

Presence of *Rhipicephalus sanguineus* (Parasitiformis: Ixodidae) Found on Shelter Dogs in Texas

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Abstract: Animal shelters are known to have a high prevalence of canine diseases harvested and transmitted within their walls because of close quarters and a high volume of traffic of various animals. *Rhipicephalus sanguineus* (L.) (Acari: Ixodidae) commonly known as the brown dog tick is unusual among ticks, because it can complete its entire life cycle within its host making it the perfect vector for diseases. In this study we looked at the prevalence of one of the main vector borne diseases in the dog population of Texas, Anaplasma. Three hundred and nine ticks were collected from six shelters around Texas. These locations were varied across the state to provide the most accurate review of this disease within different regions of the state to account for transmission and variability. Three hundred and eight were identified to be *Rhipicephalus sanguineus*, and only one *Dermacentor Variabilis* (S.) (Acari: Ixodidae) was collected. DNA was extracted from 90 ticks and analyzed using polymerase chain reactions (PCR). Positive samples were sent to a gene bank for sequencing. Eleven samples came back as positive for *Anaplasma* genus. We concluded that the prevalence of *Anaplasmosis* looks to be much higher than previously expected and that more studies need to be conducted surrounding the subject.

Keywords: Anaplasma, Canine, disease, shelters, Ticks

Canine vector-borne diseases cause significant morbidity and mortality worldwide. Texas has many locations that exhibit these diseases (Holt, 2010). *Anaplasmosis* is one of these vector-borne diseases; there are two strains of the *Anaplasma* bacteria that causes disease in

dogs. The first strain is *Anaplasma phagocytophilum* vectored mainly by the blacked legged tick *I. scapularis* (A.) (Acari: Ixodidae). This causes an infection of the white blood cells in canines. This strain is zoonotic, meaning it can infect people as well as pets (Jensen, 2007). The second

strain of *Anaplasmosis* bacteria that causes disease in canines is the *Anaplasma platys* strain; it affects blood platelets that if left untreated can lead to bleeding disorders. This strain is vectored by the brown dog tick (*Rhipicephalus sanguineus*) (Yabsley, 2008). It is important to note the developmental biology of this species of tick, as it has shown evidence of one engorged female being able to lay up to anywhere from 4,000 to 8,000 eggs usually in a place near a possible hosts resting place or sleeping place as a strategic measure for the eggs once hatched to use these hosts as their sources of food and development and therefore vectors of transmitted disease (Dantas-Torres, 2008). Based on characteristics of the vectors host (*Rhipicephalus sanguineus*), it is important to understand the patterns of oviposition of this species to further investigate the means of how the *Anaplasmosis* disease is spread from vector to canine.

Materials and Methods

Identification and Treatment

Samples were collected from 6 animal shelters: Palm Valley Animal Shelter, San Antonio Human Society, Houston SPCA,

This experiment observes the prevalence of the second strain: *Anaplasma platys*, because the strain is vectored by the most common type of tick found in animal shelters around the state. We collected ticks from 6 different shelters around Texas, accounting for movement of species and variety of data in different climates. The ticks were preserved in alcohol until they could be identified by sex and species. DNA was extracted from 90 of these samples and PCR was used to amplify the DNA. There were eleven positive samples sent for sequencing to a gene bank.

This data is used to show a clearer understanding of the prevalence of one of the most common vector borne diseases in Texas. Furthermore this experiment can provide the basis on finding ways to be able to genetically differentiate between the two strands of *Anaplasma* that cause disease in dogs.

The Human Society of Dallas County, El Paso Human Society, and the Human Society of North Texas. Each site was sampled for a quota of collecting ticks from 30 dogs per shelter. Ticks were stored in 70% ethanol (Wal-Mart, Bentonville, AR). The ticks collected were identified to

species, sex and stage using standard keys (Durdan & Keirans, 1996, Keirans & Clifford, 1978 & Sonenshine, 1979). Total DNA was extracted from 90 of the 309 ticks using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) following the manufacturer's animal tissue protocol. The ticks were chosen at random with no more than 6 ticks collected from the same dog (3 males, 3 females) preference was given to engorged ticks.

