Risk factors associated with *Coccidioides* infection in dogs

Christine D. Butkiewicz, DVM; Lisa F. Shubitz, DVM; Sharon M. Dial, DVM, PhD, DACVP

Objective—To evaluate potential risk factors for *Coccidioides* infection among dogs living in a region in which the organism is endemic (Pima and Maricopa counties, Arizona).

Design—Community-based longitudinal and cross-sectional studies.

Animals—104 healthy 4- to 6-month-old puppies (longitudinal study) and 381 4- to 18-month-old dogs with unknown serostatus (cross-sectional study).

Procedure—Dogs in the longitudinal study were tested 3 times at 6-month intervals for anticoccidioidal antibodies; dogs in the cross-sectional study were tested only once. Owners of all dogs completed a questionnaire on potential environmental exposures.

Results—In the longitudinal study, the relative risk of infection for dogs that were outdoors during the day was 4.9 times the risk for dogs that were kept indoors. Seropositive dogs in the cross-sectional study were 6.2 times as likely to have access to > 1 acre to roam as were seronegative dogs. Logistic regression analysis indicated that the odds of infection increased with age (odds ratio [OR], 1.1), amount of roaming space (OR, 2.4), and walking in the desert (OR, 2.2). Walking on sidewalks had a protective effect (OR, 0.4).

Conclusions and Clinical Relevance—Results suggest that in regions in which the organism is endemic, dogs that spend more time outdoors or have more land in which to roam are at greater risk of infection with *Coccidioides* spp. (*J Am Vet Med Assoc* 2005; 226:1851–1854)

Coccidioidomycosis is an environmentally acquired fungal disease of mammals that is caused by the dimorphic fungi *Coccidioides immitis* and *Coccidioides posadasii* (sp nov). In dogs, infection most commonly results in self-limiting respiratory tract disease, but disease can be more severe in some dogs, resulting in dissemination and death.[1] These organisms are generally limited to the lower Sonoran life zone and to other regions with similar hot, semiarid climates in the southwestern United States, Mexico, and South America.[2,3]

The life cycle of *Coccidioides* spp has 2 phases. In the saprophytic, or soil phase, mycelia grow and form arthroconidia that break off and may become airborne. Hosts inhale the arthroconidia, which then enter the tissue, initiating the parasitic phase. In the lungs, the arthroconidia transform into spherules that subsequently enlarge and undergo endosporulation. The spherules eventually rupture, releasing hundreds of endospores into the tissues. Each endospore can then form a new spherule, and the cycle is repeated.[4,5] The fungus may remain localized to the respiratory system, causing primary pulmonary infection, or it may disseminate.[5]

Environmental and host factors such as geographic location, sex, race, and occupation are well-documented risk factors for infection and illness in humans.[6-8] Unfortunately, risk factors in dogs are not as clearly defined. In a postmortem study[9] of 100 dogs with coccidioidomycosis examined prior to the advent of antifungal treatment, sex and breed were found to be associated with the risk of severe coccidioidomycosis resulting in death, with males, Boxers, and Doberman Pinschers more likely to have severe infection. A more recent retrospective study[10] of medical and laboratory records for 218 dogs with coccidioidomycosis found that after correcting for breed popularity, Boxers, Pointers, Australian Shepherds, Beagles, and Scottish Terriers had an increased risk of infection. A study[11] of medical records from multiple North American veterinary schools found an increased risk of coccidioidomycosis among terriers. To date, however, potential environmental and host risk factors for coccidioidomycosis in dogs have not been prospectively evaluated. The purpose of the study reported here, therefore, was to evaluate potential environmental and host (ie, age, sex, and breed) risk factors for coccidioidomycosis among dogs living in an area in which the causative organism is endemic (ie, Pima and Maricopa counties, Arizona). We hypothesized that activities that disturb soil near where dogs are housed would increase the risk of infection with *Coccidioides* spp. Longitudinal and cross-sectional study designs were used to recruit sufficient dogs for analysis. Results of serologic testing for anticoccidioidal antibodies in dogs in the study have been reported previously.[12]

Materials and Methods

Longitudinal study—Between May 2001 and May 2002, healthy 4- to 6-month-old puppies living in Pima or Maricopa county were enrolled in the study. A blood sample (6 to 12 mL) was obtained and submitted to a commercial laboratory[a] for a CBC and serum biochemical profile. In addition, serum was submitted to the Arizona Veterinary Diagnostic Laboratory for testing for anticoccidioidal antibodies.

