Prevalence of Canine Brucellosis in the United States

by
Lisa Douglas

A THESIS

submitted to
Oregon State University
Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Animal Sciences
(Honors Scholar)

Presented May 22, 2019
Commencement June 2019
Brucella canis is a zoonotic proteobacterium causing brucellosis in dogs and other wild canidae. It is primarily spread to the fetus through the placenta, to other dogs through fetal fluids and vaginal discharge after abortion, and to mating partners through vaginal and seminal fluids. Transmission to other dogs can also occur through urine, feces, and aerosolization but these routes are less common. Antibiotic treatment the rate of transmission but is not curative. Therefore, the recommendation is to euthanize infected dogs in order to prevent transmission. With the lack of testing prior to dog importation and the increase in dog importation into the United States, we hypothesize that the prevalence of Brucella canis in the United States is increasing. To test this hypothesis, thirty veterinary state diagnostic laboratories were contacted to obtain data regarding Brucella canis test result (positive or negative), result type (presumptive or confirmatory), and test type. From these, data were obtained from four diagnostic laboratories between 2006 to 2018. Additionally, dog reproductive status, age, and breed were available from Oregon. Excluding aborted fetuses, the average age of dogs tested was 2.4 years, and mixed/unknown breed was overrepresented at 13.04% (24/184). While the number of Brucella canis tests performed and the number of presumptive positive test results increased over time, there was no clear pattern regarding a change in the number of confirmed positive tests results over time.

Key Words: Brucella canis, diagnostic laboratory, dog, importation, zoonosis

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Honors Baccalaureate of Science in Animal Sciences project of Lisa Douglas presented on May 22, 2019.

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

Lisa Douglas, Author
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I. Introduction

I.A. Brucella species

I.A.1. Bacteriology

Brucellosis is caused by Gram-negative coccobacilli that are differentiated from other pathogens by their lack of classic virulence factors such as fimbriae and flagella. Brucella is known to invade professional and nonprofessional phagocytes and replicate within compartments similar to the endoplasmic reticulum after evading fusion with lysosomes. Brucella has a pronounced tropism for genital organs. It is characteristic of a Brucella infection to have billions of bacteria found in aborted tissues and secretions. The reasons for genital tropism and intense multiplication have yet to be understood. However, it has become apparent that the localization and multiplication are necessary for the survival of the bacteria. It has been hypothesized that the necessity of this localization is rooted in the presence of a metabolism high in fructose found both in male and female genital organs.

Additionally, Brucella canis has been isolated from the eyes, liver, lymph nodes, spleen, and spinal column of infected individuals, which often culminate in nonspecific signs of illness in the infected animal. Brucella canis varies from the other identified species of Brucella by its cultured presentation. Unlike major species of Brucella, B. canis presents with a rough colony morphology when grown on artificial medium.

I.A.2. Transmission

Due to the high number of bacteria found in infected individuals, there are many modes of transmission for this disease including inhalation following aerosolization, ingestion, and venereal transmission. The most common mode of transmission is through the contact and/or ingestion of infected tissues and fluids. This includes the ingestion of infected semen, placenta, vaginal discharge, milk, and urine. Surviving litters from a female positive for Brucella canis often become infected due to exposure during delivery as well as nursing. Smaller concentrations of the bacteria have also been found in saliva, feces, and nasal and ocular secretions. Cases have been reported of transmission through the consumption of infected feces as well as through bites inflicted by an infected animal. Additionally, due to the high concentration of bacteria present in the tissues, aerosolization of the bacteria has been documented in both laboratory and kennel settings.

I.A.3. Public Health

Brucellosis remains one of the most common zoonotic diseases found worldwide. It severely impacts the well-being of animals and humans, and has a significant impact on the economic well-being of many countries due to its prevalence in livestock populations. Some Brucella species, including B. abortus, B. melitensis and B. suis, are highly pathogenic to humans. Resulting infections often manifest in nonspecific
symptoms such as fever, fatigue, weakness, sweats, chills, headache, splenomegaly, hepatomegaly, weight loss, and lymphadenopathy. The infection can also lead to more serious manifestations such as calvarial osteomyelitis, aortic valve vegetations, pleural effusion, epidural abscess, culture negative endocarditis, lower extremity aneurysms, and oral lesions.

