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Serum banking of the Mississippi shelter dog population to estimate seroprevalence of diseases affecting animal and human health

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By

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Shelter dog populations in the United States are poorly quantified and characterized, but may be effective targets for measuring the occurrence of select diseases affecting animal and human health. Dogs in this population may have increased risk for disease due to intrinsic and extrinsic risk factors. Accurate estimates of disease in this population require sound sampling strategies within a comprehensive sampling frame.

Knowledge of the prevalence of disease in the Mississippi shelter dog population is important for diagnostic test interpretation, shelter allocation of resources, and public health risk assessment. A serum bank provides a valuable resource to investigate both zoonotic diseases in which dogs are the primary reservoir, such as canine brucellosis, and for diseases where dogs may be effective sentinels for exposure risk, such as American trypanosomiasis. Implications of this research extend beyond Mississippi through the frequent movement of shelter dogs to adoption centers across the United States.

DEDICATION

This thesis is dedicated to my parents, Cynthia and Stephen Getty, and in loving memory of Richard Hubbard. Your endless encouragement, love, and wisdom have guided each of my steps. Although I still don't know the question to life, the universe, and everything, at least we know the answer…

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CHAPTER I

LITERATURE REVIEW

Shelter dog population characteristics and disease epidemiology

Shelter dogs pose unique challenges and opportunities for disease research. This population is poorly defined with complex movement dynamics, which complicates sampling methods to measure disease prevalence. Several intrinsic and extrinsic risk factors have been proposed to explain different rates of infectious disease seen between shelter dogs and the owned dog population, however, there is little research validating these assumptions. Increased knowledge of the determinants and occurrence of disease is important for animal shelter management and public health risk assessment, with shelter dogs potentially serving both as sources of zoonotic infection as well as useful sentinels for disease.

Estimating dog populations

Estimates of the numbers of both the owned and shelter dog populations in the United States are widely quoted, but lack consistency and are subject to sample bias. The American Veterinary Medical Association (AVMA) releases nationwide statistics compiled approximately every 5 years, including the percent of US households owning pet species and the average number of pets in each household. The most recent survey, released in 2012, estimated 69.9 million dogs in 43.3 million homes.[1] Similarly, the American Pet Products Association (APPA) performs a periodic national survey to

estimate pet ownership and spending habits of pet owners. The 2013-2014 APPA survey reported 83.3 million dogs in 56.7 million homes.[2]

Discrepancies between population estimates may be partially explained by increasing pet ownership between sampled years, however, they more likely indicate variability in sampling strategy and data analysis. These estimates are produced from a small number of households selected to represent the nationwide population based on gender, age, household size, income, and geographic region. In the most recent APPA survey, a total of 505 completed surveys were used to determine all dog information.[2] The accuracy of such estimates has frequently been challenged, both informally and formally. Patronek and Rowan provide one review comparing AVMA estimates to random-digit dialing performed in select areas. The national survey resulted in marked overestimation of the dog population, and the authors cite many potentially contributing factors including low response rates, response bias among pet owners, and selection bias in household eligibility criteria.[3]

A now-dated review by Marx and Furcolow found only 6 dog population studies in the United States which could be compared on the basis of data collected and sampling method. They report an owned dog-to-human ratio range of 1:4.7 to 1:13.7, indicating that calculation of total population numbers from such simple population characteristics is likely to result in a large margin of error.[4] Similarly, studies in select communities indicate wide geographic variation in pet ownership trends and between urban and rural areas.[5-7]

More advanced methods to estimate animal populations have also been applied to shelter dog populations. An early extrapolation of AVMA data combined with regional

private data resulted in an estimated shelter dog population of 6.6 million in 1991, with 3.75 million animals euthanized.[8] Patronek and Glickman developed a population dynamics model to estimate the pet dog population, which included an estimate of 4 million dogs in animal shelters, of which, 2.4 million are euthanized.[9] Capture-recapture methodology, commonly used in ecology, has recently been applied to estimate owned dog populations as well as the US shelter dog population.[10] These methods provide more conservative estimates consistent with the apparent downward trend in the number of shelter dogs. Comprehensive data from Ohio animal shelters showed a 17% decrease in the number of dogs entering shelters and a 39% decrease in the number of dogs euthanized between 1996 and 2004.[11] The American Society for the Prevention of Cruelty to Animals estimates that 3.3 million dogs entered shelters in 2017, with euthanasia of about 670,000 dogs, but sampling methodology is not described.[12]

Additional challenges for shelter populations

Shelter animal population estimates are especially nebulous due to lack of consensus as to what constitutes an "animal shelter". Organizations with a mission to rehome unowned animals range from brick-and-mortar physical locations, operated privately or through municipal funding, to independent foster or breed-rescue groups which operate solely out of private homes and may span several states. For our purposes, a shelter is defined as a physical facility which houses dogs that are available for public adoption. This encompasses municipal facilities and private shelters, but excludes animal sanctuaries and foster-only programs.

It can be difficult to locate shelters and determine if they meet inclusion criteria, even when clearly defined. At present, animal shelter registration is required in about half

of US states, usually through the state department of agriculture, public health division, or veterinary board. States that do maintain registries differ in criteria which require facilities to be registered including population size, private versus municipal/county run facilities, and regulated inspection, making comparisons between states difficult. Past efforts to consolidate such lists have not been sustained.[13]

When registry is not required within a state, shelters must be identified through a laborious process involving internet, social media, and personal contact. Shelters that do not have a website, social media profile, or other online presence are likely to be missed, and shelter information from all sources is frequently outdated. Identified shelters and associated animal groups often require direct communication to determine if shelter inclusion criteria apply due to ambiguous descriptions of some foster-only groups, support groups for local shelters that are not separate entities, and changes in shelter name, address, or contact information.[14] Failure to identify all shelters may underestimate shelter animal populations. Alternately, failure to exclude organizations that do not meet shelter criteria may result in an overestimation. Care must be taken when extrapolating population data from known shelters and applying this "average" intake to the expected number of shelters, as readily available data may over-represent well-resourced, high-presence facilities with large populations.

The constant movement of animals into, between, and out of shelters can make it difficult to accurately quantify the shelter population. Most shelters track animal intake (including strays, owner relinquishments, or transfers) and outcome (including adoption, return to owner, transport, or euthanasia). Population estimates based on intake numbers may be inflated due to duplicate counting of animals. For example, free-ranging owned

animals may enter a shelter numerous times through animal control, with each visit counted as a separate intake unless a unique identifier such as a microchip is available. Shelters may also exchange animals with partner shelters or foster groups, resulting in animals recorded as an intake at both the destination shelter and source shelter.

There is very limited information available on the shelter animal population of Mississippi. Indeed, with a lack of mandatory registration in the state, there is not even a good estimation of the number of animal shelters and associated groups operating within the state. The Mississippi Board of Animal Health maintains a limited list of shelters that have registered for supplemental state funding received through specialty automobile license plate fees, and the Humane Society of the United States has compiled an incomplete list of animal organizations in the state. Based on personal communication with animal shelter directors across Mississippi, annual intake at most animal shelters has steadily increased over the past 5-10 years, while euthanasia rates have decreased. This indicates that more animals are exiting shelters through local adoptions or transport programs.

Shelter dog epidemiology

Dogs may enter a shelter through several routes including owner surrender, return to the shelter following unsuccessful adoption, stray capture by animal control or members of the public, or transfer/transport programs. Each of these intake sources has unique or overlapping risk factors which may contribute to unequal rates of disease exposure between these subsets of the shelter dog population and from the owned dog population. Consideration of these risk factors is important when relevant to the transmission or pathophysiology of a specific disease.

It is reasonable to assume dogs entering shelters as owner surrenders may be similar to the owned pet population. However, there may be characteristics of relinquished dogs and their homes that contribute to both increased risk for relinquishment and risk for disease exposure. Salman et al. found that top reasons for dog relinquishment to shelters included pet illness, cost of pet maintenance, inadequate facilities, and too many animals in the household/lack of homes for litter mates.[15] Thus, relinquished dogs may be at increased risk for clinical disease, lack routine veterinary preventive care, have increased exposure to overcrowding or substandard housing conditions, and be more likely to be reproductively intact compared to the general owned pet population.

Stray dogs have often been targeted as high-risk populations for disease due to lack of preventive care and increased environmental exposure to wildlife and vectors.[16-17] Urban and rural stray dogs have differing exposures to domestic and wild animal populations, however, contact is likely much higher than in corresponding owned populations. Substantial geographic differences in disease seroprevalence may occur depending on suitable disease vector and host population densities, climate, and opportunities for transmission. Stray dogs are more likely to be reproductively intact, range across large territories, and scavenge for food, increasing risk for diseases transmitted through direct or oral contact.[18] Inadequate nutrition and parasitism may decrease immunity and make stray dogs more susceptible to clinical and subclinical disease.

Finally, dogs entering shelters through transport or transfer programs may serve as unique populations in terms of disease prevalence. Transport of shelter dogs occurs

both intrastate, between partner shelters, and interstate, from overpopulated regions with historically high euthanasia rates to regions where shelter dogs are in high demand. Diseases which occur with regional specificity or with varying prevalence by region may be transported along with infected individuals. Low index of clinical suspicion for non-endemic disease may result in failure to diagnose cases and may lead to disease dissemination within the dog population or to other susceptible species, including people. Such translocation of disease has been reported following mass movement of animals following a natural disaster, but may also frequently occur with routine movement.[19] Most shelter dogs are visually screened for signs of disease and receive routine diagnostics (e.g. fecal parasite exam and heartworm test), but rarely have a comprehensive medical work-up performed prior to transport.

Movement of shelter dogs is particularly common out of the southeastern United States. Although total numbers of transported dogs have not been reported, a small program operated through the College of Veterinary Medicine at Mississippi State University has moved over 4,600 dogs out of state since it was founded in 2007. It is estimated that an additional 60 such programs exist within the state of Mississippi (personal communication). This yields a conservative estimate of over 25,000 dogs transported annually out of Mississippi alone. Dogs in the southeastern United States may have greater risk for disease due to year-round presence of arthropod vectors and socioeconomic factors affecting animal perception and care. Increased prevalence of intestinal parasitism and vector-borne disease such as canine heartworm and West Nile Virus have been documented.[17, 20-21] Reports have also found the highest prevalence of canine brucellosis in the Southeast and have linked introduction of disease into new dog

populations with interstate movement of dogs.[22-23] Shelter dogs may thus serve as a route of dissemination for many diseases.

Prevalence of disease in shelter dogs

Studies have documented dramatically different rates of disease seroprevalence between owned and stray dogs. Reasons for these differences likely include a combination of the previously noted risk factors, such as differences in environment, behavior, and food sources, however, they may also reflect intrinsic dog characteristics such as the age, sex, and breed distribution of shelter dogs compared to the owned dog population. For example, Little et al. found that shelter dogs were almost 10 times more likely to be infected with hookworms compared to owned dogs in the Southeast.[20] Authors attribute much of this difference to the age distribution in sampled dogs, with dogs over 3 years of age comprising 50% and 16% of the owned and stray dog populations, respectively. Hookworms rarely parasitize adult dogs, so the critical risk factor for being infected is likely the differing age distribution between owned and shelter dogs rather than anthelmintic treatment. A clear understanding of shelter dog demographics is therefore essential for correct risk interpretation.

Differing demographics between owned and shelter dog populations are also important for diseases with known breed predilections. For example, Macintire et al. demonstrated that *Babesia gibsoni* is found predominately in American Pit Bull Terriers in the southeastern United States.[24] This breed is overrepresented in animal shelters in the Southeast due to popularity as pets, overbreeding, and breed-specific legislation or stigma which may limit adoptability. Disease rates in shelter dogs would be expected to be correspondingly high, even though there is no evidence that classification as a shelter dog

is itself a risk factor for babesiosis. Lim et al. also noted a breed bias when evaluating vector-borne diseases between hunting and shelter dogs. Shelter dogs were not seropositive for tick-borne diseases, however, the sampled shelter was in a heavily urbanized area with over 80% of the shelter dog population composed of toy-type breeds (including Maltese, Shih Tzu, Yorkshire terriers, and poodles) typically kept in homes and not exposed to vectors through outdoor recreation.[25]

Sex, including neutered status, is often evaluated as a risk factor for disease. However, this seemingly simple classification can be difficult to apply to shelter dogs, especially in seroprevalence studies. Many animal shelters actively spay and neuter intact dogs that enter the shelter, making it very difficult to determine if disease exposure occurred before or after castration. For example, Brown et al. found that female dogs were more likely to be seropositive for *Brucella canis* than male dogs, but did not differentiate sexually intact from non-intact dogs.[26] Reproductive status is an important risk factor, with transmission primarily occurring through whelping or breeding, and because zoonotic risk is believed to be much greater in intact dogs. A cross-sectional study which evaluates dog characteristics and sample results at a single time can be misleading if disease exposure and seroconversion occurred prior to castration, or if female reproductive status is unknown; such a study would likely fail to identify being reproductively intact as a risk factor, even though it represents the most important route of exposure.

For other diseases, extrinsic factors more readily explain disease occurrence. A study of Lyme disease in Spain documented the highest seroprevalence among stray dogs and those used for hunting or herding compared to pet or watch dogs. Although breed

differences are expected within these groups, study authors attributed findings to low use of acaracides in dog groups with high exposure to ticks.[27] Similarly, *Trypanosoma cruzi* titers were increased in both rurally owned dogs and stray dogs which had exposure to vector and mammalian hosts, and seroprevalence of West Nile Virus in strays was almost double that of family dogs.[17,28] In still other cases, such as with fecal shedding of *Salmonella* and *Campylobacter* spp., an interplay of extrinsic and intrinsic factors may contribute to higher positivity rates in shelter dogs compared to owned dogs.[29] Stray dogs may have greater environmental exposure leading to infection, or stray dogs may be more susceptible to colonization and shedding due to immunosuppression from the effects of poor nutrition, stress, and concurrent disease.

Conclusions

The shelter dog population has unique risk factors that may contribute to differing prevalence of disease from the owned dog population. Knowledge of disease prevalence in this population is therefore essential for correct application and interpretation of diagnostic tests and risk assessment in animal shelters. In order to determine disease prevalence, sampling must be performed in such a way that bias is minimized, and with sufficient power to allow for assessment of intrinsic and extrinsic risk factors. This requires a representative sampling frame, which is currently unknown for Mississippi.

The author proposes a study combining methodology from several regional shelter surveys to determine the number and distribution of animal shelters and shelter dogs in the state. Components include shelter-finding, verification of inclusion criteria and direct data collection, and data analysis including standardization to minimize recognized sources of bias.[11,30-32] A census of Mississippi animal shelters will provide a

baseline for future trend monitoring of the shelter dog population within the state and serve as the sampling frame for seroprevalence research.

Brucellosis: do shelter dogs pose a significant zoonotic risk?

Introduction

Of the recognized zoonotic diseases, perhaps none is so intimately linked to human history as brucellosis. It has been the proposed etiology for vertebral lesions on an early hominin skeleton from South Africa dating back 2.5 million years ago, making it the earliest reported infectious disease of humans, and has been hypothesized as the 5th Biblical plague of Egypt.[33-34] *Micrococcus melitensis* was first isolated from British troops as the causative agent of Malta fever in 1887 by Dr. David Bruce, for whom the genus would eventually be named. Just a decade later, L. F. Benhard Bang identified a similar bacterium in cattle which would earn his moniker and become one of the most notorious diseases of veterinary medicine. The zoonotic link between human and animal health was confirmed in 1905 with the isolation of the bacteria from the milk of healthy goats, while research by Alice Evans was pivotal in the development and widespread acceptance of milk pasteurization guidelines in the United States.[35]

Despite centuries of coexistence and over 100 years of study, brucellosis remains a significant global risk to human and animal health. The World Health Organization has gone so far as to state, "We regard brucellosis as the world's most widespread of all zoonoses and apart from its toll on people, it has an enormous impact on the animal industry".[36] Though uncommon in most of the developed world, there are over half a million human cases annually which result in chronic, debilitating illness and require prolonged, multi-drug antibiotic regimens.[34] Even more alarming, this number likely falls far short of the truth, as the disease is insidious, with non-specific clinical signs, and occurs most frequently in low income regions where risk for under-diagnosis is high.[37]

Mortality rates are low, but relapse and bacterial persistence for months to years after resolution of signs is common.[38]

Taxonomy and Global Occurrence

Brucellosis is caused by gram negative, non-motile, non-spore forming bacteria in the family Brucellaceae. Following oral, aerosol, or contact exposure, the bacteria invade dendritic cells and take up intracellular residence.[39] From this immunologically protected location, the bacteria interfere with normal host cell functions including apoptosis. Thus robbed of the means to fight the bacteria directly or instigate programmed death of infected cells, hosts harbor the bacteria with high numbers present in tissues or shed in bodily fluids. In addition to horizontal transmission, the bacteria can also be vertically transmitted, with cellular targets including placenta, sperm, and the mammary gland.[37]

Although brucellosis has a significant impact on human health, it is primarily maintained within a small number of specific animal host species. Prior to 1985, six classical *Brucella* species had been identified: *Brucella melitensis* in sheep and goats, *B. abortus* in cattle, *B. suis* in swine, *B. neotomae* in rodents, *B. canis* in dogs, and *B. ovis* in sheep.[37] This static phylogeny has recently been shaken with the addition of four new species since 2007: *B. ceti* in whales and dolphins, *B. pinnipedialis* in seals and sea lions, *B. microti* from the common vole, and *B. inopinata* isolated from a human. There are additional potential species awaiting classification, including a strain isolated from Australian rats and one identified as the causative agent of abortion in non-human primates.[40]

Not only are new *Brucella* spp. being identified, there is also documentation of the classical species in new hosts. Cattle serve as the natural hosts for *B. abortus*, but they

can also be infected with and shed *B. melitensis* as well as *B. suis* in milk.[36] Wildlife is also at risk. Freshwater river fish have become infected by feeding on contaminated meat, and the elk and bison around Yellowstone National Park are an infamous lingering source of *B. abortus* in the United States.[40] *Brucella suis* has the widest host range, with documented infections in domestic dogs, bison, elk, fox, hare, African buffalo, reindeer, caribou, chamois, and ibex. The importance of these new host species is largely unknown; in some instances, such as *B. suis* in cattle, the disease appears to be relatively self-limiting, while in other cases these species serve as unexpected maintenance hosts.[36]

Brucellosis is considered a re-emerging zoonosis, with incidence of disease in human and animal populations affected by social, economic, political, and surveillance factors.[35] Global incidence is unknown, with reports in endemic areas ranging from less than 0.01 to more than 200 cases per 100,000 people.[36] In the United States, brucellosis cases have dropped from a peak of 6,321 in 1947 to about 100 per year since 1998, largely attributed to widespread milk pasteurization and a national eradication program.[35] Even within the United States, there has been a dramatic shift in brucellosis ecology. *Brucella abortus* cases were most common prior to the 1960s, followed by a predominance of *B. suis* in slaughterhouse workers in the 1970s. Today, brucellosis in the United States is mainly an imported disease, contracted while traveling abroad or through contaminated cheese and dairy products originating in Mexico and linked to the higher incidence seen in Hispanic populations in Texas and California.[34] Occasional human cases also occur through contact with feral swine or exposure to infected domestic dogs.

Diagnostic Challenges

Definitive diagnosis of brucellosis poses several challenges. Infected people and animals are often asymptomatic or have vague clinical signs, including undulant fever and arthralgia, which may go undiagnosed in non-endemic regions due to low physician awareness of the disease.[41] Laboratory tests are complicated by the close genetic similarity among the *Brucella* spp. and cross reaction with *Yersinia enterocolitica* 0:9 and other gram negative bacteria which result in false positives on common serological screening tests.[36] Serological tests may also result in false negatives due to prozoning, or antibody excess, where insufficient antigen is present in an assay to create antibody cross-linking and visible agglutination. Many brucella tests have been developed and are used in various combinations due to the inherit limitations of each.

Culture of *Brucella* spp. from the blood or tissues has been traditionally considered the gold standard, with some authors considering it "essential" for diagnosis.[42] Although infected animals have a prolonged bacteremia, intermittent periods of abacteremia may occur and result in false negatives.[43] Additionally, *Brucella* spp. are fastidious and can be difficult to culture, with low numbers of bacteria typically found in the blood.[22] In one study, culture-positive and culture-negative dogs all demonstrated histopathological lesions consistent with brucellosis, and dogs were equally likely to be positive by polymerase chain reaction (PCR) whether they were culture positive or negative.[44] Clearly, culture does not identify every infected individual, and is dependent on laboratory experience with the agent and quality of the diagnostic sample. This creates a quandry for assessing performance of other diagnostic tests when culture alone is used to determine "true" infection status.

Tests to detect Brucella antigen or DNA, including indirect fluorescent antibody tests and PCR assays, have been developed but are not readily available or well validated.[42] Individual laboratories have developed multiplex PCRs including the AMOS and Bruce-ladder PCR which can identify the 4 smooth species (*B. melitensis, B. abortus, B. suis,* and *B. neotomae*) and 6 classical species (including *B. canis* and *B. ovis*) respectively, following successful culture. More advanced analyses, such as variable number of tandem repeats, may be useful in epidemiologic trace-backs, differentiating relapse from reinfection, and to help identify vaccine candidates.[37] However, to date, these opportunities have not been realized.

