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Microsporum equinum in North America

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Microsporum equinum was isolated in Ontario, Canada, from five human and two equine cases of ringworm infection. This dermatophyte was previously recovered from North American horses on several occasions, but was considered to be *M. canis*. We regard *M. equinum* as distinct from *M. canis*. It can be differentiated from *M. canis* by the smaller size of its macroconidia, its failure to perforate hair in vitro, its poor growth and sporulation on bromocresol purple casein dextrose agar, and its incompatibility with *Nannizzia otae*, the telemorph of *M. canis*.

Microsporum equinum (Delacroix and Bodin, 1898) Gueguen, 1904, is one of the causal agents of ringworm in horses and humans that was reported frequently in the early literature. It had been the cause of severe epizootics in large stables (7, 8) as well as an important agent of equine ringworm in army horses in Belgium (22) and France (8). Equine and human infections due to *M. equinum* have been reported from other countries, such as Algeria (2), Australia (12, 26), Czechoslovakia (24), Finland (1), Great Britain (23, 27), Germany (7), Norway (20), New Zealand (9), Rumania (5), Russia (21, 28, 30), and Zaire (13).

In spite of its wide geographic distribution, *M. equinum* has never been reported from North America. Recent studies on ringworm in race horses in Caracas, Venezuela (Gladys Tapia de Fossaert, personal communication), have confirmed its occurrence as one of the agents of equine ringworm. In the North American literature, Batte and Miller (6) cursorily mentioned *M. equinum* as one of the causal agents of equine ringworm. Even though they cited it as one of the causal agents, the authors failed to isolate it from any of the 54 horses they examined for ringworm infections. Another reason for its absence in the North American literature may be that most mycologists (3, 14, 15, 29) accepted Conant's conclusion (10, 11) that *M. equinum* was a later synonym of *M. canis*. For instance, during the animal ringworm survey conducted by the Mycology Division of the Centers for Disease Control in the 1950s, Georg et al. (15) studied 26 isolates from equine ringworm. Four of the 26 isolates, provided by P. K. C. Austwick, were isolated from equine ringworm in England and Scotland and were identified as *M. equinum*. One additional isolate that Georg

TABLE 1. Isolation data on *M. equinum* and *N. otae*

Organism and accession no.	Source
M. equinum	
B-3342	Skin scrapings, human; received as OMH[a] 1468
B-3343	Skin scrapings, human; received as OMH 257
B-3344	Skin lesion, horse; received as OMH 273
B-3585	Lesion on forehead, human; received as OMH 247
B-3586	Lesion on leg, human; received as OMH 258
B-3587	Lesion on leg, human; received as OMH 654
B-3588	Skin lesion, horse; received as OMH 1469
N. otae	
B-2094 (+)	Received from A. Hasegawa, Tokyo, Japan, as VUT 74037
B-2095 (−)	Received from A. Hasegawa, Tokyo, Japan, as VUT 74039
B-3580 (+)	F1 progeny monoascospore strain from B-2094 × B-2095 cross; received from I. Weitzman as Ha3
B-3581 (+)	Received from M. Hironaga, Otsu, Japan, as VUT 77054 (+)
B-3582 (−)	Received from M. Hironaga, Otsu, Japan, as VUT 77055 (−)
B-3583 (+)	Received from M. Takashio, Antwerp, Belgium, as RV 42487 (+)
B-3584 (−)	Received from M. Takashio, Antwerp, Belgium, as RV 42488 (−)

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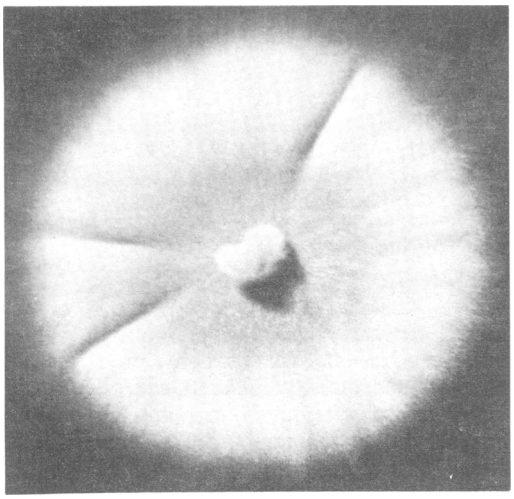

FIG. 1. Two-week-old colony of *M. equinum* on Sabouraud dextrose agar.

et al. studied was from Sabouraud's collection and also was identified originally as *M. equinum*. These five isolates were re-identified as *M. canis* by Georg et al. in accordance with Conant's conclusion. Many such isolates from ringworm in horses in the United States may have been incorrectly described in the literature as *M. canis*.

