

Seroprevalence of antibodies against *Coccidioides immitis* in healthy horses

Jill C. Higgins, DVM; Gayle S. Leith, DVM, MS, DAVBP; Ed D. Voss, DVM, DACVIM; Demosthenes Pappagianis, MD, PhD

Objective—To determine the seroprevalence of antibodies against *Coccidioides immitis* in healthy horses residing in an area in which the organism is endemic.

Design—Prospective study.

Animals—197 healthy horses (in which coccidioidomycosis had not been previously diagnosed) that resided in an area of Arizona in which coccidioidomycosis is endemic.

Procedure—Of the horses evaluated at the Arizona Equine Medical and Surgical Center during a 6-month period, 197 with no clinical signs of coccidioidomycosis were randomly selected for inclusion in the study; sera were evaluated for IgM and IgG antibodies against *C immitis* via an immunodiffusion assay (IgG-positive samples were assessed quantitatively). Within 6 months, recheck titer evaluations were attempted for all seropositive horses.

Results—Serum antibodies against *C immitis* were detected in 8 of 197 horses (seroprevalence, 4.06%). Results of serologic assays were positive for IgG antibodies and negative for IgM antibodies in 7 horses and positive for both IgG and IgM antibodies in 1 horse; reciprocal serum IgG antibody titers were low (none > 8). Follow-up serologic data were obtained from 5 horses; compared with initial findings, horses had become seronegative or titers were unchanged or decreased. Duration of residence in the area was significantly shorter for seropositive horses than for seronegative horses.

Conclusions and Clinical Relevance—Serum antibodies against *C immitis* may rarely be detected in healthy horses residing in an area in which the disease is endemic; any horse with a detectable serum antibody titer should be reevaluated after an interval of at least 3 weeks. (*J Am Vet Med Assoc* 2005;226:1888–1892)

Coccidioides immitis is a saprophytic soil fungus that grows in sandy, alkaline soils in semiarid climates, including portions of California, Arizona, New Mexico, Texas, Nevada, and Utah.¹ In the environment, *C immitis* lives as a mycelium with thick-walled, barrel-shaped arthroconidia. Infection usually occurs via inhalation of these arthroconidia when they become airborne. Once inhaled, each arthroconidium may

enlarge to form a spherule, which can then trigger inflammation in the lungs and result in the development of lesions in local lymph nodes.¹ Because of this route of exposure, the disease most commonly involves the respiratory tract; however, lymphohematogenous dissemination can occur, resulting in infections in various organs such as bone, skin, and abdominal viscera.¹ Rarely, inoculation may occur percutaneously and result in localized subcutaneous infections.²

Coccidioidomycosis has been reported¹⁻⁵ in several mammalian species. Susceptibility for primary pulmonary disease to progress to the disseminated form (because of failure of the immune system to respond appropriately) has been associated with certain equids⁶ and races of humans.³ The disease has been studied fairly extensively in humans and dogs; however, to the authors' knowledge, information regarding the disease in horses has been limited to case reports. These case reports have succeeded in defining the multiple clinical manifestations of coccidioidomycosis in horses, including interstitial pneumonia,⁷ osteomyelitis,⁸ mastitis,⁹ abortion,¹⁰ and the development of superficial and internal abscesses.¹ In most of these clinically affected horses, the disease has been severe and often fatal. The lack of original studies in horses is likely because of the low frequency with which the disease occurs and the guarded prognosis for affected animals; however, there is a need for such studies because the number of horses living in areas in which the disease is endemic is increasing and treatment options are becoming more affordable.

A diagnosis of coccidioidomycosis may be suspected on the basis of the geographic origin of the patient and detection of clinical signs associated with the respiratory tract as well as fever, chronic weight loss, and an inflammatory leukogram.¹ A confirmatory diagnosis is often made on the basis of a positive result of an assessment for serum antibodies against *C immitis* because the fungal organism can be difficult to culture and histologic examination of tissue biopsy specimens may not reveal spherules, even when *C immitis* infection is strongly suspected. The diagnostic difficulty of isolating the organism may be because of low numbers of the organism at the site of infection, the frequently inaccessible location of the lesions, or the invasiveness of diagnostic procedures such as lung biopsy.² Although isolation of the organism should be attempted, clinicians may have to rely solely on findings of serologic testing for diagnosis, evaluation of prognosis, assessment of the response to treatment, and determination of the necessary duration of treatment; therefore, correct interpretation of serologic data is imperative. Serologic evaluations have also been used in dogs and humans to track the incidence of

From the Arizona Equine Medical and Surgical Center, 1685 S Gilbert Rd, Gilbert, AZ 85296 (Higgins, Leith, Voss); and the Department of Medical Microbiology, School of Medicine, University of California, Davis, CA 95616 (Pappagianis). Dr. Higgins' present address is Loomis Basin Large Animal Services, 3901 Sierra College Blvd, Loomis, CA 95650.