Serology is most commonly used for initial screening for brucellosis. Serological tests targeting the O-antigen of the lipopolysaccharide (LPS) molecule do not differentiate among the naturally occurring smooth colony forming species, however, there is not cross reaction with rough colony forming species (*B. canis* and *B. ovis*) that lack LPS on the cell surface.[45] This may result in failure to diagnose human cases of *B. canis,* for which there is no routine screening test. Most tests used to screen livestock for brucellosis use *B. abortus* antigen, while tests used to detect canine brucellosis use either *B. ovis* or a non-mucoid variant of *B. canis* which produces less cross-reaction than traditional *B. canis* antigen tests.[42,45]

Several serological tests are approved for testing of livestock prior to international trade including the enzyme-linked immunosorbent assay (ELISA), fluorescence polarization assay (FPA), rose-bengal test (RBT), buffered acidified plate antigen (BAPA) test, and complement fixation test (CFT).[46] A variety of tests have also been used to detect disease in dogs, with the tube agglutination test (TAT) being widely used

in the 1970's after the identification of *B. canis.*[42] Tests often incorporate 2-mercaptoethanol (2-ME) to reduce disulfide bridges between immunoglobulin M (IgM) antibodies and limit non-specific agglutination with other gram negative bacteria. More specific IgG antibodies have fewer disulfide bridges and agglutinate even in the presence of 2-ME, improving test specificity and reducing false positive tests. False negative tests can occur in the first 4-6 weeks of an infection, prior to the development of a strong IgG response.[45]

Tube agglutination tests are performed via serial dilution and provide semi-quantitative measures of antibody present within a sample. Interpretation of a test as positive or negative is dependent on the cutpoint assigned. Samples with agglutination at a dilution ≥1:200 are usually considered positive for brucellosis, while those without agglutination at 1:50 are negative; samples with complete or incomplete agglutination between these values are often termed suspect with additional testing recommended.[42] This interpretation is not unanimous, making it difficult to compare studies using different cutpoints to determine seroprevalence. One noteworthy case found an overall *B. canis* seroprevalence of 67.8% in people with average exposure to dogs. The researchers considered samples positive if agglutination occurred at a dilution of 1:12,[47] with harsh criticism that this cutpoint greatly overestimated human exposure.[43] Currently, TATs or similar semi-quantitative ELISAs may be most useful as a way to monitor therapeutic response in treated animals.[42,48]

A commericial rapid slide agglutination test (RSAT) has mostly replaced the more laborious TAT for initial testing of dogs for canine brucellosis. Shortly after its development, the test earned a reputation for producing false positivies in dogs

apparently uninfected by blood culture and other serological tests, but was touted for lack of false negatives.[43] The test has retained this reputation, despite more recent evidence that the test had modest sensitivity and specificity of 70.6% and 83.3%, respectively, compared against a "true" disease status determined by a combination of clinical disease, culture, and PCR of blood and genital samples.[49] These values are reported for test performance on the commericial product, with the claim that it "immediately separates negative dogs from those potentially infected".[50] This statement is in direct contradiction to study authors who note that "the occurrence of false-negative results observed in this study indicate that these tests should be carefully employed as screening tests for canine brucellosis diagnosis, because a significant proportion of the infected dogs were not detected".[49]

Additionally, the commercial test kit does not report diagnostic performance with the addition of 2-ME following a positive test. Keid et al. found a sensitivity and specificity of 31.8% and 100%, respectively, when samples were tested with the 2ME-RSAT, however, no study has reported overall test performance when conducted in series as per test instructions.[49] Based on the work of Keid et al., the commercial RSAT, performed with follow-up addition of 2-ME to positive samples, is most useful to confirm that a dog is infected, but serves poorly as a screening test due to low diagnostic sensitivity. This stark opposition to earlier findings has unfortunately been overlooked in current diagnostic testing recommendations from organizations ranging from the American Kennel Club to public health departments. A better understanding of the RSAT test performance compared to "true" disease status is needed, along with a paradigm shift in veterinary diagnosis of canine brucellosis.

Brucellosis epidemiology in dogs

Dogs are the natural hosts for *B. canis*, but can also be infected with *B. suis*, *B. melitensis,* and *B. abortus* through contact with domestic or wild animals.[51-55] Transmission of all *Brucella* spp. occurs predominately through breeding and parturition, with the highest loads of bacteria shed in placenta and birthing fluids during abortion.[43] Infection can also occur via consumption of contaminated milk or meat, or contact with blood, urine, and saliva.[56] Infected dogs may remain bacteremic for at least 2 years.[43]

Infected dogs are often asymptomatic or show classic signs of reproductive failure including abortion and infertility. Male dogs may develop orchitis or epididymitis, with localization of bacteria within these sites leading to abnormal sperm or aspermatogenesis.[43] Infected female dogs may fail to carry a litter to term, give birth to healthy puppies, or transmit the infection vertically or horizontally through reproductive materials.[22] Intact bitches also appear to be at risk for recrudescence during estrus with a transient increase in antibodies levels measured in subsequent heat cycles.[57] Other relatively common clinical presentations include endophthalmitis and uveitis, or discospondylitis with associated neck or back pain.[22,48,58] Hematological parameters in infected dogs are usually normal or show only leukocytosis.[48]

Treatment of infected dogs is difficult and carries risk for recrudescence, as in human patients. Tetracyclines show good in vivo efficacy against *B. canis*, and have been used alone or in combination therapy.[58-61] Other common therapeutic regimens have used aminoglycosides, fluoroquinolones, and rifampin in various combinations and with variable success.[57,62] Most dogs show clinical improvement within the first two weeks of therapy and are abacteremic within 4 weeks of starting treatment.[48,58]

Serological tests may remain positive for long periods of time following treatment and resolution of clinical signs. Ledbetter et al. found a median time to seronegativity of 96 weeks with a range of 36 to 112 weeks in three dogs with unilateral uveitis.[48] Wanke et al. reported that all treated dogs in a breeding kennel were serologically negative 14 months after initial treatment, however, female dogs received additional antibotitic courses during subsequent estrus cycles.[57] Use of serology to monitor response to treatment and time to seronegativity is often recommended, however, the relationship between antibody levels and treatment success is poorly understood and guidelines are not well established.

Brucella canis in dogs in North America

Brucella canis was first described and identified in a population of breeding beagles in the late 1960's, followed shortly after by the first human case acquired from an infected dog.[63-66] Research during the following decade provided insight into pathogenesis, diagnosis, and treatment, however, *B. canis* was classified as a low zoonotic risk with clinical importance primarily limited to a causative agent of abortion and infertility in breeding kennels.[22] Positive cases are identified most often through outbreaks of disease in breeding kennels or with apparent clinical signs in companion animals.[22-23]

Serological studies measuring disease occurrence in asymptomatic dogs in North America have sporadically been reported (Table 1.1). Comparisons of prevalence between studies is difficult due to differences in populations sampled and diagnostic testing procedures performed, however, stray dogs have consistently higher seroprevalence compared to owned animals. In most studies, samples were collected

using convenience methods and may poorly represent the target population. Intrinsic risk factors for disease such as breed, sex, and age have rarely been reported. Additionally, the majority of the studies were performed shortly after the identification of *B. canis,* with very little recent information available on seroprevalence in the United States.

At least 30 cases of *B. canis* in humans have been attributed to contact with infected dogs globally, including an outbreak of 6 people who all developed disease from a single pet dog and affected litter.[67] A human case in Jackson, Mississippi, was diagnosed in 2016 following contact with a stray dog that aborted a litter of puppies while in a foster home (personal communication). Canine cases were subsequently made reportable to the Mississippi Board of Animal Health due to the zoonotic risk posed, and there is current interest in tracking surveillance information including clinical case information to assess canine risk factors.[68]

Table 1.1 Prevalence of *B. canis* in North America

Location	Population	Diagnostic Test	Prevalence	Source
FL	274 shelter dogs from 21 facilities in 16 counties	ME-TAT	3.65% (10/274)	Hoff and Nichols, 1974[69]
TN	121 stray dogs; 107 owned dogs	ME-TAT	Stray: 6.6% (8/121) Owned: 1.9% (2/107)	Fredrickson and Barton, 1974[70]
TN	235 stray dogs; 67 owned dogs	Titers (test unspecified)	Stray: 9.4% (22/235) Owned: 0%	Lovejoy et al., 1976[71]
GA	100 stray dogs from an animal shelter; 100 pets	RSAT followed by ME-TAT	Stray: 9% (9/100) Pet: 1% (1/100)	Brown et al., 1976[26]
MS	147 owned dogs and 13 stray dogs sampled from an air force base	RSAT followed by ME-TAT	Stray: 7.6% (1/13) Owned: 0%	Galphin, 1977[72]
Quebec, Canada	341 randomly sampled dogs submitted to diagnostic laboratories for unrelated testing	RSAT followed by ME-TAT	RSAT: 20.2% (69/341) ME-TAT: 1.8% (6/341)	Higgins et al., 1979[73]
WI and IL	2,572 shelter dogs from eight counties	RSAT, ME-TAT, blood culture	RSAT: 6.7% ME-TAT: 1.5% Culture: 0.2%	Boebel et al., 1979[74]
MI	499 urban stray dogs; 123 suburban stray dogs	ME-TAT	Urban: 8.6% (43/499) Suburban: 5.7% (7/123)	Thiermann, 1980[75]
Ontario, Canada	555 kennel clubs/ breeders; 1,4445 laboratory samples unrelated to brucellosis testing	RSAT followed by ME-TAT and AGID	RSAT: 5% (100/2000) TAT: 31 suspicious and 1 positive AGID: 6/100 Overall: 0.3%	Bosu and Prescott, 1980[76]
OH	200 stray dogs at a single shelter; 470 owned dogs from veterinary clinics	ME-TAT	Stray: 1.5% Owned: 0.4%	Pue, 1983[77]
WI	510 samples submitted to diagnostic lab for testing	RSAT followed by ME-RSAT	'03-'04: 4.6% (8/174) '05: 26.8% (85/317)	Brower et al., 2007[23]

Studies are listed chronologically by publication date.

Brucella suis in dogs

Brucella suis has the broadest host range among the known *Brucella* spp. (Table 1.2), although domestic and feral swine are the maintenance hosts for most biovars. The disease has been eradicated from domestic swine in the United States, but feral swine remain reservoirs for disease. Swine were first introduced into the United States during the European settlement in the 1400's as a meat source. Subsequently, feral swine have established populations through intentional release of both the Eurasian wild boar and domestic swine for hunting, by escaping from confinement operations or game reserves, and through abandonment.[78] Currently, feral swine are present in at least 39 states with an estimated population over 5 million.[79] Feral swine carry over 30 bacterial or viral diseases and 37 parasites, including 8 zoonotic diseases, in the United States, and remain as reservoirs for brucellosis, pseudorabies, and bovine tuberculosis, placing national disease-free status at risk.[79-80]

Table 1.2 *B. suis* biovar hosts and distribution

BIOVAR	HOSTS	GLOBAL DISTRIBUTION
1	Domestic and feral swine	Majority of feral swine cases in the US
2	Domestic and feral swine, European wild hare population	Low human pathogenicity; results in pathognomonic intramuscular abscesses in wild hares in Europe
3	Domestic and feral swine	Present in feral swine in the corn belt of the US and Hawaii
4	Reindeer and caribou; spill-over to rodents, foxes, wolves, and sled dogs	Zoonotic risk through consumption of raw milk, meat, and bone marrow in the Arctic
5	Rodents	Limited to Australia, Kenya, and Siberia

Information summarized from Aparicio.[80]

The prevalence of *B. suis* appears to be increasing in the United States, along with feral swine range. A 2010-2012 study found at least one serologically positive animal in 7 of 8 states surveyed, and demonstrated that under-reporting may be a serious concern as only 52% of culture positive animals tested positive by serology at a nationally certified brucellosis laboratory.[82] Feral swine have been implicated in the introduction of brucellosis to three domestic swine herds and one cattle herd, and have been associated with transmission of brucellosis to several feral swine hunters through dressing or consumption of game.[55,79,83]

Brucella suis has rarely been reported in domestic dogs, with the exception of biovar 4 in the Arctic, however, low apparent prevalence may be a result of failure to test for the disease.[55] *Brucella suis* was identified in a dog as early as 1931,[84] but the majority of canine testing is targeted at *B. canis* which does not cross-react with *B. suis*. Early experimental infection of beagle dogs with *B. suis* resulted in asymptomatic infection, but bacteria were isolated from the spleen, lymph node, kidney, and salivary gland, with hypothesized potential for human infection from infected canine urine or saliva.[56] Natural infection with *B. suis* was identified as the causative agent of a dog presenting for hind limb lameness,[51] and a recent study in Georgia identified 9 of 674 dogs serologically positive for *B. suis*.[52] Bacteria were isolated from samples submitted on two dogs in the latter study, and all serologically positive dogs were used for feral swine hunting. Transmission routes are unknown, but ingestion of carcass or birthing materials seem likely.[55]

Mississippi has both a robust feral swine population carrying highly pathogenic biovars of *B. suis*[85] and a large free-ranging dog population which may come into contact

with wildlife. Although dogs used for feral swine hunting are at greatest risk for contracting disease, the zoonotic risk posed by dogs might be highest for stray dogs entering a shelter which may then be adopted into a home and brought into close contact with family members. The zoonotic potential of *B. suis* in dogs is unknown; however, *B. suis* in natural hosts carries substantial zoonotic risk, resulting in potential transmission of a debilitating disease that is challenging to diagnose and treat.[55] Knowledge of the seroprevalence of *B. suis* in the shelter dog population may help quantify this risk.

Conclusions

Brucellosis in dogs has received little attention except as a cause for reproductive failure in breeding populations. Almost all reports of disease occurrence in dog populations were performed in the first two decades following *B. canis* identification, with little recent information available on the current epidemiology in domestic dogs in North America. Serosurveys of several dog populations, including breeding dogs, pet dogs, and stray or shelter dogs have failed to identify intrinsic dog risk factors such as sex or breed, however, there is evidence that stray dogs have considerably greater likelihood for being seropositive compared to owned dogs. A serosurvey of a single Mississippi location identified a positive stray dog,[72] while surrounding southeastern states have found a seroprevalence between 3-9% in shelter dogs (Table 1.1).

The discovery of domestic dogs in the Southeast infected with *B. canis* and recent human brucellosis cases contracted from dogs continues to provide evidence of zoonotic potential, despite relatively rare documented transmission to people. Shelter dogs may serve as an important high-risk population, with dogs frequently entering shelters as intact, free-roaming strays with increased wildlife and dog-to-dog contact. Apparently

healthy dogs infected with brucellosis may pose a local risk for human and dog populations, as well as a route for disease dissemination through interstate shelter animal transport programs which relocate animals from overpopulated shelters to regions of the country where shelter dog availability is low. Prevalence data is necessary to assess the public health risk posed by brucellosis and for correct application and interpretation of diagnostic tests. Effective control of canine brucellosis will require improved surveillance, along with education of the general public and veterinary practitioners on this difficult to diagnose disease.

American Trypanosomiasis: can shelter dogs serve as sentinels to evaluate human risk?

Impact on Human Health

Chagas' Disease

Trypanosoma cruzi is a protozoan parasite and the causative agent of Chagas' disease in people. In the century since its discovery by Carlos Chagas in Brazil,[86] Chagas' disease has been extensively researched and several large-scale control programs have been implemented. Despite these efforts, the disease still contributes the greatest burden of parasitic disease in the Americas, accounting for 40% of disability adjusted life-years lost to all parasitic and vector-borne diseases.[87] Although much less common than in South America, in the United States over 300,000 people are believed to be infected. The most common manifestation of disease is cardiomyopathy, affecting 30,000 to 45,000 Americans each year. Additionally, an estimated 63 to 315 congenital cases occur annually in the United States, contributing to the high health care costs of the disease.[88]

Humans contract Chagas' disease by one of three primary routes: stercorarian (fecal origin), congenital, or oral. Members of the Reduviidae family, commonly known as kissing bugs or cone-nosed bugs, are biological vectors and carry the parasite in their gut. Infection occurs when these nocturnal insects defecate while feeding on the blood of sleeping people. Parasites in feces enter a host through the bite, which is often located near the eyes or mouth, or across mucous membranes, and may result in the characteristic unilateral palpebral swelling known as the Romaña sign.[89] Congenital transmission may occur in up to 10% of infected mothers and represents an important route in regions where the vector is not present.[90] Oral transmission has been reported following ingestion of infected bugs or products contaminated by bugs or their feces; this route is currently

limited to the Amazon region and certain high-risk foods and drinks.[91] In the United States, most new cases result from congenital transmission or through contaminated donor products such as blood and solid organs. Competent vectors are widely present in the southern half of the United States and may serve as an important risk for stercorarian transmission, however, to date, autochthonous cases are rare.[92-93]

The majority of people infected with *T. cruzi* do not show clinical signs, while approximately 20-30% develop severe cardiac disease or gastrointestinal illness. Disease pathogenesis and reasons for variability in response to infection is poorly understood, but may reflect differences in host immune response, virulence of the infective *T. cruzi* strain, or superinfection of an individual with multiple *T. cruzi* strains.[94] Acute disease occurs 1 to 2 weeks after vector-borne transmission and is characterized by presence of trypomastigotes in the blood. Most cases are asymptomatic or present with mild fever, malaise, hepatosplenomegaly, and lymphocytosis. Swelling at the infection site (chagoma) or eyelid edema (Romaña sign) is uncommon but diagnostic. Serious infections occasionally result in meningoencephalitis or myocarditis which may be life threatening.[89] Diagnosis during the acute phase is typically made by visualization of the parasite in blood smears, culture, or positive polymerase chain reaction (PCR).[94]

Resolution of the acute phase occurs with clearance of parasitemia in 4 to 8 weeks. People then enter the indeterminate phase, which is an asymptomatic period of infection with intracellular amastigotes potentially lasting for life. Diagnosis is made via positive serology with corresponding lack of evidence of cardiac or gastrointestinal disease.[94] Progression to the determinate phase occurs in some individuals, with development of electrocardiogram (ECG) abnormalities and progressive cardiomyopathy or development

of gastrointestinal Chagas' disease characterized by motility disorders and subsequent dilation of the esophagus, colon, or both. Chagas' cardiomyopathy results in the greatest burden of disease with severe cardiac dysfunction and risk for sudden death from heart failure or thromboembolism.[95]

Treatment

Treatment options for Chagas' disease are limited. Nifurtimox and benznidazole are effective in treating *T. cruzi* infections, however, neither are readily available and dose-dependent side effects complicate use, especially in asymptomatic cases. Benznidazole has been approved for use in children 2 to 12 years of age but is currently only available through the Centers for Disease Control and Prevention (CDC). Side effects are less severe than with use of nifurtimox, especially in children, but may include allergic dermatitis, reversible peripheral neuropathy, insomnia, anorexia, and bone marrow suppression.[96] Nifurtimox is not approved for treatment of *T. cruzi* but can be obtained under investigational protocols. Gastrointestinal side effects are most common, occurring in up to 70% of patients. More serious side effects such as paresthesia and polyneuropathy have been reported.[94]

Antitrypanosomal drugs are most effective in acute disease and when used early in congenital infections, reducing both severity and duration of disease. More recently, treatment has also been recommended in chronic cases, with evidence of conversion to seronegativity in children 3 to 4 years after treatment.[94] Current recommendations also advise treatment of chronic cases in adults, however, a large randomized placebo-control trial failed to detect a reduction in the progression of cardiac disease in individuals receiving treatment.[97] Treatment of Chagas' cardiomyopathy and gastrointestinal Chagas'

disease does not differ from other causes of heart disease and idiopathic motility disorders, and guidelines have been established to monitor disease progression from the indeterminate to determinate stage.[94]

Disease control and emergence

Four large intergovernmental Chagas' disease control programs have been implemented in South America since 1991: the Southern Cone, Central American, Andean Pact, and Amazonian Initiatives.[98] These programs have achieved notable success in Latin America, with a reduction in annual new cases from 700,000 in 1990 to 41,200 in 2006.[99] Methods of vector control and subsequent interruption of transmission have included insecticide use, improvements to rural housing where the vector kissing bugs reside, public education, and intensified blood product screening.[100] Despite these positive trends, the global burden of Chagas' disease is estimated to exceed US$600 million in annual health care costs and 800,000 disability-adjusted life-years.[101]

Increasingly, Chagas' disease is recognized as an emerging disease in many parts of the world, primarily in the immigrant populations of North America, Europe, Australia, and Japan. The United States and Canada account for 18.9% of annual global health-care costs associated with Chagas' disease, a value which could increase considerably if the disease becomes endemic in areas where competent vectors are currently located.[101] In the United States, Chagas' disease remains an important public health risk for congenital transmission in Latin American immigrate populations, through possible transmission in donor blood or organs, and due to poor surveillance to detect autochthonous cases.[102]

Epidemiology of American trypanosomiasis

Life cycle and genotypic diversity

Trypanosoma cruzi has a complex life cycle, requiring a reduviid vector (subfamily Triatomae) and a mammalian host to undergo maturation through all three morphological forms.[103] In the traditional route of transmission, metacyclic trypomastigotes are passed in the feces of a kissing bug and enter a mammalian host through the bite wound or exposure to a mucous membrane. The flagellated parasites may infect macrophages or become intracellular amastigotes which replicate via binary fission and transform back into trypomastigotes once released into the blood stream.[104] Hematogenous spread can result in disseminated infection, with cardiac and neural cell trophisms. Triatomine bugs feeding on an infected mammal ingest circulating trypomastigotes, which differentiate into replicative epimastigotes in the insect midgut. Once in the hindgut, epimastigotes, characterized by a kinetoplast located anterior to the nucleus, transform into the infective trypomastigote stage, in which the kinetoplast is located posterior to the nucleus.[103]