The discovery of *Nannizzia otae*, the teleomorph of *M. canis*, by Hasegawa and Usui (16) and the availability of their tester strains provided an opportunity to study compatibility relationships between *M. canis* and *M. equinum*. Mating studies between the tester strains of *N. otae* and *M. equinum* by Padhye et al. (25) showed that the isolates were sexually incompatible with each other. Among other tests that were done, the in vitro hair perforation test provided evidence of differences between the two species. All of the isolates of *M. canis* consistently perforated hair in vitro, whereas none of the *M. equinum* isolates did so. On the basis of the negative mating and perforation tests, they (25) concluded that *M. equinum* was a distinct and good species.

The question of the occurrence of *M. equinum* in the North American literature still remained unanswered. The present report documents the occurrence of *M. equinum* as the causal agent of ringworm in five human and two equine cases from Ontario, Canada.

MATERIALS AND METHODS

Cultures. Seven cultures of *M. equinum* were isolated from clinical specimens by one of us (J.K.) during a period of 8 years. The clinical material consisted of skin scrapings or epilated hair or both that had been received from Ontario dermatologists and veterinarians by the Services Branch of the Ontario Ministry of Health, Toronto, Canada. In addition, seven tester strains of *N. otae* were included in the study for comparing the morphology and mating behavior of *M. equinum* with the anamorphs of *N. otae*. The isolation data on the *M. equinum* isolates and *N. otae* tester strains are summarized in Table 1.

Morphology. All cultures were grown on Sabouraud dextrose agar (1% neopeptone, 2% dextrose, 2% agar, and 0.05% chloramphenicol) and bromocresol purple (BCP) casein dextrose agar (19) for colony and micromorphology studies. The microscopic morphology of each isolate was also studied on slide cultures, using niger seed (*Guizotia abyssinica*) agar and medium 8 (17).

In vitro hair perforation (4) and urease broth tests (18) were performed on each isolate of *M. equinum* and *M. canis*.

Mating studies. Inocula from vigorously growing 10-day-old colonies of *M. equinum* and the tester strains of *N. otae* were used in the mating studies. All crosses were done in duplicate, using 0.1% Sabouraud dex-

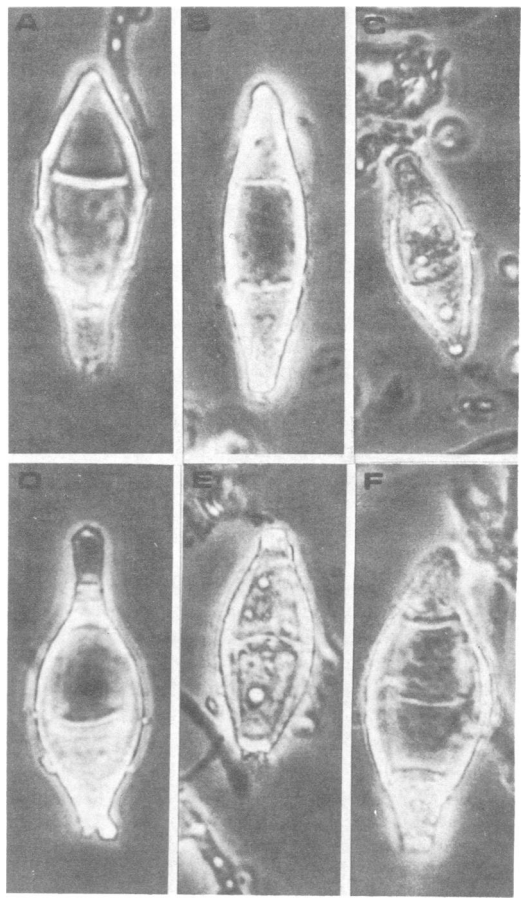

FIG. 2. Fusiform, verrucose macroconidia of *M. equinum* (×850).