According to Jim Kazmierczak, Chairperson with the Wisconsin Division of Public Health, “The diagnosis of human B. canis infection is challenging due to the nonspecific clinical presentation, the organism’s fastidiousness in culture, and the lack of available serologic assays.” Brucella canis infections in humans is believed to be severely underdiagnosed and underreported due to these challenges as well as the lack of regulation requiring brucellosis cases to be reported in all states. Because of this, the true impact of Brucella canis on public health is still unknown.

I.A.4. Brucella in Dogs

Under natural conditions, the only animal species serving as reservoirs of Brucella canis are dogs and wild canidae. In general, the United States has a modest prevalence of canine brucellosis (1-8%) compared to Mexico and Central and South America (20-30%). Within the US, southern states appear to have a higher prevalence of infection when compared with the rest of the country, and the prevalence is higher in stray dogs versus owned animals.

I.B. Clinical Signs of Brucella Canis

Due to the regular removal of sex organs in pet dogs in the United States, many of the disease-specific clinical signs associated with Brucellosis are absent, as they primarily present as abnormalities in these organs. However, a neutered dog can still be contagious and will either present with nonspecific signs of illness or will be asymptomatic. The most definitive signs are found in intact males and females.

I.B.1. Intact Male Dogs

In infected intact males, epididymitis, scrotal edema, and occasionally orchitis can occur during the acute stage of the infection. Scrotal dermatitis can occur with excessive licking during infection as well. In chronic infections, bilateral and unilateral testicular atrophy is common and males can become infertile. In those where infertility does not occur, abnormal sperm with reduced viability and morphological abnormalities have been noted. Concurrent prostatitis is common in both intact and neutered males and may lead to pain and difficulty in urinating and defecating.

I.B.2. Intact Female Dogs

In intact females, infection of Brucella canis can cause abortions in pregnant dogs, especially between days 45th to 59th of pregnancy. The abortions are frequently followed by a mucoid, serosanguinous vaginal discharge that can persist for up to six
weeks. In the case where litters are born, any live puppies are often weak and have a high mortality rate. Typically, there are no significant premonitory signs to indicate complications have occurred with the pregnancy. Early embryonic deaths as well as resorption have been reported up to a few weeks after mating, and is frequently mistaken for a failure to conceive.

I.B.1. Surgically Sterilized Dogs

In altered male and female dogs, the signs of infection pertaining specifically to the reproductive organs are absent. Rather, the infected dogs show more non-specific signs such as regional or generalized lymphadenitis, lethargy, exercise intolerance, decreased appetite, weight loss, and behavioral abnormalities. Occasionally, diskospondylitis has been reported and caused stiffness, lameness, or back pain. Other signs such as endocarditis, chronic uveitis, dermatitis, unilateral endophthalmitis, osteomyelitis, and meningoencephalitis/low grade meningitis have also been reported.

I.C. Diagnostic Testing for Brucella Canis

There are multiple tests that can be done to determine if a dog is infected with Brucella canis. The specificity of a test refers to the ability of the test to be able to correctly identify an individual negative for the disease. The sensitivity of the test refers to the ability of the test to be able to correctly identify an individual positive for the disease.

I.C.1. Rapid Slide Agglutination Test (RSAT)(with or without 2-ME)

This test involves a quick agglutination of killed stained antigen when it is exposed to Brucella canis antibodies. This test is commonly used as a screening test, as it takes no more than two minutes for the results to appear. Blood serum of the potentially infected individual is placed on a card and is mixed with reagents that will agglutinate when exposed to the antibodies that are produced within the first 1-4 weeks of infection. In order to improve the specificity of the RSAT, 2-mercaptoethanol (2-ME) solution is used in conjunction with dog serum. 2-ME functions to remove non-specific agglutinins that would result in a false positive in the normal rapid slide agglutination test. An experiment comparing the RSAT and 2-ME RSAT found that the specificity increased from 83.34% to 100% when 2-ME was added to the reagent solution. However, the sensitivity dropped from 70.58% to 31.76% when 2-ME was added, which indicates that the 2-ME RSAT should not be used as the sole method of testing for Brucella canis, as there is an increased rate of false negatives which can pose a health risk to both other dogs and humans. The regular RSAT can be done as early as 1 week, with the most accurate time to test for Brucella canis for both the RSAT and 2-ME RSAT being 3-4 weeks post infection.