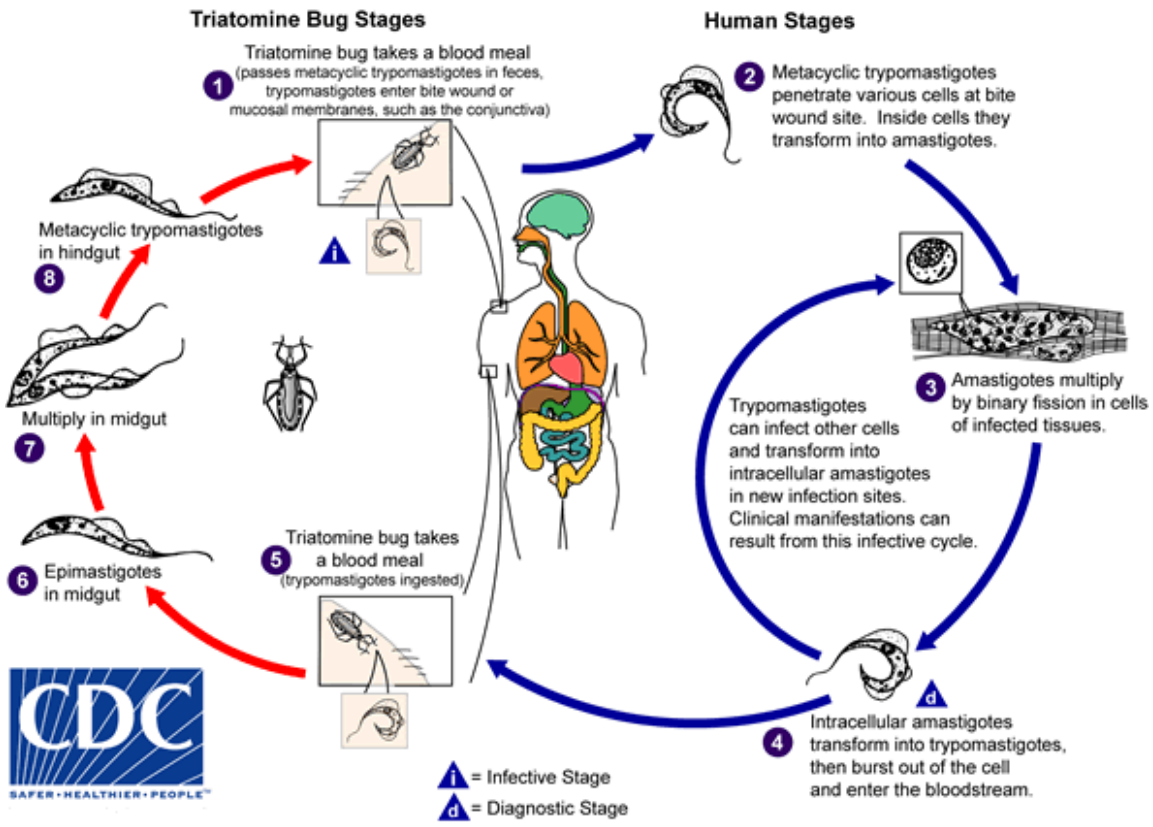

Figure 1.1 Lifecycle of *T. cruzi*

Image from the Centers for Disease Control and Prevention.[102]

The diversity of *T. cruzi* was recognized shortly after its discovery, contributing to the variability of clinical signs, severity, and occurrence of Chagas' disease. Phenotypic differences have been substantiated by molecular typing, leading to several different classification schemes for different strains of the organism.[105] Early work using multi-locus enzyme electrophoresis (MLEE) identified two distinct strain-groups,[106] which have subsequently been split into six discrete typing units (DTUs) based on 24Sα rRNA and mini-exon gene analysis.[107] Arguments have been made for the speciation of strains based on genetic variation and niche specificity, however, to date, formal reclassification has not occurred.[105]

Genotypes of *T. cruzi* demonstrate differences in epidemiology, including traditional host and vector species (Table 1.3). Two genotypes have been identified in the United States: *T. cruzi* I is believed to exist in a sylvatic cycle with Virginia opossums and has been isolated from triatomine vectors as well as autochthonous human cases, while *T. cruzi* IIa occurs in other placental mammals including raccoons, skunks, and domestic dogs.[108] Isolates from the United States show a high degree of genetic variation, providing evidence that the agent has existed in North American wildlife for a long period of time.[109] Molecular typing may prove useful in tracking disease emergence in non-endemic countries and in new peridomestic cycles where suitable hosts and vectors are located.[110]

Table 1.3 *T. cruzi* genotypic features

Genotype	Niche	Sylvatic hosts	Sylvatic vectors	Geography
TcI	Primary: arboreal, lowland topical/semi-tropical Secondary: arid rocky	Primary: American opossums, primates, arboreal rodents Secondary: terrestrial rodents	Primary: *Rhodnius* spp. Secondary: *Panstrongylus* spp., *Triatoma* spp.	Primary: Southern USA, Central and South America Secondary: North of Amazon (Central Brazil, Eastern Andean foothills)
TcIIa	Arboreal	Primates, armadillos, bats, raccoons (USA)	*Rhodnius* spp., *Panstrongylus* spp., *Triatoma* spp.	Northern South America, USA
TcIIb	Rare in sylvatic cycles	Atlantic forest primates, armadillos	?	Atlantic/Central Brazil
TcIIc	Terrestrial/ burrowing	Armadillos, rodents, marsupials, carnivores	*P. geniculatus, P. lignarius, T. rubrovaria*	Lowland South America
TcIId	Rare in sylvatic cycles	Suspect armadillos	?	Southern cone
TcIIe	Rare in sylvatic cycles	?	?	Southern cone

Adapted from Miles et al.[110] Question marks indicate unknown host and vector species.

Triatomine Vectors

In addition to genetic variation in *T. cruzi*, the epidemiology of American trypanosomiasis is complicated by the diverse feeding habits of reduviid vectors. Over 130 species of triatomine insects have been reported in North and South America, however, a relatively limited number are associated with occurrence of Chagas' disease as a result of their feeding preferences and likelihood to adapt to human dwellings.[92] All triatomine species are hematophagous, and both males and females require blood meals for maturation. Repeated blood feeding over the life of the insect results in higher prevalence of *T. cruzi* infection in adults than nymphal stages, including a greater likelihood for an insect to be infected with multiple DTUs.[111] Triatomine species occur in

distinct ecological niches, but are nonspecific in the hosts on which they feed. Utilizing a wide variety of hosts has been associated with both parasite persistence and amplification or, alternatively, reduction due to a dilution effect depending on the host-vector-agent interaction.[112]

Triatomine insects occur throughout the southern half of the United States, with eleven total reported species. The greatest variety exists in Texas, Arizona, and New Mexico with at least five species occurring in each state. Two species have the widest reported ranges: *T. protracta* occurs across the entire southwest from California to Texas, while *T. sanguisuga* occurs in the east from Texas to the Atlantic coast and as far north as Pennsylvania. Blood meal sources for these insects is highly variable, with at least 24 mammalian species serving as hosts for *T. cruzi*. Woodrats appear to be the primary reservoir of *T. cruzi* in the western United States, whereas high prevalence occurs in raccoons, opossums, armadillos, and skunks in the eastern United States.[92]

In a study performed in Texas, five triatomine species contained blood meals from nine vertebrate hosts, including woodrats, dogs, cats, cows, humans, and raccoons.[113] Few vector and wildlife field studies have been performed in the Southeast, potentially underestimating the range and variety of triatomine bugs present in this region. The single reported triatomine species in Mississippi, *T. sanguisuga,* feeds on a broad host range of sylvatic reservoirs. It has also been found in association with domestic dogs, chickens, horses, and in or near the homes of human autochthonous Chagas' disease cases in Tennessee, Louisiana, and Mississippi.[92]

Disease transmission

Complex patterns of *T. cruzi* infection occur depending on vector and host populations, transmission routes within sylvatic or domestic cycles, and agent prevalence.[114] Mammalian host species are potentially infected through many routes involving contact with triatomine insects, their feces, or infectious material from other mammals. In addition to contamination of bite sites or mucous membranes with feces, infection can occur following ingestion of infected triatomine insects, consumption of raw meat or blood from infected mammals, or through contact with infectious material such as urine or milk.[112]

Infectivity of *T. cruzi* appears to depend on host, vector, and agent characteristics. Infection rates through oral or vector-feeding transmission have been estimated for raccoons and opossums based on prevalence data,[114] but risks associated with specific host factors, such as age, gender, and health status are poorly understood.[111] Triatomine species vary in defecation behavior following feeding which may contribute to efficacy as vectors of *T. cruzi.*[115] Additionally, vector behavior, such as ability to adapt to human residences and attraction to light, may increase exposure of *T. cruzi* to domestic animal hosts and humans. Agent factors, including level of parasitemia induced in hosts (i.e. infectiveness) as well as morbidity or mortality of hosts, may significantly impact disease epidemiology.[111]

Oral transmission of *T. cruzi* is increasingly recognized as a route of disease exposure for both humans and animals. Several outbreaks in South American have been linked to consumption of sugar cane juice, acai paste or juice, or other foods products contaminated by infected triatomines.[91] These foods are often made outdoors where

insect or fecal contamination of the product can occur, especially as triatomine insects are attracted to light sources which are common around human habitations. Some researchers have proposed oral transmission as the predominant route of *T. cruzi* infection in sylvatic cycles, given the low rates of infection seen with traditional vector-feeding.[111] Many sylvatic species, including raccoons, opossums, and armadillos commonly feed on insects and oral transmission may contribute to reservoir host maintenance. Raccoons have been experimentally infected through ingestion of infected triatomine insects, however, the importance of scavenging or predation of other wildlife is less established. Research has failed to reproduce disease through these routes in other host species.[116]

Limited information is available on other routes of *T. cruzi* transmission. Congenital transmission in humans has been reported in 1 to 10% of infants from infected mothers and experimentally demonstrated in rats.[92] Naturally occurring congenital transmission has also been reported in dogs, and recognized as a limitation in the use of dogs as sentinels for disease during control programs.[117-118] Infection through transfusion of *T. cruzi* positive blood occurs in 10 to 25% of recipients, with platelet transfusion posing an apparently higher risk for transmission than packed red cells. Organ transplantation from infected donors has resulted in at least 19 documented cases.[92]

Sylvatic disease cycles

The genetic variation of *T. cruzi* indicates a long period of endemicity in wildlife from the southern United States and South America.[109] Major disease reservoirs include opossums, armadillos, and rodents, with low mortality seen in sylvatic hosts.[112,114] Different *T. cruzi* genotypes are associated with specific ecological niches and tend to circulate between a few primary host species with a primary vector species mediating

transmission.[110] Although the dynamics of disease transmission in these cycles is poorly understood, mathematical models are beginning to provide a framework for understanding how vector and host populations affect routes and rates of disease transmission.[114] The roles of superinfection, or the reinfection of an already infected host, and co-infection with multiple genotypes is poorly understood, may by contribute to new transmission scenarios between atypical vector and host species.[111]

Sylvatic disease cycles are apparent in the United States, with occasional spill-over to humans. In a seroprevalence study of six states, *T. cruzi* positive wildlife were identified in every state except California, and up to 68% of raccoons and 52% of opossums had *T. cruzi* antibodies.[119] California has documented endemic *T. cruzi*, with an autochthonous human case occurring in 1982 and reports of focal sylvatic transmission between *Triatoma protracta* and ground squirrels.[120] In the Southeast, major sylvatic cycles include raccoons, opossums, and armadillos in association with *Triatoma sanguisuga*.[121-122] An autochthonous human case in New Orleans, Louisiana, was attributed to an increase in the local armadillo population and triatomine infestation following Hurricane Katrina in 2005.[123]

Domestic disease cycles

Environmental changes such as climate shift and deforestation may lead to emergence of domestic *T. cruzi* cycles. Triatomines are generalists and readily feed on many species, which may include domestic animals and humans when sylvatic host populations are reduced.[111] Dogs, cats, and guinea pigs have all been identified as amplifying hosts and linked to increased peridomestic disease transmission in South America. Other domestic animals, including goats, sheep, pigs, and chickens, may serve

as blood-meal sources for triatomine bugs and thus increase vector populations, but have low infection rates and do not significantly contribute to domestic cycles.[112] In addition to availability of suitable hosts, domestic cycles require establishment of vector populations near human habitations. Adobe and thatch housing, common in South America, pose a high risk for triatomine infestation and disease transmission.[92]

American trypanosomiasis in domestic dogs

The role of dogs in domestic transmission cycles

In South America, dogs play a crucial role in domestic *T. cruzi* transmission. A study in Colombia identified domestic dogs as potential bridge vectors, bringing *T. cruzi* genotypes typically found in sylvatic cycles into domestic environments and resulting in increased transmission to humans.[124] Several studies have identified the presence of infected dogs to be the greatest risk factor for occurrence of peridomestic transmission,[112-113,125] with human infection rates 4.5 to 4.7 times greater when a seropositive dog is present in a home.[126] Reasons for this association include heavy vector feeding on dogs, which increases the prevalence of infected dogs and vector population size, and greater infectiousness of dogs to triatomine insects compared to humans or other domestic animals.[125,127] Not all dogs pose equal risk, however, as dogs do not display a homogeneous rate of infectiousness. In one study, younger dogs infected a greater proportion of feeding insects than older dogs, and about 1/3 of dogs were "super-spreaders", infecting 40% or more of feeding insects compared to the majority of dogs that infected less than 10%.[127]

The importance of dogs in domestic transmission within the United States is poorly understood. Beard et al. reported a focal domestic transmission cycle between

domestic dogs and *Triatoma gerstaeckeri* in southern Texas, identified after three dogs from a single property died from acute cardiomyopathy.[128] Three of four remaining dogs on site were seropositive for *T. cruzi*, and 24 of 31 live triatomines of various life stages collected on-site were infected. Neither person living on-site was seropositive. Additional work has shown widespread infection in the dog population in Texas, including 8.8% seroprevalence in shelter dogs from across the state[16] and 537 clinical cases diagnosed between 1993 and 2007.[129]

At least 10 species of triatomine vectors have been reported in Texas, with documented occurrence in 97 of 254 counties and three vector species infected with *T. cruzi* found in or near houses.[130-131] Seroprevalence studies in wildlife have identified many infected host species and disease is also reported in humans, although differentiating autochthonous from imported cases can be difficult.[130] Transmission cycles in Texas, although still poorly defined, have been studied more intensively than any other region in the United States. Other states across the southern half of the country have unknown endemic cycles, resulting in occurrence of seropositive wildlife, vectors, and domestic dogs, alongside documented cases of clinical disease in dogs and people.

Clinical disease in dogs

Domestic dogs have been proposed as both sentinels for human disease[16,112,118] and as animal models for Chagas' cardiomyopathy,[132] due to similarities in the clinical course of disease in dogs and humans. Acute disease, characterized by parasitemia, occurs between 3 and 17 days post-infection. Clinical signs may include lymphadenopathy, acute myocarditis, lethargy, pale mucous membranes, and splenic or hepatic enlargement, with damage occurring as trypanomastigotes rupture infected

cells.[103] Dogs less than 1 year of age tend to have more serious illness and higher mortality due to severe cardiac disease, while older dogs may not demonstrate clinical signs.[92] About 80% of experimentally infected dogs developed transiently abnormal electrocardiograms (ECGs).[132]

Following recovery from the acute phase, dogs enter the indeterminate phase which is asymptomatic and may persist for the duration of the dog's life. Parasitemia usually ends by 30 days post-infection, at which time ECG findings are typically normal, although exercise may induce arrhythmias. Unlike in humans, sudden death due to heart failure is uncommon during the indeterminate phase.[103] Progression to chronic disease occurs with development of cardiac dilation and eventual right-sided or bilateral heart failure. Dogs infected at 2 years of age or younger have rapid development of heart disease within 1-2 years, while older dogs survive 3-5 years after infection.[103] Experimentally infected dogs developed chronic diffuse fibrosing cardiomyopathy as seen in human Chagas' cardiomyopathy, which may be immunologically mediated.[132] Meningoencephalitis is a less frequent clinical presentation resulting in weakness, ataxia, and hyperreflexia which can be mistaken for canine distemper.[103]

Treatment for dogs with American trypanosomiasis is similar to that used for people. Benznidazole results in fewer adverse side effects than nifurtimox and is typically given in conjunction with prednisone for acute disease, although no drugs have been approved by the FDA for use in dogs. Current protocols reduce parasitemia but may not result in serorecovery or prevent progression to chronic disease.[112] Treatment during the chronic stage is focused on mediating the signs of heart failure. Dogs diagnosed with trypanosomiasis generally have a poor prognosis, and euthanasia may be warranted due

to zoonotic risk.[103] Prevention and control measures include reduction of exposure to vectors or sylvatic hosts. Insecticides applied to dog housing areas or directly administered have shown varying efficacy, with subcutaneous ivermectin purportedly being more effective than fipronil impregnated collars.[112] Integrated pest management, including use of barriers, altering outside lighting, and housing dogs inside at night, may allow for implementable risk reduction.[129] Serological screening of blood donor dogs and breeding bitches is recommended to reduce transmission in endemic areas.[103]

Diagnostic testing

Diagnostic testing is similar for both dogs and humans, and is dependent on the clinical stage of disease. In acute disease (or early congenital infection), circulating trypanomastigotes can be observed on blood smears, however, observation of the buffy coat stained with Wright's or Giemsa improves diagnostic sensitivity.[103] Hemoculture and PCR techniques have also been developed but are not widely available and increase diagnostic time.[92] Acute disease is most commonly recognized in dogs with sudden death due to cardiomyopathy and may be confirmed via histopathology.[129]

Serology is used to diagnosis chronic infection, with development of detectable IgG antibody within 4 weeks of infection in dogs.[28] Clinical signs which may prompt testing for chronic Chagas' disease in dogs include cardiomegaly, decreased activity or appetite, ascites, abnormal ECG findings, or other signs of cardiomyopathy.[129] Several serological tests have been used for diagnosis, including an immunofluorescent antibody assay (IFA), enzyme-linked immunosorbent assay (ELISA), and radioimmunoprecipitation assay (RIPA), however, all are prone to cross-reaction with *Leishmania* spp.[103] In humans, serological tests targeting two different antigens or using

two different methods are applied in series (for blood screening) or parallel (for clinical disease) to improve diagnostic specificity and sensitivity, respectively.[92] The US Food and Drug Administration has approved two tests to screen human blood donors: an ELISA in 2006 and a recombinant chemiluminescent immunoassay in 2010. Both tests require repeat-reactive results to remove a blood donor from eligibility, and positives are usually confirmed via RIPA. Human clinical cases are diagnosed by the CDC through a combination of tests including an in-house IFA, a commercial ELISA, or an immunoblot assay.[93]

In addition to conventional testing, recent studies have investigated the use of immunochromatographic tests in people, domestic dogs,[16,133-135] and wild canids.[136-137] These screening tests are rapid, simple to perform in the field, and do not require specialized equipment or technical skills.[133] A commercial canine dipstick test using recombinant *T. cruzi* antigens (Trypanosoma Detect™ MRA Rapid Test; Inbios International Ltd., Seattle, Washington) showed a sensitivity and specificity of 91% and 98%, respectively, in experimentally infected dogs from the United States.[135] A human commercial cassette test using recombinant protein conjugated to dye (Chagas STAT-PAK; Chembio Diagnostic Systems, Medford, New York) had perfect agreement with the IFAT performed by the CDC on 50 canine serum samples, and performed slightly better than the dipstick test which had perfect sensitivity and 95% specificity.[134] Both tests are practical, economical alternatives for serological screening of dog populations.

Prevalence of T. cruzi in dogs in the United States

Prevalence of *T. cruzi* is poorly documented for dogs in the United States. Reports of population level prevalence vary widely based on the population tested and diagnostic

test(s) used, making comparisons across studies difficult (Table 1.4). In addition, several previous reports have used convenience sampling which may not be representative of the overall dog population. Studies may also suffer from lack of geographic resolution due to low statistical power; Tenney et al. did not detect any regional differences in dogs across Texas, however, sample sizes from each district were small (<30 dogs).[16] Vector-borne diseases such as Chagas' may occur at uneven rates, with pockets of hyperendemicity occurring where there is convergence of suitable vector habits, high populations of reservoir hosts, and higher rates of disease and transmission.[21] Systematic, intensive sampling may be required to detect important differences in regional transmission and dog risk factors for disease.

Table 1.4 Seroprevalence of *T. cruzi* in dogs in the United States

State	Population	Test Used	Sample size	Prevalence	Citation
SE US	Random samples collected from patients at 3 institutions; samples were from GA (309), SC (29), NC (19), VA (4), LA (2), FL (1), WV (1)	Direct agglutination (DA), positives tested with complement fixation (CF)	365	6.6% for DA, 1.9% for CF	Tomlinson et al., 1981[138]
LA	85 dogs from rural environment with host exposure; 103 dogs from rural without host exposure; 176 dogs from urban animal shelter; 100 pet dogs from urban housing	ELISA	464	4.7% in rural with known host contact, 2.3% in shelter	Barr et al., 1991[28]
VA	Mother and 7 of 8 puppies positive; 12 dogs from area of index case; 52 dogs in the county	ELISA and RIPA	64 plus index case and litter	3.8% (2 of 52 dogs sampled from county)	Barr et al., 1995[117]
OK	Owned and impounded stray dogs (selection criteria undefined)	RIPA	304	3.6%	Bradley et al., 2000[139]
TX	Convenience sample of healthy dogs >6 months of age in Harris County, TX and surrounding regions	ELISA + flow cytometry	356	2.6%	Shadomy et al., 2004[140]
LA	Group 1: three kennels with previous positive dogs; Group 2: convenience samples from veterinary clinics in area with reported Chagas' in dogs	IFAT	Group 1: 31 total (15, 8, 8 from each of 3 kennels) Group 2: 91	Group 1: 51.6% (60%, 25%, 62.5%, respectively) Group 2: 12%	Nieto et al., 2009[134]
TX	Dogs from 7 shelters across TX	STAT-PAK	205	8.8%	Tenney et al., 2014[16]

Studies are listed chronologically by publication date.

