

University of Nevada, Reno

**The Role of Epizootic Bovine Abortion Agent on Mule Deer Populations of the Mojave National Preserve in California and the Starkey Experimental Forest and Range in Oregon**

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Bachelor of Science in Veterinary Science

by

Afton F. Timmins

Dr. Kelley Stewart, Thesis Advisor  
Dr. Mike Teglas, co-Thesis Advisor

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We recommend that the thesis  
Prepared under our supervision by

**AFTON F. TIMMINS**

Entitled

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Requirements for the degree of

**BACHELOR OF SCIENCE, VETERINARY SCIENCE**

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Kelley Stewart, Ph.D., Thesis Advisor

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Mike Teglas, DVM, Ph.D., co-Thesis Advisor

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Tamara Valentine, Ph.D., Director, Honors Program

May, 2017

**ABSTRACT:**

Ticks, and the diseases they transmit play an important role in the health of wildlife and domestic animal populations. They can decrease the health of an animal through their effects on body condition and in extreme instances for survival (Merino et al. 2005). Ticks infesting deer populations cause deer to become anemic, and lose hair, weight, and muscle mass (McCoy et al. 2014). Ticks are also vectors for diseases such as Epizootic Bovine Abortion (EBA), which infects cattle (Teglas et al. 2005). In cattle, the agent of EBA (aoEBA) causes females to abort their fetus, but effects of the disease on mule deer (*Odocoileus hemionus*) are uncertain (Brooks et al. 2016). **This research will examine exposure to the EBA agent among mule deer populations from the Mojave National Preserve in California and the Starkey Experimental Forest and Range in Oregon. Effects on mule deer body condition will also be determined in individuals who have been exposed to the EBA pathogen and in individuals with tick infestation.** Results from this thesis conclude that in the two studied populations (Mojave National Preserve in California, and Starkey Experimental Forest and Range (hereafter Starkey) in Oregon) mule deer in the Mojave Desert were positive for the aoEBA, which was not present in mule deer at Starkey. Mule deer in the Mojave Desert also had higher tick infestations than mule deer at Starkey. Additionally, *Ornithodoros coriaceus*, the vector for the aoEBA, was not found on any Starkey deer. My results also indicated that the presence of the aoEBA was not directly correlated with tick infestation. Mojave mule deer, on average, had lower body condition scores than the Starkey mule deer. Although no correlation between aoEBA and body condition or tick infestation and body condition was evident, I suspect that increased abundance in tick infestation and more individuals with exposure to disease could

negatively affect body condition of mule deer. Research in this field needs to be conducted to determine the true effects of infection with the aoEBA on mule deer health.

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## **Introduction:**

Ticks of the genera *Ixodes*, *Rhipicephalus*, *Amblyomma*, *Dermacentor*, *Hyalomma*, *Haemaphysalis*, *Argas*, and *Ornithodoros* (Merino et al. 2005) are obligate parasites that parasitize vertebrates and often are vectors for a number of infectious agents including bacteria, viruses, and parasites (Main, 1977). Ticks are most commonly found in areas where host abundance is high and cause threats to wildlife populations, domestic animal populations, and even human populations (Merino et al. 2005). Nonetheless, tick abundance in the environment can be limited through climate, vegetation, and host abundance (Merino et al. 2005). Because ticks carry infectious pathogens, disease is easily transmitted throughout host populations causing decreased health in their hosts (Main, 1977). Infestation by ticks leads to decreased health in a host by affecting body condition, increasing energy expenditure, and transmitting pathogens that cause disease. Nutritional condition also can be impacted through damage to hair follicles, leading to hair loss in the host and a concurrent reduction in the ability to thermoregulate (Mooring & Samuel, 1998). Ticks also cause the host to become emaciated because the nutrients that the host needs are taken up by the tick, through a blood meal, and the host has to increase energy expenditure to compensate for that loss; and in extreme infestations may lead to decreased body weight and loss of muscle definition in the host (Samuel, Wilke, & Welch, 1991; McCoy et al. 2014). Tick infestation also leads to physiological problems resulting from anemia, in which body functions are diminished, including the ability to produce offspring (Mooring & Samuel, 1999).

Infestation of ticks in mule deer (*Odocoileus hemionus*) and other wild ungulates, can lead to decreases in populations through increased disease transmission (Yamada & Urabe, 2007). Diseases transmitted by ticks, including Lyme disease, Rocky Mountain spotted fever,

and anaplasmosis may become zoonotic, crossing over into human populations (Merino et al. 2005). Ticks also may transfer diseases between livestock and wildlife populations. Because medical treatment of diseases in wildlife species is difficult at best, wildlife populations can become reservoirs for those diseases.

Epizootic Bovine Abortion (EBA) is carried by soft tick *Ornithodoros coriaceus* and is a common cause of abortion among cattle in the foothill areas of California (Brooks et al. 2016). In the past few decades this disease has been identified in cattle in parts of southern Oregon, and areas of northern Nevada (Teglas et al. 2005) (Figure 1). Since infection with aoEBA can cause late term abortion in cattle, this disease can be devastating for beef producers within the western United States (Brooks et al. 2016). The only known vector for this disease is the soft tick *Ornithodoros coriaceus*, a tick that has been found in the areas in which this disease is most prevalent, and feeds primarily on the blood of mule deer and cattle (Teglas et al. 2005). Larvae, nymph and adult *Ornithodoros coriaceus* are usually found in soil and foliage around bedding and grazing sites of both cattle and deer. Nymphs, larvae, and adult ticks feed by taking a blood meal from a host. The tick will feed until full and then mate, depositing its eggs in the environment. Those eggs will hatch and the cycle continues (Teglas et al. 2005). A host becomes infected with the bacterial pathogen that causes EBA, *Pajaroellobacter abortibovis*, through the bite of an infected *Ornithodoros coriaceus* tick (Brooks et al. 2016).



**Figure 1:** Distribution of Mule Deer and *Ornithodoros coriaceus* Ticks. Mule deer distribution is designated by the green shade. This distribution overlaps with the distribution of the habitat of the *Ornithodoros coriaceus* tick designated as the striped area. Study areas of where mule deer were captured are also shown.

(Heffelfinger, 2006; <http://thetickwithatophat.weebly.com/vector.html>)

Cattle are the only animals known to be affected by this disease, following exposure to the etiological agent of EBA, *Pajaraoellobacter abortibovis* (Brooks et al. 2016). Mule deer also serve as an important host for *Ornithodoros coriaceus* and can, therefore, maintain tick numbers and potentially serve as a reservoir host for the disease (Teglas et al. 2006). Mule deer distribution overlaps with the habitat distribution of the *Ornithodoros coriaceus* tick and the aoEBA (Figure 1). However, there has been no evidence that aoEBA also infects mule deer or affects mule deer health. Whether the aoEBA causes abortion in mule deer is unknown. Nevertheless, mule deer serve as a reservoir host for this disease and pass the etiological agent along to cattle where the effects are devastating for cattle producers (Brooks et al. 2016). Because mule deer may act as a reservoir for aoEBA, research of aoEBA in mule deer is important to determine how the disease is distributed geographically, and how the etiological agent may affect body condition and reproduction of individual mule deer. Unfortunately, the only research conducted on EBA has been in cattle (Brooks et al. 2016; Chen et al. 2006; Stott et al. 2002; Teglas et al. 2005; Teglas et al. 2006); there is little to no research on the geographical distribution of aoEBA in mule deer or the corresponding effects on mule deer health.

