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# The prevalence of immediate and delayed type hypersensitivity reactions to *Microsporum canis* antigens in cats

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Spontaneous recovery from *Microsporum canis* infections in cats is thought to be dependent on the development of a competent immune response. The purpose of this study was to determine the prevalence of positive delayed type hypersensitivity reactions in cats with and without dermatophytosis. Four groups of cats were intradermally skin tested with *M canis* extract and test sites were evaluated both subjectively and objectively at 0, 24 and 48 h after injection. Delayed intradermal testing (IDT) reactions were absent in cats not exposed to dermatophytosis ($n$=20); infected–recovered cats ($n$=38 culture negative lesion negative and $n$=43 lesion negative but culture positive) had significantly larger IDT reactions than unexposed cats and cats that were still actively infected ($n$=18). Based on the results of this study, IDT with *M canis* extract can be used to assess the cellular immune response of cats with dermatophytosis.

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## Introduction

In cats, dermatophytosis is a contagious superficial fungal skin disease most often caused by *Microsporum canis*. As in many species, recovery from infection is believed to be dependent on the development of a competent immune response; in particular, a cell-mediated immune (CMI) response, although this has not been conclusively proven (DeBoer and Moriello 1993, DeBoer et al 1991, Sparkes et al 1995). The lack of development of an adequate CMI response to a dermatophyte has been suggested as a possible cause of chronic dermatophytosis in some cats (Moriello and DeBoer 1995).

CMI responses in cats to *M canis* have been evaluated using lymphocyte blastogenesis testing (LBT) and intradermal testing (IDT) (DeBoer and Moriello 1993, DeBoer et al 1991, Otto et al 1993, Sparkes et al 1995). LBT still remains a research tool and, even when available, is not a practical clinical diagnostic test. In a clinical setting, evaluation of delayed type hypersensitivity (DTH) reactions via IDT is a more practical diagnostic test of CMI response. It is an easy, inexpensive way to assess CMI response. In addition, because it is an *in vivo* diagnostic test, it evaluates multiple aspects of the CMI response.

IDT with fungal antigens and evaluation of DTH reactions have been used for years as a measure of CMI response in man and other species. In recent years, IDT with fungal antigens has been used in a small number of cats to study and document a CMI response to *M canis* infections (DeBoer et al 1991). Currently, IDT with *M canis* fungal antigens is still an experimental tool; however, it may have clinical applications in the diagnosis and management of dermatophytosis. The clinical usefulness of this test will become more obvious as more information is obtained on the prevalence of positive IDT reactions in cats exposed to *M canis*.

The purpose of this study was to determine the prevalence of positive cutaneous DTH reactions to *M canis* extract antigens in several groups of cats with varying dermatophyte infection status.

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## Materials and methods

### Cats

Healthy unexposed adult cats from a research colony with no known history of dermatophytosis were used as controls. Exposed–recovered or actively infected cats (>6 months old) were recruited from catteries, multiple-cat private homes, and/or multiple cat research colonies. This protocol was reviewed and approved by the School of Veterinary Medicine, University of Wisconsin Animal Care Committee, and written permission was obtained from the owners for this procedure. None of the cats was receiving systemic anti-fungal drugs at the time of IDT, and none had a recent drug history that would preclude IDT. The skin of each cat was carefully examined for clinical signs suggestive of, or compatible with, dermatophytosis, ie, hair loss, scaling, and broken hairs. Care was taken to classify each cat as *lesional* (actively infected) or clinically normal (exposed–recovered). Each cat was examined with a Wood's lamp and, where possible, microscopy of plucked fluorescent hairs was performed. Each cat was fungal cultured using the toothbrush technique as subsequently described.

### Fungal cultures

A toothbrush fungal culture was performed on each cat as follows. A new toothbrush was removed from its package and combed over entire hair coat of the cat for at least 2 min or until the bristles were full of cat hair. The hairs were inoculated onto fungal culture plates by gently stabbing the bristles of the toothbrush onto the surface of a dual fungal culture plate (Sab-Duets, Bacti-lab, Mountain View, CA, USA). Plates were sealed with paraffin film and incubated at room temperature for 30 days. Colony growth was examined microscopically to confirm species identification.

### M canis *antigen*

As previously described, two strains of *M canis* were used to prepare a glycoprotein fungal antigen extract for IDT. This antigen had not been found to be an irritant in a previous study (DeBoer et al 1991).