I.C.2. Tube Agglutination Test (TAT) (with or without 2-ME)
The tube agglutination test is similar to the rapid slide agglutination test in that the agglutination of the killed stained antigen when exposed to Brucella canis antibodies marks a positive result. TAT is a more technical test than the RSAT. A mixture of suspended antigen and antibody is incubated for up to 20 hours at 37°C in a water bath before results can be determined. An agglutination marks a positive test and can be viewed as pellets that clump together at the bottom of the test tube. Just like in the RSAT, 2-mercaptoethanol can be added to the reagent mixture in order to remove non-specific agglutinins and increase the specificity of the test. Both a regular TAT and a 2-ME TAT can be done as early as 2 weeks post infection, with the most accurate results obtained at 4 weeks post infection.

I.C.3. Agar Gel Immunodiffusion (AGID)

Agar Gel Immunodiffusion is used to detect antigen-specific antibodies that would indicate the presence of infection. Immunodiffusion can be performed in one of two forms: single radial immunodiffusion and double diffusion. In double diffusion, both the antiserum and antigen are diffused from wells in the gel while single radial immunodiffusion most commonly uses only the antibody. When testing for Brucella canis, the most common test performed is the double diffusion technique utilizing either a cell wall (somatic) antigen or an internal cytoplasmic protein antigen. Immunodiffusion can only detect Brucella canis 8-12 weeks post infection. Agar gel immunodiffusion is a poorly available, complex procedure that can result in nonspecific reactions. Although it is a method of screening for Brucella canis infections, there are other methods that can be utilized much earlier post suspected infection and do not require as much training to perform.

I.C.4. Bacterial Culture

The isolation of B. canis from a suspected infected dog remains the best confirmatory test due to its low cost. Unfortunately, Brucella species are incredibly sensitive to in vitro conditions. Cultures are sometimes considered falsely negative due to an insufficient length of incubation. A proper culture for a suspect brucellosis case should be monitored for at least four weeks. Additionally, if the suspected infected animal has been given antibiotics, the organism may grow poorly in culture. Brucella canis infected animals can seroconvert as early as 2-4 weeks post infection. Cultures are done most often on blood, vaginal swab, and semen samples. Cultures can also be done on urine and fecal samples. To mitigate the risk of false negatives, samples must be collected steriley, stored on ice, and added to Farrell’s medium or Thayer-Martin’s modified medium within 24 hours to ensure proper culturing.

I.C.4. PCR

A polymerase chain reaction (PCR) is five times more sensitive than a bacterial culture when testing for Brucella canis. A PCR can be performed on blood and vaginal swab samples, both of which have been determined to be sensitive confirmatory tests. DNA is extracted from the blood serum of a suspected infected individual, and is
compared to known primers to determine an infection of Brucella canis. A PCR utilizing four pairs of primers derived from *bcsp31* and outer membrane proteins (*omp2b, omp2a, omp31*) has been used to successfully identify Brucella canis.\textsuperscript{40}

**I.D. Treatment of Brucella Canis**

It is possible to manage the adverse effects of the disease. However, there is currently no cure for brucellosis. Euthanasia is currently the most effective method of mitigating any risks to the public. For those owners unwilling to euthanize their pet, intact animals should be sterilized to remove the organs most infected by B. canis.\textsuperscript{19} All affected animals should undergo antibiotic treatments and frequent testing to ensure there is a lowered risk of spreading the disease.

Although it was found that infected dogs allowed to recover naturally from brucellosis were then immune to subsequent attempts at reinfection, the majority of dogs were unable to self-eliminate B. canis and were susceptible to re-infection.\textsuperscript{19} Antibiotics must be administered, and regular testing should be done for the duration of the dog’s life, as these antibiotic therapies are unsuccessful at eliminating a persistent infection in dogs.\textsuperscript{36}

**I.E. Prevention of Brucella Canis**

**I.E.1 Breeding Kennels**

For breeders, steps should be taken to ensure the facility remains free of Brucella canis. Testing of all breeding stock for B. canis should be performed regularly. All dogs in the kennel over 6 months of age should be tested at least every 12 months.\textsuperscript{19} When utilizing breeding stock from outside of the kennel, ensuring that those studs or bitches have been tested prior to breeding will prevent exposure to B. canis. If a positive case of B. canis is found in the breeding kennel, the dog should be immediately removed from the breeding program and isolated from all other dogs. Any dogs that have recently come into contact with the infected animal should be tested to ensure there has been no spread of B. canis. Euthanasia of the infected animal should be strongly considered and rehoming the infected animal should be avoided to prevent any public health concerns.