The authors thank Romelo Delossautos and Cheryl McVean for performance of the serologic assessments.

Address correspondence to Dr. Higgins.

exposure and disease in populations over time. The Arizona Department of Health Services reported an incidence of 43 clinical cases/100,000 humans (0.043%) in 2001, which represented an increase of 186% from the reported incidence 6 years earlier.¹¹ Coccidioidomycosis is becoming more prevalent in areas in which the disease is not endemic because of travel; in regions in which the disease is endemic, extensive urban development and construction and the continuous influx of both immunologically naïve people and animals contribute to this increasing disease prevalence. The seroprevalence of antibodies against *C immitis* in humans is not truly known because individuals without clinical signs are not routinely tested.

The purpose of the study reported here was to determine the seroprevalence of antibodies against *C immitis* in healthy horses in an area in which the disease is endemic. Further, seropositive healthy horses were monitored over time in an attempt to determine the natural kinetics of the antibody response. Because the interpretation of low anti-*C immitis* antibody titers can be difficult (as determined in a retrospective study by Ziemer et al¹ in which 30% of horses with clinical signs of coccidioidomycosis that were confirmed as having the disease via fungal culture or histologic evaluation of tissues had a reciprocal serum antibody titer ≤ 16), a primary goal of the study was to determine how to interpret titer values obtained from horses (with or without clinical signs of coccidioidomycosis) that reside in areas in which the disease is endemic.

Materials and Methods

Sample population—From the approximately 2,000 horses that were examined at the Arizona Equine Medical and Surgical Center in Gilbert, Maricopa County, Arizona, from January to June 2004, 197 were randomly selected for inclusion in the study; of these horses, 169 were healthy and underwent evaluation as part of routine preventative care, 22 were examined because of noninfectious diseases, and 6 were examined prior to elective surgical procedures. Blood samples were collected from the horses between January and June 2004. Informed consent was obtained from each owner for collection of data and blood samples. The horses resided in the counties of Maricopa and Pinal in the area surrounding metropolitan Phoenix; coccidioidomycosis is known to be endemic in these 2 counties.¹¹

Study design—For each horse, a complete physical examination was performed; blood was collected to provide a serum sample. Horses in which coccidioidomycosis had been diagnosed previously were excluded from the study. Details of the horses' medical histories were obtained, specifically in regard to clinical signs associated with coccidioidomycosis as well as any other medical problems. Information was also collected regarding housing and present and prior geographic residences of the animals. Serum samples were analyzed for the presence of IgG and IgM antibodies against *C immitis* according to the method described by Pappagianis.¹² Briefly, qualitative immunodiffusion tests were performed on a single plate to screen for an IgM reaction (the early precipitin antibody) and an IgG reaction (the complement fixation antibody that is present later in the course of the immune response) in undiluted serum. For all serum samples in which IgG antibodies were detected, quantitative immunodiffusion assays were performed on serial dilutions until the end titer was reached (reciprocal titers reported). Complement fixation is available but was not used

in the present study because of the suspicion of one of our group that some horses, like dogs, have anticomplement properties in their sera that may lead to false-positive test results. Attempts were made to recheck anti-*C immitis* antibody titer values in all seropositive horses within 2 to 6 months of the initial evaluation to assess the kinetics of the antibody response. Recheck serum samples were paired with initial serum samples and evaluated simultaneously to minimize laboratory variations. To screen for inflammatory changes consistent with active coccidioidomycosis infection, a CBC was performed in each horse in which serum anti-*C immitis* antibodies were detected at any test dilution (ie, greater than undiluted serum, such as 4 or 8). Descriptive statistics were calculated for the data to determine significant differences in age or duration of residence in the area of interest between seropositive horses and the remaining seronegative horses. A *t* test was used, and a value of $P < 0.05$ was considered significant.

