RESPONSES OF THE GULF COAST TICK TO ODORANTS TO ENHANCE FIELD COLLECTION AND A KNOWLEDGE, ATTITUDE AND PRACTICES SURVEY OF TICKS WITH OKLAHOMA BEEF PRODUCERS

By

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Abstract:

The Gulf Coast tick, *Amblyomma maculatum*, Koch, is an arthropod of emerging medical, veterinary, and economic importance. Dragging, flagging, and CO_2 trapping produce low capture rates despite populations existing within economic thresholds. Tick responses to host or conspecific associated chemicals were evaluated using a Y-tube olfactometer bioassay. Semiochemicals tested included ammonium hydroxide, squalene, 1-octen-3-ol, CO_2, 2,6-dichlorophenol, 2-nitrophenol, and ear exudate and rumen fluid from cattle. We hypothesized that rumen fluid would be most attractive to *A. maculatum* ticks. Of all tested, only rumen fluid showed strong responses in the lab assays. Squalene (0.1%) had repellent properties and 2,6-dichlorphenol (5%) failed to attract any ticks. When field tested, rumen fluid did not demonstrate definable attraction. This was the first time rumen fluid was shown to be attractive to *A. maculatum* in a laboratory setting. Further research is needed to evaluate its role as a tick attractant, its potential to improve trapping success, and its role as a host cue facilitating parasitism of cattle. Additionally, farmers are a vulnerable population at increased risk for tick bites and tick-borne illnesses. Oklahoma beef producers (n=198) were surveyed to determine their attitudes, knowledge and perceptions about ticks and the risks they pose to cattle and humans, the tick prevention methods used, and where producers get information. Producers (68.9%) believed ticks were at most a moderate problem for cattle, whereas, only 42.1% thought ticks were only somewhat of a problem for people. Rocky Mountain spotted fever (78.7%) was of most concern for humans while only 9.3% indicated concern for ehrlichiosis. Respondents checked their body for ticks more often than wearing protective clothing. Chemical control methods were used most often to treat ticks on cattle and 30% use injectable dewormer. Veterinarians were the main source of information for producers. Most frequently requested additional information was for prevention and control of ticks on their cattle. Ticks were perceived to be a greater risk for cattle than for humans, though ticks vector more pathogens to humans in Oklahoma. This survey will assist in the development of educational tools used by extensions services.

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CHAPTER I

INTRODUCTION

The hard ticks (Ixodidae) are parasites of a wide array of animals and are important parasites impacting the health and well-being of humans, companion animals, and livestock world-wide. Diseases in humans, livestock and other animals are caused by pathogens vectored by Ixodid ticks. Farmers, especially, are at a higher risk for tick bites (Arikan *et al.* 2010; Kisomi *et al.* 2016) Additionally, attachment and feeding can have undesired economic impacts in livestock production systems via livestock animal death by vectored pathogens; physically damaging to body of animals; or weight loss and reduction in daily weight gains of animals. Cattle and calf sales amount to $76.4 billion in the United States and account for 19% of all annual agricultural revenue (USDA 2015). Between 2007 and 2012, Oklahoma ranked as one of the top five cattle and calf production states boasting an inventory of 1.7 million cattle generating $1.6 billion in sales (USDA 2015). The economic success of the cattle industry relies on development and maintenance of healthy and productive animals.

The Gulf Coast tick (*Amblyomma maculatum* Koch) is one such hard tick emerging as an arthropod of medical, veterinary, and economic importance. Current monitoring methods (dragging, flagging, and carbon dioxide trapping) produce low capture rates despite populations existing in pastures within economic thresholds. Information about *A. maculatum*'s life history in Oklahoma is decades old and in need of updating to match current changes in the ecology of the species. In addition, little information exists in the United States or in Oklahoma in regards to interactions between humans, cattle and ticks. In other parts of the world, knowledge, attitude and perception (KAP) surveys and questionnaires are commonly used to address these interactions. The focus of this study was to expand on these two areas of missing knowledge. These studies were done to better understand *Amblyomma maculatum* in Oklahoma and to gain an understanding of what information is known about cattle-tick interactions by Oklahoma beef producers.

First Objective. Bioassays were conducted to determine the attractiveness of chemicals of biological origin to *Amblyomma maculatum* in both a laboratory and field settings. Eight putative attractants previously show to be attractive to other ixodid ticks species were evaluated: CO_2, 2,6-dichlorophenol, 1-octen-3-ol, 2-nitrophenol, ammonium hydroxide, squalene, ear exudate from the ears of cattle, and fluid from the rumen of a cow. The hypothesis was that rumen fluid would elicit the strongest positive response when compared to the other chemicals of biological origin.