Infestation of ticks leads to decreased health in a host by affecting nutritional and physiological condition. Body condition of individual deer becomes compromised as tick infestation causes damage to hair follicles, resulting in alopecia (McCoy et al. 2014). When deer experience hair loss, their ability to thermoregulate is compromised, which can reduce their survival, especially in colder climates where a coat is important for maintenance of body heat (Samuel, Wilke, & Welch, 1991). Body condition also becomes compromised when large infestations remove blood that is needed for homeostasis (McCoy et al. 2014). When blood loss is high, deer experience anemia, weakness, malnutrition, and poor body condition. When large

blood meals are taken from an individual deer, that deer also expends more energy than normal to account for the blood loss. That animal may become emaciated and loses more energy to ticks than what it can obtain from feed (McCoy et al. 2014). This effect is especially intense during winter when forage availability and body condition are low already. In these circumstances mule deer become emaciated, muscle mass is lost, and survival becomes a challenge (Samuel, Wilke, & Welch, 1991). Despite the importance of tick infestation, especially in the transmission of pathogens and the threat ticks pose to mule deer hosts, the only studies directly correlating tick abundance on the body condition of deer and survival have been conducted on winter ticks (*Dermacentor albipictus*) in moose (*Alces alces*). Published studies regarding the impact of ticks on mule deer, moose, white-tailed deer (*Odocoileus virginianus*) and black-tailed deer (*Odocoileus hemionus*) have shown that ticks have a negative effect on coat quality (McCoy et al. 2014; Mooring & Samuel, 1998; Mooring & Samuel, 1999; Samuel, Wilke, & Welch, 1991; Yamada & Urabe, 2007). Decrease in coat quality results in increasing difficulty for deer to survive harsh winters, resulting in their overall decreased health, which leads to increased mortality rates in populations of those species.

No study has been conducted on infection rates of aoEBA or the effect of aoEBA on mule deer populations. Further research needs to be performed regarding the geographical distribution of aoEBA in mule deer as well as the impacts that this disease has on mule deer populations. Mule deer are considered an important host for the *Ornithodoros coriaceus* tick (Schmidtman et al. 1976; Stott et al. 2002; Teglas et al. 2006; Chen et al. 2006; Brooks et al. 2016). Thus aoEBA infected mule deer populations could cause devastation in the form of disease transfer to domestic cattle populations as a result of the role of mule deer as a potential reservoir host. Diminished cattle reproduction through decreased calf survival can in turn lead to

negative impacts, such as decreased cattle populations, on beef production in California, Oregon, and Nevada, regions of the U.S. where the disease is considered endemic. This is the area in which the vector of the etiological agent of EBA has been captured. The impacts, however, on mule deer and the role mule deer play as a reservoir host for aoEBA are not discussed in great length throughout these studies.

**My research will focus on the impacts of tick infestations and the impacts that Epizootic Bovine Abortion bacterial agent may have on mule deer body condition, as well as the geographical location of aoEBA among mule deer populations located in the Mojave National Preserve in California and the Blue Mountains region of Northeastern Oregon. I** hypothesize that the body condition will be lower in mule deer infected with the aoEBA and in those deer with greater tick infestations. I predict that an overall negative effect will be seen in the health of individual mule deer infested with ticks. Additionally, I also hypothesize that the aoEBA in mule deer will have a similar geographical distribution to the region in which *Ornithodoros coriaceus* ticks are common, and where disease outbreaks have been seen among domestic cattle populations. Results from this study will provide information about the importance of tick infestations. The damage ticks cause to body condition and the role of ticks as vectors for diseases. The significance of EBA as a potential disease in those mule deer populations will also be discussed.

### **Literature Review:**

The focus of this research will be based on three major topics: tick abundance on free ranging wild ungulates including mule deer, elk (*Cervus canadensis*), and moose. I will specifically address presence or absence of aoEBA exposure in mule deer populations, and the

effect that both tick abundance and aoEBA have on mule deer body condition. A number of studies have been published on tick abundance on free ranging wild ungulates, especially the impact of the winter tick *Dermacentor albipictus* in moose populations (Mooring & Samuel, 1998; Yamada & Urabe, 2007). Heavy tick infestation causes the hair follicles of the moose to turn white. The moose will rub the hair off their bodies thereby removing their coats, which substantially reduces their ability to thermoregulate, such individuals are described as ‘ghost moose’ (Samuel, Wilke, & Welch, 1991).

Ticks are a common parasite present throughout the world and feed on the blood of wild and domestic animals as well as humans. They can also be vectors for zoonotic diseases such as Lyme disease, anaplasmosis, and Rocky Mountain spotted fever, which can be transferred to animals as well as humans (Merino et al. 2005). Tick-borne diseases are widespread throughout the United States and play a role in transmitting the pathological agent of diseases in to various hosts from which ticks collect a blood meal (Main, 1977). Vertebrate hosts are particularly important in their role as a reservoir for diseases carried by ticks (Main, 1977). Some of the major tick genera that play roles in the transmission of vector-borne diseases include *Ixodes*, *Rhipicephalus*, *Amblyomma*, *Dermacentor*, *Hyalomma*, *Haemaphysalis*, *Argas*, and *Ornithodoros* (Merion et al. 2005). These genera serve as vectors for zoonotic diseases such as Lyme disease, anaplasmosis, and Rocky Mountain spotted fever (Merino et al. 2005).

Environmental conditions supporting tick survival are widespread throughout the United States (Merino et al. 2005). Ticks, like animals, only survive in specific environments that can vary among genera and species. Geographically, habitats in which certain tick species can be found, are dependent on climate, humidity, vegetation, and abundance of hosts (Merino et al. 2005). Tick species usually favor specific conditions that limit the geographic distribution of

many tick-borne pathogens (Merion et al. 2005). For example, one of the more common genera of ticks in the western part of the United States infesting wild ungulates is *Dermacentor* (Yabsley et al. 2005). *Dermacentor* species play an important role in the transmission of anaplasmosis in their hosts (Yabsley et al. 2005). *Dermacentor* species have also been found in significant numbers in the southern part of the United States (Cortinas & Kitron, 2006). Another example of tick species dependent on climate is, *Ornithodoros coriaceus*, a soft tick that is most prominently found in regions of California, Southeastern Oregon, and Northern Nevada (Teglas et al. 2006) (Figure 1). *Ornithodoros coriaceus* commonly feeds off of cattle and mule deer and is known to be a vector for the aoEBA, which causes abortions in pregnant cows (Teglas et al. 2006).

Nevertheless, climate, vegetation, and humidity are not the only factors influencing where abundance of certain tick species is common (Cortinas & Kitron, 2006; Merino et al. 2005). Another factor that affects tick abundance is host density (Cortinas & Kitron, 2006; Merino et al. 2005). For example, in areas where there are fewer wild ungulates or cattle, *Dermacentor* and *Occidentalis* ticks are generally less abundant than in areas where there are large populations of wild ungulates and cattle (Cortinas & Kitron, 2006).

Climate change may also have impacts on the distribution of ticks in the environment. Arthropods, including ticks, are extremely vulnerable to change in climate (Gilbert, 2010). Heating and cooling of climate, as well as abundance of water to an environment causes certain species of ticks to relocate or even disappear (Eisen et al. 2017). This change in distribution also may have an effect on the transmission of diseases. Diseases can be introduced to areas where the disease has never been seen and can possibly cause greater harm to more vulnerable populations (Gilbert, 2010). For example, animals that have never been exposed to a certain

disease have not yet developed antibodies against the antigen that the disease produces would be much more vulnerable to the pathogen than those with evolutionary history of exposure. This naiveté often causes the infected animal to be more susceptible to the disease and may result in high mortality rates of the infected population (Cox, 2004).