Results

One hundred ninety-seven horses were included in the study. The breeds of horse were Quarter Horse (n = 86 [43.7%]), Arabian (31 [15.7%]), American Paint (28 [14.2%]), Thoroughbred (13 [6.6%]), warmblood (6 [3.0%]), pony (6 [3.0%]), mule (5 [2.5%]), Appaloosa (5 [2.5%]), Mustang (4 [2.0%]), American Miniature Horse (4 [2.0%]), Missouri Fox Trotting Horse (3 [1.5%]), Tennessee Walking Horse (3 [1.5%]), unknown (2 [1.0%]), and Andalusian (1 [0.5%]). Of the 197 horses, 109 (55.3%) were geldings, 79 (40.1%) were mares, and 9 (4.6%) were stallions. The horses were 2 months to 30 years old (mean \pm SD, 11.1 \pm 7.04 years). Horses resided at 99 different premises; there were 1 to 9 horses/site (mean, 1.99 horses/site). The duration of residency in Maricopa or Pinal Counties ranged from 6 weeks to 30 years (mean \pm SD, 6.61 \pm 6.3 years).

Among the 197 serum samples, serologic testing of 189 samples yielded negative results for both IgM and IgG antibodies against *C immitis*; sera obtained from 8 horses yielded positive results for either IgM or IgG antibodies (or both) against *C immitis* (seroprevalence, 4.06%; **Appendix**). Rectal temperature and pulse and respiratory rates were within reference limits in all seropositive horses, and these horses were considered healthy at the time of blood sample collection. The 8 seropositive horses were of various breeds, including Arabian (n = 3), Quarter Horse (3), Thoroughbred (1), and Mustang (1); there were 5 mares and 3 geldings. In 6 of the 8 horses, evaluation of an undiluted sample of serum yielded positive results for IgG antibodies and negative results for IgM antibodies against *C immitis*. Analysis of serum obtained from another horse revealed an IgG antibody titer of 4 with concurrent positive results for IgM antibodies against *C immitis*. The remaining horse had a serum IgG antibody titer of 8, but no serum IgM antibodies against *C immitis* were detected. Complete blood counts were performed on the horses with a titer of 4 or 8, and findings were within reference limits without any indication of inflammatory disease. Five of the 8 seropositive horses were available for follow-up; 2 had become seronegative within 2 to 6 months, 2 had a stable unchanged antibody titer (ie, IgG antibodies against *C immitis* detected in undiluted serum) 2 months later, and 1 had

a decrease in antibody titer value from 8 to 4 at a recheck 2 months after the initial blood collection. The seropositive horses were 4 to 16 years old (mean \pm SD age, 7.88 ± 4.08 years); in comparison, the mean age of seronegative horses was 11.1 ± 7.04 years, but the difference between the 2 groups was not significant ($P = 0.079$). The duration of residence of seropositive horses in an area in which coccidioidomycosis was endemic was 7 months to 9 years (mean \pm SD residency, 2.98 ± 2.64 years); this was significantly ($P = 0.007$) shorter than the duration of residence of seronegative horses (mean residency, 6.61 ± 6.3 years). Seven of the 8 seropositive horses were housed on a dirt paddock at least 50% of the time, whereas 135 of the 189 seronegative horses had similar access to dirt paddocks.

Discussion

Although coccidioidomycosis is not readily preventable in any species, a better understanding of the epidemiology of the disease in horses can assist veterinarians in the interpretation of serologic data, allowing for earlier diagnosis of the disease and application of appropriate medical management. During the period of the present study, seroprevalence of antibodies against *C immitis* in healthy horses (in which coccidioidomycosis had not been previously diagnosed) that resided in an area of Arizona in which the disease is endemic was 4.06%. In dogs and humans, the prevalence of infection with *C immitis* is known to vary throughout the year; in Arizona, most clinical infections become apparent during the cool months (November to February) following exposure to the organism during prolonged drought periods associated with dry, dusty conditions.^{11,13} Among the 8 seropositive horses evaluated during the winter and spring months in the present study, only 1 had serum IgM antibodies against *C immitis* (a finding consistent with recent exposure). It may be assumed that the other 7 horses were exposed earlier in the year during the drier, dustier seasonal conditions.