### Intradermal test procedure

Intradermal testing was performed using a standard protocol. Briefly, cats were restrained in lateral recumbency and hair from the lateral thorax was clipped. Each cat was injected intradermally with 0.1 ml of 1:100,000 histamine phosphate (positive control), phosphate buffered saline (negative control), ragweed pollen extract (Greer Laboratories, Lenoir NC) (irrelevant antigen control), and *M canis* extract (1 mg/ml). Immediate hypersensitivity reactions were read at 15 min after injection. Delayed hypersensitivity reactions were read at 24 and 48 h after IDT. Caretakers and/or owners observed the cats during the 24 and 48 h post injection; they were instructed to contact us if the cat exhibited self-mutilation at the site and/or if any skin irritation developed. Cat did not wear any restraint devices to keep them from licking the sites.

For immediate reactions, injection sites were scored subjectively using a 0 to +4 scale; erythema, induration, and wheal size of the positive and negative controls were used as reference points. Subjective scoring of delayed reactions was done differently because the positive control was no longer visible. For delayed reactions, investigators first determined as to whether or not there was any clearly visible reaction at the site. If found so, the following scale was used for scoring sites: 1, small, erythematous patch, no induration; 2, small but well circumscribed erythematous patch, palpable induration; 3, large and well circumscribed erythematous patch, palpable induration; and 4, large well circumscribed erythematous patch, marked palpable induration.

Both immediate and delayed reactions also were scored objectively. Injection sites were scored objectively by measuring the vertical and horizontal wheal diameters. The mean wheal diameter was used as the final score. Injections were administered in duplicate and the mean subjective or objective score was used for data analysis.

Cats that did not respond to the positive control (histamine phosphate), for some reason, were not included in the study. Cats were tested by one of two teams of investigators (KM/MF and GK/KK) using the described criteria for subjective and objective scoring. Because of the concern for contagion and zoonosis, investigators were not blinded as to whether or not the cats were *M canis* naïve or *M canis* exposed. The same team of investigators tested the control cats (KM/MF).

The investigators were prepared to use sedation to decrease stress and/or pain if necessary. However, we found this to be unnecessary, most likely due the fact that every effort was made not to stress the cats. Investigators traveled to the cats

**Table 1**. Percentages of control and exposed cats with immediate and delayed IDT reactions to *M canis* extract

| Test antigen | Control cats ($n$=20) | | | Group 2 ($n$=38) | | | Group 3 ($n$=43) | | | Group 4 ($n$=18) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 h | 24 h | 48 h | 0 h | 24 h | 48 h | 0 h | 24 h | 48 h | 0 h | 24 h | 48 h |
| | % ($n$) | % ($n$) | % ($n$) | % ($n$) | % ($n$) | % ($n$) | % ($n$) | % ($n$) | % ($n$) | % ($n$) | % ($n$) | % ($n$) |
| Saline control | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Histamine control | 100 (20) | 0 (0) | 0 (0) | 100 (38) | 0 (0) | 0 (0) | 100 (43) | 0 (0) | 0 (0) | 100 (18) | 0 (0) | 0 (0) |
| *M canis* | 0 (0) | 0 (0) | 0 (0) | 5 (2) | 87 (33) | 87 (33) | 16 (7) | 74 (32) | 79 (43) | 17 (3) | 38 (7) | 50 (9) |
| Ragweed pollen | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 5 (2) | 0 (0) | 0 (0) | 17 (3) | 0 (0) | 0 (0) |

Data represent scores of +2 or greater.

and examined, tested, and scored the cats in their 'home' environment (research room or cattery area). More often than not owners and/or caretakers familiar to the cat were available to briefly restrain the cat for the clipping, testing, and lesion measurement. The presence of the owners and/or caretakers clearly had a calming effect on the cats. We noted that the majority of cats 'kneaded and purred' during the brief diagnostic procedure and or during restraint for measurements. Furthermore, the limited number of injections ($n$=8), compared with 20 to 50 in a standard IDT, greatly shortened the testing procedure and limited stress. Cats excluded from the study because they did not have a positive histamine control were not particularly excitable or visibly distressed.

### Statistical analysis

Statistical analyses on IDT data for *M canis* extract injection sites were performed using a commercially available computer program (JMP2.20, SAS Institute, Cary, NC, USA). For the purposes of analyses, the mean sums of the IDT scores for each group were used. A General linear models procedure was used to analyze subjective and objective data for differences among and between the individual groups: mean sums of the IDT scores for each group, mean sums of IDT scores at 0, 24, and 48 h, and a comparison of IDT scores between groups at 0, 24 and 48 h. Differences were considered significant at $P$<0.05. Values reported in this text are mean±SEM.

