AJVR

Currents in One Health
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AVMA

Antibody response over time correlated with treatment outcome in 30 dogs naturally infected with *Brucella canis* (2017–2022)

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Received January 20, 2023.
Accepted February 21, 2023.

doi.org/10.2460/ajvr.23.01.0014

OBJECTIVE
Brucella canis is a zoonotic bacterial pathogen of dogs that is notoriously difficult to diagnose and treat. Humans can become infected with *B canis* when an infected pet dog is brought into their home. Our objectives were to describe the clinical presentation and outcomes in dogs treated for *B canis* and evaluate the performance of the quantitative serologic canine *Brucella* multiplex (CBM) assay for monitoring treatment response.

ANIMALS
Diagnostic records from the Animal Health Diagnostic Center at Cornell University were retrospectively reviewed (2017–2022) for dogs that underwent repeat *B canis* serologic testing. Medical records were requested to compare the clinical presentations and outcomes for dogs that underwent treatment for *B canis*. Changes in CBM antibody values were compared between dogs with and without resolution of clinical signs.

RESULTS
While treatment protocols varied in the 30 treated dogs meeting the inclusion criteria, poly-antimicrobial therapy was prescribed in 97% (29/30) of cases. Gait abnormalities, spinal pain, and discospondylitis were the most common clinical abnormalities. A difference (*P* value = .0075) in the percent decrease in CBM assay PO1 antibody values was found in dogs with resolved clinical signs.

CLINICAL RELEVANCE
Young dogs presenting with recurring lameness or back pain should be screened for *B canis* infection. A 40% decline in CBM assay values 2 to 6 months posttreatment can be supportive of response to treatment. Further prospective studies are needed to determine the ideal *B canis* treatment regimen and the magnitude of public health risks associated with maintaining neutered *B canis*-infected animals as pets.

Introduction

Brucella canis is a zoonotic bacterial pathogen of dogs that is notoriously difficult to diagnose and treat.[1] Its intracellular nature and many mechanisms for evasion and multiplication within the host are implicated when dogs fail to respond to antimicrobial therapy or when relapse occurs following cessation of treatment.[2] Due to their perceived risk to public health, dogs diagnosed with *B canis* are often euthanized. When treatment is attempted, there is no universally accepted antimicrobial protocol for veterinarians to follow. While combination antimicrobial therapy has been shown to be more effective than monotherapy protocols, the ideal combination of antimicrobials and duration of treatment required to mitigate clinical signs and reduce the risk of recrudescence has not been adequately defined, as addressed in the companion Currents in One Health by Pinn-Woodcock et al, *JAVMA*, April 2023.[1]

Prompt diagnosis of *B canis* in dogs and monitoring for recrudescence of infection following treatment may decrease human exposure.[3–9] Gold standard diagnosis of *B canis* is made by isolation of the bacteria using culture; however, due to intermittent shedding, antigen detection techniques have poor sensitivity. Instead, the initial diagnosis is often made using qualitative screening serologic assays, such as an indirect fluorescent antibody (IFA) test, enzyme-linked immunosorbent assay (ELISA), or rapid slide agglutination test (RSAT). These assays have poor specificity and require confirmation using an agar gel immunodiffusion assay (AGID II) that employs the cytoplasmic components of a

B canis (M-) strain. The highly specific AGID II is available only through reference laboratories. The AGID II is commonly run in parallel with a 2ME-RSAT, an RSAT that employs 2-mercaptoethanol to reduce nonspecific agglutinins. The reagents for the AGID II are challenging to produce, requiring the growth of this biosafety level 3 organism and a substantial volume of serum from *B canis*-infected dogs. A novel alternative screening assay has recently been developed, the canine *Brucella* multiplex (CBM) assay, a fluorescent bead-based assay that simultaneously quantifies antibodies directed against 2 *Brucella*-specific antigens, BP26 and PO1.[10] The CBM assay has advantages in that it is produced with synthetic reagents that are efficiently manufactured and provides quantitative results.