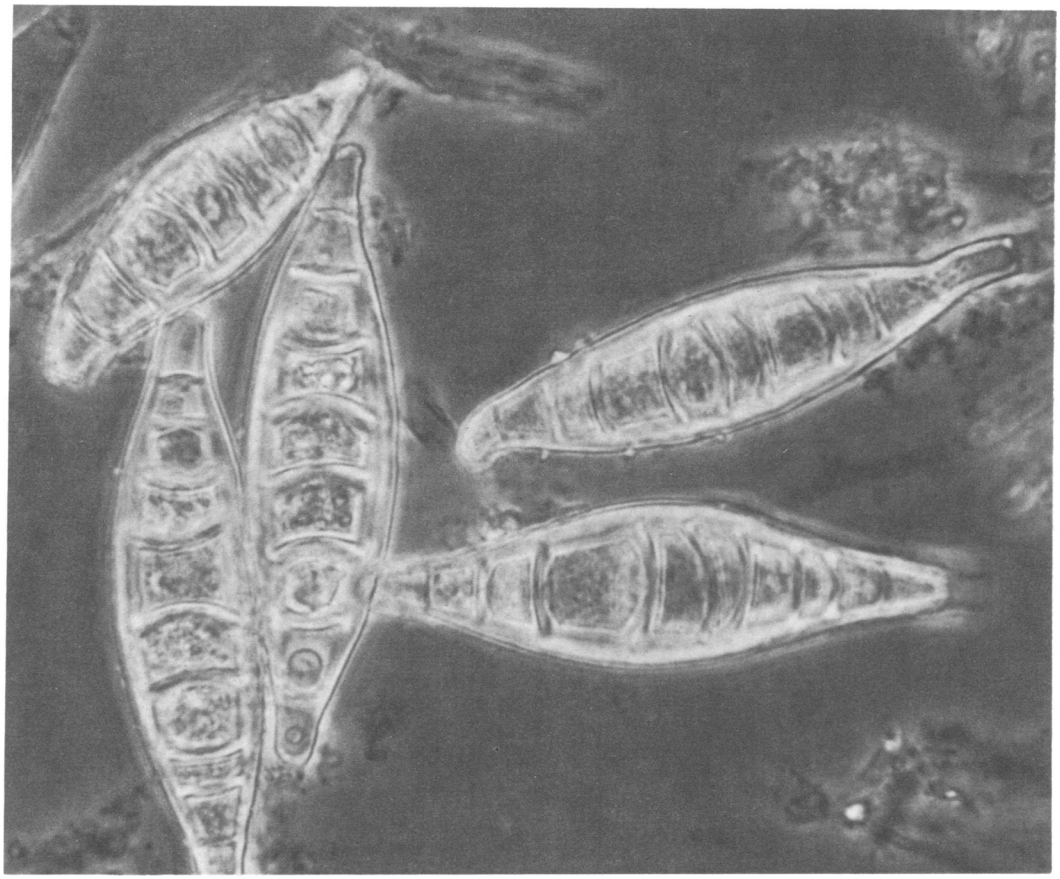

FIG. 3. Spindle-shaped, verrucose, thick-walled macroconidia of *M. canis* (×850).

trose agar with salts (31) and niger seed agar-medium 8 (17). They were incubated at 25°C in the dark for 4 to 6 weeks and were examined weekly for the presence of gymnothecia or pseudogymnothecia.

RESULTS

Morphology on Sabouraud dextrose agar. Colonies of the seven isolates of *M. equinum* measured 60 to 70 mm in diameter after 2 weeks at 25°C. Their surfaces ranged from downy or velvety to finely powdery, especially in the central areas, and they were white or pale buff to pale salmon. Four of the 7 cultures developed radial grooves (Fig. 1). The reverse of the colonies varied from buff through amber to salmon.

Macroconidia were not readily produced by any of the isolates on Sabouraud dextrose agar. However, slide cultures with niger seed agar-medium 8 supported production of both micro- and macroconidia. The microconidia were pyriform to clavate, 3 to 8 by 2 to 3 μm, one celled, smooth, and sessile or on short conidiophores, and they were borne laterally on simple hyphae. Macroconidia were generally numerous. They were elliptical to broadly fusiform, 18 to 60 by 5 to 13 μm, with verrucose, thick walls (up to 3 μm thick at the center) and predominantly two to four celled (Fig. 2A to F). They were borne terminally or on short lateral conidiophores, generally singly and rarely on more complexly branched conidiophores, to form clusters of macroconidia.

