14,500	
1-12-32	Well and active		103.0	13,600	
1-13-32	Killed with chloroform	2,440	103.0	12,600	41.0

* *M. candida*

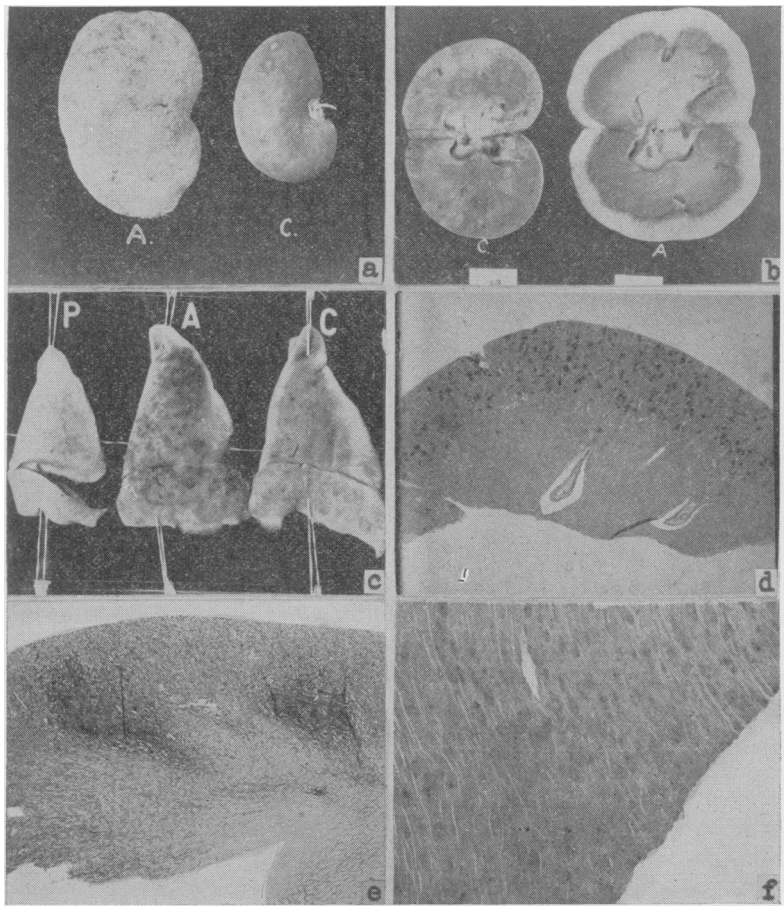

FIGURE I

a. A: *M. albicans* infected kidney; weight of rabbit, 1,585 gm.; inoculated intravenously with 62 million cells; killed on 5th day.
C: *M. candida* infected kidney; weight of rabbit, 1,665 gm.; inoculated intravenously with 229 million cells; killed on 9th day.

b. A: *M. albicans* infected kidney; weight of rabbit 1,820 gm.; inoculated intravenously with 54 million cells; died on 8th day.
C: *M. candida* infected kidney; weight of rabbit 1,855 gm.; inoculated with 229 million cells; killed on 13th day.

c. Differential characteristics of lungs of rabbits inoculated intravenously with three species of monilia and killed 6 hours after inoculation.
P: *M. parapsilosis,* dose 3,000 million cells, very rare petechial hemorrhage.
A: *M. albicans;* dose 60 million cells; numerous petechial hemorrhages.
C: *M. candida;* dose 275 million cells; few petechial hemorrhages.

d. Kidney of rabbit inoculated intravenously with 50 million *M. albicans* cells and killed 2 days later. Miliary abscesses in cortex.

e. Kidney of rabbit inoculated intravenously with 335 million *M. candida* cells and killed on the 19th day. Wedge shaped granulomatous areas involving medulla and lower zone of the cortex.

f. Miliary abscesses in skeletal muscle (thigh muscle). Rabbit died on 4th day after intravenous inoculation with *M. albicans.*