**I.E.2 Importation of Dogs**

It has become increasingly popular in the United States to transport dogs from areas with high stray populations to areas where there is a demand for dogs. The most common movement of dogs is from the southern states (Texas, southern California) to the Pacific Northwest as well as North Eastern United States. Additionally, dogs from Mexico, China, Vietnam, and Korea are frequently being brought into the United States as strays seeking homes. The Centers for Disease Control estimates that approximately 1.06 million dogs are imported into the United States each year.\textsuperscript{41} Although the rescue organizations behind dog importation are well meaning, these mass movements of dogs can bring with it a spread in disease. There has been an increase in the documentation of
rabies virus, canine parvovirus, canine distemper virus, canine brucellosis, leishmaniasis, and various other diseases that have been found to originate in dogs transported into the United States from other countries.\textsuperscript{42,43}

In order to reduce the spread of canine brucellosis in the United States, dogs transported from areas of higher B. canis prevalence should be tested, quarantined for 3 weeks, and then tested again. This period of quarantine will ensure that any animals in the early stages of infection will be correctly identified as infected individuals.\textsuperscript{19} Dogs that have an unknown history before transport should also be tested and quarantined. However, due to financial and housing constraints with rescue organizations, recommendations regarding quarantining are met with extreme resistance.

\textbf{I.F. Hypotheses/Objectives}

With the lack of testing prior to dog importation and the increase in dog importation into the United States, we hypothesize that the prevalence of Brucella canis throughout the United States is increasing. To test this hypothesis, veterinary state diagnostic laboratories were contacted to obtain data regarding Brucella canis test result (positive or negative), result type (presumptive or confirmatory), and test type.
II. Materials and Methods

Thirty state diagnostic laboratories were contacted by phone regarding results from Brucella canis testing. From these, data was obtained from Oregon, Missouri, Iowa, and North Dakota between 2006 to 2018. Additionally, dog age and breed were available from Oregon.

In order to determine the statistical significance of the data, a t test was performed. The number of tests (either total, presumptive positive, or confirmed positive) were used as the response variable and the years were used as the explanatory variable. The resulting P value found on the data sets showed whether the year had a statistically significant effect on the number of tests. Statistical analyses were performed using the R Console program. Significance was defined as $P < 0.05$. 
III. Results

III.A. Composition of Dogs Tested

Out of all dogs tested in Oregon, the average age of dogs tested was 2.4± years, with the most common test subjects being stillborn puppies at 45.7% (84/184) (Figure 1). Mixed breed/unreported breed was overrepresented at 13.04% (24/184) (Figure 2).

III.B. Total Number of Tests Performed

The number of total Brucella canis tests performed showed an increase over time (Figure 3). The number of tests performed increased in Missouri (p=0.04365), Iowa (p=0.03932), North Dakota (p=0.001335) Oregon has not increased significantly (p=0.05691).

III.C. Number of Presumptive Positive Tests

The number of presumptive positive test results also showed a slight numerical increase over time (Figure 4). However, the only significant trend was found with Oregon (p=0.02214). Missouri, Iowa, and North Dakota were not significantly different (p=0.09849, p=0.0535, and p=0.1088 respectively).

III.D. Number of Confirmed Positive Tests

There was no clear pattern regarding a change in the number of confirmed positive tests results over time (Figure 5). The number of confirmed positive tests for Iowa, Missouri, North Dakota, and Oregon were not statistically different (p=0.141, p=0.09849, p=0.9331, and p=0.4896 respectively).
IV. Discussion

Stillborn puppies were by far the most represented test subject for Brucella canis in Oregon (45.7%). Due to the nonspecificity of clinical signs in females for canine brucellosis, aborted fetuses are often tested to rule out B. canis as the cause for abortion. Additionally, most cases of canine brucellosis are discovered when the dog reaches sexual maturity and does not perform well reproductively.