Evaluating the effectiveness of antibiotic treatment for a variety of different bacterial infections can be challenging. Quantitative or semiquantitative antibody values can be used to support effective treatment for other bacterial pathogens, where successfully treated individuals demonstrate a > 40% decline in antibody values by 6 months posttreatment.[11,12] Utilizing a quantitative serologic assay to measure *B canis* antibody titers has been suggested as the best means of monitoring for successful treatment of *B canis*[1]; however, evaluation of the CBM assay for this purpose has not yet been performed.

Our objectives were to describe the diagnostic workup, treatment, and clinical outcome in dogs treated for *B canis* infection and to evaluate the performance of the CBM assay for monitoring response to treatment in these cases. We hypothesized that the resolution of clinical signs would correlate with a significant decline in CBM antibody values.

Methods

Case selection criteria

Diagnostic records from the laboratory management software at the Cornell University Animal Health Diagnostic Center (AHDC) in Ithaca, NY, were retrospectively reviewed for dogs that underwent repeat *B canis* serologic testing over a 5-year period, from January 1, 2017, through August 1, 2022. Dogs were considered for study inclusion only if they had at least 3 *B canis* serologic submissions, tested positive at least once on both the 2ME-RSAT/AGID II, and tested positive on either test on a follow-up submission. From this initial group of dogs, medical records were requested from the veterinary practice associated with the most recently requested *B canis* serologic testing. Furthermore, only dogs with medical records that included *B canis* treatment information were considered for further analysis.

Medical records review

Medical records of dogs included in this study were reviewed, each dog was given a unique identification number, and the following information was recorded: signalment, clinical signs, methods of diagnosis, duration to diagnosis, *B canis* serologic test results, diagnostic imaging findings, antimicrobial and analgesic treatment protocols, age when spayed or neutered, comorbidities, clinical outcome, length of survival postdiagnosis, whether a dog was considered lost to follow-up, and whether the case was reported to state officials. Duration to diagnosis was defined as the number of days lapsed between the onset of relevant clinical signs and *B canis* positive 2ME-RSAT/AGID II or culture. Relevant clinical signs included abortion, gait abnormalities, weakness, pain upon palpation of the cervical or thoracolumbar spine, anorexia, weight loss, lethargy, depression, and exercise intolerance. Animals were considered "lost to follow-up" if they were noted to be alive, but their last veterinary examination or prescription fulfillment was more than 6 months prior to the receipt of medical records.

Diagnostic testing

The canine *Brucella* 2ME-RSAT/AGID II tests and the CBM assays were performed by the AHDC in accordance with their standard operating procedures. The Cornell AHDC began utilizing the CBM assay for clinical submissions in June 2022.[10,13] At least 3 banked serum samples from all dogs meeting the study's inclusion criteria were tested on the CBM assay.

Statistical analysis

The signalment, clinical signs, treatment, and outcome characteristics were analyzed using descriptive statistics. Statistical analysis was performed in GraphPad Prism version 6.07. Changes in CBM antibody values, measured as percent decrease from the initial diagnosis to a time point 2 to 6 months postinitiation of treatment, were compared between groups using an unpaired *t* test with 2-tailed *P* value; $P < .05$ was considered statistically significant.

Results

Signalment

A total of 98 dogs were identified for potential inclusion in this retrospective study, of which medical records for 30 dogs meeting the inclusion criteria were obtained from veterinary practices. Gender distribution at the time of diagnosis consisted of 1 intact female, 16 spayed female, 5 intact male, and 8 castrated male dogs. All the intact dogs were neutered within 3 months of their *B canis* diagnosis. The breed distribution is presented **(Table 1)**. The median age at diagnosis was 1.65 years (mean = 2.54 years, minimum = 0.59 years, and maximum = 11.72 years). The median duration of survival since *B canis* diagnosis was 3.11 years (mean = 3.29 years, minimum = 0.30 years, and maximum = 7.47 years). Medical records indicated that 73% (22/30) of dogs remained alive; 1 dog died (dog 22) at 14 years of age, which was 2.2 years after *B canis* diagnosis; and 7 dogs (dogs 8, 14, 17, 19, 21, 23, and 26) were considered lost to follow-up.