Because of the possibility of conversion back to seronegative status in healthy, *C immitis*-exposed horses, overall exposure during the lifetime of horses in an area in which coccidioidomycosis is endemic may be greater than we are able to detect at a single point in time. A possible reason is that the serum antibody concentration that developed in response to a previous exposure may remain at a residual level that is below the lowest concentration measurable by use of the diagnostic test. It had previously been reported¹² that most humans with residual coccidioidal nodules and 50% of patients with chronic coccidioidal pulmonary cavities were assessed as seronegative by use of tube precipitin and complement fixation tests. However, by use of more modern methods of serum concentration and immunodiffusion assays, it was possible to enhance the detection of low serum concentrations of anti-*C immitis* antibody in some of those patients. Such low serum antibody concentrations may be similar to those detected in previously exposed horses.

In the present study, 3 of the seropositive horses were from 1 location, whereas 2 of the other seropositive horses were boarded together. These findings sup-

port the idea of focal "hotspots" existing within regions in which coccidioidomycosis is endemic¹⁴ or nonendemic.¹⁵ The location from which the 3 horses originated was surrounded by ongoing construction activities and soil turnover, whereas the site at which 2 horses were boarded was adjacent to a new freeway that had recently been constructed. In the present study, a greater percentage of seropositive horses were reportedly kept in dirt paddocks, compared with seronegative horses. Exposure to *Coccidioides* organisms in association with soil turnover has previously been reported⁸ in a horse with signs of coccidioidomycosis; the horse lived in an area in which severe windstorms and flooding, and resultant soil turnover, had occurred 1 month prior to the development of clinical signs.

The mean age of seropositive horses was less than that of seronegative horses, but the difference was not significant. The duration of residence in an area in which coccidioidomycosis was endemic was significantly shorter for seropositive horses than for seronegative horses. On the basis of these data, we assume that the initial serologic response to *C immitis* in naive horses, such as young horses and horses recently introduced to an area in which the disease is endemic, is strong and that the serologic response in older horses and long-term residents may decrease to an undetectable level.

In our study, Arabians appeared to be overrepresented (37.5%) in the seropositive population of horses, although they comprised only 15.7% of the total sample population (197 horses). However, there was no significant ($P = 0.2$) difference between the number of Arabians in the seropositive group and the number of Arabians in the total sample group. Similar findings were reported by Ziemer et al¹; in their study of coccidioidomycosis in horses, Arabians comprised 33% of the clinical cases but only 14% of all horses admitted to the hospital. In our study, 5 of the 8 seropositive horses were mares, yet mares comprised only 40.1% of the sample population. In the study by Ziemer et al,¹ there were more clinically ill female horses than male horses, even though the ratio of males to females evaluated at that hospital was 2:1. However, the number of horses included in either the present or that previous study are still too small to ascribe greater susceptibility or exposure rate to 1 breed or sex.

One of the main goals of our study was to develop recommendations for the interpretation of titers of serum antibodies against *C immitis* in horses living in areas in which coccidioidomycosis is endemic. In our study, seropositive horses without clinical signs of coccidioidomycosis were identified, and our data suggest that a serum IgG anti-*C immitis* antibody titer of 8 or less may be consistent with exposure to the organism without subsequent development of disease. This indicates that subclinical infection with *C immitis* followed by healing (without medical intervention) does appear to occur in horses. However, none of the healthy horses had a serum antibody titer ≥ 16 ; therefore, it could be assumed that a titer of this magnitude is consistent with active disease. In humans, serum antibody titers ≥ 16 are associated with dissemination of *Coccidioides* organisms to bone or skin,³ but in dogs, the magnitude