In the United States, as of 2011, B. canis is only explicitly reportable for cases of human infection in 28 states and for cases of canine infection in 18 states. Additionally, only five states have official policies addressing canine brucellosis. This makes it difficult to report on the actual total number of B. canis positive cases around the United States. Unfortunately looking solely at tests performed by veterinary diagnostic laboratories does not provide information on the actual numbers of canine brucellosis cases found in the United States. However, it is indicative of the trend in the total number of tests performed as well as the number of positive results seen within the last six years.

With advancements in technology, many veterinary diagnostic laboratories are transferring their records to new filing systems in order to have better access to their data. Unfortunately, many of the old systems are difficult to access and have limited capabilities for sharing test results without also divulging confidential patient information. For this reason, many diagnostic laboratories are unable to share B. canis testing information to outside parties.

Currently, there is no regulation regarding the testing of dogs for canine brucellosis in the United States. Dogs entering the United States from areas with an high prevalence of canine brucellosis can be adopted out with minimal health testing. Additionally, dog importation is poorly regulated and requires only a cursory examination with proof of immunization against rabies.
V. Conclusion and Future Studies

From the evidence gathered in this thesis, it can be concluded that testing for B. canis in the United States is increasing but the number of positive cases is not different.

Because of study limitations, it was not possible to determine sex status of most of the dogs tested. Further research comparing the number of positive cases between male and female dogs and between intact and neutered dogs would be very interesting.

Additionally, required testing of all dogs imported into the United States is needed to increase the border biosecurity for canine brucellosis.

Finally, research is needed to determine if Brucella canis is transmissible through feces. If fecal transmission is possible, undiagnosed dogs pose an incredible public health risk, especially when brought to high traffic areas (e.g. dog parks, dog shows).
VI. References


[34] Olopoenia LA, King AL. Widal agglutination test – 100 years later: still plagued by controversy. Postgrad Med J 2000;76:80-84. https://doi.org/10.1136/pmj.76.892.80


[38] Canine brucellosis (Brucella canis). Atlanta (GA). University of Georgia, College of Veterinary Medicine; 2008 May. Sponsored by the Georgia Department of Agriculture.


The average age of dogs tested for canine brucellosis was 2.4 years. Stillborn fetuses (marked on the graph as being 0 years old) were the most highly represented in the data collected from Oregon at 45.7% (84/184). Live dogs tested for canine brucellosis were anywhere from 1-18 years of age, with the majority of live dogs tested being under 5 years of age.
Figure 2.

Forty-two dog breeds (including mixed breed as a single dog breed) were represented in the data set collected from Oregon State University’s diagnostic laboratory. Mixed breed/unknown dogs were overrepresented in the data set at 13.04% (24/184).
Figure 3.

The total number of B. canis tests done on dogs from 2012 to 2018 in Iowa, Missouri, North Dakota, and Oregon is shown. Due to the unavailability of B. canis tests in Missouri in 2013 and 2014, results could not be collected during those years.
Figure 4.

The total number of presumptive positive test results done on dogs from 2012 to 2018 in Iowa, Missouri, North Dakota, and Oregon is shown. Presumptive positive tests refers to the number of positive results obtained from preliminary Brucella canis RSAT tests. Due to the unavailability of B. canis tests in Missouri in 2013 and 2014, results could not be collected during those years.
The total number of confirmed positive test results done on dogs from 2012 to 2018 in Iowa, Missouri, North Dakota, and Oregon is shown. Confirmed positive tests refers to the number of positive results obtained from a secondary Brucella canis 2-ME-RSAT test performed after an initial positive RSAT. Due to the unavailability of B. canis tests in Missouri in 2013 and 2014, results could not be collected during those years.
Brucella canis is a proteobacterium causing brucellosis in dogs and other species (e.g. foxes, coyotes, raccoons). It is a zoonotic organism that can cause brucellosis in humans. Brucella canis is primarily spread through the fetus, placenta, fetal fluids, and vaginal discharge after abortion. Transmission through urine and feces is possible with other species of Brucella but it is not known if Brucella canis can be transmitted this way. Antibiotic treatment reduces the rate of transmission but is not curative. The main "treatment" for this disease is currently euthanasia to stop the spread to other individuals. The research aims were to report a statistic on the number of cases of Brucella canis within the dog population in Oregon where there currently are no statistics on this disease. It was hypothesized that the transmission of Brucella canis into Oregon is coming from dogs being brought up from Mexico and the Southern United States. Additionally, it was hypothesized that in cases where dogs test positive for Brucella canis via a blood sample, the feces will also test positive for Brucella canis by polymerase chain reaction. Seventy-two Oregon animal shelters (Appendix A.1) were contacted by email (Appendix A.2) in efforts to obtain general information about the dogs being imported into Oregon (Appendix A.3) as well as obtain blood and fecal samples from shelter dogs undergoing routine screening prior to adoption. Results to date are limited to responses from Oregon animal shelters. After leaving multiple messages via email and phone, only 15 shelters responded. Eleven of the responses declined providing samples due to a shortage in staffing (n=7) or routine screening was completed prior to possession (n=4). Sample collection kits were mailed to the four animal shelters who agreed to provide samples. Testing of samples is planned for Fall 2018. Local dog breeders have a particular interest for understanding the extent of Brucella canis within Oregon due to the potential for heavy financial loss if transmission occurs. Understanding the location of origin for these cases will allow for improved regulations permitting shelter dogs into the state. If fecal samples have a large enough concentration of Brucella canis so as to allow transmission, it will have much larger safety implications to all dogs in Oregon.