Clinical presentation and *B canis* diagnosis

Back or hip pain (63%, 19/30) and lameness or limb weakness (63%, 19/30) were the most common

Table 1—Breed distribution.

Breed	n
Miniature Schnauzer	1
Golden Retriever	2
Beagle	1
Australian Cattle dog	1
Pit Bull	1
French Bulldog	1
Rhodesian Ridgeback	1
Labrador Retriever	2
Siberian Husky	1
Labrador Retriever mix	6
German Shepherd mix	5
Pit Bull mix	1
Heeler	1
Boxer Mix	2
Border Collie mix	1
Australian Cattle dog mix	1
Unknown mix	2

physical exam abnormalities. Pain on musculoskeletal palpation was specified as lumbar (n = 5), thoracolumbar (3), cervical/thoracolumbar (1), lumbar/cervical (1), hip (1), or back/spinal (8) pain. Forty-two percent (8/19) of the dogs with lameness or limb weakness also had a history of shifting or recurring lameness. Tibial plateau leveling osteotomy surgery was recommended for dog 29 and performed on dogs 11 and 12 prior to *B canis* diagnosis. Bilateral triple pelvic osteotomy surgery was performed on dog 26, but persistent lameness returned after surgical recovery and discospondylitis was subsequently diagnosed.

Comorbidities identified by clinicians at the time of *B canis* diagnosis or afterward included atopic dermatitis in 33% (10/30) of dogs and ophthalmic abnormalities in 13% (4/30) of dogs including 1 case each of blepharitis, conjunctivitis, chemosis, and corneal ulceration.

For all cases, *B canis* diagnosis was made based on combined positive results on the 2ME-RSAT/AGID II test. *B canis* was isolated from blood culture in only 33% of tested cases (2 positives/6 tested).

The mean time lapsed from the onset of relevant clinical signs to diagnosis was 191 days (median = 92.5 days, minimum = 0 days, and maximum = 982 days). A radiographic diagnosis of discospondylitis (n = 27) was the most common impetus for *B canis* testing. Other catalysts for *B canis* testing included abortion (n = 1) or *B canis* diagnosis in a housemate (1) or littermate (1). Testing to diagnose *B canis* was not immediately performed in many cases, including for 2 dogs with a history of discospondylitis; dog 4 was diagnosed with discospondylitis and was not tested for *B canis* until 6 months later, and dog 13 was diagnosed with severe spondylosis at the lumbosacral junction but was not tested for *B canis* until 2.25 years later. The initial radiographs of dog 1 and dog 6 failed to reveal discospondylitis, delaying diagnosis for 2 months and 9 months, respectively. The delay in diagnosis for dog 7 was related to a delay in seroconversion, where an in-clinic RSAT was positive initially, but the 2ME-RSAT in-clinic test did not produce a positive result for nearly 7 months. Dog 30 was not conclusively diagnosed for several months because of repeated inconclusive 2ME-RSAT/AGID II results. Dog 12 was tested following *B canis* diagnosis of another dog in the home, after having presented with persistent hindend lameness in the preceding months. Dog 2 was tested because of an alert from the state veterinarian that a littermate tested positive, after having presented with lameness or difficulty walking at 4 clinic visits over the preceding year.

Antibiotic and analgesic therapy

All dogs included in this study received antibiotic treatment following a serologic diagnosis of *B canis* infection. Combination antimicrobial therapy was prescribed at the time of *B canis* diagnosis in 93% (28/30) of dogs in this study, with delayed initiation in 1 additional dog. Doxycycline was a component of the antimicrobial therapy for 90% (27/30) of dogs. The antibiotic combinations administered are listed **(Table 2)**. The aminoglycosides used were gentamicin (n = 5), amikacin (3), or streptomycin (1) and were administered SQ for 7 days on weeks 1 and 4 (8) or every 3 to 4 weeks (1); for 4 dogs, aminoglycoside therapy was repeated after the first

Table 2—Antibiotic combinations prescribed for treatment.