Data from Mississippi is lacking for Chagas' disease. No studies have measured seroprevalence in dogs within the state, and limited information is available on occurrence in wildlife and triatomine vectors.[92] There have been comparatively few

human Chagas' cases in Mississippi found during blood donation screening, with only 9 of the 2281 confirmed positives in the AABB Biovigilance Network reported from Mississippi in the past 10 years.[141] However, these low numbers likely reflect a smaller Latin America immigrant population in which the majority of positives occur. Of note, two Mississippi natives were identified as probable autochthonous cases shortly after widespread blood donor screening was implemented and triggered The United States *Trypanosoma cruzi* Infection Study by the CDC.[93] Human cases in Mississippi may therefore represent a greater occurrence of indigenously acquired disease than most other states. Data on the seroprevalence of *T. cruzi* in transmission cycle components across the state, including domestic dogs, triatomine insects, and wildlife hosts, may prove useful in assessment of public health risk.

Conclusions

Many authors have recognized the potential of domestic dogs as sentinels for American Trypanosomiasis in both South America and the United States.[16,112,118,130] Humans are primarily infected through domestic transmission cycles, and dogs are ideal sentinels for these cycles in that they are comparatively easy to sample and diagnostic tests are increasingly available. Prevalence of disease, and therefore efficiency of surveillance testing to detect areas where disease is endemic, is likely greater in dogs than in humans. In particular, free-ranging dogs or those housed predominately outdoors may have greater rates of infection due to triatomine exposure, both by insects feeding on dogs and alternative routes of transmission such as ingestion of triatomines. It is logistically challenging to perform surveillance sampling on free-ranging dogs, however, animal

shelters provide convenient, large populations of dogs which can be easily and economically sampled.

An appropriate sampling strategy is required to approximate the true seroprevalence within a population. Inclusion criteria must be carefully defined to prevent introduction of bias; many of the *T. cruzi* seroprevalence studies done in the United States either do not define inclusion criteria for sampled dogs or have excluded dogs less than 6 months of age. Information on this subset of the population is therefore unknown. A better understanding of risk factors for being seropositive, such as age, source (stray versus owner surrender), and apparent health status, may reveal important criteria for targeted surveillance of high-risk individuals when the goal is to determine if disease is present, rather than to evaluate seroprevalence. Additionally, clustering of disease may occur by geographic region or even at a local level depending on disease transmission factors. To capture these differences, sampling must be systematic and provide high enough statistical power to detect differences. Stratified random testing of shelter dogs on a state-wide level may help resolve important questions on *T. cruzi* prevalence in Mississippi as an indication of autochthonous disease risk for both dogs and humans.

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CHAPTER II

MISSISSIPPI ANIMAL SHELTER CENSUS AND CANINE SERUM BANK FOR POPULATION-BASED SEROPREVALENCE RESEARCH

(Prepared for submission to the Journal of the American Veterinary Medical Association)

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Abstract

Objective: To develop a representative serum bank for population-based seroprevalence studies

Design: Census of animal shelters followed by cross-sectional collection of serum samples

Population: 61 shelters in 45 counties identified in census; 571 dogs over 8 weeks of age proportionately sampled from 9 geographic districts for serum bank

Procedures: Organizations believed to be animal shelters in Mississippi were compiled from existing lists and web-based searches. Information on animal intake and shelter practices was obtained by phone or other contact. Organizations with a physical facility and offering public adoptions were classified as shelters, and used to determine dog intake for 9 geographic districts during 2015. Blood and physical examination information was collected for randomly selected dogs in 18 shelters, proportionately sampled from each district based on the shelter dog population identified in the census. Serum was frozen in aliquots for future seroprevalence research. Summary statistics for animal shelters and sampled dogs are presented.

Results: The 61 animal shelters in Mississippi had a combined intake of over 56,000 dogs in 2015. Shelters varied widely in size, and dog intake was correlated to human population by district (R^2=0.91). Over half of shelters used foster homes for animals, and 37% of shelters had transport programs for dogs. Average annual dog adoption rate by shelter was 55%.

A serum bank was established containing 571 dog samples, of which 36% came from puppies (less than 6 months of age). Dogs had a variety of health abnormalities, with coat and skin problems being most common.

Conclusions and Clinical Relevance: This is the first report of the shelter dog population and distribution in Mississippi, providing a baseline to monitor future trends in intake and adoption. Additionally, banked sera provides a rare opportunity for disease prevalence estimation from randomly collected samples to minimize bias. Knowledge of the prevalence of diseases in the Mississippi shelter dog population should guide public policy and shelter risk management.

Key Words: animal shelter, census, canine serum bank

Introduction

Shelter animals are a unique population for disease surveillance with multiple risk factors frequently contributing to higher rates of disease than seen in owned companion animals. Animals often enter shelters as free-roaming strays with greater exposure to other domestic and wild animal populations, are more likely to be sexually intact, and may lack preventative care including vaccination and parasite control prior to shelter intake.[1] Additionally, these populations are often maintained in high density facilities with variable levels of biosecurity and isolation. Although previous research has identified risk factors contributing to animal relinquishment and shelter outcome,[2-3] limited data is available on the health of animals within the shelter environment. Knowledge of the prevalence of disease within this population is necessary to assess the public health risk posed to animal shelter employees and adopters, and for effective shelter allocation of resources to minimize disease transmission and occurrence.

Accurate measurements of disease require representative sampling of the population of interest, which is dependent on an accurate sampling frame. Animal shelters and their populations have been described as a "statistical black hole," with multiple and inconsistent estimates of both the number of animal shelters and the animal population they house in the United States.[4] Much of this confusion results from lack of mandatory shelter facility registration in some states, the often transient nature of volunteer-run or locally-financed operations, and lack of funding and organization to maintain multistate lists. Additionally, a wide variety of facilities may house unowned animals including humane societies, municipal animal control departments, animal sanctuaries, and foster-based or breed-specific groups. Without a clear understanding of

the characteristics and distribution of the animal shelter population, disease estimates are usually determined by convenience sampling at one or a few shelters, which may not be representative of the general shelter animal population. Furthermore, lack of wide-spread systematic sampling may fail to identify important areas of high disease endemicity within areas of lower occurrence.[5]

The Mississippi shelter dog population is highly mobile with both intrastate and interstate travel for adoption through foster homes and transport programs with partner shelters. Transported animals are usually screened for visible signs of disease and receive routine diagnostic testing (e.g. heartworm and fecal parasite testing), but often do not have comprehensive medical workups prior to transportation.[6] Movement of these animals may introduce diseases common in the southeastern United States to new areas, or to low prevalence areas, and may pose a zoonotic disease risk. Previous reports have shown higher rates of canine heartworm,[5] canine brucellosis,[7-9] and erlichiosis[5] in the Southeast. Also, there is some evidence for endemic canine leishmaniasis,[10] Chagas' disease,[11] and babesiosis[12] with competent vectors and wildlife reservoir species present in the Southeast.

Prevalence of these diseases in the Mississippi shelter dog population is largely unknown. Therefore, the objective of this study was to quantify and determine distribution of the Mississippi shelter dog population in order to develop a representative serum bank. Serum samples collected from shelter dogs across the state of Mississippi provide a valuable research tool for population-based seroprevalence investigation of infectious and zoonotic diseases.

Materials and Methods

Prospective Shelter List

Registration of animal shelters is not currently required by the state of Mississippi, thus a complete list of shelters in the state was not available. Two incomplete lists of animal shelters and other animal organizations were obtained from the state Board of Animal Health; one included shelters that had applied for funding from a specialty license plate program operated through the Board of Animal Health and the other was compiled by the Humane Society of the United States. A third list was generated by study authors using multiple internet searches performed between December 2015 and March 2016 with the keywords “animal shelter”, “humane society”, and “animal control” for each of the 82 counties in Mississippi.

Shelter Census

A phone census was attempted for each organization on the prospective shelter list. One of three individuals conducted each survey following a written script which introduced the caller, requested participation from the shelter director or other staff member able to provide requested information, and gathered data on shelter contact information, animal intake and adoption, and record keeping. Shelters were asked to consult records, or, if records were unavailable, to provide a best estimate of the number of dogs: which entered the shelter in 2015, were adopted to the public in 2015, were currently housed at the shelter, and the sources from which dogs were received (owner surrender, animal control/stray, transport/exchange, or other). Some organizations requested a paper copy of the survey to aid in record analysis, and this was provided via email. If repeated telephone contact attempts were unsuccessful, addition methods of

communication were attempted, including email and private messaging on social media sites.

Serum Bank Sampling

Stratified sampling was performed within the nine public health districts in Mississippi to reflect the geographic distribution of dogs in the state. The percentage of the total shelter dog population located in each district was used to proportionately sample ~500 dogs for the serum bank. Sample size was selected to provide adequate precision for a disease of low prevalence, specifically, canine brucellosis with an estimated prevalence of 5% and desired precision of 1.5%. Clopper-Pearson exact confidence intervals, chosen for increased accuracy at extreme values, are shown across prevalence levels that may be present in future seroprevalence research (Figure 2.1). One to three shelters were sampled per district based on previously established working relationships, shelter willingness to participate, and logistic feasibility. For each shelter, trained study personnel collected samples on a single day (a single shelter was sampled twice to fill district quota, while ensuring that no dog was resampled on the second collection visit).

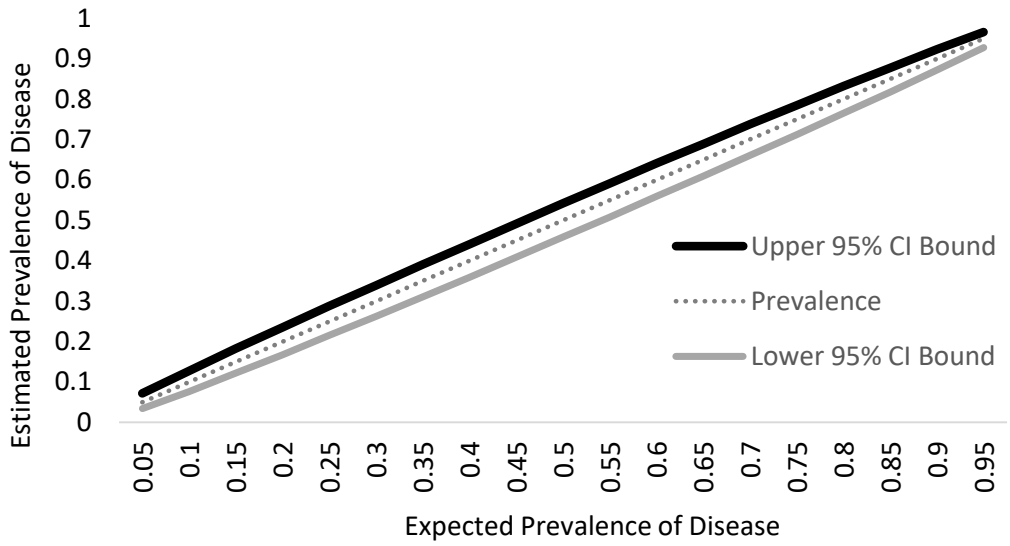

Figure 2.1 Clopper-Pearson exact 95% confidence intervals for serum bank Seroprevalence disease estimate 95% CI based on a serum bank containing 571 samples. Sample size was selected to provide precision of 0.015 for a disease with an expected prevalence of 0.05.

Dogs were eligible for sampling if they were owned by the shelter (not in a required hold period) and over 8 weeks of age. Within a shelter, dogs were randomly selected for sampling; each eligible dog was assigned a consecutive number based on housing location within the shelter, and a random number generator[a] was used to select dogs. In some cases, the randomization scheme had to be modified. Individual dogs to be sampled within group housing (of up to 4 dogs) were arbitrarily selected. If a sample could not be safely obtained using mild manual restraint, the next randomly selected dog was sampled as a replacement.

A brief physical examination was performed on all sampled dogs including an estimate of the dog's age, weight, and breed. Recorded information included sex, body condition score (1-9), and a description of any examination abnormalities. A whole blood sample of up to 20 milliliters was collected by vacutainer from the jugular or cephalic

vein based on animal size and compliance. Samples were stored on ice during transportation and processed within 24 hours of collection. Serum was collected after centrifugation at 3000 rpm for 10 minutes and stored in 1 ml aliquots at -80°C.

Census Data Analysis

Following the census, all organizations were either designated a Mississippi animal shelter or excluded from further analysis. For inclusion in our study, shelters must have had a "brick-and-mortar" facility and offered animal adoptions to the public. Organizations were excluded if they were duplicate entries under different names, were foster-based only with no physical location, did not operate within the state of Mississippi, were no longer active, or if web-based contact information failed to connect to the organization.

Not all information was available for every shelter. Notably, some shelters did not provide information on intake sources or record keeping systems. One shelter reported a greater number of dogs adopted than received; this shelter was not included in analysis of adoption rate since we do not know if this reflects data error, animals taken in during previous years, or if this shelter does not consider some sources such as transported animals as "received."

Shelter locations were mapped using open-source geographic information software.[b] Summary statistics were calculated using a commercial spreadsheet program.[c] Multivariable linear regression of dog intake by district was modeled using candidate explanatory variables of human population and average median household income by district.[d] County level data were obtained from the 2015 estimates from the United States Census Bureau,[13] however, data were analyzed by district since many Mississippi

counties did not have an animal shelter and these counties likely utilize nearby animal shelters. Average household income by district was calculated from county median household income weighted by county population.

Serum Bank Data Analysis

Physical examination data included both objective and subjective assessment at the time of sample collection. Age was estimated by tooth eruption and wear, but was also dichotomized during data analysis as puppy (<6 months of age) or adult (≥ 6 months of age) based on eruption of secondary canine teeth. Empirical assessment of predominant breed, recorded during examination, was used to classify dogs into the seven American Kennel Club breed groups for analysis. Dogs with a body condition score of 4 or 5 out of 9 were considered ideal, with scores <4 and >5 classified as underweight and overweight, respectively. Categorical statistics for sampled dogs, including sex, age, breed, body condition, and health abnormalities, were reported as percentages. Data from sampled dogs, along with corresponding source shelter information, were stored in a database for serum bank management and detailed analysis of risk factors during future seroprevalence research.

Results

Census

Of the 124 organizations on the initial prospective list, 61 facilities were determined to be brick-and-mortar buildings which housed animals and offered public adoption, meeting our definition for an animal shelter (Figure 2.2). Shelters were present in 45 of Mississippi's 82 counties, and each of the nine geographic districts contained

between 4 and 10 shelters. The 61 shelters reported intake of 56,886 dogs in 2015 (Table 2.1). Shelters varied widely in size with a minimum of 45 and a maximum of 7,539 dogs received during 2015 (Figure 2.3). Dog intake was associated with human population, with shelters taking in 23 dogs per 1,000 people (SE=0.0028, R^2=0.91, p<.0001), but was not associated with median household income (p=0.68).

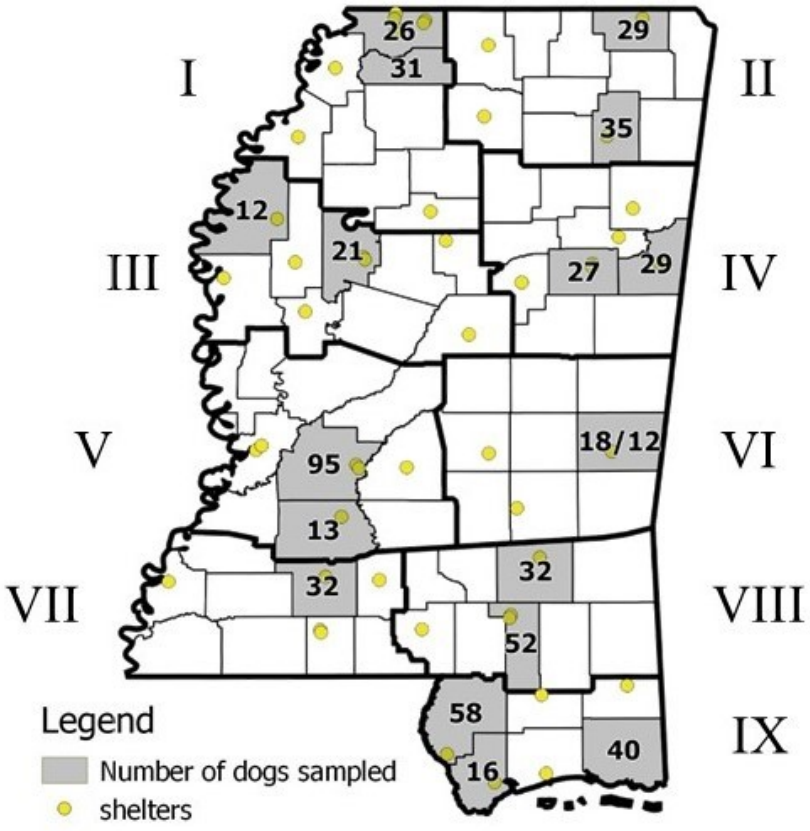

Figure 2.2 Distribution of 61 Mississippi animal shelters present in 2016

Shaded counties display the number and distribution of dogs sampled from 18 shelters for the serum bank, proportionately sampled by shelter dog population within nine health districts (Roman numerals).

Table 2.1 Census shelter dog intake and dogs sampled for serum bank

	Census Results			Serum Bank	
District	# of shelters	Dog Intake	% of intake	Dogs sampled	% of sampled
1	10	5120	9.0	57	10.0
2	4	6316	11.1	64	11.2
3	9	3090	5.4	33	5.8
4	8	4727	8.3	56	9.8
5	8	12682	22.3	108	18.9
6	6	3095	5.4	30	5.3
7	5	3006	5.3	32	5.6
8	5	7086	12.5	77	13.5
9	6	11764	20.7	114	20.0
TOTAL	**61**	**56886**		**571**	

Dog intake for Mississippi animal shelters in 2015 by geographic district, and number of dogs sampled by district for inclusion in the serum bank to reflect shelter dog distribution across the state.

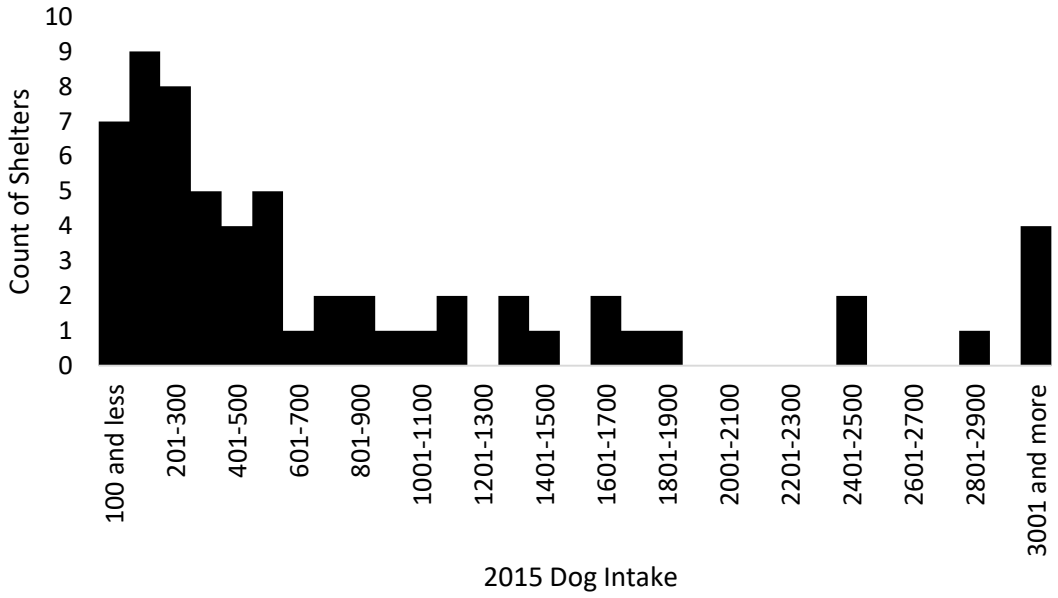

Figure 2.3 Frequency distribution of 61 shelters in Mississippi by 2015 dog intake

Dog intake sources were available for 49 shelters. Owner surrender dogs were accepted at 48 shelters (98%), and stray dogs were accepted at 48 shelters (98%); the shelter which did not accept owner surrender did accept strays and vice versa. Thirty-seven percent of shelters utilized transport programs for dogs (18/49), and over half of shelters (34/61) reported housing animals in foster homes, however, shelters were not asked to distinguish between foster home placement of dogs and cats. Information on the number of dogs adopted during 2015 was available from 40 shelters. Adoption rate ranged from 5% to 100% of dogs received, with an average shelter adoption rate of 55% (Table 2.2).

Table 2.2 Mississippi animal shelter adoption rates for dogs in 2015

Adoption rate	**Shelters Adopting Dogs**
25% and less	6/40 = 15%
25.1% to 50%	12/40 = 30%
50.1% to 75%	11/40 = 27.5%
Greater than 75%	11/40 = 27.5%

Forty-three shelters provided information on record keeping. Forty-nine percent of shelters used only paper records, 16% used a shelter database system, 19% used some other method of record keeping, and 16% used paper records in conjunction with another type of record. Fifty-four of the 61 shelters identified had a website (89%), and 55 used social media (90%).

Serum Bank

A total of 571 dogs were sampled from 18 shelters (Figure 2.2). The proportion of dogs sampled by district ranged from 5.3% (30 dogs) in district 6 to 20.0% (114 dogs) in district 9 to reflect the distribution of shelter dogs based on the 2015 census (Table 2.1).