As mentioned, tick infestation can lead to diseases such as anaplasmosis, Rocky Mountain spotted fever, and Lyme disease in mule deer populations which can be transferred into domestic animal populations and vice versa. Those diseases may cause large mortality rates or large medical bills in infected animals or both. One disease that has serious implications for domestic cattle, is EBA. Stott et al. (2002) discusses EBA, its geographic location within California, Oregon and Nevada, as well as the possibility of the tick, *Ornithodoros coriaceus* being the only vector for disease transmission. Cows that are infected with aoEBA either abort the fetus or give birth to a weak calf (Stott et al. 2002). The presence of the aoEBA is seen mostly within parts of California, but within the past couple decades the disease has also been identified in parts of Nevada and Oregon (Stott et al. 2002) (Figure 1). The tick's distribution is concurrent with the distribution of the disease in California, Oregon, and Nevada (Stott et al. 2002). Teglas et al. (2006) showed that cattle were diagnosed with aoEBA within the costal ranges and the Sierra Nevada Mountains of California, as well as in regions of northern Nevada, and the Klamath Basin in Southern Oregon. This study further reported that a higher abundance of the *Ornithodoros coriaceus* tick was found in the Cascade region of Oregon than in the study areas of Nevada and California (Teglas et al. 2006). The results suggest that although aoEBA was primarily seen in areas of California and the Sierra Nevada Mountain ranges, the disease occurs in areas of Oregon (Stott et al. 2002; Teglas et al. 2006; Chen et al. 2006; Brooks et al. 2016). In this way, the potential for mule deer (which also share a similar distribution to that of

the aoEBA) to become exposed to the etiological agent becomes an important issue for research (Figure 1).

Stott et al. (2002), showed that 9 heifers out of 17 exposed to aoEBA via tick bite, aborted their fetuses. About 53% of the infected cows aborted a fetus, but no significant conclusions were made from this study (Stott et al. 2002). Because of the inconclusiveness of these results, research on EBA in cattle did not progress and many questions about the effect of the disease on cattle were not answered (Stott et al. 2002).

Similarly, Stott et al. (2002) and Chen et al. (2006) also discussed the presence of cattle exposed to the aoEBA in California, Nevada, and Oregon and reinforced the hypothesis that *Ornithodoros coriaceus* is the only known vector for the transmission of the aoEBA in deer and cattle populations. In this study, heifer cows were experimentally fed upon by *Ornithodoros coriaceus* ticks; similar to Stott et al. (2002) about 50% of exposed heifers developed EBA and aborted their fetus. Conversely, Chen et al. (2006) mentioned that limitations arose within their study: a low percentage of aoEBA-infected ticks in nature, and the ability for ticks to remain infected in the laboratory setting, without a reservoir host. It is possible in this study, that cows who did not develop EBA were not bitten by a tick that was carrying the pathogen. Chen et al. (2006) provided further incentive to find a reservoir host for the aoEBA and to continue research on the disease and the effect of the etiological agent on cattle.

Antigens against *Pajaroellobacter abortibovis* can be detected by performing an indirect fluorescent antibody test (IFAT) described by Blanchard et al. (2014). In previous studies, diagnosis of aoEBA had been achieved through gross necropsy findings, immunohistochemical staining, and polymerase chain reaction assays (Stott et al. 2002; Chen et al. 2006). Blanchard et al. (2014) described the validity of using an IFAT assay for the detection of aoEBA antibodies in

cattle and confirmed that the IFAT assay was capable of determining low concentrations of aoEBA antibodies in infected cows (Blanchard et al. 2014). The protocol used in this study was adapted to determine aoEBA presence in mule deer populations. The methods used by Blanchard et al. (2014) will be discussed further in the methodology of this thesis.

Tick abundance and the presence of the antibodies made against the aoEBA in mule deer also affect body condition of individual mule deer. Although no studies have been performed on the effect of aoEBA infection on mule deer body condition, there have been studies on the impact of ticks, in general, on body condition in mule deer. In one study, Samuel, Wilke, and Welch (1991) discuss body condition of tick- infested ungulates and observed declines in several aspects of body condition: weight loss, muscle definition, hair loss (alopecia), anemia, and in severe cases where disease was involved these conditions were heightened. Research has shown that the most common effect of heavy tick infestations on body condition in ungulates is from alopecia (Samuel, Wilke, & Welch, 1991). Samuel, Wilke, & Welch, (1991) also reported that the ungulates, moose, elk, mule deer, and white-tailed deer, that were studied in their research had some hair loss from tick infestation. Ungulates that lose hair, especially in colder environments, are subject to increased energy use to thermoregulate and are more susceptible to other illnesses, like disease, which could result in death (Samuel, Wilke, & Welch, 1991). When entire herds are infested with ticks, population numbers can decrease. A sudden decrease in a population has major impacts on environment as well as on other animals in the area. Environmentally, forages that are usually grazed upon by wild ungulates can overgrow and become fire hazards in dry conditions when a population experiences a considerable decrease (Kie, Bowyer, & Stewart, 2003). Additionally, impacts on other animals can be possible as well. Wolves, common predatory species of ungulates, can starve if an ungulate population

dramatically decreases, as their source of food disappears (Kie, Bowyer, & Stewart, 2003). Deer are keystone species in ecosystems they inhabit (Kie, Bowyer, & Stewart, 2003) and are therefore important in research on tick infestation, through their role as a host for ticks, which are the vector of many zoonotic diseases such as Lyme disease, Rocky Mountain spotted fever, and anaplasmosis (Merino et al. 2005).

Mooring and Samuel (1998) discussed the importance of grooming in tick removal from the coats of ungulates. On average animals infested with ticks resulted in a weight loss between 10 - 44kg per year, from blood loss, and tick-induced anorexia (Mooring & Samuel, 1998). This severe decrease in weight causes winter mortality to increase because ungulates do not obtain the nutritional requirements they need to replace the energy they lost to the ticks. Because energy expenditure is higher in the winter than other seasons, these animals can rapidly decline in body condition, resulting in malnutrition and emaciation, and ultimately death in severe cases (Mooring & Samuel, 1998). Ticks can be removed from the coat through grooming, however, grooming can have consequences when done excessively: saliva is lost, teeth are worn, vigilance against predators decreases, and winter coats disappear (Mooring & Samuel, 1998; Yamada & Urabe, 2007; Samuel, Wilke, & Welch 1991). In addition, Yamada and Urabe (2007) reported on the importance of grooming by sika deer (*Cervus nippon*) as a way to rid ticks from coats. In that particular study, results suggested that increased grooming was correlated with the increase of tick abundance (Yamada & Urabe, 2007). These studies suggest that tick infestations can result in decreased body condition from an increase in grooming, further impacting other social and health issues among infested animals (Mooring & Samuel, 1998, Yamada & Urabe, 2007). Ultimately, overabundance of ticks on an ungulate's coat can be detrimental to the animal, and

can cause poor body condition just from the tick itself, and even from the animal trying to rid itself of the parasite.

By conducting research on tick abundance and the effect of the aoEBA on mule deer, better insight will be provided in the geographical distribution and movement of disease in mule deer populations, as well as provide understanding of the impacts of ticks and the EBA agent on body condition of mule deer. Clarification of these questions can further promote research on the effect of the bacterial agent of EBA in mule deer and the possible role of mule deer in the passage of disease into cattle populations, which are important economically for humans, since the cattle industry is a major source of income and food for many individuals.