of the serum IgG anti-*C immitis* antibody titer does not appear to be consistently associated with severity or extent of disease.⁵ In our study, most of the horses in the area in which coccidioidomycosis is endemic were seronegative for antibodies against *C immitis* as determined via immunodiffusion assay. Because of the rarity of detectable anti-*C immitis* antibody titers in the general equine population at a given point in time, any serum antibody titer that is positive at any dilution (including 4 or 8) should be considered seriously, especially in a horse with clinical signs consistent with coccidioidomycosis. A subsequent recheck serum sample should always be assessed to detect a possible increasing serum antibody titer, which might be suggestive of dissemination of or progressive infection with *Coccidioides* organisms, even in an apparently healthy horse that is being examined for any reason. Screening of healthy horses for *C immitis* infection may be requested by owners residing in an area in which coccidioidomycosis is endemic, and guidelines for proper interpretation of serologic data are necessary. It appears that serum antibody titers in healthy horses that are not actively developing infection with *Coccidioides* organisms may remain detectable for 2 to 6 months and will likely decrease or remain stable with each recheck serologic assessment during that period. Recheck serologic assessments should be performed at intervals of at least 3 weeks because there is usually no notable change in serum antibody titers over shorter periods due to the half-life of IgG.¹⁶ In horses with a low serum antibody titer, a decision to administer antifungal treatments should be based on hematologic evidence of inflammation, weight loss, clinical signs of infection such as fever, and a rising serum IgG anti-*C immitis* antibody titer. Low antibody titer values are difficult to interpret in many species; for example, in a study by Johnson et al,⁵ 6 of 14 dogs with clinical disease were reported to have a serum IgG anti-*C immitis* antibody titer < 8. In 1 of those 6 dogs, *C immitis* spherules were detected histologically in biopsy specimens, yet that patient had a serum IgG anti-*C immitis* antibody titer of only 2. Obviously, serologic data have to be interpreted in light of other findings including clinical signs, isolation of the organism, and an

increase or decrease in serum antibody titer over time. Nonetheless, monitoring of the serologic status can provide useful information for use in the evaluation and management of horses with clinical signs of coccidioidomycosis, especially when one considers that serologic testing revealed serum antibodies against *C immitis* in only 4.08% of the healthy horses included in our study.

References

1. Ziemer E, Pappagianis D, Madigan J, et al. Coccidioidomycosis in horses: 15 cases (1975–1984). *J Am Vet Med Assoc* 1992;201:910–916.
2. Kerl M. Coccidioidomycosis. *Vet Clin North Am Small Anim Pract* 2003;33:732–737.
3. Crum N, Lederman E, Stafford C, et al. Coccidioidomycosis: a descriptive survey of a reemerging disease. Clinical characteristics and current controversies. *Medicine (Baltimore)* 2004;83:149–172.
4. Fowler M, Pappagianis D, Ingram I. Coccidioidomycosis in llamas in the United States: 19 cases (1981–1989). *J Am Vet Med Assoc* 1992;201:1609–1614.
5. Johnson L, Herrgesell E, Davidson A, et al. Clinical, clinicopathologic, and radiographic findings in dogs with coccidioidomycosis: 24 cases (1995–2000). *J Am Vet Med Assoc* 2003;222:461–466.
6. Terio K, Stalis I, Allen J, et al. Coccidioidomycosis in Przewalski's horses (*Equus przewalskii*). *J Zoo Wildl Med* 2003;34:339–345.
7. Maleski K, Magdesian KG, LaFranco-Scheuch L, et al. Pulmonary coccidioidomycosis in a neonatal foal. *Vet Rec* 2002;151:505–508.
8. Kramme P, Ziemer E. Disseminated coccidioidomycosis in a horse with osteomyelitis. *J Am Vet Med Assoc* 1990;196:106–109.
9. Walker R, Johnson B, Jones K, et al. *Coccidioides immitis* mastitis in a mare. *J Vet Diagn Invest* 1993;5:446–448.
10. Stoltz J, Johnson B, Walker R, et al. *Coccidioides immitis* abortion in an Arabian mare. *Vet Pathol* 1994;31:258–259.
11. Increase in coccidioidomycosis—Arizona, 1998–2001. *MMWR Morb Mortal Wkly Rep* 2003;52:109–112.
12. Pappagianis D. Serologic studies in coccidioidomycosis. *Semin Respir Infect* 2001;16:242–250.
13. Reed R, Converse J. The seasonal incidence of canine coccidioidomycosis. *Am J Vet Res* 1966;27:1027–1030.
14. Crum N, Lamb C, Utz G, et al. Coccidioidomycosis outbreak among United States Navy SEALs training in a *Coccidioides immitis*-endemic area—Coalinga, California. *J Infect Dis* 2002;186:865–868.
15. CDC. Coccidioidomycosis in workers at an archeologic site—Dinosaur National Monument, Utah, June–July, 2001. *MMWR Morb Mortal Wkly Rep* 2001;50:1005–1008.
16. Pappagianis D, Zimmer B. Serology of coccidioidomycosis. *Clin Microbiol Rev* 1990;3:247–268.