DNA Collection

All ticks were tested for the presence of *A. phagocytophilum* and *A. platys* using PCR

reactions. Subsequently we sequenced any positive samples. A PCR enzyme kit was used in all assays (PCR Supermix, Invitrogen, Carlsbad, CA), and water was used as a negative control in all assays. All assays were run in a 50 µl reaction volume. DNA from infected laboratory colony *I. scapularis* nymphs provided by D. Fish at Yale University was used as positive controls. For sequencing, all positive products were purified using Qiagen PCR Purification Kit (Qiagen, Valencia, CA) and sequences were determined on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Results

Out of the samples collected, only ticks from Palm Valley Animal Shelter and The Human Society of Dallas County tested positive (Table 1.). In total only 0.1% of the ticks that were tested, were positive for the *Anaplasmosis* bacteria. The numbers of ticks shown to test positive were only present within the Three Hundred and Eight ticks

that were collected within the *Rhipicephalus sanguineus* species. From this data we were unable to sequence the positive sample to species we were only able to identify them as *Anaplasma spp.* Further testing on the samples in future testing to sequence the presence of the *Anaplasma spp.* is necessary for investigating the migration or transmission patterns of this species for future use.

Table 1. Shows all the positive samples, an * means that the band in the electrophoresis gel was very faded.

Species	Gender	PCR result
Rhipicephalus Sanguineus	Female	Pos
Rhipicephalus Sanguineus	Female	Pos
Rhipicephalus Sanguineus	Male	Pos
Rhipicephalus Sanguineus	Female	Pos
Rhipicephalus Sanguineus	Male	Pos*
Rhipicephalus Sanguineus	Female	Pos*
Rhipicephalus Sanguineus	Female	Pos
Rhipicephalus Sanguineus	Male	Pos*
Rhipicephalus Sanguineus	Female	Pos*
Rhipicephalus Sanguineus	Female	Pos*

Discussion

In this experiment the goal was to learn more about the degree to which canines have been exposed to a panel of parasites and pathogens and the quantity of animals that are hosts for these agents. The main interest was in the prevalence of *Anaplasma* in canines derived from the *Rhipicephalus sanguineus* species due to its zoonotic capability and medical importance to human disease research. The Brown Dog Tick, can harbor a disease called *Ehrlichiosis*, leading to detrimental health effects upon a human vector, and it has been presented that bites within the United States are limited, but have been recorded in portions of North Central Texas and Oklahoma, making further research upon this species to avoid illness and spread of diseases necessary

(Goddard, 1989). With information showing that human infections of this disease and more interaction of the Brown Dog Tick biting more humans, it is obvious that a more human adapted species of the *Rhipicephalus sanguineus* species is developing. Our results showed that *Anaplasma* is present in the Dallas and McAllen area. Limitations were that we were unable to sequence our positive samples to species. To further this experiment, instead of implementing solely the use of PCR, further experimentation may use other DNA sequencing methods to discover DNA information to the species level. This is not to say that the data in this experiment was any less relevant because of the success in finding the presence of this

species alone. According to EDDEX last year there were 3,274 reported cases of *Anaplasmosis* (Beall, 2008). This data was derived from veterinarians in the state, which shows that this number might be much higher, since it does not take into account the stray population in the state. Studies have shown that the average animal shelter only tests for heartworms when they first admit any new animal into the shelter. *Ehrlichia* and *Anaplasmosis* testing is only conducted in about 50% of the animal shelters in the country (Ravnik, 2011). The current experiment showed results that

Anaplasmosa is present in the shelter dog population; our hope is that this data exemplifies that *Anaplasma* is something that is present in Texas, providing awareness to the population that dogs in animal shelters should be tested for more than just heartworms. This disease if left untreated can be deadly for dogs therefore it is imperative to emphasize that more research be done surrounding this topic in order for prevention and treatment to become advanced.

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Appendix

Figure 1. Illustrates the characteristics of the brown dog tick by stage.

 Tick Encounter Resource Center

***Rhipicephalus sanguineus* (Brown Dog Tick)**