## **Materials and Methods:**

In order to complete this research, I obtained blood samples and ticks from individual mule deer from samples previously collected during ongoing mule deer projects in two locations, the Mojave National Preserve in California and the Starkey Experimental Forest and Range in the Blue Mountains of Northeast Oregon (Figure 1). All aspects of capture and handling of mule deer were approved by the Institutional Animal Care and Use Committee at UNR (Mojave: Protocol #538, exp. 1/23/2018, Starkey #563, exp. 1/15/2019) and were consistent with guidelines established by the American Society of Mammologists for research on wild mammals (Sikes et al. 2011).

## **Study Area:**

Thirty-one mule deer were captured from the Mojave National Preserve in San Bernardino, California, USA (Figure 1). The boundaries of the Mojave National Preserve are

designated by interstate Highway 15 to the north, Interstate Highway 40 to the south, and the state boarder of California and Nevada to the east (McKee et al. 2015). Research and mule deer capture was concentrated in three main areas of the Mojave National Preserve; New York Mountains, Mid Hills, and Cima Dome. Twenty-seven mule deer were captured from the Starkey Experimental Forest and Range in the Blue Mountains of Union County, Oregon, USA (Figure 1). Starkey is located 35 km southwest of La Grande, Oregon. The area in which mule deer were captured is a typical representation of national forests in the intermountain west (Morano et al. 2013). The Starkey Experimental Forest and Range is also enclosed by a 2.4 m tall fence, creating a closed population of mule deer that do not immigrate or emigrate (Morano et al. 2013).

### **Mule Deer Capture:**

Mule deer samples of serum and collected ectoparasites were provided from a previous study led by Dr. Kelley Stewart, researcher and Associate Professor in the Department of Natural Resources and Environmental Science at the University of Nevada Reno. In the late winter to early spring, 2013-2016, adult female mule deer were captured in the the Mojave National Preserve. Mule deer capture was accomplished by using a net gun fired from a helicopter (Krausman, Hervert, & Ordway, 1985; McKee et al. 2015). Deer were taken to a processing station where they were given uniquely numbered ear tags for individual identification. Ectoparasites were collected from immobilized animals and placed in 70% ethanol solution for preservation. Using a syringe, 50 ml of blood was collected via the jugular vein from each animal. Blood was spun down to separate serum from red blood cells. The serum was immediately placed in a freezer and frozen. At this time, maximal depth of rumpfat was

measured at the point of the hip via ultrasonography (McKee et al. 2015). After data collection, mule deer were released from the processing station.

At the Starkey Experimental Forest and Range in Union County, Oregon, mule deer were captured individually during winter of 2016. These deer were captured in panel traps baited with hay (Rowland et al. 1997). Ectoparasites were removed from each of these deer and placed into tubes with 70% ethanol solution to keep preserved. Using a syringe, 50ml of blood was collected from the jugular vein of each individual. Blood was spun down to separate serum from red blood cells and the serum was immediately frozen. Subcutaneous fat on the rump was also measured from these deer using ultrasonography (McKee et al. 2015). After data collection these mule deer were released.

#### **Tick Identification:**

I counted and identified ticks ( $n = 75$ ) collected from mule deer from the Mojave and Starkey study areas to determine genus and species (Table 1). All ticks collected from an individual deer had been preserved in the same tube containing 70% ethanol, labeled with the date and unique animal ID number and stored. Identification of ticks was achieved through use of a dichotomous key, in which a number of identification steps were performed in order to determine the final genus and species of the tick being studied (Furman & Loomis, 1984). Ticks were placed under a dissecting microscope and observed for key structures such as, scutum, festoons, and shape of spiracular plate, to determine genus and species. Number of ticks as well as genus and species of ticks were recorded according to the mule deer from which the ticks were collected.

#### **Indirect Fluorescent Antibody Test:**

Protocol for the indirect fluorescent antibody test was adapted from Blanchard et al. (2014). Serum collected from mule deer blood samples were briefly vortexed and centrifuged at 13,300 rpm for 30 seconds. The deer serum was then diluted 1:100 with 2 $\mu$ l of serum and 198 $\mu$ l of phosphate buffered saline (PBS). After the dilutions were prepared the samples were briefly vortexed again to ensure homogeneity of serum and PBS. The resulting serum dilutions were then organized in the same order on a rack in which they would be applied to the 12-well slides coated with the aoEBA antigen and fixed with acetone (slides were provided by M. Blanchard, University of California, Davis).

Following preparation of serum dilutions, acetone fixed slides were allowed to air dry for 1 minute. After thawing, the slides were placed in a PBS bath for 2 minutes and allowed to rehydrate. The slides were then removed from the bath, and the Teflon borders of the slide were quickly dried using thick filter paper. Once the borders were dried, 15 $\mu$ l of the primary antibody (deer serum and known positive and negative dilutions) were added to the wells using a 20 $\mu$ l multichannel pipette. Mule deer serum sample Mojave DE16022 (MIDHILLS) was used as the known negative control, and mule deer serum sample Carson Range 1759 was used as the known positive control.

The slides with the added primary antibody (the prepared serum dilutions) were incubated in the dark at room temperature for exactly 20 minutes. After the 20-minute incubation the diluted serum in each well was carefully removed using a vacuum. The wells were then gently washed with PBS using a transfer pipette; to ensure that cross contamination between the wells while washing did not occur. The slides were then placed in a PBS bath for 5 minutes and removed from the bath. The Teflon borders were dried using thick filter paper.

Once the borders of the slide were dried, 15 $\mu$ l of the secondary antibody was added to each well using a 20 $\mu$ l multichannel pipette. The secondary antibody used was KPL #02-31-06: Rb anti-Deer IgG (H&L); Lot #120992; Reconstruction Date: 12/13/16. The secondary antibody was diluted 1:10; 45 $\mu$ l of secondary antibody and 405 $\mu$ l of PBS. This dilution was then vortexed to ensure homogeneity. The diluted antibody was distributed equally between 6 dilution tubes with 75 $\mu$ l aliquots. The slides were then incubated at room temperature for 15 minutes. After the 15 minutes of incubation the serum dilution was gently removed from each well using a vacuum and the slides were gently rinsed with PBS using a transfer pipette. Once washed, the slides were then placed in a PBS bath for 5 minutes.

After removing the slides from the PBS bath, I dried the boarders with a thick piece of filter paper. A drop of Shandon Immu-mount (mounting media) was then added to each well. After adding the mounting media, I also placed a coverslip (24x60 mm) over the slide and gently tapped down using forceps, allowing for the media to spread and removing air bubbles that might have formed over the wells. The slides were then turned on their sides to remove excess media from the slide. Once slides no longer contained excess media, the slides were placed in the dark and allowed to incubate for 15 minutes at room temperature and the slides were viewed using a fluorescent microscope.

### **Slide Viewing:**

After I performed the indirect fluorescent antibody test (IFAT) the resulting slides were viewed under a fluorescent microscope capable of emitting and detecting Alexa Fluor 488 dye. Visual observation of fluorescent flecks on the slides containing primary antibody bound to

secondary antibody tagged with a fluorophore, allowed for detection of aoEBA antibodies and ultimately indicating whether or not individual mule deer had been exposed to the aoEBA.

Slides were first viewed at 10x and examined for consistent fluorescent flecks. I looked for consistency in flecks meaning that they were all of similar size and shape, and they were equally bright. Additionally, to be considered consistent, these flecks were evenly spread out within the well. Examination started along the edges of each well, where fluorophores were easier to view and then moved along throughout the well to confirm consistency or inconsistency. After viewing the entire slide at 10x, I viewed the slides again at 20x. At 20x the fluorescence was easier to visualize and presence or absence of antibodies (designated by consistency in fluorescent flecks) could be confirmed. Observations of exposure to the aoEBA were recorded as + or - and notes were added in cases of uncertainty.