The serum bank included 204 samples from puppies <6 months of age (35.7%). Sporting, hound, terrier, and herding-type breeds predominated, and most dogs were in good body condition at the time of sampling (Table 2.3).

Table 2.3 Summary of categorical variables for 571 dogs in the serum bank

Variable	Category	Number	%
Sex	Female	302	52.9
	Spayed	80/302	(26.5)
	Male	257	45.0
	Neutered male	66/257	(25.7)
	Not recorded	12	2.1
Age	Puppy	204	35.7
	2 to 3 months	121/204	(21.2)
	4 months	49/204	(8.6)
	5 to 6 months	34/204	(6.0)
	Adult	354	62.0
	0.5 to 1 year	139/354	(24.3)
	1 to 2 years	100/354	(17.5)
	2 to 5 years	98/354	(17.2)
	5 to 10 years	14/354	(2.5)
	10+ years	3/354	(0.5)
	Not recorded	13	2.3
Breed	Sporting	188	32.9
	Hound	117	20.5
	Terrier	104	18.2
	Herding	99	17.3
	Working	22	3.9
	Toy	9	1.6
	Non-sporting	4	0.7
	Not recorded	28	4.9
***Condition (1-9)**	Underweight (<4)	34/333	10.2
	Normal (4 or 5)	276/333	82.9
	Overweight (>5)	23/333	6.9

*Body condition data is not available for all dogs and is reflected in the denominator.

Estimated weight ranged from 3 to 50 lbs for puppies with an average weight of 15 lbs (SD 10.0), and from 10 to 110 lbs for adults with an average weight of 43 lbs (SD 14.1). Physical examination at the time of sample collection revealed a variety of abnormalities, with coat and skin problems most commonly identified (Figure 2.4). Two dogs were pregnant, 2 were nursing, and 3 were showing visible signs of estrus at the time of sample collection.

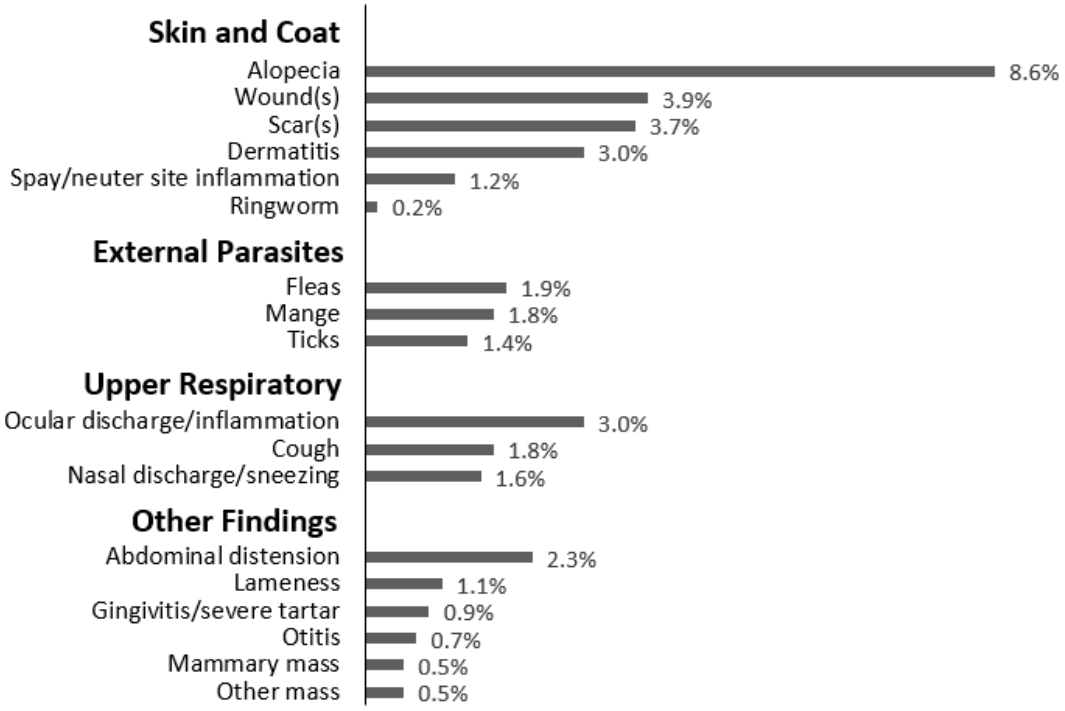

Figure 2.4 Percent of dogs showing health abnormalities at the time of serum bank sample collection

Discussion

This paper is the first comprehensive assessment of the Mississippi shelter dog population. We identified 61 animal shelters in 45 counties in Mississippi, accounting for

intake of over 56,000 dogs in 2015. Shelters varied widely in size and by adoption rate. Frequent movement of dogs occurred through foster and transport programs both within and outside of Mississippi. This report provides a baseline for monitoring future trends in the Mississippi shelter dog population.[14]

Information on the location, size, and characteristics of animal shelters is limited in the United States, and this information is difficult to collect in states where facility registration is not required. We used three different sources to compile our sampling frame, including funding sources and internet-based searches. The vast majority of shelters had a website or social media account, however, this could be a result of selection bias in our search method. Shelters without an internet presence are very difficult to find and may have been missed in our attempted census. Additionally, there is wide variability in the type of organization called an "animal shelter". We excluded organizations without a physical facility and those which did not offer public adoptions, however, often this could only be determined by speaking with a representative of the organization. Similarly, many support and volunteer groups working with one or more shelters appeared on our lists and represented duplicate entries. These could be difficult to identify and verify. Despite our best efforts, our census likely failed to capture some shelters within the state, especially local animal control offices which offer public adoption of animals when available.

Much of the information available on animal disease prevalence is based on convenience sampling rather than random sampling and may not be representative of the population, especially when disease occurs in clusters.[5,15-16] Our serum bank represented the geographic distribution of dogs across nine regions of the state. By randomly

sampling dogs within each shelter, we captured a cross-section of the Mississippi shelter dog population. We used minimal exclusion criteria to reduce selection bias, and believe the serum bank is a good representative of our target population. However, as with all samples, potential sources of bias remain and must be identified and accounted for when the serum bank is applied to a research question.

Shelters were selected for sample collection based on willingness to participate and previously established relationships, rather than at random. Some shelters were reluctant to provide detailed information during the phone census and did not wish to participate in random sampling. Although we have no reason to believe that sampled shelters differ in meaningful ways from non-sampled shelters, each shelter represents a unique environment and may differ in disease prevalence. In general, larger shelters were included in order to collect the required number of samples, however, we sampled from 2 shelters with an annual dog intake below 300, and half of sampled shelters had intake below 1200.

Although samples were collected over 15 months, we were not sampling by season. Almost 60% of all samples were collected during the summer, so selection bias may be present if the dog population housed at shelters differs by season for characteristics such as age or intake source (stray versus owner surrender). The proportion of dogs sampled that were puppies (<6 months) did not differ by season, with the exception of spring. Only two shelters were sampled in the spring, so it is unknown whether the greater proportion of puppies sampled at those shelters was a result of season or inherent to the shelters. Similarly, we did not have intake source data on enough dogs to determine if season was associated with intake source.

There is high variability in the daily shelter dog population at many shelters, which may be reflected in our one-time sample collection at each shelter. For example, we sampled very few toy breeds for the serum bank. This may represent low intake of these breeds into animal shelters, or may indicate high demand of these breeds with rapid adoption. A similar scenario might occur for disease; dogs with clinical signs of illness or documented infection such as canine heartworm may be less adoptable, have longer shelter stays, and therefore have a greater likelihood to be sampled compared to healthy dogs. This might result in increased prevalence within the sampled shelter dog population.

Despite these limitations, our serum bank is a relatively unique tool for investigation of disease prevalence, risk factors, and diagnostic test validation. Knowledge of the Mississippi shelter dog population and distribution obtained through the state census of shelters allowed us to reduce many potential sources of bias. Our ultimate goal, accurate measurement of the disease burden in the Mississippi shelter dog population, is necessary to guide evidence-based public policy regarding zoonotic diseases and for shelter prioritization of risk management within animal populations. Impacts of disease in this population are not limited to Mississippi, but may extend across the United States with the frequent, high volume movement of shelter animals through transport programs and into homes.

Acknowledgements

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Footnotes

a. Random.org Random Sequence Generator, Randomness and Integrity Services Limited, Dublin, Ireland, https://www.random.org/sequences/.

b. Quantum GIS Geographic Information System version 2.14, Open Source Geospatial Foundation Project, http://qgis.osgeo.org

c. Microsoft Excel version 2013, Microsoft Corporation, Redmond, WA

d. PROC MIXED, SAS, version 9.4, SAS Institute Inc., Cary, NC

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CHAPTER III

SEROPREVALENCE OF BRUCELLOSIS IN MISSISSIPPI SHELTER DOGS

(Prepared for submission to Preventive Veterinary Medicine)

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Abstract

Canine brucellosis is an emerging disease and compatible with a One Health management approach. Previous research has found higher *Brucella canis* seroprevalence in stray dog populations than in owned animals, and shelter dogs may represent a zoonotic risk to pet owners. Dogs may also contract other *Brucella* spp., including *Brucella suis*, which is carried by some feral swine in the United States and poses a public health risk.

Diagnostic tests for *Brucella* spp. are imperfect. Misclassification of disease status can result in serious repercussions for canine and human health including the unnecessary euthanasia of falsely positive dogs or failure to identify and remove falsely negative dogs from susceptible populations. Correct interpretation of any diagnostic test requires knowledge of the pre-test probability of disease in the population, therefore the objective of this study was to estimate the seroprevalence of *B. canis* and *B. suis* in Mississippi shelter dogs to guide evidence-based diagnostic testing and inform policy recommendations.

Banked serum samples from 571 dogs collected in 2016-2017 as a representative sample of the Mississippi shelter dog population were tested for *B. canis* using a commercial rapid slide agglutination test (RSAT) and for *B. suis* using a buffered acidified plate agglutination test. No dogs were seropositive for *B. suis* antibodies. Twenty-eight dogs (4.9%) were seropositive for *B. canis* antibodies on the RSAT, with 13 dogs (2.3%) remaining positive when retested with the addition of 2-mercaptoethanol to increase specificity. Test prevalence by shelter ranged from 0 to 8.6%. True prevalence was estimated using stochastic modeling to account for test performance and clustering of

dogs by shelter. Approximately 65% of modeled shelters did not have seropositive dogs. For shelters where *B. canis* was present, the mean modeled seroprevalence was 17.8%.

This study reveals important information regarding the distribution of *B. canis* seroprevalence in Mississippi shelter dogs. Current diagnostic tests lack the sensitivity needed to correctly identify individual infected dogs, but population testing may provide a reasonable estimate of disease. Eradication or control measures should focus on the small number of shelters where canine brucellosis occurs to effectively minimize transmission among dogs and to humans.

Keywords: Canine brucellosis, seroprevalence, animal shelter

Abbreviations:

BAPA: Buffered acidified plate agglutination

LPS: lipopolysaccharide

NVSL: National Veterinary Services Laboratory

RSAT: Rapid slide agglutination test

2ME-RSAT: 2-mercaptoethanol rapid slide agglutination test

Introduction

Brucellosis is a global animal disease with significant zoonotic potential and an ideal example of the importance of the One Health initiative because of the interface between wildlife, domestic animal, and human populations. *Brucella* spp. are classically identified by their natural host species, with dogs serving as the natural host for *B. canis*. Domestic dogs can be infected with three additional *Brucella* spp.: *B. suis, B. abortus*, and *B. melitensis,* typically following exposure to swine, cattle, and small ruminants, respectively.[1]

A recent serosurvey in Georgia found 1.3% of dogs seropositive for *B. suis,* with speculated transmission occurring through recreational hunting of feral swine.[2] Additionally, *B. canis* infection in dogs became reportable in Mississippi in 2016, following a human case linked to a stray dog.[3] These previously under-recognized zoonotic risks have created a need for veterinary practitioner education on appropriate testing strategies and interpretation, which is dependent on the prevalence of disease in the dog population.

In the United States, a higher prevalence of *B. canis* has been reported in stray and free-roaming dogs, particularly in the rural southeast.[4-5] This subset of the dog population has a greater proportion of reproductively intact dogs compared to owned dogs, facilitating transmission through reproductive contact.[6] Stray dogs may also have increased exposure to and predation of wildlife, which can serve as a source of brucellosis infection.[7] Free ranging feral swine, present in at least 39 states including Mississippi, remain a recognized source for *B. suis*.[8] Seroprevalences of 0.3 to 52.6% have been reported in feral swine, varying with geographic region, season, and animal

factors.[9] Transmission of *B. suis* to domestic swine, dogs, cattle, and people has been demonstrated in the United States, with feral swine serving as an important source for introduction of the disease into atypical host species. [2,8,10-13]

Diagnosis of brucellosis is complicated by vague or absent clinical signs and imperfect tests. Common human and livestock serological screening tests detect antibodies against the lipopolysaccharide (LPS) component of the outer cell membrane. These tests do not differentiate *B. abortus*, *B. melitensis*, and *B. suis*, all of which typically form smooth phenotype colonies and contain complete LPS molecules. Rough colony forming species, including *B. canis,* lack the LPS O-side chain and do not cross-react with smooth species.[1] Instead, a commercial rapid slide agglutination test (RSAT) is available for in-house *B. canis* testing. Samples showing agglutination are retested with the addition of 2-mercaptoethanol to improve test specificity, but may fail to detect some positives.[14] To detect other *Brucella* spp., dogs may be tested using smooth-strain antigen such as that employed in the buffered acidified plate antigen (BAPA) test recommended by the OIE for *B. suis* screening of livestock and wildlife.[15]

Considering these diagnostic challenges, identifying infected animals is difficult. Shelter dogs may serve as a bridging population, bringing potentially infected dogs into intimate contact with human family members. This poses an unquantified risk for human health, especially for the 20% of the population most immunologically susceptible, including children and the elderly, for whom shelter animals often provide companionship.[16] The zoonotic potential of *B. canis* is well recognized, though human cases are rare.[5,17] *Brucella suis* has greater zoonotic potential than *B. canis*, dependent on biovar and host, with urine and saliva speculated as vehicles for the transmission of *B.*

suis from dogs to humans.[18] Isolated cases of natural infection of dogs with *B. suis* have been documented,[2,19-20] but no additional information is known on prevalence of the disease in dog populations. Mississippi has a large feral swine population with potential transmission of *B. suis* to dogs through predation of feral swine or birthing materials, especially dogs used for feral swine hunting or free-ranging dogs which may enter shelters as strays.[2,8]

In addition to posing a local zoonotic risk, undiagnosed dogs may also contribute to spread of brucellosis from areas of higher endemicity to new populations. Shelter dogs are highly mobile both within the state of Mississippi as well as nationally through foster networks and transport programs, which move animals from overcrowded shelters to adoption centers where animals are in greater demand. Animals are typically screened for visible signs of disease, but a comprehensive diagnostic workup is usually not performed due to financial limitations and lack of requirements for such testing before interstate movement. Infected dogs moved to historically low-risk areas may be more likely to remain in the population if clinicians have a low index of suspicion for the disease, even in dogs showing consistent clinical signs. Infected dogs may serve as a source of *Brucella* spp. for other animals and people during the bacteremic phase which may exceed two years.[21]

A stray dog seropositive for *B. canis* was identified during a 1976 survey of a single Mississippi site,[22] however, to date, no systematic sampling of Mississippi dogs has been performed to identify if, and to what extent, *B. canis* and *B. suis* are present in this population. Therefore, the objective of our study was to estimate the seroprevalence of these pathogens to guide evidence-based risk assessment and public policy.

Materials and Methods

A cross-sectional study was performed on samples collected from shelter dogs across Mississippi between 2016 and 2017 as a representative serum bank (Chapter 2). In brief, dogs were proportionately sampled from one, two, or three shelters within each of the nine state public health districts to reflect the geographic distribution of dogs across the state, determined from a statewide census of animal shelters. Whole blood samples were collected from 571 randomly sampled dogs over 8 weeks of age from 18 participating shelters. Serum was separated and stored at -80°C until testing. A sample size of ~500 dogs was selected to provide precision of 0.03 when using the 2ME-RSAT (sensitivity 0.32, specificity 1),[14] an expected true prevalence of 0.04, and a confidence level of 0.95.[23]

B. canis testing

Serum samples were tested for the presence of *B. canis* antibodies using a commercially available RSAT (D-TEC® CB, Synbiotics) according to kit instructions. Samples with visible agglutination were retested with the addition of 2-mercaptoethanol (2ME-RSAT) to remove nonspecific agglutinins and improve test specificity.[21] Dogs were considered positive for *B. canis* if both the RSAT and the 2ME-RSAT showed visible agglutination.

B. suis testing

A BAPA test was performed using *B. abortus* antigen according to the procedure obtained from the National Veterinary Services Laboratory (NVSL) to detect *B. suis* or other smooth *Brucella* spp.[24] Positive and negative *B. suis* controls from the NVSL were

run on each plate, and a sample was considered positive if it showed macroscopic agglutination similar to the positive control.

Data analysis and stochastic modeling

B. canis apparent seroprevalence was calculated as the proportion of 2ME-RSAT test positive dogs out of total tested dogs. Descriptive statistics, including apparent prevalence by district, shelter, and several dog characteristics, were performed using a commercial spreadsheet program.[a] Risk factors for 2ME-RSAT seroprevalence were assessed using manual forward selection in multivariable logistic regression, with shelter included as a random effect in all models.[b] Age was recorded as a categorical variable but analyzed as a binomial variable (dogs ≤2 years of age versus dogs >2 years of age) due to low accuracy of age estimation in older dogs. Sex was recorded as intact female, spayed female, intact male, or neutered male, but intact and altered animals were grouped for each sex for analysis since spayed female dogs could not be reliably identified and we were unable to determine temporal relationships between time of sterilization, entry into shelter, and seroconversion. Shelter source, when available, included animals surrendered by owner and stray animals (including intake through animal control services). Breed group was categorized by predominant breed identified during physical exam and further consolidated by dog size and historical breed purpose (e.g. hunting-type breeds). Due to the expected low prevalence of *B. canis*, significance was defined *a priori* at $\alpha=0.1$.

To account for imperfect test performance and clustering by shelter, true prevalence was estimated using stochastic models.[c] Binomial parametric distributions were used for input variables and included two parameters: total samples tested and probability of a positive test derived from the number of test positive animals (Table 3.1).

The distributions for test sensitivity and specificity were defined from literature reports of 2ME-RSAT performance.[14] The distribution of prevalence by shelter was defined using total dogs sampled at each of 18 shelters and the corresponding apparent prevalence by shelter.

Convergence tolerance was set at 1% (with a 95% confidence interval), and Latin hypercube sampling with 5,000 iterations was conducted to meet the convergence criteria (i.e. the change in the median of main outputs converged at 1.0% or less). Outputs included test sensitivity, test specificity, apparent prevalence for each of 18 shelters, and total apparent prevalence over all shelters (Table 3.2). Overall true prevalence and true prevalence for each of the 18 modeled shelters was calculated for each of the 5,000 iterations as:

$$P = \frac{P^T + specificity - 1}{sensitivity + specificity - 1} \quad (3.1)$$

where P^T is the test, or apparent, prevalence,[23] and 2ME-RSAT specificity and sensitivity are 100% and 31.76%, respectively.[14]

Table 3.1 Model parameters, number seropositive (m), number sampled (n), prevalence (p), commands and distributions

Parameter	m	n	p	Command and distribution
*2ME-RSAT Sensitivity	27	85	0.3176	RiskBinomial(85,0.3176)
*2ME-RSAT Specificity	42	42	1	RiskBinomial(42,1)
Shelter 1	0	32	0.0000	RiskBinomial(32,0)
Shelter 2	0	12	0.0000	RiskBinomial(12,0)
Shelter 3	1	29	0.0345	RiskBinomial(29,0.0345)
Shelter 4	0	13	0.0000	RiskBinomial(13,0)
Shelter 5	1	29	0.0345	RiskBinomial(29,0.0345)
Shelter 6	1	26	0.0385	RiskBinomial(26,0.0385)
Shelter 7	0	12	0.0000	RiskBinomial(12,0)
Shelter 8	0	16	0.0000	RiskBinomial(16,0)
Shelter 9	0	52	0.0000	RiskBinomial(52,0)
Shelter 10	0	18	0.0000	RiskBinomial(18,0)
Shelter 11	0	21	0.0000	RiskBinomial(21,0)
Shelter 12	3	95	0.0316	RiskBinomial(95,0.0316)
Shelter 13	1	27	0.0370	RiskBinomial(27,0.0370)
Shelter 14	1	58	0.0172	RiskBinomial(58,0.0172)
Shelter 15	1	31	0.0323	RiskBinomial(31,0.0323)
Shelter 16	1	25	0.0400	RiskBinomial(25,0.0400)
Shelter 17	3	35	0.0857	RiskBinomial(35,0.0857)
Shelter 18	0	40	0.0000	RiskBinomial(40,0)

*Data from Keid et al.[14]

Table 3.2 Model simulated values and final outputs

Simulated values	*2ME-RSAT sensitivity*: number positive (m) from RiskBinomial(85,0.3176)/85 *2ME-RSAT specificity*: m from RiskBinomial(42,1)/42 *Shelter B. canis*: $m_{1\text{-}18}$ from RiskBinomial for each of 18 shelters
Model 1 Outputs: **Overall Prevalence**	*Total number seropositive*: $\sum(m_1 \ldots m_{18})$ *Apparent prevalence*: $\sum(m_1 \ldots m_{18})/571$ *True prevalence*: $P = \frac{P^T + specificity - 1}{sensitivity + specificity - 1}$ Frequency distribution (Figure 3.1) shows true prevalence from 5,000 iterations of total number seropositive
Model 2 Outputs: **Prevalence by Shelter**	*Apparent prevalence*: Simulated (m)/n for each of 18 shelters *True prevalence*: calculated as above for each of 18 shelters Frequency distribution (Figure 3.2) shows true prevalence by shelter from 5,000 iterations each of 18 shelters (90,000 simulated shelters)

Results

Serum samples from 571 dogs were tested for the presence of brucellosis antibodies. No animals were seropositive for *B. suis* on the BAPA. Twenty-eight samples (4.9%) were initially positive for *B. canis* on the RSAT. Thirteen samples remained positive on the 2ME-RSAT for an apparent prevalence of 2.3% in this population. Apparent prevalence by district ranged from 0 to 6.3%, and apparent prevalence by shelter ranged from 0 to 8.6% (Table 3.3). Of the 18 sampled shelters, 9 shelters had no dogs positive for brucellosis, 7 shelters had a single positive dog, and the remaining 2 shelters each had 3 seropositive dogs.