Seronegative dogs for which results of the screening CBC and serum biochemical profile were within reference limits were enrolled in the study. Owners of these dogs were

From the Department of Veterinary Science and Microbiology, University of Arizona, Tucson, AZ 85721.

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Address correspondence to Dr. Shubitz.

asked to complete a questionnaire regarding possible environmental risk factors of their dogs and to return their dogs twice at 6-month intervals for follow-up serologic testing; owners completed new copies of the environmental risk factor questionnaire during each follow-up visit. Owners of all dogs enrolled in the study provided informed consent.

Cross-sectional study—Between March 2002 and March 2003, primary care veterinarians in Pima and Maricopa counties were encouraged to submit single serum samples from 4- to 18-month-old dogs for serologic testing for anticoccidioidal antibodies. Dogs were enrolled in the study regardless of health status at the time of serum collection. Owners gave informed consent and completed the environmental risk factor questionnaire.

Environmental risk factor questionnaire—The environmental risk factor questionnaire was designed to obtain information on signalment of the dog and possible environmental exposures to *Coccidioides* spp. Information collected included the address at which the dog resided and its age, sex, and neuter status. Additional questions included location and age of the home, approximate size of the home's yard, and how frequently and where the dog engaged in exercise. Exposure to dust was evaluated through questions about proximity of the home to new construction, amount of time spent outdoors, yard cover, and frequency of digging observed by the owner.

Serologic testing—Agar gel immunodiffusion assays for IgG and IgM against *Coccidioides* spp were performed as described.[12,13] Plates were prepared with commercial-grade gellan gum[b]; IgG was detected with F antigen,[c] and IgM was detected with TP antigen.[d] Appropriate positive control antisera were used. All assays were performed at the Arizona Veterinary Diagnostic Laboratory. Dogs were considered to be infected if IgG or IgM anticoccidioidal antibodies were detected, regardless of titer.

Statistical analyses—Data were collected on scannable forms that were read with document scanners.[e,f] Data were manually checked for accuracy. **Odds ratios** (**OR**; cross-sectional study) and relative risks (longitudinal study) were calculated along with 95% **confidence intervals (CI)** and *P* values with standard statistical software.[g] Categorical variables with low response rates were collapsed into dichotomous variables as appropriate. To correct for the large number of tests run and reduce the likelihood of type 1 errors, values of $P < 0.005$ were considered significant. Multivariate logistic regression[g] was also performed to evaluate the relationship between seropositivity and all potential dependent variables. Variables with values of $P < 0.1$ were evaluated for correlation. Highly correlated variables were removed, and the final model was evaluated for significance and goodness of fit.

Results

A total of 124 dogs were enrolled in the longitudinal portion of the study, of which 104 (84%) returned for follow-up testing 6 and 12 months later. Results of serologic testing and questionnaire data obtained at the 12-month recheck were used for analyses. There were slightly more female (n = 56 [54%]) than male (48 [46%]) dogs, but these proportions were not significantly different. Eighty of the 104 (77%) dogs had been neutered by the 12-month recheck. Seventy-nine (76%) dogs were reported to be purebred. Because of the small number of dogs of each individual breed, dogs were grouped according to American Kennel Club groups. There were 22 (21%) sporting dogs, 19 (18%) working dogs, 11 (11%) hounds, 11 (11%) herding dogs, 9 (9%) terriers, 4 (4%) toy dogs, and 3 (3%) dogs of nonsporting breeds. The remaining 25 (24%) dogs were of mixed breeding. By the time of the 12-month recheck, 25 of the 104 (24%) dogs had seroconverted; only 3 of these dogs were clinically ill.

When owners were asked to indicate how much time their dogs spent outdoors each day, 10 said their dogs were outdoors ≥ 90% of the time, 34 said their dogs were outdoors during the day, and 60 said their dogs were indoors ≥ 80% of the day. Compared with dogs that were indoors ≥ 80% of the day, dogs that were outdoors had a significantly ($P < 0.001$) increased risk of infection with *Coccidioides* spp (relative risk, 4.9; 95% CI, 1.91 to 12.31). No other significant risk factors for infection with *Coccidioides* spp were found.