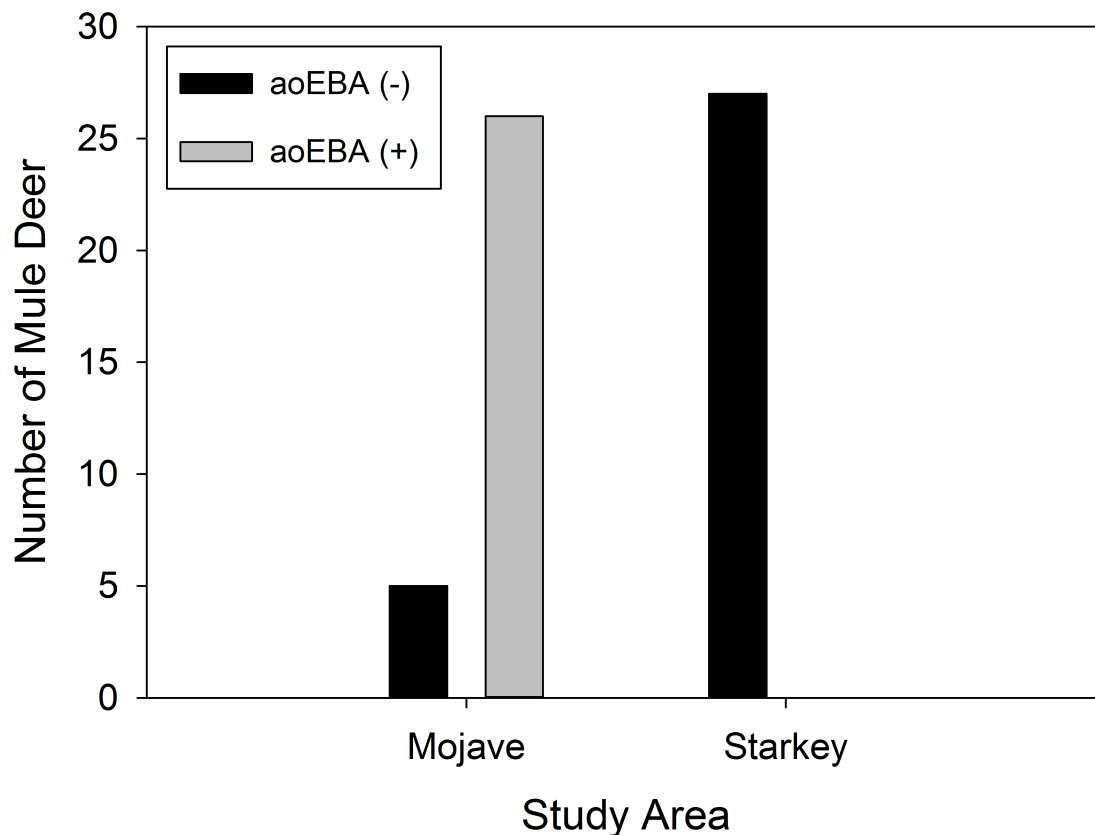
### **Statistical Analysis:**

Chi squared and Fisher's exact tests were used to compare groups of deer by the following: Geographic location of deer and aoEBA antibody presence; presence of ticks and aoEBA antibody presence; deer location and tick infestation; tick infestation and aoEBA presence or absence for statistical significance. Analysis of variance (ANOVA) was used to determine statistical difference in body condition scores between populations. For all analyses ( $p \leq 0.05$ ) was considered significant and the null hypothesis was rejected (Zar, 2010).

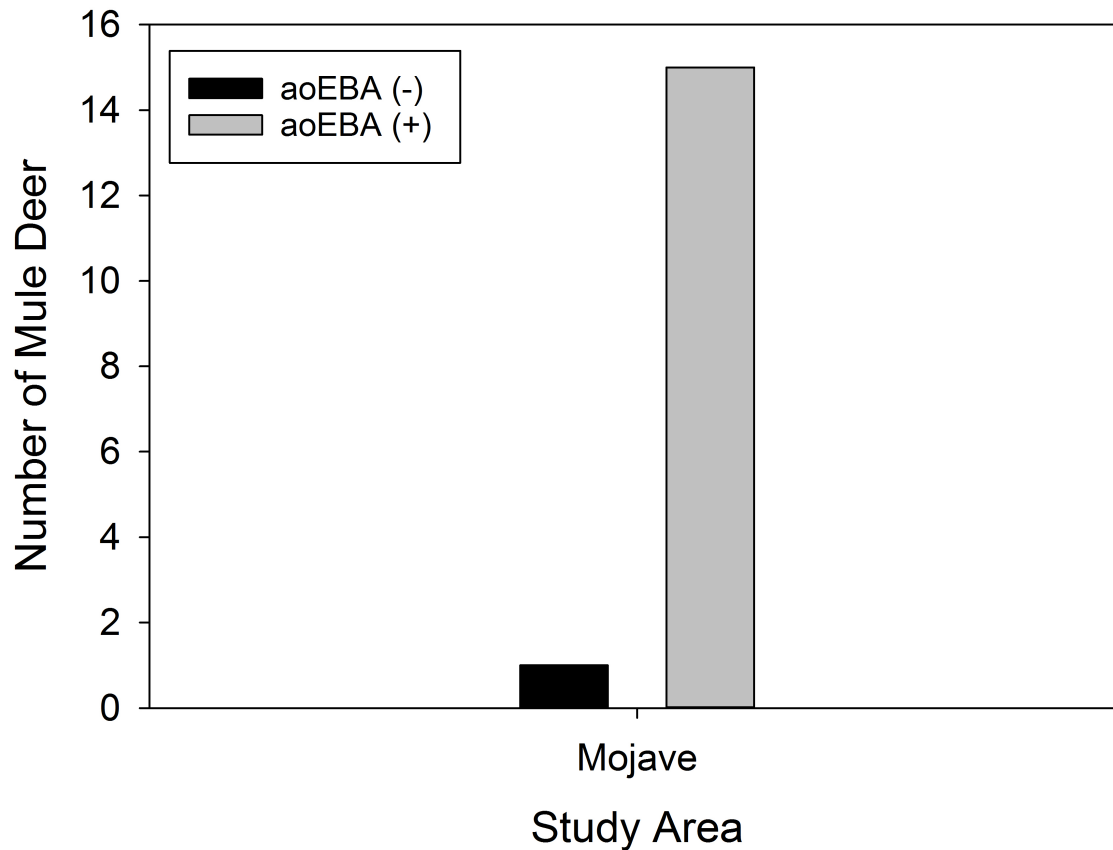
### **Results:**

#### **Geographic Distribution aoEBA:**

Mule deer captured in the Mojave and Starkey regions significantly differed in antibody prevalence against *Pajaroellobacter abortibovis* ( $X^2= 41.8$ ,  $p<0.001$ ). The bacterial agent of EBA was only documented within the Mojave Desert population. None of the deer we sampled at the Starkey Experimental Forest and Range had presence of aoEBA antibodies (Figure 2). In addition, infestation of ticks increased likelihood of aoEBA antibodies in Mojave mule deer ( $X^2= 28.0$ ,  $p<0.001$ ; Figure 3).