Table 3.3 Apparent prevalence of *B. canis* by geographic district and individual shelter using the 2ME-RSAT

			Shelter 1		Shelter 2		Shelter 3	
District	**No. sampled**	***B. canis* pos (%Prev)**	**n**	***B. canis* pos (% Prev)**	**n**	***B. canis* pos (% Prev)**	**n**	***B. canis* pos (% Prev)**
1	57	2 (3.5)	26	1 (3.8)	31	1 (3.2)	-	-
2	64	4 (6.3)	35	3 (8.6)	29	1 (3.4)	-	-
3	33	0 (0)	21	0 (0)	12	0 (0)	-	-
4	56	2 (3.6)	29	1 (3.4)	27	1 (3.7)	-	-
5	108	3 (2.8)	95	3 (3.2)	13	0 (0)	-	-
6	30	0 (0)	18	0 (0)	12	0 (0)	-	-
7	32	0 (0)	32	0 (0)	-	-	-	-
8	77	1 (1.3)	25	1 (4.0)	52	0 (0)	-	-
9	114	1 (0.9)	58	1 (1.7)	40	0 (0)	16	0 (0)
Total	**571**	**13**						

Sex, source, and breed were not associated with odds for being *B. canis* seropositive, however, age was significant (Table 3.4). Adult dogs had 14.4 times greater odds for being seropositive compared to puppies (95% CI: 1.81, 114.24). All 13 positive samples came from adult dogs, for an apparent prevalence of 3.6% in this population. Multivariable logistic regression using manual forward selection did not improve model fit or identify other significant risk factors.

Table 3.4 Logistic regression analysis of individual dog risk factors for *B. canis*

Variable	*Levels*	n	*B. canis* pos *(%Prev)*	p	OR	Lower 95% CI	Upper 95% CI
Age	>2 years	155	6 (5.2)	0.03	3.43	1.13	10.43
	≤2 years	443	7 (1.6)	Ref			
Sex	Male	255	8 (3.1)	0.27	1.90	0.61	5.89
	Female	298	5 (1.7)	Ref			
Source	Owner Surrender	49	3 (6.1)	0.19	5.07	0.45	56.53
	Stray	68	1 (1.5)	Ref			
Breed	Terrier/Toy	113	5 (4.4)	0.08	4.37	0.83	23.06
	Herding/Working/ Non-sporting	125	4 (3.2)	0.20	3.10	0.56	17.26
	Hound	117	1 (0.9)	0.87	0.81	0.07	9.12
	Sporting	188	2 (1.1)	Ref			

Not all information was available for every dog. Models contain the following number of observations: age (n=558), sex (n=553), source (n=117), breed (n=543). Shelter included as a random effect in all models (PROC GLIMMIX).

Stochastic modeling of *B. canis* true prevalence within the Mississippi shelter dog population resulted in a distribution exhibiting right skew with a mean of 7.4% and a 95% credible interval of 3.5% to 12.8% (Figure 3.1). However, the model produced a bimodal distribution of *B. canis* seroprevalence by shelter with no *B. canis* present in 64.6% of shelter iterations. Of the remaining 35.4% of shelters, mean seroprevalence in a shelter was 17.8%. Seroprevalence by shelter exhibited right skew, with a median seroprevalence of 13.3% (Figure 3.2).

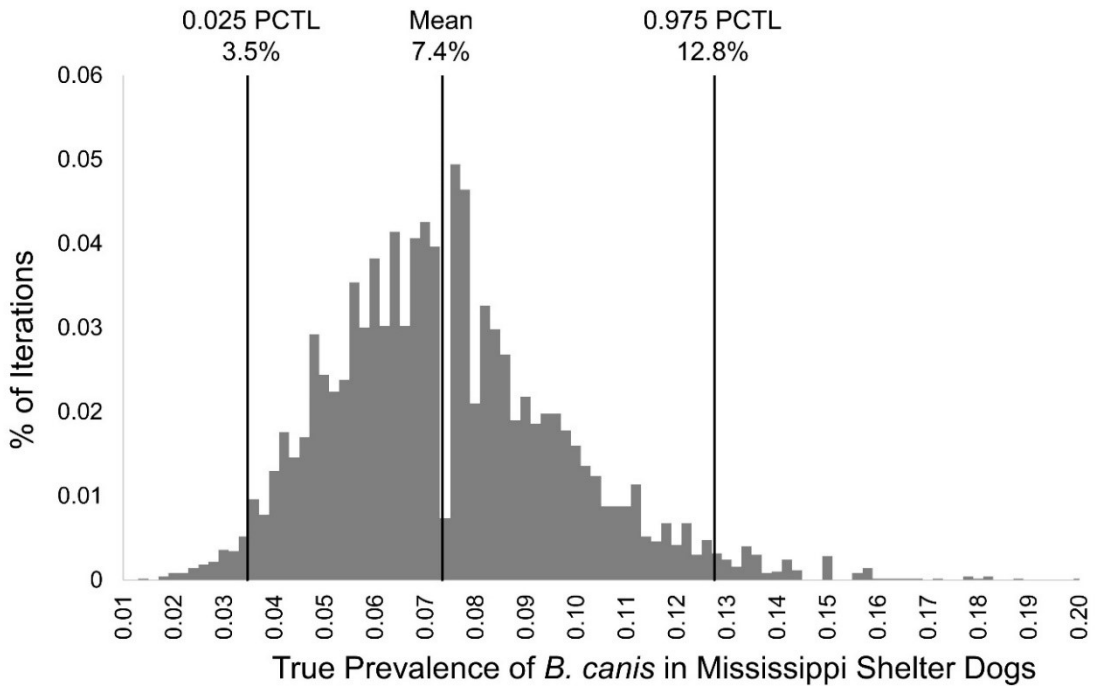

Figure 3.1 Modeled true prevalence of *B. canis* in the Mississippi shelter dog population

Stochastic model includes 5,000 iterations. The shaded portion represents the 95% credible interval.

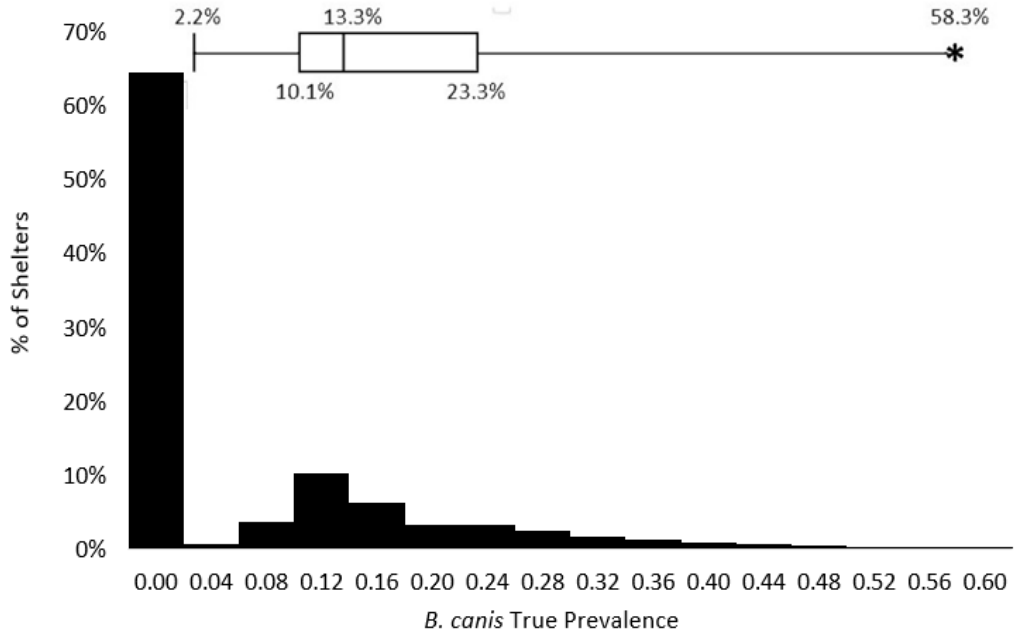

Figure 3.2 Modeled true seroprevalence of *B. canis* by shelter

Modeled are 5,000 iterations for each of 18 sampled shelters. The box and whisker plot denotes the minimum, first quartile, median, third quartile, and 99th percentile for the 35.4% of shelters with *B. canis*. The maximum modeled value, prevalence of 1.0, is not shown to improve graph clarity.

Discussion

Our seroprevalence estimates are consistent with previous reports of brucellosis in shelter dog populations. Although we did not detect *B. suis*, we expected very low prevalence in the state and did not selectively sample hunting dogs which have the greatest risk of exposure.[2] Rather, we were able to confirm that shelter dogs do not pose a meaningful *B. suis* zoonotic risk. The apparent *B. canis* prevalence of 2.3% in this study is similar to other findings, with the slightly lower prevalence likely due to differences in serological tests used and sampling strategy. We included puppies in our testing because infection can result from exposure during whelping or nursing from an infected bitch,

however, all positive dogs in our sample were adults which we expect to be at greatest risk due to predominately venereal transmission of *B. canis*.[5] Apparent prevalence in adult dogs sampled was 3.6%.

Previous studies have not reported prevalence corrected for imperfect diagnostic test performance. Challenges in serologic diagnosis of brucellosis are well recognized,[21,25-26] and the commercial RSAT has poor diagnostic sensitivity when used in series with the 2ME-RSAT.[14] Reports of apparent prevalence are therefore likely to underestimate true prevalence of *B. canis*. Calculations for true prevalence are straightforward and should be applied when assessing disease risk, however, more advanced statistical methods, such as stochastic modeling, may be needed to determine a confidence interval around the true prevalence when accounting for clustering or other effects of sampling strategy.

The mean seroprevalence we obtained from our model of overall prevalence is similar to that expected when correcting for test performance. Our model produced a narrower 95% credible interval than the corresponding 95% confidence interval of true prevalence calculated by the standard, but conservative, equation using the normal approximation.[23] More importantly, the bimodal distribution from our stochastic model by shelter indicates that *B. canis* prevalence in our target population is not adequately described by a singular mean value. Mississippi animal shelters do not have an "average" prevalence of brucellosis, rather, the majority of shelters do not have *B. canis*, while a small number have a much greater prevalence of disease.

Additional work is necessary to determine if these disease clusters result from our sampling process, increased transmission of brucellosis in some shelters, or differing

levels of brucellosis in communities surrounding shelters. Shelter seroprevalence was measured on a single sampling day and may not be repeatable or reflective of an individual shelter or community. However, if certain shelters consistently contain seropositive dogs, eradication or control efforts may be most effective when resources are allocated to identify and minimize disease at the individual shelter level. Interventions for shelters with brucellosis may include management practices, such as eliminating group housing of intact dogs if transmission occurs within the shelter, or community education and policy concerning owned and stray dogs if dogs are already seropositive at shelter intake.

Currently available diagnostic tests misclassify some individual dogs. The most likely outcome for test positive dogs in a shelter is euthanasia, so a highly specific test is desirable to prevent false positives. Use of the 2ME-RSAT test to diagnose positive dogs improves test specificity, but results in decreased test sensitivity and a greater proportion of false negatives which remain in the population. Based on our stochastically estimated population prevalence of 7.4%, removal of 2ME-RSAT test positives results in an absolute risk reduction of 2.2% and requires 44 dogs to be tested to identify a positive. Assuming a cost of $26.00 per test, the cost to identify a positive dog is ~$1150, a considerable investment for animal shelters which are often resource poor, especially considering that 5% of dogs in the population remain positive.

Effective brucellosis control cannot be achieved by individual dog testing, however, population testing may be useful to estimate if brucellosis is present in individual shelters. Our model demonstrates that most shelters should not be prioritizing limited resources toward brucellosis control, but a small number of shelters may have a

high seroprevalence of brucellosis that could pose a risk for transmission to other dogs, shelter workers, or adopters. Similar to breeding kennels, identification of these high-risk shelters depends on recognition of clinical signs or requires population testing at several time points, with limited risk reduction and high cost. Other preventive measures, such as spay/neuter of all dogs prior to adoption and public education including clinical signs of disease and good hygiene practices, may feasibly reduce transmission risk to other dogs and to humans and be more viable options for brucellosis control in animal shelters.

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Footnotes

a. Microsoft Excel, version 2013, Microsoft Corporation, Redmond, WA

b. PROC MIXED, SAS, version 9.4, SAS Institute Inc., Cary, NC

c. @risk, version 7.5, Palisade Corporation, Ithaca, NY

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CHAPTER IV

AMERICAN TRYPANOSOMIASIS (CHAGAS' DISEASE) SEROSURVEY OF MISSISSIPPI SHELTER DOGS

(Prepared for submission to Emerging Infectious Diseases)

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Abstract

American trypanosomiasis, caused by the parasite *Trypanosoma cruzi*, is uncommon in the United States, but may result in serious cardiac disease in infected people and animals. Domestic dogs are important hosts in domestic cycles in South America, and naturally occurring canine cases have been reported in the United States. Triatomine insects, the primary biological vector, are present across the southern United States and endemic disease has been described in wildlife. Dogs may be a useful sentinel for human disease risk due to similarities in disease between the two species. Free-ranging dogs or those housed primarily outside may have greater exposure to feeding vectors and through ingestion of infected insects.

A serum bank containing samples from 566 shelter dogs proportionately sampled from nine geographic districts in Mississippi was tested for the presence of *T. cruzi* antibodies using a commercial immunochromatographic assay validated for use in domestic dogs. Forty-two of 566 dogs were seropositive for *T. cruzi* (7.4%, 95% CI: 4.7, 10.1%). Prevalence by shelter ranged from 0 to 25%, but neither shelter nor district was significantly associated with the probability of being seropositive. Although 6 puppies <6 months of age were seropositive for *T. cruzi*, adult dogs had 3.6 times greater odds for being seropositive. Accounting for the random effect of shelter, the greatest model-adjusted *T. cruzi* seroprevelance by age was seen in dogs 3-5 years of age ($\hat{p}$=0.17, SE=0.05). Other risk factors including sex, breed, and source were not significant in logistic regression models containing shelter as a random effect.

This is the first report of the prevalence of *T. cruzi* in the Mississippi dog population. Shelter dogs may serve as useful sentinels and provide an initial estimate of

typanosomiasis occurrence and distribution within the state. These data help us assess the risk for dogs to be infected with *T. cruzi* as well as the public health risk posed by *T. cruzi* in Mississippi.

Keywords: American trypanosomiasis, Chagas' disease, shelter dogs

Introduction

Chagas' disease is classified as one of five neglected parasitic diseases by the Centers for Disease Control and Prevention due to the limited surveillance and prevention measures currently in place.[1] People and dogs show very similar courses of disease when infected with *Trypanosoma cruzi,* with the exception of frequent sudden death due to cardiomyopathy seen in dogs less than 1 year of age. In older dogs and people, the disease is often asymptomatic in acute cases, although severe disease may rarely result in myocarditis or encephalitis.[2]

Chronic infections result in severe cardiac or gastrointestinal disease in 20-30% of people,[3] while over 80% of experimentally infected dogs showed abnormal electrocardiograms (ECGs) three months after disease induction.[4] Treatment options are limited and carry a high complication rate, contributing to the large economic burden of the disease in endemic areas.[2,5] Although autochthonous human cases are rare in the United States, implementation of blood donor screening has increased identification of chronic carriers.[6] Since screening begin in 2007, two Mississippi donors have been identified with suspect locally-acquired infections.[7]

T. cruzi is endemic in much of the southern United States, and the disease can be carried by over 100 animal species. Raccoons and opossums have the highest reported prevalence, ranging from 1.5-63% and 8-33% respectively.[8] Transmission occurs primarily through the bite of triatomine bugs, commonly called kissing bugs, 11 species of which are found in the United States. *Triatoma sanguisuga* is broadly distributed across the entire Southeast, including Mississippi, and has a wide host range including wildlife, dogs, chickens, and humans.[2]

Dogs are recognized hosts for Chagas' disease in South and Central America, and local transmission to dogs has been reported in the United States.[9-11] Dogs may acquire the infection through ingestion or from the bite of an infected triatomine insect, and may pose a zoonotic risk by serving as a source of infection for other triatomine insects or through direct contact with an infected dog's blood such as by an accidental needle-stick.[10] Dogs may also serve as a sentinel for human disease, as they often have greater exposure to infectious vectors and they have a shorter incubation time with clinical manifestations recognized in domestic dogs before identification of disease in humans.[9-10]

Prevalence of *T. cruzi* is poorly documented for dogs in the United States. Although several studies have reported population level prevalence, studies vary widely in the population tested and diagnostic test(s) used, making comparisons across studies difficult. [10,12-17] In addition, several previous reports have used convenience sampling which may not be indicative of the overall dog population. Studies may also suffer from lack of resolution; vector borne diseases such as Chagas' may occur at uneven rates, with pockets of hyperendemicity occurring with convergence of suitable vector habits, high populations of reservoir hosts, and higher rates of disease and transmission.[18] Systematic, intensive sampling may be required to detect important differences in regional transmission and dog risk factors for disease.

No studies have measured seroprevalence in Mississippi dogs, and limited information is available on occurrence in wildlife and triatomine vectors.[2] There have been comparatively few human Chagas' cases in Mississippi found during blood donation screening, with only 9 of the 2281 confirmed positives in the AABB Biovigilance Network reported from Mississippi in the past 10 years.[19] However, these

low numbers are likely reflective of a smaller Latin America immigrant population in which the majority of positives are measured. Of note, two Mississippi natives were identified as probable autochthonous cases shortly after widespread blood donor screening was implemented, and triggered The United States *Trypanosoma cruzi* Infection Study by the CDC.[6] Human cases in Mississippi may therefore represent greater occurrence of indigenously acquired disease than many other states. Stratified random testing of shelter dogs on a state-wide level may help resolve important questions on *T. cruzi* prevalence in Mississippi as an indication of autochotonous disease risk for both dogs and humans.

Materials and Methods

Serum samples were banked from a cross-sectional study of shelter dogs across Mississippi (Chapter 2). In brief, samples were obtained following a census of animal shelters within the state of Mississippi to determine shelter dog population and distribution. A total of 571 dogs were proportionately sampled from the nine Public Health Districts within the state to represent geographical population distribution. In each district, 1-3 shelters were sampled based on previously established relationships and willingness to participate. Each dog in the shelter over 8 weeks of age was assigned a consecutive number, and dogs selected for sampling were chosen via a random number generator. Whole blood samples, not exceeding 10% of the dog's circulating blood volume, were collected, kept on ice during transportation then refrigerated until processing within 24 hours of sampling. Blood tubes were centrifuged at 3000 rpm for 10 minutes, followed by serum collection using a pipette. Sera was banked in 1.0ml aliquots at -80°C. Each sample was labeled with an identification number, dog name or number

assigned by the shelter, collection date, and shelter of origin. Brief physical exam information for each sampled dog was recorded in a database, along with quantity of serum banked and any test results obtained on serum samples.

Available banked serum samples (n=566) were tested for the presence of *T. cruzi* antibodies using a commercial immunochromatographic assay (Chagas' STAT-PAK, Chembio Diagnostic Systems, Inc., Medford, NY, USA) designed for human blood screening but validated for use in domestic dogs.[16] Apparent *T. cruzi* seroprevalence and 95 percent confidence interval accounting for clustering by shelter was calculated for the Mississippi shelter dog population based on the results of the immunochromatographic test.[20] Apparent seroprevalence for each of 18 sampled shelters was mapped using open source geographic information system software.[a]

The association between seropositive status and various dog characteristics was tested using logistic regression models.[b] Candidate categorical variables included geographic region (1-9), shelter (1-18), sex (male or female), intake source (owner surrender or stray/animal control), and breed (primary identified breed classified into the 7 American Kennel Club breed groups and further consolidated into 4 groups due to low numbers of toy, non-sporting, and working group dogs sampled). Age was analyzed as a binary variable (puppies <6 months of age or adults >6 months of age based on eruption of secondary canine teeth) to investigate the probability of dogs less than 6 months of age being seropositive, a population which has rarely been included in previous serosurveys. Additionally, association between seropositive status and age was analyzed for young dogs (<2 years of age) and mature dogs (>2 years of age), as in a previous study; separate models were prepared for all dogs tested in the serum bank and with the removal of

puppies less than 6 months of age.[17] Finally, age was analyzed as a categorical variable with 4 levels based on researchers' confidence in estimating dog age (<6 months, 6 months to 2 years, 3-5 years, >5 years of age), and displayed as the model-adjusted prevalence by age. Shelter was included as a random effect in all univariable models and in multivariable model assessment using manual forward selection. Because of an expected low prevalence, an alpha of 0.1 was selected *a priori* for assessing risk factors.