A total of 381 dogs were enrolled in the cross-sectional portion of the study. Of these, 105 lived in Pima County and 276 lived in Maricopa County. Proportion of dogs that were female (n = 209 [55%]) was not significantly different from the proportion that was male (172 [45%]). Two hundred fifty of the 381 (66%) dogs were neutered, and 270 (71%) were purebred. There were 109 (29%) sporting dogs, 65 (17%) working dogs, 52 (14%) herding dogs, 37 (10%) toy dogs, 36 (9%) hounds, 35 (9%) terriers, and 29 (8%) dogs of nonsporting breeds. Thirty-two of the 381 (8%) dogs were seropositive, of which 13 were clinically ill. The remaining 19 were otherwise healthy and were classified as subclinically infected.

When asked about the area in which their dogs could roam, 290 owners indicated that their dogs had access to < 1 acre, 59 indicated that their dogs had access to > 1 acre, and 10 indicated that their dogs ran loose (owners of 22 dogs did not respond). Because of the low number of dogs that reportedly ran loose, these dogs were combined with dogs that had access to > 1 acre for statistical analyses. Compared with dogs with access to < 1 acre, dogs with access to > 1 acre in which to roam were significantly ($P < 0.005$) more likely to be seropositive for antibodies against *Coccidioides* spp (OR, 6.2; 95% CI, 2.97 to 13.00).

For both the longitudinal and cross-sectional portions of the study, age of the home, proximity to new construction, sex, American Kennel Club group, frequency of digging, and yard cover were not significantly associated with whether dogs were seropositive. Because of the low number of dogs that were clinically infected (3 dogs in the longitudinal study and 13 in the cross-sectional study), risk factors were not separately assessed for clinically infected versus subclinically infected dogs.

Logistic regression was performed on data for dogs included in the cross-sectional portion of the study. Each category on the questionnaire as well as age in months, sex, and neuter status was evaluated for use in the model. Neuter status was removed because it appeared highly associated with age of the dog. How frequently the dog engaged in exercise appeared highly associated with the amount of time spent outdoors, so it was not considered. Dependent variables significantly associated with increased odds of seropositivity were as follows: age in months (OR, 1.1; 95% CI, 1.036

to 1.220; $P = 0.005$), amount of roaming space (OR, 2.4; 95% CI, 1.231 to 4.500; $P = 0.01$), and being walked in the desert (OR, 2.2; 95% CI, 0.979 to 4.901; $P = 0.046$). Being walked on sidewalks had a protective effect (OR, 0.4; 95% CI, 0.159 to 0.802; $P = 0.013$).

Logistic regression was also performed on data for dogs in the longitudinal portion of the study. For these dogs, age was excluded as dogs were selected to be within a 2-month age range at enrollment. Significant dependent variables were the same as those found for dogs in the cross-sectional portion of the study. Because age was a significant risk factor for seroconversion for dogs in the cross-sectional portion of the study, results of logistic regression for dogs in the longitudinal portion of the study were not presented.

Discussion

Results of the present study suggested that age and various environmental factors were associated with the risk of infection with *Coccidioides* spp among dogs living in a region in which the organism is endemic. In particular, risk of infection increased with age, and dogs that spent more time outdoors or had more land in which to roam were at greater risk of infection.

Previous studies[1,10,14] have shown that environmental factors affect the risk of coccidioidal infection in dogs and people. For example, outbreaks of coccidioidomycosis in humans living in regions in which the organism is endemic have been associated with high dust exposure.[15,16] Additionally, early skin test surveys of recruits on central California air bases showed that instituting dust control and promoting activities with low dust exposure reduced the risk of asymptomatic infection and illness.[17] Disruption of soil is also believed to be the explanation for an increased number of cases, despite the possibility of previous exposure to *Coccidioides* spp, reported in Ventura County, Calif, after the Northridge Earthquake in 1994, which created a widespread dust cloud.[18] Thus, whereas lack of previous exposure is a risk factor in naïve populations visiting regions in which the organism is endemic, large disruptions in soil in such regions can also result in substantial increases in the rates of infection and disease.

We found similar results in dogs in the present study. Dogs in the longitudinal portion of the study that were outdoors during the day were 4.9 times as likely to be seropositive as were dogs that lived predominantly indoors. Similarly, seropositive dogs in the cross-sectional portion of the study were 6.2 times as likely to have access to > 1 acre in which to roam as were seronegative dogs. Both of these results suggest that increased exposure to the environment increased the risk of infection.

Logistic regression analysis of data for dogs in the cross-sectional portion of the study also suggested that environmental exposure was a risk factor for infection. Dogs with access to > 1 acre in which to roam and dogs that walked in the desert were significantly more likely to be seropositive, whereas dogs that were walked on the sidewalk were significantly less likely to be seropositive. Possibly, dogs that were walked on the sidewalk lived in more urban environments with less dust exposure. It would have been interesting to classify these dogs as living in urban or rural environments and reexamine the data, but we were unable to obtain geographic and census data for Maricopa County.