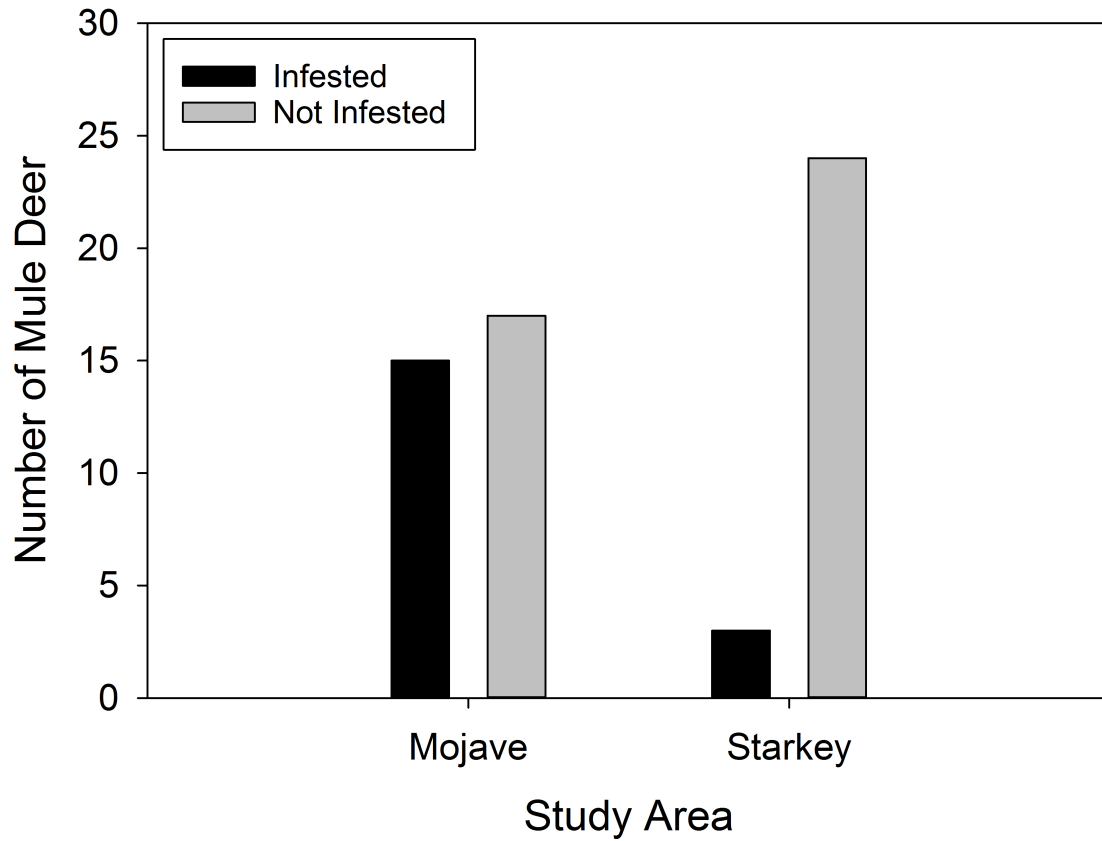
**Figure 2:** The presence of the aoEBA in mule deer in Mojave National Preserve and Starkey Experimental Forest and Range during winter 2016. Mule deer in the Mojave Desert had more individuals exposed to the aoEBA compared to the Starkey area. We did not detect evidence of infection with the aoEBA at Starkey.



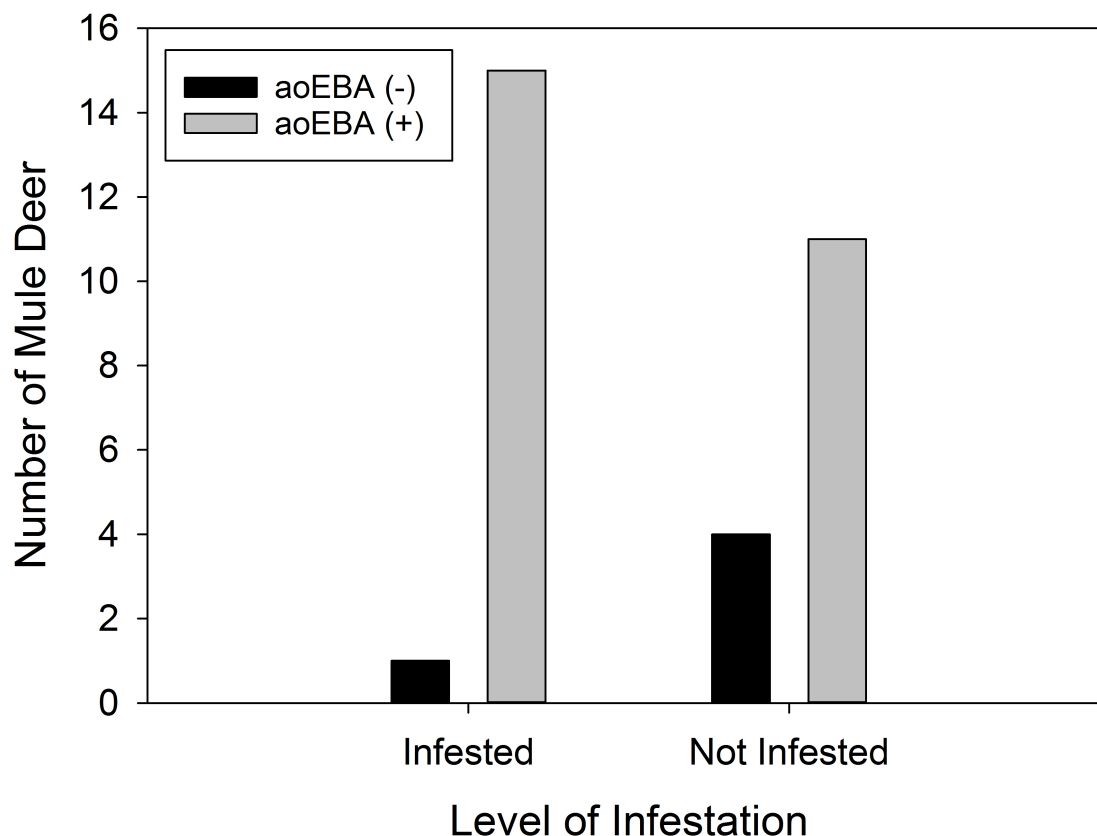
**Figure 3:** Presence of the aoEBA on mule deer with presence of  $\geq 1$  tick at capture in the Mojave Desert, 2016. Tick infested mule deer that showed exposure to the aoEBA was more prominent in the Mojave Desert area.

#### **Tick Infestation:**

Tick infestation was substantially greater in the Mojave population of mule deer compared to those from Starkey ( $X^2 = 8.83$ ,  $p = 0.0042$ ; Figure 4). I suspect there was a direct relationship between tick infestation and presence of aoEBA antibodies, but no significant difference was observed between tick infestation and aoEBA positive mule deer ( $X^2 = 2.39$ ,  $p = 0.1719$ ; Figure 5).



**Figure 4:** Tick infestation ( $\geq 1$  tick) in mule deer individuals from the Mojave National Preserve (n=32) and Starkey Experimental Forest and Range (n=28), winter 2016. Tick infestation in mule deer was seen to have higher abundance on individuals from Mojave than those from Starkey.



**Figure 5:** Tick infestation ( $\leq 1$  tick) and IFAT results in the Mojave mule deer population, 2016. More aoEBA positive individuals were shown to be infested with ticks whereas more aoEBA negative individuals were not infested with ticks.