## Results

### Cats

A total of 119 cats were included in this study and were divided into four groups based on infection status. The presence or absence of clinical lesions was used as the criterion for determining whether or not a cat was considered normal, recovered, or actively infected. Group 1 ($n$=20) consisted of *unexposed-healthy culture negative cats*. Group 2 ($n$=38) comprised previously infected cats that were *recovered*, *clinically normal*, and *culture negative*. Group 3 ($n$=43) consisted of previously infected cats that were *recovered*, *clinically normal*, but *culture positive*. On close examination, eight of the 38 cats in Group 2 had weak positive Wood's lamp examinations (<5 fluorescing hairs per cat) that were positive for ectothrix spores upon microscopy. These cats were placed in Group 3 because they were lesion-free and appeared clinically normal. Finally, Group 4 ($n$=18) consisted of cats that were considered *actively infected* (lesional, culture positive, Wood's lamp positive and ectothrix spore positive). These cats had obvious clinical lesions consistent with dermatophytosis.

### Intradermal test reactions

All of the 119 cats used in this study had positive subjective and measurable IDT reactions to histamine phosphate within 15 min after injection. None of the saline control sites evoked a positive reaction in any of the cats. Immediate positive reactions that scored 2+ or greater were considered positive. DTH reactions were more dramatic and consisted of well-demarcated, firm, indurated areas of erythema; IDT reactions scored as 2+ or greater were considered positive. None of the IDT sites at 0, 24, and 48 h appeared to be self-traumatized and, thus, were considered non-pruritic.

### Ragweed antigen

Five of the 119 cats (0.6%) had immediate positive reactions (+2 or more) to ragweed pollen antigen (Table 1). None of the cats had a delayed reaction to ragweed pollen antigen.

**Table 2**. Comparison of *M canis* IDT scores between groups at 0, 24, and 48 h

| Hours | Subjective score | SD | Objective score | SD |
|---|---|---|---|---|
| *0 h* | | | | |
| Group 1 | 0.100±0.195 | NSD | 1.000±1.114 | 1<2 |
| Group 2 | 0.342±0.141 | NSD | 5.467±0.808 | 2>4,1 |
| Group 3 | 0.532±0.133 | NSD | 3.470±0.759 | NSD |
| Group 4 | 0.6111±0.205 | NSD | 0.944±1.174 | 4<2 |
| *24 h* | | | | |
| Group 1 | 0.000±0.195 | 1<2,3,4 | 0.000±1.114 | 1<2,3,4 |
| Group 2 | 3.000±0.141 | 2>1,3,4 | 12.197±0.808 | 2>1,4 |
| Group 3 | 2.383±0.133 | 3>1,4 | 11.779±0.759 | 3>1,4 |
| Group 4 | 1.139±0.206 | 2,3>4>1 | 3.847±1.174 | 2,3>4>1 |
| *48 h* | | | | |
| Group 1 | 0.333±0.191 | 1<2,3,4 | 0.000±1.114 | 1<2,3,4 |
| Group 2 | 3.105±0.141 | 2>1,3,4 | 16.743±0.808 | 2>1,3,4 |
| Group 3 | 2.465±0.133 | 3>1,4; 3<2 | 14.052±0.759 | 3>1,4; 3<2 |
| Group 4 | 1.444±0.206 | 2,3>4>1 | 7.402±1.74 | 2,3>4>1 |

Values are shown as mean ±SEM. Column 'SD' indicates significant differences ($P<0.05$) between groups (eg, 1<2, 3, 4 IDT scores were significantly greater in Groups 2, 3, and 4 when compared with Group 1). 'NSD' indicates no significant difference.

### M canis *antigen extract*

In Group 1, none of the cats had immediate or delayed positive reactions to *M canis* (Table 1).

In Groups 2, 3, and 4, the prevalence of cats with an immediate positive IDT reactions to *M canis* extract was 5, 16, and 17%, respectively (Table 1). The means of the subjective IDT scores for cats in Groups 2, 3, and 4 were: 2.0±0.0, 2.3±0.4, and 2.7±0.7, respectively.

The prevalence of positive delayed IDT reactions (+2 or more) at 24 h was 87, 74, and 38% in Groups 2, 3, and 4, respectively. At 48 h, the prevalence of delayed IDT reactions were 87, 79, and 50% in Groups 2, 3, and 4, respectively. The means of the subjective DTH scores at 24 and 48 h were: 3.4±0.7 and 3.6±0.6 (Group 2), 3.2±0.8 and 3.1±0.8 (Group 3), and 2.4±0.4 and 2.3±0.5 (Group 4).

### *Comparison of the overall mean sums of immediate IDT scores*

Using both scoring methods, significant differences were found among the mean sums of the IDT scores of the four groups ($P=0.00010$). In cats exposed to *M canis*, IDT reactions to *M canis* antigen were significantly greater than in *M canis* naïve cats. When individual groups were compared, IDT scores in exposed–recovered *clinically normal* cats (Groups 2 and 3) were significantly greater than those of Group 4 cats (*actively infected*). There was no significant difference between the IDT scores of Groups 2 and 3.