Results

Forty-two of 566 dogs tested positive for antibodies to *T. cruzi,* for an apparent prevalence of 7.4% (95% CI: 4.7, 10.1%) in the Mississippi shelter dog population. Prevalence by shelter ranged from 0 to 25%, but was not associated with geographic district (Figure 4.1). Risk factors including shelter of origin, sex, breed group, and source were not significant (Table 4.1). However, adult dogs (>6 months of age) had 3.60 times greater odds for being seropositive than puppies (95% CI: 1.49, 8.73). Dogs >2 years of age had 2.98 times greater odds for being seropositive than dogs <2 years of age (95% CI: 1.51, 5.88), which remained significant with the removal of puppies less than 6 months of age (OR=2.68, 95% CI: 1.23, 5.86; Table 4.2). Age was significant when assessed as a categorical variable, with higher *T. cruzi* seroprevalence in dogs between 3-5 years of age than in dogs <6 months or between 6 months and 2 years (Figure 4.2).

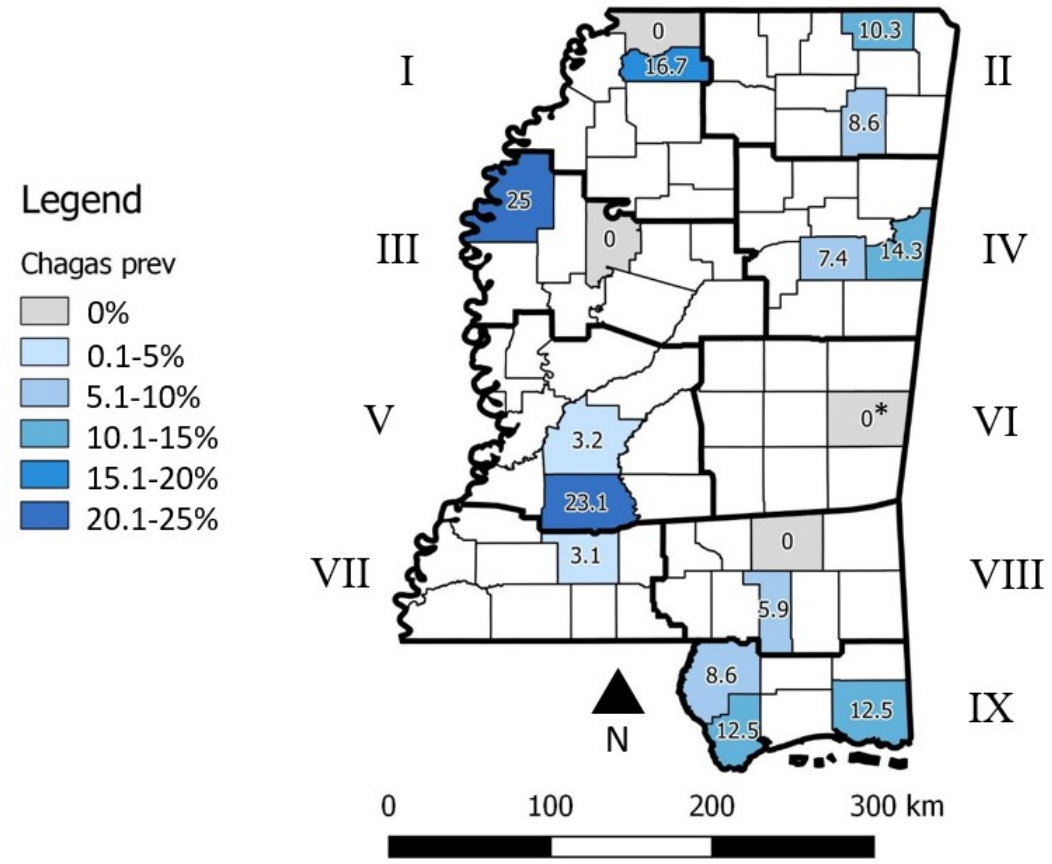

Figure 4.1 Seroprevalence of *T. cruzi* in 566 sampled dogs from 18 shelters

*Represents two shelters sampled in a single county, with no positive dogs sampled in either. Roman numerals depict the nine public health districts in Mississippi.

Table 4.1 Prevalence of *T. cruzi* in sampled dogs and univariable analyses of risk factors for seroprevalence

Risk Factor	n	No. pos (%)	OR	95% CI	Estimate	SE	p
District							0.73
Intercept					-2.10	0.43	<.0001
I – Northwest	54	5 (9.3)	0.83	0.24-2.92	-0.18	0.63	0.78
II – Northeast	64	6 (9.4)	0.85	0.26-2.80	-0.17	0.61	0.78
III – Delta Hills	33	3 (9.1)	0.82	0.19-3.52	-0.20	0.74	0.79
IV – Tombigbee	55	6 (10.9)	Ref	Ref	0	-	Ref
V – West Central	108	6 (5.6)	0.48	0.15-1.57	-0.73	0.60	0.22
VI – East Central	30	0 (0)	<.001	<.001-	-13.47	438.1	0.98
VII – Southwest	32	1 (3.1)	0.26	0.03-2.31	-1.33	1.10	0.23
VIII – Southeast	76	3 (3.9)	0.34	0.08-1.41	-1.09	0.73	0.14
IX – Coastal Plains	114	12 (10.5)	0.96	0.34-2.72	-0.04	0.53	0.94
Shelter							0.63
Intercept					-2.36	0.47	<.0001
1	24	0 (0)	<.001	<.001-	-14.21	807.5	0.99
2	30	5 (16.7)	2.12	0.56-8.02	0.75	0.68	0.27
3	29	3 (10.3)	1.22	0.27-5.54	0.20	0.77	0.79
4	35	3 (8.6)	0.99	0.22-4.46	-0.006	0.76	0.99
5	12	3 (25.0)	3.53	0.71-17.5	1.26	0.81	0.12
6	21	0 (0)	<.001	<.001-	-14.21	863.3	0.97
7	28	4 (14.3)	1.77	0.43-7.19	0.57	0.71	0.43
8	27	2 (7.4)	0.85	0.15-4.69	-0.16	0.87	0.85
9	13	3 (23.1)	3.18	0.65-15.5	1.16	0.81	0.15
10	95	3 (3.2)	0.35	0.08-1.51	-1.06	0.75	0.16
11	12	0 (0)	<.001	<.001-	-14.21	1142.1	0.99
12	18	0 (0)	<.001	<.001-	-14.21	932.5	0.99
13	32	1 (3.1)	0.34	0.04-3.10	-1.07	1.12	0.34
14	51	3 (5.9)	0.66	0.15-2.93	-0.41	0.76	0.59
15	25	0 (0)	<.001	<.001-	-14.21	791.2	0.99
16	16	2 (12.5)	1.51	0.26-8.68	0.41	0.89	0.64
17	40	5 (12.5)	1.51	0.41-5.63	0.41	0.67	0.53
18	58	5 (8.6)	Ref	Ref	0	Ref	Ref
***Sex**							0.48
INTERCEPT					-2.62	0.28	<.0001
Female	275	25 (8.3)	1.27	0.67-2.42	0.23	0.33	0.46
Male	238	17 (6.7)	Ref	Ref	Ref	Ref	Ref
***Breed**							0.71
INTERCEPT					-2.76	0.35	
Sporting	187	11 (5.9)	Ref	Ref	Ref	Ref	Ref
Hound	116	10 (8.6)	1.59	0.66-3.79	0.50	0.45	0.30
Herding/Working	120	9 (7.5)	1.21	0.47-3.10	0.18	0.49	0.69
Terrier/Toy/Non-sporting	115	10 (8.7)	1.52	0.63-3.72	0.38	0.46	0.35
***Source**							0.39
INTERCEPT					-2.77	0.52	0.006
Owner Surrender	49	5 (10.2)	1.82	0.46-7.23	0.60	0.70	0.39
Stray/Animal Control	68	4 (5.9)	Ref	Ref	Ref	Ref	Ref

*Univariable models include shelter as a random effect.

Table 4.2 Univariable analyses for *T. cruzi* seroprevalence association with age

Risk Factor	n	No. pos (%)	OR	95% CI	Estimate	SE	p
Age 1	564						0.005
Intercept					-3.46	0.43	<.001
Puppy (<6 months)	202	6 (3.0)	Ref	Ref	Ref	Ref	Ref
Adult (>6 months)	362	36 (9.9)	3.59	1.47-8.77	1.28	0.45	0.005
Age 2	553						0.002
Intercept					-2.87	0.26	
Young (<2 years)	439	24 (5.5)	Ref	Ref	0	-	Ref
Adult (>2 years)	114	16 (14.0)	2.98	1.51-5.88	1.09	0.35	0.002
Age 3	333						0.014
Intercept					-2.77	0.31	
Young (<2 yrs, no pups)	219	13 (5.9)	Ref	Ref	0	-	Ref
Adult (>2 years)	114	16 (14.0)	2.68	1.23-5.86	0.99	0.40	0.014
Age 4	553						0.009
Intercept					-2.94	0.34	<.001
1 (<6 months)	220	11 (5.0)	Ref	Ref	0	-	Ref
2 (6m-2yr)	219	13 (5.9)	1.14	0.49-2.66	0.13	0.43	0.75
3 (3-5 yrs)	78	13 (16.7)	3.87	1.63-9.20	1.35	0.44	0.002
4 (5+ yrs)	36	3 (8.3)	1.81	0.47-6.91	0.59	0.68	0.39

Shelter is included as a random effect in all models.

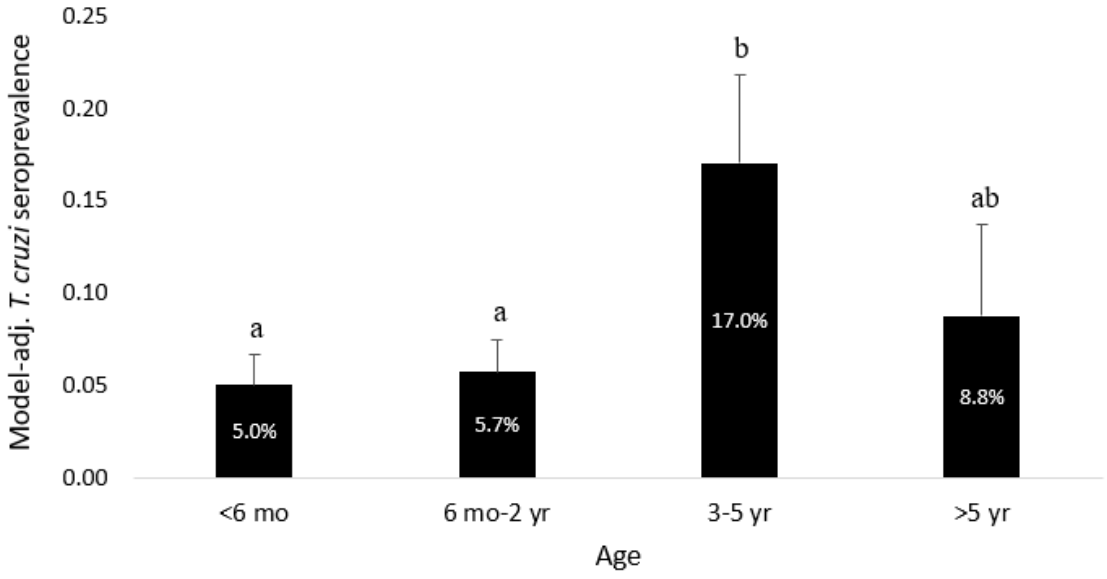

Figure 4.2 Model-adjusted *T. cruzi* seroprevalence by dog age

Different superscripts represent significant differences in probability for *T. cruzi* seropositivity by age at α=0.1. Error bars represent one standard error.

Discussion

This study provides the first estimate of the probability for dogs being seropositive for *T. cruzi* in Mississippi. We found an apparent seroprevalence of 7.4% in Mississippi shelter dogs, consistent with findings from a similar study reporting 8.8% seroprevalence in Texas shelter dogs.[17] Other research has detected a lower seroprevalence of 2.3% in Louisiana shelter dogs,[13] and prevalence rates between 2.6% and 12% in owned dogs from southern states.[10,12,14-16] This variation may reflect differences in disease endemnicity rates, exposure risks between owned and shelter dog populations, or diagnostic test performance.

Seropositive shelter dogs were found in 8 of 9 geographic districts in the state, and in 13 of 18 sampled shelters, indicating widespread exposure to infectious insect vectors or mammalian hosts. Additionally, geographic districts showed wide variation in seroprevelance between shelters. Reasons for these differences may include focal pockets with higher disease occurrence, transmission within a shelter, or may be an artefact of our sampling strategy. Samples were collected from each shelter on a single day, and seroprevalence at the individual shelter level may not be a consistent and repeatable measure.

Age was significantly associated with the probability of being seropositive in our study. Infection with *T. cruzi* has been detected in dogs as young as 6 weeks of age,[21] however, little work has been done to measure disease in dogs less than 6 months of age. Young dogs (<1 year) are at increased risk for sudden death or severe signs of disease,[11] but our study also indicates a low prevalence of subclinical infection. Odds for being seropositive were 3.6 times greater in dogs >6 months of age, and the model-adjusted

prevalence by age was greatest for dogs between 3-5 years of age. A similar age association has been reported in populations with very high seroprevalence in endemic areas of South America, where prevalence of infection was associated with increasing dog age.[22]

Targeted surveillance to detect *T. cruzi* occurrence may be most effective in dogs greater than 6 months of age with potential for vector exposure, however, identification of young infected dogs may be important to minimize transmission and zoonotic risk. The same study found that 100% of dogs less than 1 year of age were infectious to bugs, while only 50% of dogs >7 years of age were infectious, and that young dogs infected a greater number of feeding vectors.[22] Although authors report that age may be a surrogate for acuteness of infection rather than a true age association with infectiousness, young dogs may contribute to parasite burden in vectors and transmission of disease if not identified.

Future work will include confirmatory testing of positive samples using an indirect fluorescent antibody (IFA) test for titer quantification. Results of the IFA will be compared against categorical scores (strong, medium, or weak positive) based on the color saturation of the immunochromatographic test to determine if color intensity reflects titer and if weakly positive samples should be considered seropositive. Prior work by other researchers found that only 4 of 11 faintly positive samples had detectable antibody levels on an IFA.[17] Our seroprevalence may therefore be an overestimate; if only strong positives on the immunochromatographic test are positive by IFA, the apparent seroprevalence of *T. cruzi* will decrease to 3.7%, with 1% of puppies and 5.3%

of adult dogs in our sample being seropositive. This information may guide future interpretation of immunochromatogrphic test results when determining seroprevalence.

Little information is available on the occurrence of *T. cruzi* in Mississippi. Our study suggests widespread exposure in the shelter dog population, with potential for exposure of owned dogs and humans in the state. Additional work is needed to determine routes of infection within these populations and risk factors for disease. Although only a single species of kissing bug, *Triatoma sanguisuga*, is reported to be widespread within the state, seroprevalence is similar to Texas where numerous competent vectors are present. Mississippi may, therefore, represent an important area of disease endemicity within the United States. Veterinarians should consider *T. cruzi* as a differential in dogs with chronic cardiomyopathy or for sudden death in young dogs when history suggests possible exposure. Surveillance of clinical and seropositive canine cases within the state may help identify risk factors for autochthonous Chagas' disease in people.

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Footnotes

a. QGIS, Version 2.14, QGIS Development Team, Open Source Geospatial Foundation, URL http://http://qgis.osgeo.org

b. PROC GLIMMIX, SAS for Windows, Version 9.4, SAS Institute Inc., Cary, NC, USA

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CHAPTER V

CONCLUSIONS

Shelter dogs are a unique subset of the United States dog population, with different risk factors and prevalence of disease than owned dogs. Knowledge of the prevalence of disease in this population is necessary for the correct application and interpretation of diagnostic tests and for effective allocation of resources within animal shelters. Additionally, shelter dogs may be a useful population to assess public health risks from zoonotic diseases such as canine brucellosis, in which dogs are the primary reservoir, as well as effective sentinels for exposure risk for diseases such as American trypanosomiasis.

In the second chapter, a cross-sectional study describes a census of animal shelters in Mississippi and establishment of a serum bank. Many previous seroprevalence studies have relied on convenience sampling, resulting in potential for bias and either under- or over-estimation of disease. Random sampling eliminates many sources of bias but requires an accurate sampling frame of animal shelters, which is not readily available for much of the United States, including Mississippi. The census was a necessary first step in order to quantify the shelter dog population in the state and to establish the distribution of shelters and dogs in Mississippi. Based on this census, over 500 dogs were randomly sampled from 18 shelters to represent the Mississippi shelter dog population.

Sera from these dogs were banked for use in the studies reported in subsequent chapters as well as for future seroprevalence research.

The third chapter reports an estimate of the seroprevalence of canine brucellosis in the Mississippi shelter dog population. A previous study in Georgia found a small number of dogs positive for *B. suis*, a highly zoonotic disease, following exposure to feral swine. We demonstrated that shelter dogs do not pose a significant risk for transmitting *B. suis* despite the presence of infected feral swine in Mississippi, but an estimated 7.4% of shelter dogs are seropositive for *B. canis*. Additionally, this study is the first to report a bimodal distribution of *B. canis* in animal shelters, with the majority of shelters having no infected dogs and a small number of shelters having a much higher proportion of infected dogs. This study also addresses diagnostic testing limitations by reporting true prevalence of disease accounting for test sensitivity and specificity. The following appendices contain educational material developed for the public and veterinarians regarding canine brucellosis prevention and ongoing surveillance in the state.

The fourth chapter contains an estimate of the seroprevalence of *T. cruzi*, the causative agent of Chagas' disease, in the Mississippi shelter dog population. Rare autochthonous cases of Chagas' disease have been reported in dogs and people in Mississippi, but little is known about the occurrence of disease in domestic dogs, insect vectors, or wildlife mammalian hosts. We found 7.4% of dogs seropositive for *T. cruzi,* with positive dogs identified in 8 of 9 geographic districts and 13 of 18 sampled shelters, indicating widespread exposure in Mississippi. Additionally, we found that while older dogs are at greater risk for being seropositive, a small proportion of young puppies were also seropositive and may contribute to disease transmission through insect vectors.

The research included in this thesis has important implications for dog and human health in the state of Mississippi and beyond. Thousands of dogs are transported from shelters in the state to adoption centers across the United States each year and may result in dissemination of disease. The studies included in this thesis, along with future seroprevalence research utilizing the serum bank, will assist in the development of evidence-based public policy and disease control programs aimed at safeguarding animal and human populations.

APPENDIX A

WHITE PAPERS CREATED IN CONJUNCTION WITH THE MISSISSIPPI BOARD OF ANIMAL HEALTH FOR PUBLIC EDUCATION ON CANINE BRUCELLOSIS

How is brucellosis spread?

Dog-to-dog spread of brucellosis occurs most often through breeding and by contact with vaginal discharges, semen, birthing fluids, and urine. Contact with an infected dog's blood, milk, saliva, and feces are less common sources of infection.

Brucellosis can spread from dogs to people through contact with an infected dog's birthing fluids and vaginal discharge or infected puppies. This is why dog breeders and veterinarians are at higher risk. Rarely, brucellosis can be spread from family pets to people through contact with urine, saliva, and other bodily fluids from infected dogs.

What are the symptoms?

In female dogs, the most common symptoms are aborted pregnancies, stillbirth, and inability to become pregnant. In males, the primary symptom is the inability to sire puppies. Both sexes may have swollen lymph glands, eye disease, and infections of the spine. However, most infected dogs appear normal and show no symptoms except for infertility. Female dogs can deliver healthy-appearing, but infected puppies.

Brucellosis in people normally causes a fever, headache, swollen lymph glands, night sweats, joint and muscle aches, fatigue, weight loss, and swollen liver and/or spleen. Some people may not have any symptoms.

For more information, contact:
Mississippi Board of Animal Health,
601-359-1170, www.mbah.ms.gov.

This information was adapted from the Canine Brucellosis and Foster-Based Rescue Dog Brochure by the Minnesota Department of Health

& created in conjunction with

MISSISSIPPI STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

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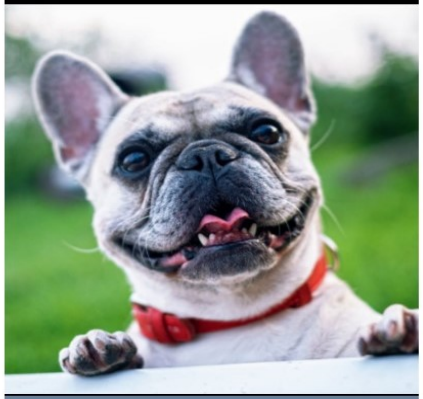
Canine Brucellosis: Information for Animal Shelters



Mississippi Board of Animal Health

James A. Watson, D.V.M.
State Veterinarian

Figure A.1 Canine Brucellosis: Information for Animal Shelters (Page 1)

What is canine brucellosis?

Canine brucellosis is a disease found in dogs caused by a type of bacteria called *Brucella canis*. Infected dogs can also spread the disease to people.

Who gets brucellosis?

Dogs and people can become infected with brucellosis.

Dogs that have not been spayed or neutered (intact) are at higher risk of having brucellosis. This includes dogs in breeding programs and intact stray or owned free-roaming dogs.

In people, dog breeders and veterinarians are most at risk of infection because of their work with birthing puppies (whelping). People can also get brucellosis from their family pet, but this is much less common.

How do you test for brucellosis?

Testing for brucellosis can be very difficult. Not all infected dogs will test positive, and dogs may require several tests to decide if they have brucellosis. Your veterinarian may recommend testing dogs that are sick or those which have been in contact with an infected dog.