#### **Tick Identification:**

Ticks were identified according to species and genera. Ticks that were manually collected from immobilized mule deer in the Mojave and Starkey. Out of the thirty-one captured mule deer from the Mojave National Preserve, sixty-nine ticks were collected and identified. Four species of ticks were identified in mule deer from the Mojave National Preserve: 1 larvae from *Ornithodoros coriaceus*; 44 adults, 6 nymphs from *Dermacentor albipictus*; 15 adults, 2 nymphs from *Dermacentor occidentalis*; 1 larvae from *Ornithodoros turicata* (Table 1). From the twenty-seven mule deer captured from the Starkey Experimental Forest and Range, six ticks

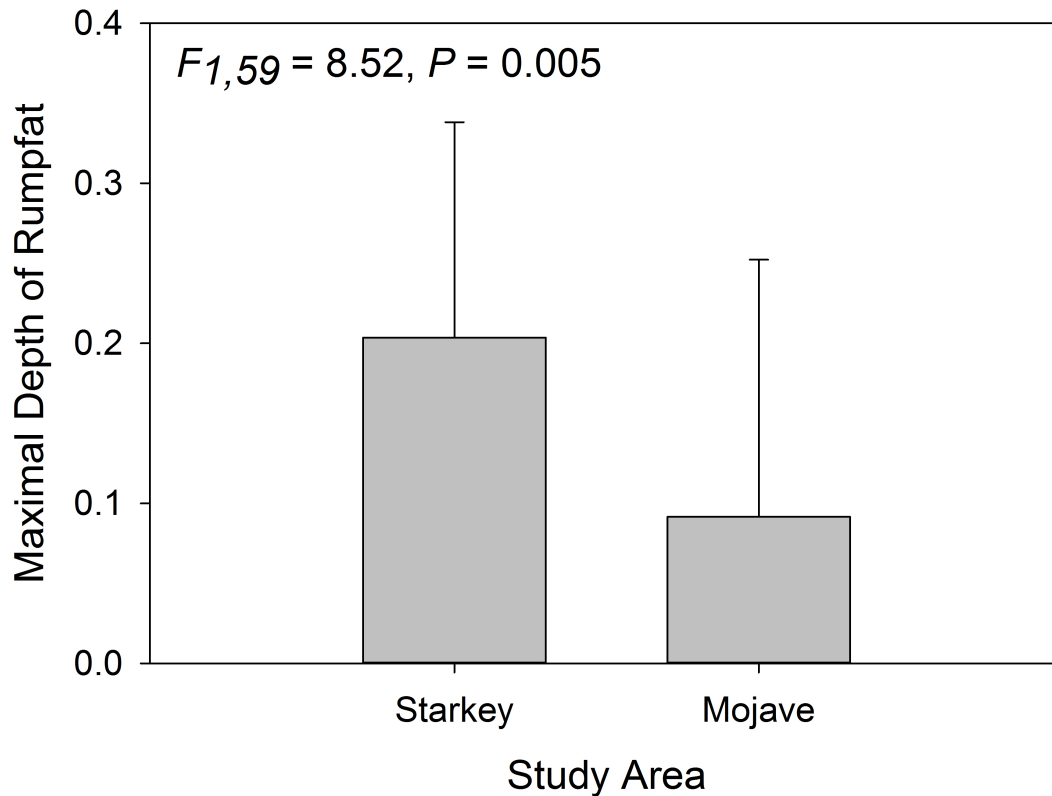
were collected and identified. Ticks identified among mule deer from the Starkey Experimental Forest and Range included: 3 adults from *Dermacentor occidentalis*; 3 adults from *Dermacentor albipictus* (Table 1).

**Table 1: Number of Identified Tick Species Collected from Mule Deer of the Mojave National Preserve vs. Those Collected off Mule Deer from Starkey Experimental Forest and Range.**

Tick Species Collected	Mojave (# of ticks)	Starkey (# of ticks)
<i>Ornithodoros coriaceus</i>	1	0
<i>Dermacentor albipictus</i>	52	0
<i>Dermacentor occidentalis</i>	18	3
<i>Ornithodoros turicata</i>	1	0

### Body Condition:

Body condition of mule deer were examined in the Mojave Desert and at Starkey by measuring the thickness of subcutaneous fat over the rump. Overall, subcutaneous fat measurements of Mojave mule deer were lower than those of mule deer at Starkey (ANOVA  $F_{1,59} = 8.522$ ,  $P = 0.0049$ ; Figure 6). In general, Mojave mule deer had consistently smaller subcutaneous fat measurements compared to those from Starkey. The average rumpfat measurements for Mojave was  $0.0915 \pm 1.507$  (SD) and for Starkey  $0.2035 \pm 1.507$  (SD).



**Figure 6:** Subcutaneous Fat Measurements (cm) of Mojave and Starkey mule deer 2016. Rumpfat measurements of the Mojave mule deer are noticeably lower individuals from the Starkey mule deer.

### Discussion:

I observed geographical variation in the presence of the aoEBA in the Mojave study area, but did not observe presence of the aoEBA in mule deer from the Starkey area. Distribution of the *Ornithodoros coriaceus* has mainly been described in California and Northern Nevada, with a small habitation area in Southeastern Oregon (Figure 1). The Mojave National Preserve study area is within this habitat distribution of the *Ornithodoros coriaceus* tick (Figure 1). However, the habitat of the tick has not extended as far north as the Starkey Experimental Forest and

Range (Figure 1). Since the habitat of the *Ornithodoros coriaceus* does not extend as far north as Starkey, it is understandable that the aoEBA was not detected in any Starkey mule deer.

I observed no direct correlation between aoEBA presence and tick infestation in the Mojave where the aoEBA was present. This effect was likely due to higher tick abundance in the *Dermacentor* genus (70 ticks) (*Dermacentor albipictus* and *Dermacentor occidentalis*) than in the *Ornithodoros* genus (3 ticks) (*Ornithodoros coriaceus*), the vector for *Pajaroellobacter abortibovis*, the etiological agent of EBA (Table 1). The *Ornithodoros turicata* tick was also captured off of a mule deer from the Mojave National Preserve (Table 1). This tick is a vector for *Borrelia turicatae*, the etiological agent of relapsing fever (Kim et al. 2017). Due to the presence of this tick on a mule deer from the Mojave, further research may show mule deer exposure to *Borrelia turicatae* antibody.

Subcutaneous fat measurements, measured through ultrasonography, indicated that body condition of mule deer was significantly different between populations and that average mule deer body condition scores of the Mojave were less than those from Starkey. This difference in body condition may be correlated with tick infestation, although my sample size of deer did not support that observation. Other possible reasons for the difference in average body condition measurements between populations may have been due to timing of capture. Mule deer from the Mojave National Preserve were caught in late February in 2016. Since these deer were just coming out of winter, the most energy demanding season, it is reasonable that these deer had smaller average maximal depth of rumpfat. The Starkey mule deer were captured in November, December, and early January in 2016. These deer were just entering the cooler season about 1-3 months before the Mojave individuals, so it is not surprising that those deer had higher average body condition measurements than the Mojave mule deer (Figure 6). Had mule deer from each

study area been captured close to the same time, body condition measurements could have had a lower correlation than what was shown in this study.

Interesting results from this thesis were obtained that offer further insight into the current research on EBA. Although no studies involving EBA have focused on mule deer, there were still some similarities between this research and past research in this field. Research from this thesis suggested that aoEBA is still widely abundant in California, however, there was no presence of the aoEBA in the population from northeastern Oregon (Figure 2). Past research suggests that the aoEBA had migrated from California into Southern Oregon (Teglas et al. 2006). It is possible that the aoEBA has not yet spread as far north as the Starkey Experimental Forest and Range, where we did not observe the tick vector, *Ornithodoros coriaceus*. With warming climate, the pathogen could move north in distribution if the tick vector also expands north.