### *Comparison of the mean sum of IDT scores at 0, 24, and 48 h*

Using both scoring methods, a significant difference among the scores at 0, 24, and 48 h was found ($P=0.0001$). When individual times were compared, the IDT scores at 24 and 48 h were significantly larger than those at 0 h. No significant difference between IDT scores was found at 24 and 48 h when subjectively scored. When objective scores were compared, IDT reaction sites at 48 h were significantly larger than at 24 h.

### *Comparison of IDT scores between the groups at 0, 24, and 48 h*

Table 2 summarizes the mean sum of all the IDT scores between groups at 0, 24, and 48 h. No significant differences were found between the mean subjective scores of the four groups at 0 h. When objective scores were compared, mean IDT scores were significantly larger in Group 2 (exposed–recovered culture negative) than in Groups 1 (control) and 4 (actively infected culture positive) at 0 h.

At 24 and 48 h, differences between the mean scores for both objective and subjective data were almost identical (Table 2). Mean subjective and objective IDT scores were significantly larger in

Groups 2, 3, and 4 (*M canis*-exposed cats) when compared with those in Group 1 (*M canis* naïve cats). At both 24 and 48 h, mean subjective and objective IDT scores for cats in Groups 2 and 3 (exposed–recovered) were significantly greater than those in Group 4 (actively infected).

At 24 and 48 h, a few differences were found between Groups 2 (exposed–recovered culture negative) and 3 (exposed–recovered culture positive). At 24 h, objective scoring found no significant differences between Group 2 and 3; however, subjective scoring depicted a difference, with Group 2 scores being significantly larger. At 48 h, both subjective and objective scoring depicted the same trend; scores in Group 2 were significantly larger than those in Group 3.

## Discussion

The goal of this study was to determine whether IDT with *M canis* extract could be used to demonstrate CMI response in *recovered–exposed* cats. In this study, we found that IDT with a glycoprotein extract of *M canis* is not irritant, as evident by the lack of positive reactions in control cats. In addition, we found that *M canis*-exposed cats had significantly larger mean DTH scores than *M canis* naïve cats. Also, DTH scores in exposed–recovered cats (Groups 2 and 3) were significantly larger than those of actively infected cats (Group 4). The larger IDT reactions in the exposed–recovered groups probably reflect the presence of CMI response, resulting in recovery from infection. From these findings, it appears that IDT with a non-irritating *M canis* extract could be a practical alternative to LBT for assessing the development of CMI response to *M canis* infection in cats.

These findings are similar, but not identical, to those of a smaller study from our laboratory involving 10 normal cats and five infected cats (DeBoer et al 1991). In our first study, as in this one, there was a low prevalence of immediate hypersensitivity reactions (2/10 or 20%), and there were no DTH reactions in the control cats. The difference was in the prevalence of immediate hypersensitivity reactions in exposed/recovered cats between the studies and the time period when DTH reactions were most likely observed. In the first study, 100% (5/5) infected cats had immediate hypersensitivity reactions, while only 12% (12/99) of our cats had immediate hypersensitivity reactions. Also, DTH reactions were more commonly seen at 24 h (4/5 cats) than at 48 h (2/5 cats). In contrast, we observed a large number of DTH reactions at both times with the highest proportion at 48 h.

The same stored lot of *M canis* extract was used in both the studies, making a reagent difference unlikely as an explanation. The cats in the first study were sedated, while those in this were not. It is possible that excitation interfered with the development of immediate type hypersensitivity reactions to *M canis* antigen even though only cats with appropriate positive and negative controls were included in study. It is possible that the reactions were too subtle to be detected. It is also equally possible that these differences reflect a large patient pool (five vs 99 cats). Excitation would have little influence on the presence or absence of DTH reactions. The increased proportion of DTH reactions in the cats in this study again most likely represented a combination of greater familiarity of IDT in cats and a larger patient pool.

Two factors, while conducting this study, might have introduced some bias and/or affected the results. First, it was impossible for all of the cats to be subjected to IDT and scored by the same investigator. Because we were attempting to accrue as large a patient population as possible, multiple investigators with access to catteries and research facilities were needed and this was simply not feasible. It is possible this had some impact on the IDT scores, particularly on the subjective scores. Second, the investigators were not blinded to the status of the cat (normal or exposed). We believe that inclusion of an objective-scoring criterion (size measurement) in the evaluation largely eliminated this bias.

With the exception of fungal culture status and the presence or absence of obvious clinical lesions, no attempt was made to correlate the data with patient signalment, duration of infection, severity of infection, etc.

Based on the findings of this study, it appears that IDT with *M canis* extract can be used to investigate the immune status of cats with dermatophytosis. There are many questions regarding the immune status of chronically infected cats, cats prone to re-infection, and the carrier cats. The availability and use of a field test, such as IDT with a fungal extract, for CMI response to *M canis* could answer some of these questions.

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