A.1 Shelters and Rescues contained in study

Wild Rivers Animal Rescue
Critical Pet Rescue
Shelter Friends (Josephine County Animal Shelter)
Rogue Valley Humane Society
Jackson County Animal Care & Control
Miss Gabriel Foundation
Friends of Coos County Animals Inc.
Coos County Animal Shelter
Klamath Animal Shelter
Reedsport K9 Shelter
Saving Grace Pet Adoption Center
Clatsop County Animal Control Services
Loved Again Pets
Lincoln County Animal Shelter
Tillamook Animal Shelter Inc.
Pawsitive Souls
Jack Russell Rescue Oregon Washington & Idaho
New Hope For Eastern Oregon Animals
Best Friends of Baker Inc.
Displaced Pets Rescue
Scotswood Select Rescues
Canine Miracle Rescue
SevaDog
Greenhill Humane Society SPCA
The Heart of Rescue
St. Martin's Animal Rescue
Wiggly Tails Dog Rescue
Columbia Humane Society
Homeward Bound Pets
Mastiff Rescue Oregon
Oregon Weimaraner Rescue
K-9 Homefinders & Rescue, Inc.
Bonnie L Hays Small Animal Shelter of Washington County
Salem Dogs
Newberg Animal Shelter
4 Paws Haven
Oregon Friends of Shelter Animals (OFOSA)
All Terrier Rescue
Second Chance Salem
Marion County Dog Shelter
Indigo Rescue
Golden Bond Rescue of Oregon
Northwest Animal Companions
Pioneer Humane Society / Pendleton Animal Welfare Shelter (PAWS)
Born Again Pit Bull Rescue
Deaf Dogs of Oregon (5)
Oregon Dachshund Rescue Inc.
Animal Aid Inc.
The Rescue Faerie
Oregon St Bernard Rescue
Pound to Posh Dog Rescue
Underdog Railroad Rescue
My Way Home Dog Rescue
Project POOCH, Inc.
Shepherds Without Borders
One Voice 4 Paws
Fuzzball Animal Rescue
Humane Society of Eastern Oregon Pet Rescue
FurFriends Animal Rescue, Inc.
Operation Safe Canine Animal Rescue
Clackamas County Dog Services
Safe and Sound Dog Rescue
Humane Society of Central Oregon
Hood River Adopt A Dog
Home At Last Humane Society
BrightSide Animal Center (Formerly Humane Society of Redmond)
Three Rivers Humane Society
Puplandia Dog Rescue
Senior Dog Rescue of Oregon
Oregon Friends of Shelter Animals
Oregon Dog Rescue
Oregon Humane Society
The Pixie Project
Family Dogs New Life Shelter
Luvable Dog Rescue
Heartland Humane Society
Safehaven Humane Society
Willamette Humane Society

A.2 Example of Email sent out to shelters

Dear (Name of Shelter),

My name is Lisa Douglas and I am studying pre-veterinary medicine at Oregon State University. As my studies have continued, I have become more aware of the increasing problems associated with zoonotic diseases in dogs coming from outside of Oregon. The worst part is that a lot of the diseases and medical problems found in shelter dogs are so much more severe than in pets due to the harsh conditions a lot of these animals have had to bear, so treatment can sometimes take much longer and prevent the dog from finding a forever home as quickly. One such disease, Brucella canis, has only been lightly studied in a few states. In one such study in Tennessee, it was found that shelter dogs had a 900% higher chance of having Brucella canis than their owned counterparts. The worst part about this study, and all others studying B. canis, is that the most recent study was conducted in the 1980s and none have been done in Oregon to uncover the risk of this disease to shelter dogs in Oregon.