Reasons for the absence of this disease in Starkey may also have to do with the ecosystem of Starkey in comparison to that of the Mojave Desert. Past research has indicated that climate has a large effect on the presence or absence of certain tick species (Cortinas & Kitron, 2006; Merino et al. 2005). *Ornithodoros coriaceus*, the soft tick that serves as a vector for the transmission of aoEBA, is mainly found in California, Southern Oregon, and Nevada (Teglas et al. 2006). The Starkey Experimental Forest and Range is further north than the described range of the *Ornithodoros coriaceus* tick. Possibly the climate this far north is not suitable for the tick vector. The Mojave Desert, on the other hand, located in southern California and Nevada is an ecosystem that supports the habitation of the *Ornithodoros coriaceus* tick (Teglas et al. 2006) (Figure 1) The possibility of climate change could alter the distribution of *Ornithodoros coriaceus* and in turn the bacterial agent of EBA. Since tick abundance is dependent on climate, any drastic fluctuations in temperature may risk survival of certain tick species in an area.

Research from this thesis also concluded that there was no correlation between aoEBA presence and tick infestation. Past researchers have found that ticks are vectors for many diseases, and that the correlation between tick abundance and disease presence would be likely (Merino et al. 2005; Main, 1977; Stott et al. 2002; Teglas et al. 2006; Chen et al. 2006; Brooks et al. 2016). Reasons for this thesis not displaying any significant results could have to do with inadequate sample size or mule deer that had been bitten by a tick that had since dropped off the individual. With a larger sample size this correlation may have been more likely.

Challenges with this research came from the mule deer sample sizes that were used to obtain data. Data were collected from 31 mule deer in the Mojave and 27 mule deer from Starkey. Perhaps if 50 more individuals had been captured in each study area there would be greater effect sizes on analysis, but I do appreciate that adding more samples would have been a huge increase in cost and time. However, out of the 27 mule deer from Starkey that were tested for aoEBA, none of them came back positive, providing good evidence for either absence or very low rates of exposure to the aoEBA in northeastern Oregon. Additionally, the vector for the aoEBA, *Ornithodoros coriaceus*, was not collected off any deer from Starkey. The only tick collected off those deer was *Dermacentor occidentalis*. Therefore, including more deer from Starkey into our dataset, would most likely not have had profound effects on results. Since, aoEBA was present in many of the mule deer from the Mojave National Preserve, it may have been beneficial to catch more members of the population to increase the significance of the results relative to effects of tick abundance or exposure to the aoEBA on body condition of mule deer.

When determining significance in body condition between the Mojave and Starkey mule deer populations, challenges also occurred in individual mule deer variation as well as seasonal

variation. Due to the numerous amount of variables that affect body condition of mule deer, no correlation could be determined between the aoEBA and body condition, or of tick abundance and body condition. With more defined variables, this correlation could have been made.

Additionally, seasonal variation of when mule deer were captured could have also had an effect on the average body condition measurements that were recorded in this thesis. By performing mule deer capture in both study areas during the same season could have had concluded different results. Nevertheless, methods for capturing mule deer in each study area varied as a function of habitat type. Helicopter capture is not possible at Starkey therefore researchers are forced to use the slower method of trapping individuals over a longer period of time.

Issues also arose with the accuracy of interpreting the indirect fluorescent antibody test (IFAT) results. This test is subjective in nature and presence or absence of fluorescence is based on the observer's visual and observational skills. When determining presence of antibody, consistency among fluorescent flecks is sought. Although some samples showed a clear presence of antibodies; consistency was sometimes difficult to determine in other samples. These challenges were overcome by visualizing the sample at 10x on the fluorescent microscope and then again at 20x. Additionally, mistakes were avoided by having two researchers look at the IFAT results and coming to a consensus on presence or absence of antibody. When presence was uncertain, notes were also taken to avoid misinterpretations.

The effect of *Pajaroellobacter abortibovis* on mule deer has not been studied to the same extent as the disease's effect on cattle, however, there has been sufficient amount of past research on the effect of tick infestations on mule deer body condition. Mule deer, and many ungulates in general, with heavy tick infestations can develop health and physiological problems (McCoy et al. 2014; Mooring & Samuel, 1998; Mooring & Samuel, 1999; Samuel, Wilke, &

Welch, 1991; Yamada & Urabe, 2007). Studies have noted that tick infestations can lead to anemia, damage to hair follicles in winter coats, weight loss, muscle loss, reduced rates of reproduction, and reduced overall health (Samuel, Wilke, & Welch, 1991). Results from this thesis showed that body condition from the Mojave Desert mule deer were lower, on average, than those from Starkey. Whether this is due to tick infestation, the presence of aoEBA, or some other factor, cannot be determined. Since both aoEBA presence and tick infestation is high in the Mojave, it is possible that these factors have a toll on body condition; however, no relationships were able to be studied due to absence of body condition measurements for one animal and low sample size. The correlation of these factors could be determined in future studies on this topic.

The conclusions from this thesis provides some very interesting results that can be applied to future studies and management policies. Mule deer in the Mojave Desert of California are at risk for high tick infestations and infection with the bacterial agent of Epizootic Bovine Abortion. This effect can lead to large numbers of animals that can serve as a reservoir for *Ornithodoros coriaceus* to pass the disease along to other individuals including healthy mule deer and cattle. Although, the effects of this disease on mule deer health and body condition are not known at this point in time, there is possibility that this disease can be a real threat to mule deer survival. Evidence from Stott et al. (2002) and Chen et al. (2006) have already shown the effect of aoEBA on cattle to be severe in cows infected with the etiological agent of EBA aborting their fetuses. The inability to control this disease could lead to significant losses for people who rely on cattle for income. Loss of both mule deer and cattle can have severe costs ecologically and economically to beef producers in California, Nevada, and Southern Oregon. By becoming aware of the presence of aoEBA in mule deer and cattle and better understanding the implications of this disease, more can be done to prevent disease from spreading.

**Future Directions:**

As is noted by this research, the aoEBA poses a large threat to not only domestic cattle populations, but also potentially to other wildlife populations where the disease is endemic. Mule deer in the Mojave National Preserve had more individuals that tested aoEBA positive and more individuals with higher tick loadings. Results also showed that tick infestations were directly correlated with the presence of aoEBA in that population. Mojave deer had lower body condition scores than the Starkey mule deer. As is stated in our results, Mojave also had more individuals that were exposed to the aoEBA.

Although results showed that the aoEBA is present in Mojave mule deer populations, it can not be determined for certain that the aoEBA has a negative impact on body condition or overall health of individuals. In reality, the actual effect that the aoEBA has on mule deer health is still not certain. Further research needs to be conducted in this area to determine if similar to cattle, mule deer populations are also at risk when infected with the aoEBA.

Research in this field is extremely important not only to mule deer populations, but also to domestic cattle populations in the dangerous effects that the aoEBA has on cow fetuses. As mule deer contract the disease they can continue to spread the aoEBA from California, Nevada, and Oregon into untouched areas and into uninfected cattle herds, as well as to uninfected mule deer herds. Epizootic Bovine Abortion can possibly become more widespread and more difficult to control in future. Further research still needs to be done on how the aoEBA affects these animals and the consequences it may have on physiology, anatomy, and general health. Additional research also needs to be done to determine how this disease can be controlled or eliminated from both wildlife and domestic animal populations, so as to put an end to a possible spreading disease.

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