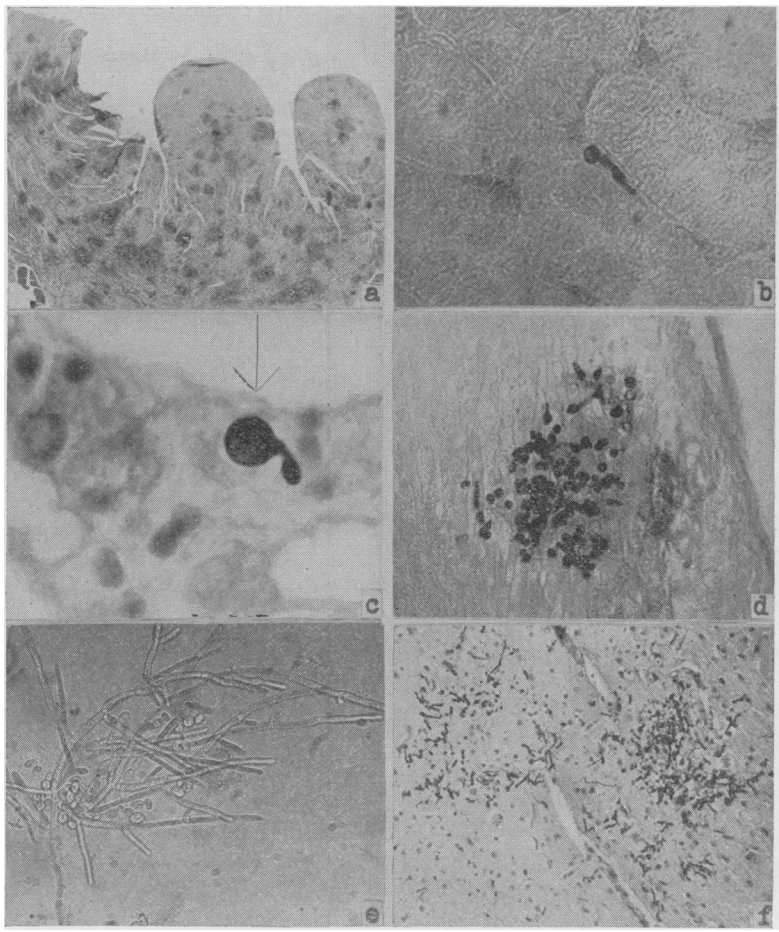

FIGURE II

a. Myocardium of rabbit inoculated intravenously with 25 million *M. albicans* cells. Killed on the 5th day.
b. Early formation of a mycelium with terminal conidia in the intertubular capillary of the kidney. Rabbit killed 4 hours after intravenous inoculation with *M. albicans*. Gram stain.
c. Budding *M. albicans* in alveolar wall of a rabbit's lung. Gram stain.
d. *M. albicans* lesion in gall-bladder wall of a rabbit inoculated intravenously. Budding cells and elongated forms. Gram stain.
e. Bile aspirated from the gall-bladder of a rabbit inoculated with *M. albicans* into the left kidney. Luxuriant growth of mycelia.
f. Budding cells and mycelia in brain lesion. Rabbit inoculated with *M. albicans* locally into left kidney. Died on 4th day. Gram stain.

skeletal muscle (Figure Ib), brain (Figure II,f) and spleen and lungs. The striking thing is that in spite of this widespread manifestation of infectivity of this organism the liver rarely showed lesions. This organ appears to be highly resistant to infection although the gall bladder often was the site of abscesses (Figure II,d) and the bile nearly always contained abundant yeast-like cells and much long mycelium (Figure II,e).

The intensity of the reaction was revealed more clearly in the kidneys and lungs. The kidneys were 2 to 2½ times normal size, the capsule was tense and the surface appeared greyish white and granular, due to numerous pinhead to pinpoint size abscesses (Figure I,a). The cut surface showed the cortex to be markedly thickened and whitish in appearance due to numerous small abscesses. The medulla was deeply congested and contained only a few small abscesses (Figure I,b).

The lungs were characterized by numerous sub-pleural petechial hemorrhages (Figure I,c). The cut surface also showed some small hemorrhagic spots and thrombosis of the large vessels was occasionally seen; 8 per cent of these animals died of pulmonary thrombosis.

The lesions produced by *M. candida* were so much fewer and less intense in reaction that after a few post-mortems we could distinguish the species of organism with which the animal had been inoculated without consulting our notes.

The lungs revealed in the case of the *M. candida* animals relatively few sub-pleural hemorrhages (Figure I,c) and the kidneys showed no increase in size; the surface contained only an occasional small abscess (Figure I,a). The cut surface revealed a cortex which was not thickened and contained only an occasional small wedge-shaped greyish-white area which extended through the medulla (Figure I,e). The medulla contained a few greyish-white areas but did not appear congested. No other tissues or organs contained lesions.