Antibiotic combination	N	Dog identification number
Enrofloxacin/doxycycline	14	1,*,[a] 2,* 4,* 6, 7,[a] 9,* 10, 19,* 21,[a] 22, 25,[a] 27,*,[b] 29,*,[b] 30*
Aminoglycoside/doxycycline	6	8, 13, 14, 15,*,[c] 16, 26
Aminoglycoside/enrofloxacin/doxycycline	3	5, 24,*,[a] 28[d]
Ciprofloxacin/doxycycline	2	11,[e] 12[e]
Amoxicillin-clavulanic acid/enrofloxacin	1	23*,[f]
Rifampin/doxycycline	1	18*
Marbofloxacin/doxycycline	1	17[g]
Cefalexin/enrofloxacin	1	20*,[f]
Enrofloxacin	1	3*

*Clinical signs resolved.

[a]Maintained for > 1 year on a combination of enrofloxacin/doxycycline. Dog 7 alternated monotherapy weekly. [b]Maintained on doxycycline indefinitely, > 1 year. Dog 29 had remained on combination therapy for 6 months. [c]Initiated enrofloxacin monotherapy 8 months after discontinuing treatment based on continued positive 2ME-RSAT/AGID II results. [d]Continual therapy with doxycycline, along with intermittent enrofloxacin and streptomycin for > 2 years until switching to indefinite marbofloxacin/azithromycin. [e]Indefinite combination treatment lasting > 2 years. [f]Maintained on enrofloxacin indefinitely, > 2 years. [g]Initially treated with and maintained on doxycycline alone for 8 months.

month. Twelve dogs were administered long-term (> 4 mo duration) antibiotic treatment, as indicated, despite the resolution of clinical signs in 6 of these dogs. Antibiotic treatment was terminated in 2 dogs because of negative serology, 1 of which subsequently tested positive at a 3-month follow-up, and the other was lost to follow-up.

All except 1 dog, dog 2, received analgesia for pain management at or before the diagnosis of *B canis.* Carprofen and gabapentin were prescribed most frequently for 80% (24/30) and 73% (22/30) of dogs, respectively. Opioids were prescribed for pain management in 47% (14/30) of dogs. Following a diagnosis of *B canis*, 27% (8/30) of dogs were prescribed continuous analgesia, and the same number were managed intermittently with analgesics on an as-needed basis. Resolution of clinical signs with cessation of analgesia was achieved in 47% (14/30) of dogs.

Serologic monitoring during and after treatment

The 2ME-RSAT/AGID II assay had been used to monitor the serologic response in all dogs included in this study. We aimed to investigate whether the CBM assay could be used to monitor the response to treatment more effectively, given the quantitative nature of the assay. Stored serum samples obtained from the time of *B canis* diagnosis were available for the 26 dogs in this study diagnosed after January 1, 2017, all of which were nonnegative on the CBM assay. Serum from all 26 dogs contained antibodies directed against the PO1 antigen, while only 50% (15/30) were found to have antibodies directed against the BP26 antigen.

Effective antimicrobial treatment should be associated with a substantial decline in antibody values. We found that the quantity of antibodies directed against the PO1 antigen decreased by > 40% in 12 of these dogs by 3 to 6 months posttreatment and in an additional 3 dogs by 8 to 12 months posttreatment. Of note, 1 of the dogs, dog 26, had an initial decrease of > 40% in PO1 antibody values at 3 months, but antibody values rebounded 2 months later, suggestive of recrudescence, but the dog was subsequently lost to follow-up.