What can animal shelters do to reduce risk of canine brucellosis?

Animal shelters may have dogs infected with brucellosis since stray dogs are at greater risk, however, dogs only infect others through direct contact, usually during breeding. Ways to prevent spread in animal shelters include:

Prevent Breeding – House intact dogs alone or with other dogs of the same sex.

Spay and Neuter - Although spayed or neutered dogs can be infected, they are not as likely to spread the disease to other dogs or people.

Monitor – Monitor all dogs daily for signs of disease and promptly isolate any that are sick. Any items such as food bowls, beds, and leashes should not be used for healthy dogs until disinfected.

Recognize Risks - Dogs that have aborted or stillborn puppies may have brucellosis. Wear gloves and thoroughly disinfect whenever a litter is born and use extra caution if the mom or puppies are sick.

Cleaning and Disinfection - The bacteria which causes brucellosis can survive for months in dirty environments, but is readily killed by common disinfectants when applied to clean surfaces.

Can brucellosis be treated?

In dogs brucellosis is very difficult to treat and relapses are common. Treatment includes spaying or neutering, giving antibiotics for several months, and frequent blood tests to monitor treatment progress. Treatment is typically not attempted for dogs in breeding kennels and when dogs can not be regularly tested and isolated from other dogs and people, potentially for the life of the dog.

Brucellosis is treatable in people. Treatment involves taking antibiotics daily for several months, regular monitoring, and may also involve surgery.

Figure A.2 Canine Brucellosis: Information for Animal Shelters (Page 2)

How is brucellosis spread?

Dog-to-dog spread of brucellosis occurs most often through breeding and by contact with vaginal discharges, semen, birthing fluids, and urine. Contact with an infected dog's blood, milk, saliva, and feces are less common sources of infection.

Brucellosis can spread from dogs to people through contact with an infected dog's birthing fluids and vaginal discharge or infected puppies. This is why dog breeders and veterinarians are at higher risk. Rarely, brucellosis can be spread from family pets to people through contact with urine, saliva, and other bodily fluids from infected dogs.

What are the symptoms?

In female dogs, the most common symptoms are aborted pregnancies, stillbirth, and inability to become pregnant. In males, the primary symptom is the inability to sire puppies. Both sexes may have swollen lymph glands, eye disease, and infections of the spine. However, most infected dogs appear normal and show no symptoms except for infertility. Female dogs can deliver healthy-appearing, but infected puppies.

Brucellosis in people normally causes a fever, headache, swollen lymph glands, night sweats, joint and muscle aches, fatigue, weight loss, and swollen liver and/or spleen. Some people may not have any symptoms.

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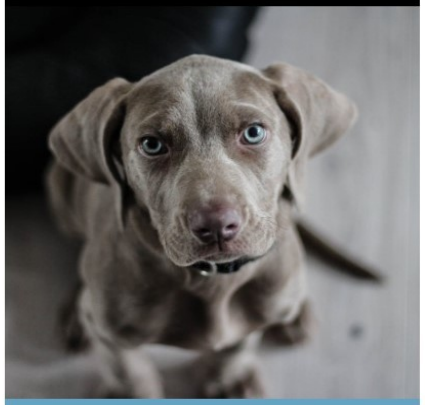
Canine Brucellosis: Information for Breeding Kennels



Mississippi Board of Animal Health

James A. Watson, D.V.M.
State Veterinarian

Figure A.3 Canine Brucellosis: Information for Breeding Kennels (Page 1)

What is canine brucellosis?

Canine brucellosis is a disease found in dogs caused by a type of bacteria called *Brucella canis*. Infected dogs can also spread the disease to people.

Who gets brucellosis?

Dogs and people can become infected with brucellosis.

Dogs in breeding programs are at higher risk of having brucellosis due to increased reproductive contact. Infected puppies can be born to apparently healthy moms.

In people, dog breeders and veterinarians are most at risk of infection because of their work with birthing puppies (whelping). People can also get brucellosis from their family pet, but this is much less common.

How do you test for brucellosis?

Testing for brucellosis can be very difficult, and not all infected dogs will test positive. Breeding animals should come from kennels without a history of brucellosis or reproductive losses. Isolating and testing dogs before they enter a kennel may identify some infected dogs, however, common tests may fail to identify half or more of infected dogs.

If one dog tests positive in a breeding kennel, it can be very difficult to determine which other dogs in the kennel have been infected even with testing. Some dogs may test negative on blood tests due to early infection or because the bacteria is in other parts of the body. Often, all dogs in the kennel are considered infected due to the risk to other dogs and people.

What can breeding kennels do to reduce risk of canine brucellosis?

All dogs purchased for breeding or being sold from a breeding kennel should be inspected by a licensed veterinarian. By law, all dogs traveling across states lines must have a valid health certificate (certificate of veterinary inspection). Work with your veterinarian to develop a preventive program to reduce the risk of brucellosis and other diseases from entering your kennel. Plans should include:

Biosecurity – Limit movement of people and dogs into and out of the kennel. Ensure that dogs entering the facility are apparently healthy and come from disease-free kennels.

Cleaning and Disinfection - The bacteria which causes brucellosis can survive for months in dirty environments, but is readily killed by common disinfectants when applied to clean surfaces.

Monitoring – Monitor all dogs daily for signs of disease and promptly isolate any that are sick. Ill dogs should not have contact with other dogs, including using the same outdoor spaces unless cleaned and disinfected after use. Any items such as food bowls, beds, and leashes should not be used for healthy dogs until thoroughly disinfected.

Can brucellosis be treated?

Brucellosis is very difficult to treat and relapses are common. Treatment is typically not attempted for dogs in breeding kennels because even the best treatments may fail, are expensive, and may require medication and follow-up testing for months. Some dogs remain test positive for two years or more even with treatment and may need to be retreated multiple times. Euthanasia of breeding dogs should be considered in infected kennels.

Figure A.4 Canine Brucellosis: Information for Breeding Kennels (Page 2)

How is brucellosis spread?

Dog-to-dog spread of brucellosis occurs most often through breeding and by contact with vaginal discharges, semen, birthing fluids, and urine. Contact with an infected dog's blood, milk, saliva, and feces are less common sources of infection.

Brucellosis can spread from dogs to people through contact with an infected dog's birthing fluids and vaginal discharge or infected puppies. This is why dog breeders and veterinarians are at higher risk. Rarely, brucellosis can be spread from family pets to people through contact with urine, saliva, and other bodily fluids from infected dogs.

What are the symptoms?

In female dogs, the most common symptoms are aborted pregnancies, stillbirth, and inability to become pregnant. In males, the primary symptom is the inability to sire puppies. Both sexes may have swollen lymph glands, eye disease, and infections of the spine. However, most infected dogs appear normal and show no symptoms except for infertility. Female dogs can deliver healthy-appearing, but infected puppies.

Brucellosis in people normally causes a fever, headache, swollen lymph glands, night sweats, joint and muscle aches, fatigue, weight loss, and swollen liver and/or spleen. Some people may not have any symptoms.

For more information, contact:
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This information was adapted from the Canine Brucellosis and Foster-Based Rescue Dog Brochure by the Minnesota Department of Health

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Canine Brucellosis: Information For Pet Owners

Mississippi Board of Animal Health

James A. Watson, D.V.M.
State Veterinarian

Figure A.5 Canine Brucellosis: Information for Pet Owners (Page 1)

What is canine brucellosis?

Canine brucellosis is a disease found in dogs caused by a type of bacteria called *Brucella canis*. Infected dogs can also spread the disease to people.

Who gets brucellosis?

Dogs and people can become infected with brucellosis.

Dogs that have not been spayed or neutered (intact) are at higher risk of having brucellosis. This includes dogs in breeding programs and intact unowned stray or owned free roaming dogs.

In people, dog breeders and veterinarians are most at risk of infection because of their work with birthing puppies (whelping). People can also get brucellosis from their family pet, but this is much less common.

How do you test for brucellosis?

Testing for brucellosis can be very difficult. Not all infected dogs will test positive, and dogs may require several tests to decide if they have brucellosis. Your veterinarian may recommend testing your dog if it is sick or has had contact with a brucella-infected dog.

What are the recommendations or rules in Mississippi for canine brucellosis?

- All dogs crossing state lines must be inspected by a licensed veterinarian and have a valid health certificate (certificate of veterinary inspection).
- Dogs over three months old must be vaccinated for rabies.
- *Brucella canis* is reportable in MS but there are no specific regulations in MS pertaining to the disease. Your veterinarian can provide additional information on canine brucellosis and ways to protect your dog.
- If your dog tests positive, consult your veterinarian to discuss options for treatment and monitoring.
- People who assist with puppy birthing or breeding dogs should wear gloves and thoroughly wash hands when working with litters and during cleaning/disinfection.

Can brucellosis be treated?

In dogs brucellosis is very difficult to treat and relapses are common. Treatment includes spaying or neutering, giving antibiotics for several months, and frequent blood tests to monitor treatment progress. Treatment is typically not attempted for dogs in breeding kennels and when dogs can not be regularly tested and isolated from other dogs and people, potentially for the life of the dog.

Brucellosis is treatable in people. Treatment involves taking antibiotics daily for several months, regular monitoring, and may also involve surgery.

Figure A.6 Canine Brucellosis: Information for Pet Owners (Page 2)

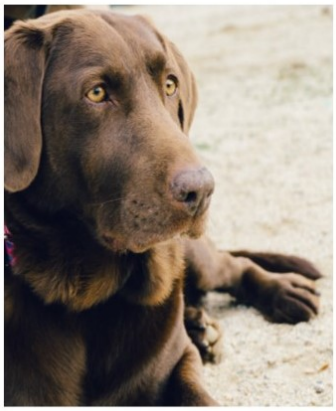
APPENDIX B

WHITE PAPERS CREATED IN CONJUNCTION WITH THE MISSISSIPPI BOARD OF ANIMAL HEALTH FOR VETERINARY EDUCATION ON CANINE BRUCELLOSIS AND CONTINUED SURVEILLANCE

CANINE BRUCELLOSIS: INFORMATION FOR VETERINARIANS

Mississippi Board of Animal Health 1.31.2018

Canine Brucellosis in Mississippi



Brucellosis is caused by gram negative, non-motile, non-spore forming, intracellular bacteria of the family Brucellaceae. *Brucella canis* is endemic in the dog population with an estimated prevalence of 1-8% in the United States. Prevalence of the disease in the Mississippi dog population is unknown and is a current topic of research within the state.

Risk Factors and Transmission: Dogs in breeding programs, stray dogs that have not been spayed or neutered, and unowned or free-roaming dogs are at higher risk of having brucellosis. Transmission occurs most often through breeding or contact with vaginal discharges, semen, and birthing fluids. Bacteria may also be transmitted in blood, urine, milk, and saliva.

Clinical Signs: Infected dogs are often asymptomatic. Reproductive signs including aborted or stillborn puppies, infertility, and orchitis/epididymitis are most common. Other signs may include uveitis and neck or back pain due to discospodylitis. Routine blood work is often normal or shows only a mild leukocytosis, even during acute disease.

Zoonotic Risk: Although human cases of canine brucellosis are rare, veterinarians and dog breeders are at increased risk due to frequent contact with reproductive fluids. Symptoms in people are nonspecific and may include fever, headache, swollen lymph nodes, night sweats, joint and muscle aches, fatigue, weight loss, and hepatic or splenic enlargement.

Diagnostic Testing

Testing for brucellosis can be confusing and frustrating. Common tests include:

⇒ Serology: Rapid Slide Agglutination Test (RSAT), Tube Agglutination Test (TAT), Agar Gel Immunodiffusion (AGID)

⇒ Agent: Culture, PCR

The RSAT Explained

The commercial rapid slide agglutination test (RSAT) detects *B. canis* antibodies. The test has a high rate of false positives, so dogs which test positive are usually retested with 2-mercaptoethanol (2ME). This disassociates nonspecific IgM and improves test specificity, however, test sensitivity is decreased.

Test Interpretation:

RSAT +, 2ME-RSAT + : the dog has circulating antibody and is likely infected

RSAT +, 2ME-RSAT - : the RSAT result may be a cross-reaction with another gram negative bacteria or the dog may be early in the course of infection → repeat test in 4 to 6 weeks to differentiate

Diagnostic Limitations

Testing individual dogs with current tests may result in false positives & negatives.

Highly specific tests like the 2ME-RSAT and the AGID may confirm a diagnosis, but it can be very difficult to definitely rule out brucellosis due to intermittent bacteremia and unpredictable titer responses.

Take extra precautions when working with sick dogs, including appropriate use of PPE for diagnostic sample collection.

Consider consulting a public health veterinarian for zoonotic risk assessment and appropriate risk management on difficult cases.

Testing in kennels...

Testing individual animals is inefficient!

The commercial RSAT is a poor screening test as it may fail to identify half or more of infected dogs when used according to label directions. Testing all dogs in a breeding kennel annually is a better way to demonstrate freedom of disease. Breeders should source dogs from kennels without a history of brucellosis and have good biosecurity and cleaning/disinfection protocols to reduce risk for brucellosis.

What if a breeding dog tests positive?

A positive serological test should be confirmed by culture of all dogs in the population. If brucellosis is found in the kennel, it is nearly impossible to distinguish infected from uninfected dogs and all should be considered potentially infected.

Figure B.1 Canine Brucellosis: Information for Veterinarians (Page 1)

CANINE BRUCELLOSIS

Treatment and Clinical Course of Disease

Therapy

Treatment of canine brucellosis is difficult. All treatment options carry risk for treatment failure, including possible recrudescence of disease months to years after apparent recovery. The following references include specific treatment protocols that have been effective in some dogs:

- Zoha & Walsh; Effect of a two-stage antibiotic treatment regimen on dogs naturally infected with *B canis*. *JAVMA* 180(12):1474-1475, 1982.
- Ledbetter et al.; *Brucella canis* endophthalmitis in 3 dogs: clinical features, diagnosis, and treatment. *Veterinary Ophth* 12(3):183-191, 2009.
- Wanke et al.; Use of enrofloxacin in the treatment of canine brucellosis in a dog kennel (clinical trial). *Theriogenology* 66: 1573-1578, 2006

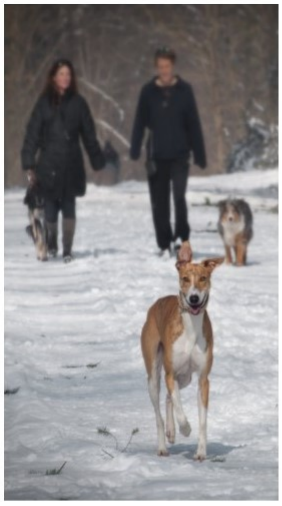
Response

- Dogs typically show clinical improvement within the first two weeks of therapy, however, there are no good guidelines as to when a treatment course should be ended.
- Treated dogs rapidly become abacteremic, but non-apparent carrier states may develop. Male dogs may be at increased risk due to sequestration in the prostate/epididymides.
- Female dogs may be at risk for recrudescent during subsequent heat cycles. Wanke et al. demonstrated a rise in antibody levels before estrus in treated breeding bitches.

Monitoring

- Follow-up testing and titers may be useful to monitor response to therapy, however, dogs may remain seropositive for months after initial treatment. Ledbetter et al. found a median time to seronegativity of 96 weeks in treated dogs.
- Titer responses can be unpredictable and difficult to interpret. Wanke et al. found that titers declined at variable rates in a treated breeding kennel; acute cases may demonstrate an increase in titer even when treated.
- Appropriate intervals for repeat testing are unclear. Ledbetter el al. retested dogs at 4-8 weeks intervals and ended therapy 8 weeks after all serology tests become negative (total treatment times ranged from 44 to 120 weeks).

Prevention and Zoonotic Risk Reduction



Prevention in Dogs

Canine brucellosis is reportable to the State Veterinarian in Mississippi (601-359-1170)!

Breeding Kennels: Work with dog breeders to develop a preventive program to reduce risk of brucellosis and other infectious diseases. Plans should include: biosecurity measures to prevent introduction and disease spread, monitoring for any clinical signs of disease and isolation of sick animals, and an effective cleaning and disinfection protocol. Annual testing of all dogs in a kennel is a better measure of freedom of disease than individual dog testing prior to breeding.

Prevention in People

Reduce exposure: All bodily fluids from animals should be treated as potentially infectious. Many zoonotic agents including brucellosis, leptospirosis, and cryptosporidiosis may be present in commonly collected samples. Use of appropriate personal protective equipment, designated sample testing areas, and thorough disinfection protocols will reduce risk of exposure.

Recognize risks: Apply extra precautions to animals showing signs of illness. Follow good biosecurity practices including segregation, isolation, and decontamination. Bodily fluids from ill animals including blood, urine, birthing fluids, and fetal tissues should be treated as biological hazards. Prompt disinfection of contact surfaces and workers will reduce spread of disease.

React to unusual findings: Unexplained illness in a veterinary clinic worker or pet owner should raise suspicion of a possible zoonosis, especially if recent contact with an ill animal has occurred. Inform your physician of any concerns and seek prompt medical care.

For more information, contact: Mississippi Board of Animal Health, 601-359-1170, www.mbah.ms.gov.

Recommendations are adapted from the Canine Brucellosis and Foster-Based Rescue Dog Brochure by the Minnesota Department of Health and developed in conjunction with the Mississippi State University College of Veterinary Medicine

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Phone: 601-359-1170
Fax: 601-359-1170
Email: msstatevet@mdac.state.ms.us

Figure B.2 Canine Brucellosis: Information for Veterinarians (Page 2)

Mississippi Board of Animal Health
P.O. Box 3889
Jackson, MS 39207

James Watson, D.V.M., Director
msstatevet@mdac.state.ms.us
www.mbah.state.ms.us

Canine Brucellosis Case Report Form

Please answer the following questions and email this form to Dr. Jim Watson, State Veterinarian, at msstatevet@mdac.state.ms.us. If you have any questions, the MBAH office number is 601-359-1170.

CLINIC INFORMATION

Clinic: Clinic Name

Veterinarian: Veterinarian Name

Address: Clinic Address

Phone: Clinic Phone Number

Date of Form Completion: Select Date

Person Completing Form: Enter Name

OWNER INFORMATION

Owner Name: Owner Name

Owner Address: Owner Address

Phone: Owner Phone Number

County of Residence: Enter County

DOG INFORMATION

Name: Dog Name

Breed: Dog Breed

Age: Enter Number ☐months ☐years

Sex: ☐Male ☐Female Intact: ☐Y ☐N

How long has the dog been owned by current owner? Enter Number ☐months ☐years

Where did the current owner get the dog? ☐Breeder ☐Shelter ☐Family/Friend ☐Other: Describe Other

CLINICAL INFORMATION AND RISK FACTORS

Clinical Signs and Date of Onset:

Y	N		Onset	Y	N		Onset
☐	☐	Apparently Healthy	Select Date	☐	☐	Uveitis	Select Date
☐	☐	Abortion/Stillbirth	Select Date	☐	☐	Lameness	Select Date
☐	☐	Orchitis/Epididymitis	Select Date	☐	☐	Back/Neck pain	Select Date
☐	☐	Fever	Select Date	☐	☐	Describe Other	Select Date

Current status of dog: ☐Alive and well ☐Alive and ill ☐Euthanized

How is/was the dog primarily housed? ☐Indoors ☐Outside kennel ☐Free-ranging ☐Describe Other

Is/was the dog used for breeding? ☐Y ☐N

Last date of breeding: Select Date

Does the owner have additional dogs? ☐Y ☐N

Are other dogs on-site used for breeding? ☐Y ☐N

Figure B.3 Canine Brucellosis Surveillance: Case Report Form (Page 1)

Mississippi Board of Animal Health
P.O. Box 3889
Jackson, MS 39207

James Watson, D.V.M., Director
msstatevet@mdac.state.ms.us
www.mbah.state.ms.us

DIAGNOSTIC TESTING

Date Tested: Select Date Location: ☐In-clinic ☐Lab: Enter Lab Name

Test Performed: Enter Test Name (e.g. RSAT or AGID) Test Result (Titer if applicable): Enter Test Result

Reason for Testing: ☐Pre-breeding ☐Sale/Movement ☐Signs of Illness ☐Other: Describe Other

Additional tests (please attached results): ☐CBC ☐Chem ☐UA ☐Radiographs ☐Other: Describe Other

Is the client willing to pursue additional confirmatory testing on this dog? ☐Y ☐N On additional dogs? ☐Y☐N

Please describe additional confirmatory testing: List additional tests performed or planned

TREATMENT AND FOLLOW-UP

Treatment and duration (check all that apply): ☐Castration ☐Currently under treatment ☐Completed treatment ☐Not treated

Drug 1: Enter Drug Enter Dose mg/day Enter Duration days
Drug 2: Enter Drug Enter Dose mg/day Enter Duration days
Drug 3: Enter Drug Enter Dose mg/day Enter Duration days

Please describe any additional treatment: List additional treatment

Is the client willing to pursue follow-up testing after treatment? ☐Y ☐N

Please describe follow-up testing: List additional tests performed or planned

OUTCOME AND FOLLOW-UP

Perceived likelihood of human exposure (including owner, veterinary staff, etc): ☐Low ☐Medium ☐High

Would you like the Board of Animal Health to do any of the following (select all that apply):

- ☐ Contact the owner regarding zoonotic disease education and prevention
- ☐ Provide clinic with a letter to send to the client including information on canine brucellosis
- ☐ Contact veterinarian regarding additional information on diagnosis and treatment

Thank you for your assistance. Please email completed form to msstatevet@mdac.state.ms.us.

Figure B.4 Canine Brucellosis Surveillance: Case Report Form (Page 2)