Host factors are also important in the development and severity of coccidioidomycosis in humans. Demographic risk factors in people are well studied, with males, African-Americans and Filipinos, older individuals, pregnant individuals, and individuals with an underlying disease at higher risk.[2,5,19] Comparable information is not available in the veterinary literature to aid in risk assessment and diagnosis in companion animal species.

The questionnaire used in the present study was designed to determine whether common environmental factors associated with dust in southern Arizona communities and host factors such as sex and breed affected the infection rate in dogs. No significant associations were found between subclinical infection and demographic factors that were studied, including breed, sex, and neuter status. We were unable to analyze associations between clinical illness and demographic variables because only 16 of the 485 dogs included in the study were clinically infected.

On the other hand, age was found to be significantly associated with the risk of *Coccidioides* infection. Logistic regression analyses indicated that even after controlling for roaming space and walking location, increasing age was significantly associated with an increased risk of infection, with the odds of infection increasing by 1.1 for each month of life under observation. Although we were only able to study this population of dogs for a relatively short period, our findings were consistent with statements by veterinarians in the region that clinical illness seems to occur most commonly in young adult dogs. One study[11] of the prevalence of disease found that higher rates of disease existed among dogs between 4 and 6 years old. Thus, it would have been interesting to follow up the study dogs to evaluate infection and disease rates for a longer period.

Data for dogs in the longitudinal and cross-sectional portions of the present study were analyzed separately because of the different proportions of seroconversion (longitudinal study, 23%; cross-sectional study, 8.4%). We speculate that the difference in infection was primarily age-related because the median age of dogs in the cross-sectional portion of the study was 8 months, whereas dogs in the longitudinal portion of the study were approximately 16 to 18 months of age at the time of analysis. A second explanation for the different infection rates could be the location of the dogs. It is possible that dogs in the cross-sectional portion of the study were more likely to live in urban environments, but without geographic information from Maricopa County, we were unable to explore this.

Surprisingly, 2 of the factors that we believed would be strongly associated with the likelihood of infection—frequency of digging and proximity to new construction—were not found to be significant. It is possible that owners erroneously misclassified the amount of digging by their dogs, but when dogs that were reported to dig at all were compared with dogs

that reportedly never dug, a significant association still was not found. Nevertheless, we speculate that with a larger sample size, digging may have become a significant risk factor for exposure.

We also believe that analysis of environmental risk factors was hindered by low and focal participation in both counties. We enlisted veterinarians from the entire metropolitan areas of Tucson and Phoenix to obtain a representative sample of dogs in both counties. However, veterinarian participation was limited, with 35% of the dogs in both studies enrolled through 3 veterinary hospitals. This complicated the statistical analysis, since most owners tend to choose a veterinary hospital near their home. Roughly mapping the locations of dogs in both counties showed that enrolled dogs tended to cluster in certain areas, whereas few dogs were enrolled from other areas. Dogs living in approximately the same region may have had similar exposures in regard to particular risk factors, such as proximity to new construction, age of home, and location of walks, which would introduce bias into our results and decrease our ability to detect significant associations. Additionally, without good background data on odds of *Coccidioides* infection as a function of location, further analysis of the data was hindered.

Being able to recognize potential risk factors for *Coccidioides* infection is useful to veterinarians practicing in regions in which the organism is endemic. Our data showed increased rates of seroconversion among dogs that spent more time outdoors or had more land in which to roam, which is consistent with findings in the human literature that increased exposure to dust is associated with a greater risk of infection. Surveying dogs from a wider area in both counties would have greatly aided our analysis. Evaluation of risk factors for *Coccidioides* infection in dogs will provide additional information that could assist owners and veterinarians in reducing the risk of coccidioidomycosis.

a. Antech Diagnostics Inc, Phoenix, Ariz.
b. Gelrite gellan gum, Sigma Chemical Co, St Louis, Mo.
c. Gibson Laboratories Inc, Lexington, Ky.
d. Meridian Diagnostics Inc, Cincinnati, Ohio.
e. DSMobile USB scanner, Pentax, Golden, Colo.
f. Teleform 5.1, Cardiff Software, Sunnyvale, Calif.
g. Intercooled Stata 7.0, Stata Corp, College Station, Tex.

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