It has previously been proposed that 2 sequential negative results on the AGID II assay are supportive of elimination of infection.[14] Only 1 dog, dog 3, seroconverted to negative on 2 sequential 2ME-RSAT/AGID II assays, while 4 dogs returned to negative on the CBM assay **(Figure 1)**. Dog 1 and dog 29 achieved a negative CBM result approximately 3 months postinitiation of treatment, while dog 28 and dog 3 did not achieve a negative result until 1 and 3 years posttreatment, respectively. Clinical signs had resolved in all 4 of these dogs at the time of the last serologic sample. Dog 28 was reported to have had recrudescence of clinical signs 1.75 years later; however, serologic samples from that period were not available. Three of these dogs were maintained on antibiotics indefinitely (dog 1, dog 28, and dog 29), while dog 3 received 2 finite courses of enrofloxacin, with the first 104-day course concluding after a negative AGID II results and the second 180-day course beginning after a positive AGID II result was received 4 months later.

Resolution of clinical signs can be an indication of treatment success, so we were interested in evaluating whether a decrease in antibody values on the CBM assay was associated with the resolution of clinical signs in the months following treatment. Stored serum samples from day 60 to 185 postinitiation of antimicrobial treatment were available for 22 of these dogs. Of these, 55% (12/22) of dogs demonstrated resolution of clinical signs, while 45% (10/22) of dogs were reported to have persistent clinical signs, including lameness or pain, requiring long-term analgesic treatment. We found a significant difference (t = 2.989, df = 19, *P* value = .0075) in the percent decrease in PO1 antibody values between these 2 groups **(Figure 2)**.

One specific case we want to highlight is that of dog 17. This dog was initially administered long-term monotherapy with doxycycline after *B canis* diagnosis. Eight months after treatment began, the dog presented to the emergency room with severe pain in the hind limbs, difficulty walking, and dribbling urine. Surgery was recommended, and an L4-5, L5-6 hemilaminectomy was performed the following day. The owners reported that they had been administering

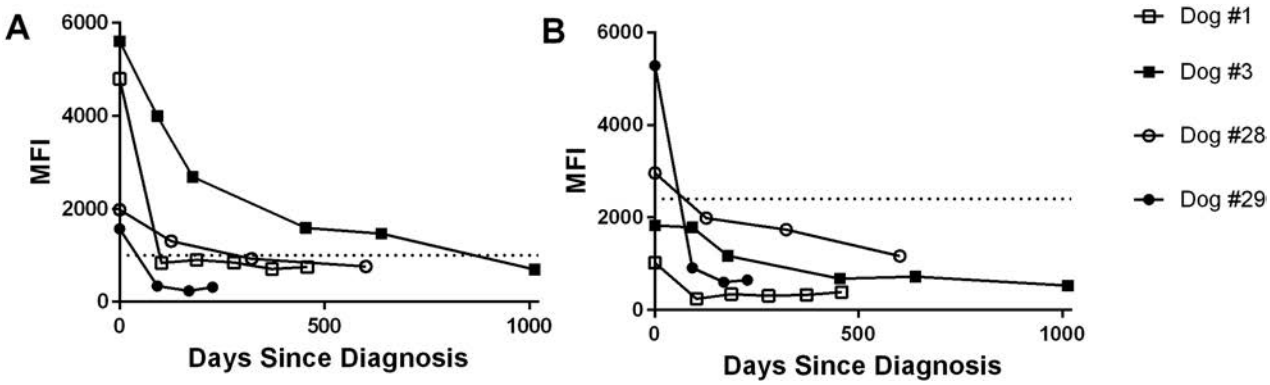

Figure 1—CBM assay results for 4 dogs that seroconverted to negative during or after antimicrobial treatment. Results from serum samples tested in the CBM assay are presented. Dotted lines represent the lower cut-off values for each antigen PO1 (A) and BP26 (B).