Colonies of *N. otae* tester strains measured 65 to 75 mm in diameter after 2 weeks at 25°C. At first, the colonies were mostly submerged, with a thin surface, strongly radiating, with a buff, powdery area developing in the center. The microconidia were clavate to pyriform, smooth, one celled, 3.5 to 8.0 by 1.5 to 3.5 μm, and sessile or on short conidiophores. They were borne along the sides of simple hyphae. The macroconidia were fusiform, variable in size, 35 to 110 by 12 to 25 μm, and up to 14 celled. Their verrucose walls were thick (up to 4 μm at the center) (Fig. 3). They were borne terminally on short conidiophores at an acute angle to the parent hyphae.

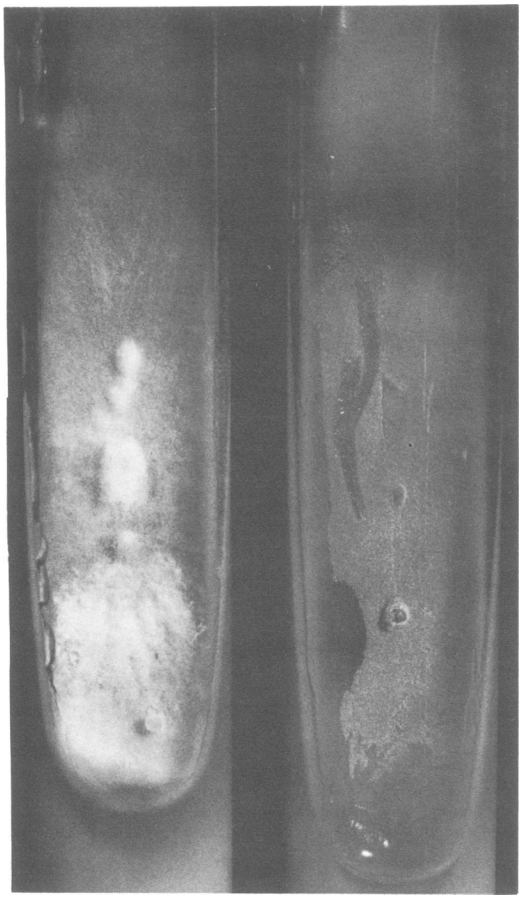

FIG. 4. Comparison of profuse growth of *M. canis* on BCP casein dextrose agar (left slant) with reduced growth of *M. equinum* (right slant) after 2 weeks.

Morphology in BCP casein dextrose agar. Colonies of *M. equinum* isolates grew poorly on BCP casein dextrose agar. The aerial mycelium was scanty and short. The growth induced a change in the pH of the medium, making it alkaline and resulting in a color change in the medium from grey to blue. Microscopically, *M. equinum* isolates did not sporulate on BCP casein dextrose agar.

Colonies of *N. otae* tester strains, on the other hand, showed good aerial growth on BCP casein dextrose agar. The mycelium was white to yellowish green and velvety to fine powdery. Microscopically, numerous macroconidia and a moderate number of microconidia were produced. The tester strains of *N. otae* did not change the pH of the medium or cause a change in color (Fig. 4).

Urease test. All of the *M. canis* strains hydrolyzed urea broth by 8 to 12 days. The seven isolates of *M. equinum* accomplished this within 8 to 14 days.

In vitro hair perforation test. All of the *M. canis* strains perforated hair in vitro, but none of the *M. equinum* isolates had perforated hair by the end of a 4-week incubation period.

Mating studies. None of the 49 crosses between seven *M. equinum* isolates and the seven tester strains of *N. otae* produced fertile gymnothecia or pseudogymnothecia or showed growth stimulation at the end of 6 weeks. These results confirmed our previous findings (25) that *M. equinum* was a species distinct from *M. canis*.

DISCUSSION

The isolation of *M. equinum* from ringworm infections in humans and equines in Ontario, Canada, demonstrated that *M. equinum* occurs in North America. Georg et al. (15) recognized that *Trichophyton equinum* was the most common cause of equine ringworm in the United States, Canada, South America, and Europe. They recognized the following dermatophytes, in order of frequency, as being associated with equine ringworm: *M. canis*, *T. mentagrophytes* var. *mentagrophytes*, *T. verrucosum*, and *M. gypseum*. *M. equinum*, rather than *M. canis*, may prove to be the second most common incitant of equine ringworm in North America.

M. equinum can be easily differentiated from *M. canis* in a diagnostic laboratory by its morphological and physiological characteristics. The smaller size of the macroconidia produced by *M. equinum*, its failure to perforate hair in vitro, and its poor growth and lack of sporulation on BCP casein dextrose agar provide accurate tests to identify *M. equinum*.

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