From this description of the gross pathology one cannot escape the strong suggestion that the process is primarily thrombotic, but that it is purely thrombotic does not seem likely. For instance, why do the arterioles of the glomeruli of the kidney collect the fungus cells in the case of *M. albicans* and not those of *M. parapsilosis* or *M. candida?* Why does *M. albicans* produce lesions in the arterioles of the skeletal muscles and the other 2 do not? Why do the capillaries of the liver escape the plugging effect of even albicans? These questions we undertook to answer by a determination of the relative size of the cells of organisms of the 3 groups, and by a determination of the growth capacity of the 3 species within the animal body. The result of determination of the size of the cells is shown in Table II.

It is apparent that there is not sufficient variation in the size of the cells to explain the difference in the species virulence. While it is true that *M. parapsilosis* has cells which average slightly smaller than either albicans or candida, still candida has an average cell size larger than albicans. It is apparent, therefore, that the difference in the size of the cells does not offer

TABLE II

Type	*Culture Number*	*Largest* micra	*Smallest* micra	*Average* micra	*Total Average* micra
I	50858	7.1	2.1	4.5	4.63
	35221	7.1	2.1	4.73	
	38746	6.0	3.2	4.65	
II	4135	12.0	2.8	6.1	6.03
	801	9.7	2.4	6.0	
	33691	9.5	3.1	6.0	
III	750	9.9	3.4	6.4	6.37
	14999	8.7	4.2	6.43	
	23669	11.0	3.0	6.29	

an explanation for the difference in infectivity of the organism and that the process cannot be entirely thrombotic.

To investigate still further the significance of the size of the cells in the pathology of the experimental disease we next made up suspensions of the different species in normal salt solution and killed them with formaldehyde. When they were proven to be dead by culture the cells were again measured and intravenous injections done. This experiment resulted in no illness in any animal and post-mortem examinations revealed no gross lesions. The microscopic examination of these tissues revealed no reactions of even a mild type.

At this point we felt certain that simple thrombosis as an explanation of our post-mortem findings had been ruled out but so far we had no positive explanations to account for the pathology. We now undertook to determine the growth capacity of 3 species in the animal body. We expected that we might be able to accomplish this demonstration by making blood cultures and urine and bile cultures of animals killed at intervals varying from 2 hours following the injection up to 6 days. We expected that if one species showed a greater capacity for reproduction in the blood stream than another, the organism showing the least capacity would disappear from the blood stream first, that urine and bile cultures would show fewer positive results and, further, that Gram stain of the microscopic sections of the tissues would reveal in the case of the most vigorous reproducers more abundant mycelium. Table III illustrates the results.

It is apparent from this table that *M. parapsilosis* died very shortly after introduction into the blood stream; that *M. albicans* remained in the blood stream as viable organisms for a comparatively long time and showed evidence of being able to invade tissue by its appearance in both the urine and bile. The milder property of *M. candida* in these particulars is indicated by the much lessened frequency with which it appeared in the urine and bile and its inability to maintain itself for as long a period in the blood stream. This difference was still further substantiated by the examination of tissue removed at varying intervals following injection. *M. parapsilosis* showed, shortly after inoculation, signs of degeneration by the appearance of Gram-positive bodies in the tissue which were beginning to break up and it was never possible in these tissues to demonstrate either budding forms or mycelium. *M. albicans,* on the other hand, demonstrated vigorous powers of reproduction in the capillaries by the appearance in sections of tissue removed at 2 and 4 hours of many budding forms of Gram-positive fungi and frequent strands of mycelium, and as the disease progressed mycelium became more abundant. (Figure II, b and c.) *M. candida* showed Gram-positive yeast-like cells some of which were budding but it never produced mycelium.

Our next experiments were designed to test the evidence obtained that *M. albicans* alone of these organisms was able to invade tissue. Animals were injected subcutaneously, intra-muscularly, intra-pleurally, and into the nasal

TABLE III

	M. parapsilosis	*M. albicans*	*M. candida*
Septicemia	None	Always	Rarely
Blood cultures	Positive up to 18 hours	Positive 2 to 4 days	Positive up to 36 hours
Urine cultures	Negative throughout	Positive 95%	Positive 35%
Bile cultures	Negative	Positive 50%	Positive 2%