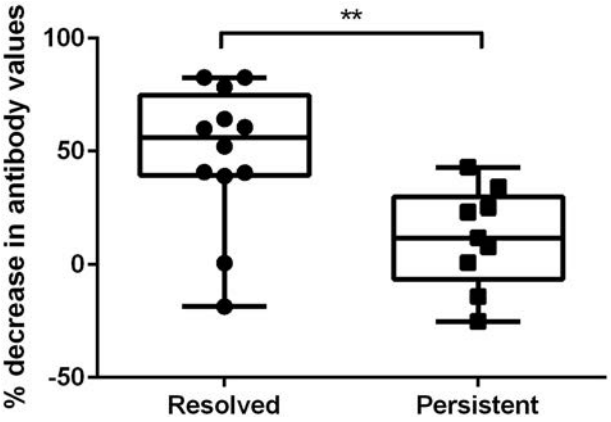

Figure 2—Percent decrease in antibody values according to clinical outcome. The percent decrease in anti-PO1 antibody values, as measured by the CBM assay, from the initial serum sampling to a serum sample taken 2 to 6 months after the initiation of treatment is presented. Dogs that were considered to have resolved clinical signs (n = 12) demonstrated a more substantial percent decrease in PO1 values in this timeframe than dogs that were reported to have persistent clinical signs (9) (*P* value = .0075).

amoxicillin, in addition to the previously prescribed doxycycline, in the preceding weeks for a suspected urinary tract infection. The PO1 antibody values had decreased by only 11% of the initial value at 3 months postinitiation of doxycycline monotherapy and did not decrease further at 6 months; a sample taken several weeks after initiating polytherapy (amoxicillin/doxycycline) revealed a further 37% decrease in PO1 antibody values as compared to the 6-month post-treatment value.

Discussion

The decision to initiate treatment for a dog diagnosed with *B canis* should not be made without careful consideration. The expense to treat an infected dog, and the need for substantial follow-up to identify recrudescence is significant; owners should be made aware of this, as well as of the associated zoonotic risks and quarantine recommendations. In particular, in cases of a discospondylitis diagnosis associated with *B canis* infection, owners should also be cautioned about the potential for recrudescence.[14] Human infections from pet dogs have been reported, with some cases leading to hospitalization.[8,9,15] However, symptoms in humans may be nonspecific, including intermittent fever and fatigue, or even unapparent.[16] Diagnosis of infection in exposed pet owners or veterinary clinic employees is challenged by the lack of validated *B canis* serologic tests for humans.[15]

Canine brucellosis regulations vary by state, and in some localities, clinicians are required to report the diagnosis of *B canis* in dogs. Only 47% (14/30) of records indicated that the case was reported to state officials. Reporting of *B canis* diagnosis to state officials can be of value, as exemplified in the case of dog 2, where the dog had previously presented with lameness concerns and the diagnosis was only made based on the contact tracing associated with the report of a positive littermate. Prompt reporting of *B canis* cases allows public health officials to assess public health risks and implement treatment, testing, or prevention where warranted.

Our study revealed substantial delays in the diagnosis of *B canis* following the initial presentation of back or hip pain and gait abnormalities, and most of these dogs were < 5 years of age. A recent retrospective study[17] of *B canis* discospondylitis (BDS) in 33 dogs similarly revealed that dogs with BDS typically present at a young age and have a long duration of clinical signs leading up to their diagnosis. Taken together, these studies support the need to screen young dogs that present with signs of chronic back or hip pain, or recurring lameness, for *B canis.*

A diagnosis of discospondylitis was made in 90% of dogs in this study and was the most common indication for *B canis* testing. While *B canis* is a well-described cause for discospondylitis in dogs, other pathogens associated with the disease process include *Staphylococcus pseudintermedius*, *Aspergillus terreus, Escherichia coli*, *Pseudomonas aeruginosa, Enterococcus faecalis,* and *Staphylococcus epidermidis.*[18,19] Delayed diagnosis of discospondylitis can be detrimental to the prognosis, as both the vertebral lesion and neurological status can worsen with time.[18] Changes consistent with infection can be detected at earlier stages when using magnetic resonance imaging or computed tomography, compared with radiography[20–22]; however, these imaging modalities can be cost prohibitive. Often the progression of disease at the time of presentation is substantial, warranting pain management. In some cases, even with effective treatment, some irreversible damage may occur, causing continued pain. The findings of our study suggest that clinical signs suggestive of discospondylitis should be sufficient to warrant serologic screening for *B canis.*

Prompt in-clinic *B canis* screening has previously been available using the D-Tec CB kit,[23] allowing clinicians to implement preliminary biosecurity protocols or prevent breeding until a confirmatory test result could be obtained. Unfortunately, this assay became unavailable in 2022, but referral laboratories across the United States offer a variety of other screening assays. Further information about the various serologic tests available and the limitation of serologic testing is addressed in our companion Currents in One Health by Pinn-Woodcock et al, *JAVMA*, April 2023. When *B canis* is highly suspected, a negative result on a single test should not be accepted as proof of freedom from infection and additional diagnostics should be pursued.