Appendix on page 1892.

Appendix

Data regarding 8 horses that had detectable serum antibodies against *Coccidioides immitis* among 197 horses with no clinical signs of coccidioidomycosis that resided in an area in which the disease is endemic.

Signalment (age)	Duration of residence in study area	Results of initial serologic evaluation for antibodies against <i>C immitis</i>	Follow-up information (signs of coccidioidomycosis and results of serologic reevaluation)
Quarter Horse mare* (8 years)	7 months	IgG positive (undiluted sample)	No clinical signs at 5 months; unable to recheck serum antibody titer
Quarter Horse mare* (16 years)	7 months	IgG positive (undiluted sample)	No clinical signs at 5 months; seronegative for IgG antibodies
Quarter Horse mare (9 years)	9 years	IgG positive (undiluted sample)	No clinical signs at 2 months; seronegative for IgG antibodies
Thoroughbred gelding (12 years)	2 years	IgG positive (undiluted sample)	No clinical signs at time of sampling; lost to follow-up
Mustang gelding (4 years)	8 months	IgG positive (titer = 4) and IgM positive	No clinical signs at 1 month; unable to recheck serum antibody titer
Arabian gelding† (4 years)	4 years	IgG positive (titer = 8)	No clinical signs at 2.5 months; IgG positive (titers = 4)
Arabian mare† (6 years)	3 years	IgG positive (undiluted sample)	No clinical signs at 2.5 months; IgG positive (undiluted sample)
Arabian mare† (4 years)	4 years	IgG positive (undiluted sample)	No clinical signs at 2.5 months; IgG positive (undiluted sample)

*Horses moved to same premise from Colorado. †Horses from same premise.



Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Evaluation of xylazine and ketamine for total intravenous anesthesia in horses
Khursheed R. Mama et al

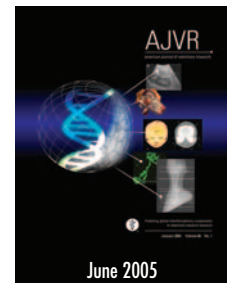
Objective—To evaluate the use of xylazine and ketamine for total IV anesthesia in horses.

Animals—8 horses.

Procedure—Anesthetic induction was performed on 4 occasions in each horse with xylazine (0.75 mg/kg, IV), guaifenesin (75 mg/kg, IV), and ketamine (2 mg/kg, IV). Intravenous infusions of xylazine and ketamine were then started by use of 1 of 6 treatments as follows for which 35, 90, 120, and 150 represent infusion dosages ($\mu\text{g}/\text{kg}/\text{min}$) and X and K represent xylazine and ketamine, respectively: X35+K90 with 100% inspired oxygen (O_2), X35+K120- O_2 , X35+K150- O_2 , X70+K90- O_2 , K150- O_2 , and X35+K120 with a 21% fraction of inspired oxygen (ie, air). Cardiopulmonary measurements were performed. Response to a noxious electrical stimulus was observed at 20, 40, and 60 minutes after induction. Times to achieve sternal recumbency and standing were recorded. Quality of sedation, induction, and recovery to sternal recumbency and standing were subjectively evaluated.

Results—Heart rate and cardiac index were higher and total peripheral resistance lower in K150- O_2 and X35+K120-air groups. The mean arterial pressure was highest in the X35+K120-air group and lowest in the K150- O_2 group (125 ± 6 vs 85 ± 8 at 20 minutes, respectively). Mean PaO_2 was lowest in the X35+K120-air group. Times to sternal recumbency and standing were shortest for horses receiving K150- O_2 (23 ± 6 minutes and 33 ± 8 minutes, respectively) and longest for those receiving X70+K90- O_2 (58 ± 28 minutes and 69 ± 27 minutes, respectively).

Conclusions and Clinical Relevance—Infusions of xylazine and ketamine may be used with oxygen supplementation to maintain 60 minutes of anesthesia in healthy adult horses. (*Am J Vet Res* 2005;66:1002–1007)



See the midmonth issues of JAVMA for the expanded table of contents for the AJVR or log onto www.avma.org for access to all the abstracts.