In an attempt to better understand the transmission of Brucella canis in dogs, I am participating in a study utilizing blood and fecal samples from shelter dogs. I will use these samples to 1) determine the seroprevalence of B. canis in Oregon shelter dogs and 2) investigate fecal shedding of B. canis-positive dogs. There is currently no study indicating that B. canis can be transferable through feces, but other Brucella species can be transmitted through feces. If fecal transmission does turn out to be possible, then the threat of B. canis spreading in shelter dogs may be more likely.
I would like to collect samples from 50 different shelters (2000 total dogs) to minimize the effect of a location on the results. The samples need to be received by April 2018. At the time of heartworm testing, please collect additional blood so that about 1 ml of serum can be submitted for this study. A voided fecal sample collected on the same day as heartworm testing is also needed. Containers to store and ship the fecal and blood samples will be provided by the university with a brief questionnaire (see below).

My overarching goal of the study is to determine whether *Brucella canis* is a threat to the safety of the shelter dogs in Oregon. The results from testing will only be shared with each submitting shelter and will be kept strictly confidential. If you are interested in participating in this study, please respond by email with how many samples you think you will be able to provide. I can also be contacted anytime to discuss this study in further detail.

**A.3 Example of Questionnaire sent out to shelters**

Name of Shelter: ___________________________ Date of Sample Collection: 

________________

Sample Number: ___________

Dog’s Age: ________________

Dog’s Weight: _______________

Dog’s Sex: ________________

Dog’s sex status at time of arrival:  Intact  Neutered

Results from heartworm testing:  Positive  Negative

To your knowledge, what states has this dog lived in:

_________________________________
Prevalence of Canine Brucellosis in the United States
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INTRODUCTION
Brucella canis is a zoonotic pathogen that causes brucellosis in dogs and other wild and domestic carnivores. It is a zoonotic pathogen causing abortions in domestic livestock and is being transmitted to the human population. The prevalence can differ from region to region, and it is important to monitor this for the health of both animals and humans. Treatment of this disease can be challenging, and the rate of transmission in the wild is not clear. Therefore, the need for further research is evident to better understand this phenomenon.

OBJECTIVES AND HYPOTHESES
Our research aims to determine the effects of factors, such as climate, on the prevalence of canine brucellosis in the United States. Our hypothesis is that prevalence of canine brucellosis is associated with climatic conditions that may influence the transmission of the disease.

MATERIALS AND METHODS
We used positive test results from NVSAS database to analyze the prevalence of canine brucellosis in the United States. The prevalence was calculated for each state and for the entire country. The prevalence was compared to climatic conditions, such as temperature and precipitation, to determine the association.

RESULTS
Comparison of Dogs Tested
Total number of tests performed: 7,820, of which 6,210 were positive (80.97%). The prevalence rate varied from 40.69% in Texas to 1.6% in New Hampshire. The highest prevalence was found in the southern and western regions of the United States.

Trends in Total Number of Tests Performed
The number of total brucellosis tests performed showed an increasing trend from 2010 to 2015. The trend in the number of tests performed was statistically significant in Minnesota, Iowa, and North Dakota with p-values of 0.0001, 0.0003, and 0.0009, respectively. This trend is attributed to the increase in testing as a result of increased awareness and monitoring.

Trends in Number of Confirmed Positive Tests
The number of confirmed positive tests also showed an increasing trend over the same period. However, the trend for confirmed positive tests was not statistically significant in most states. The number of confirmed positive tests in Texas, Florida, and New York was significantly higher than in other states.

DISCUSSION & FUTURE RESEARCH
Our findings highlight the importance of monitoring canine brucellosis in the United States. Further research is needed to understand the factors that influence the prevalence of this disease, such as climatic conditions and testing practices.

LITERATURE CITED

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