The variation in the treatment protocols used for dogs in our study limited any conclusions that could be made regarding the efficacy of particular *B canis* treatment protocols. One important conclusion,

however, is that monotherapy with doxycycline is not appropriate, and poly-antimicrobial therapy is commonly pursued. The 1 dog in this study, dog 17, that had been prescribed doxycycline monotherapy was the only dog that developed progressive clinical signs within the months following initiation of treatment, leading to hemilaminectomy surgery before resolution of clinical signs could be achieved. The efficacy of the poly-antimicrobial therapy subsequently administered in this case was associated with a decrease in the quantitative antibody values on the CBM assay.

The quantitative nature of the CBM assay makes it an excellent tool for monitoring immune response over time, and this study supports the use of this assay for this purpose. A 40% decline in PO1 antibody values at 2 to 6 months posttreatment could be considered supportive of response to treatment. Antibody values may continue to decline after antibiotic treatment is discontinued, and it may take years for values to return to negative, as shown by the results of dog 3 (Figure 1). It is essential to continue to monitor the immune response after treatment is discontinued, as exemplified by the rebound of antibodies observed for dog 26. Recrudescence is not uncommon for *B canis,*[14] and stimulation of the immune response could be recognized by the CBM assay prior to the recurrence of clinical signs.

One limitation of this study is the lack of antigen detection through culture or PCR in 93% (28/30) of dogs, which is the gold standard means of *B canis* infection confirmation. However, since the AGID II assay is considered to have > 99% specificity,[24,25] we can be fairly confident that all dogs included in the study were infected with *B canis.* Due to the intermittent bacteremia and shedding of the organism in bodily fluids, culture or PCR has very poor diagnostic sensitivity in these cases and negative antigen detection diagnostics alone should never be used to rule out the possibility of *B canis* infection or for confirmation of response to treatment. Likewise, antigen detection testing (PCR and culture) should not be used to make regulatory quarantine decisions, due to its poor sensitivity, which was unfortunately the case with dog 2 who was allowed to live outside of quarantine if he tested negative every 6 months on whole blood PCR.

An additional limitation of this study was that the time points for follow-up serology were not consistent across cases; however, sufficient samples and time points were available to identify trends. The variability in treatment protocols, both antimicrobial drug combinations, doses, and duration varied substantially, preventing any meaningful conclusions about increased efficacy for any specific protocol. A well-designed prospective clinical study monitoring both the antibody response on the CBM assay and the presence of antigen over time in bodily fluids is warranted to better understand the efficacy of specific treatment protocols and the public health risks associated with keeping *B canis*-infected dogs as pets.

Conclusions

Overall, our study revealed that treatment and follow-up recommendations varied substantially between clinicians, and there is a need for a consensus on the best treatment and follow-up practices. Importantly, we identified substantial delays in diagnosis from the initial clinical presentation with back pain and/or lameness. We recommend screening for *B canis* in all young dogs that present with recurring lameness and/or back pain. Serologic monitoring after treatment to evaluate for recrudescence is necessary and appropriate, and the CBM assay is a valuable tool for this purpose. Further study is needed to determine the magnitude of the public health risks associated with maintaining *B canis*-infected animals as pets.

Acknowledgments

We express our gratitude to all the clinicians and clinic staff, rescue organizations, as well as referral laboratories and their staff, who were willing and able to provide patient records for inclusion in this study. We also acknowledge all the dedicated pet owners who were agreeable to have their pet's records shared for this investigation.

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