TABLE IV

Number	*Source*	*Clinical Description of Case and Remarks*
		Type I M. parapsilosis
35221	Sputum	Mild but persistent bronchitis. Sputum repeatedly negative for tubercle bacilli. Wassermann negative. No further report.
38746	Sputum	Male, age 40, farmer; lost 15 lb. weight; sick 3 years; 2 oz. of sputum in 24 hours. Wassermann negative; sputum negative for tubercle bacilli. Died. No autopsy.
M. parapsilosis		Furnished by J. H. Lamb, Johns Hopkins University, Department of Pathology.
50858	Sputum	Male, age 36, a laborer. Four months before pulmonary symptoms had ringworm on cheek; sick 2 years; X-ray diagnosis: interlobar empyema or abscess. Reported year later blastomycosis from another laboratory. Sputum negative; Wassermann negative. Recovered.
36255	Sputum	Chronic bronchitis. No response to inquiry about patient.
		Type II M albicans
33691	Sputum	Male, age 45, sick 2 months; sputum negative for tubercle bacilli; severe cough; large ulcer on soft palate, upper and lower lip; fungus in sputum, and fluid from ulcers on lips; dullness over lower right chest posteriorly and anteriorly. Died. No autopsy.
22353	Sputum	Male, age 35, mild bronchitis with elevation of temperature for 2 months. Recovered.
4135	American Type Culture	M. pseudo-tropicalis Castellani.
2112	American Type Culture	M. albicans; Natl. Coll. Type Cul., Lister Institute, 714. Isolated by Craik from case of thrush.
2117	American Type Culture	M. psilosis Ashford. Natl. Coll. Type Culture Lister Inst. From J. T. Duncan, London School for Trop. Medicine. Isolated from feces of acute case of sprue.
		Type III M. candida
23669	Sputum	No record of patient.
14999	Buccal mucosa	Membrane covered the mucosa of the cheek, gums and lips to the edge of the skin. Thrush. Patient anemic; membrane present 1 year.
2113	American Type Culture	M. candida Bonorden. Natl. Coll. Type cultures; Lister Institute, 922, Tanner Collection.
1369	American Type Culture	C. Neuberg, Berlin, Germany (Bonorden Handb., p. 76, Fig. 86, 1857) Thom and Church collection, 4472–2.
750	American Type Culture	M. tropicalis Castellani; Aldo Castellani, Tulane Univ., isolated 1909.

accessory sinuses with standard doses, already described, of *M. albicans* and *M. candida*. *M. parapsilosis* was not used in these experiments since it had been shown that even in large intravenous doses it had no pathogenicity.

The subcutaneous inoculations of *M. albicans* cultures resulted in abscesses which healed in 10 to 12 weeks. The injections into muscle, pleura, and nasal sinuses resulted in septicemia and multiple lesions throughout the body, particularly the kidneys.

M. candida cultures produced by these methods of injection neither local nor general reactions.

The cultures used in these experiments are described in Table IV.

We selected these cultures because of their association with certain clinical manifestations in human cases and because some of them are well known in the literature. The classification as *M. parapsilosis, M. albicans* and *M. candida* is our own and was arrived at by methods previously described. As already indicated under the discussion of dosage arrived at by cell counts in a hemocytometer, individual cultures of *M. albicans* vary somewhat in virulence but never so much as to be at all confusing with the other species of lower virulence, *M. candida* or with *M. parapsilosis,* which showed no pathogenicity for rabbits. We made no attempt to step up virulence by animal passage before using the culture in an experiment. It is apparent that some of these cultures have been cultivated on artificial media for many years; *i.e.,* culture No. 4135, American Type Culture Collection, *M. pseudo-tropicalis* Castellani, was one of the most virulent cultures we used; killing in smaller doses and causing more widespread lesions when inoculated locally into various tissues. Our own cultures had been on artificial media for 5 or 6 years without animal passage.

SUMMARY

In our experience, and from what we can get from the literature, *M. albicans* appears to be the organism most frequently associated with human cases of moniliasis. Many organisms described as different species of monilia have in our hands proved to be cultures of *M. albicans*. The animal experiments reported in this paper demonstrate that organisms belonging to the species of which *M. albicans* is typical are the only ones which can multiply vigorously in the animal body and invade tissue. Although the organisms of which *M. candida* is typical *have* shown some feeble power of reproduction in the animal body they have demonstrated a very low degree of virulence, and this no doubt accounts for the frequency of *M. albicans* in the case reports found in the literature. Organisms of the *M. parapsilosis* species have shown no pathogenicity for rabbits.

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