The specific objectives were as follows:

1. Identify volatile compound(s) attractive to the Gulf Coast tick in a laboratory setting using a two-choice selection Y-tube bioassay.
2. Use lab identified compound(s) to recapture marked and released Gulf Coast tick at higher rates than traditionally used dry-ice baited CO_2 traps.

Second Objective. A KAP survey was administered to Oklahoma beef producers using fifteen questions in a paper survey format. Questions were multiple-choice, multiple choice with write-in options and open-ended questions. Data such as location, production type, perception of ticks as a problem, perceived risks of ticks, tick bite preventative behaviors, tick biology, source of information, and follow-up opportunity was gathered.

The specific objectives were as follows:

1. Survey Oklahoma beef producers to better understand the attitudes and knowledge they have in regard to ticks and the risks they pose to their cattle and themselves, their methods of prevention both personal and on their cattle, and where they get their information.

Highlight useful information gathered for the creation of educational materials to be used by Extension specialists that are targeted toward issues producers feel are important.

CHAPTER II

RESPONSES OF *AMBLYOMMA MACULATUM* TO ODORANTS IN LABORATORY AND FIELD ASSAYS

ABSTRACT

The Gulf Coast tick, *Amblyomma maculatum* Koch, is emerging as an arthropod of medical, veterinary, and economic importance. Current monitoring methods (dragging, flagging, and carbon dioxide trapping) produce low capture rates despite populations existing within economic thresholds. The responses of mixed-sex adult *A. maculatum* to chemicals associated with hosts or conspecifics were evaluated using a Y-tube olfactometer selection bioassay. We hypothesized that rumen fluid would elicit the strongest positive response when compared with the other chemicals of biological origin. Host-associated semiochemicals tested: ammonium hydroxide, squalene, 1-octen-3-ol, and CO_2 in addition to the known conspecific semiochemicals, 2-6-dichlorophenol and 2-nitrophenol, components of tick pheromone. Host-associated substances included exudate collected from the ears of cattle and rumen fluid. Only rumen fluid elicited strong

responses in the lab assays. Squalene at 0.1% had repellent properties and 5% 2,6-dichlorphenol failed to attract any ticks. When field tested, rumen fluid did not show attraction in the field. This was the first time rumen fluid was shown to be attractive to *A. maculatum* in a laboratory setting. Further research is needed to evaluate its role in tick attraction and its potential in tick trapping regimens or its role as a cattle host cue for wild populations of *A. maculatum*.

INTRODUCTION

Ixodid ticks play an important role in the health and well-being of humans, companion animals, and livestock around the world. Ixodid ticks have been shown to vector several pathogens known to cause disease in humans and other animals. Attachment and feeding by ticks can lead to undesired physical and physiological responses of the hosts. Additionally, tick parasitism can have economic impacts in livestock production systems: through loss of life due to vectored pathogens; physical damage to the body or hide of animals; reduction in weight of animals; or through production cost increases due to more expensive or more frequent pesticide applications and treatment for diseases (Williams *et al*. 1977; Stacey *et al*. 1978; Pérez de León *et al.* 2010; Edwards 2011).

The Gulf Coast tick (*Amblyomma maculatum*) is one of the larger species of hard ticks in the United States, belonging to the family Ixodidae. Carl Ludwig Koch originally collected the type specimen for *A. maculatum* in “Carolina” in 1844 (Teel *et al*.2010).

Evidence suggests *A. maculatum* is emerging as an arthropod of medical, veterinary, and economic importance. It has been implicated as a major livestock pest, producing conditions such as "gotch ear" (Edwards 2011) and causing weight reduction of drylot steers, leading to economic loss for producers (Williams *et al*. 1977). Its experimental ability to vector potentially fatal heartwater disease (*Ehrlichia ruminantium*), currently an issue in areas as close as the Caribbean, increases its importance as an emerging veterinary livestock pest (Uilenberg 1982; Uilenberg *et al*. 1984). Additionally, *A. maculatum* is the invertebrate host for *Hepatozoon americanum*, the parasite known to cause American canine hepatozoonosis, a severe and sometimes fatal disease of companion dogs and other canids (Mathew *et al*. 1999; Ewing *et al*. 2000). *A. maculatum* also has the ability to parasitize humans. It is not only an irritant and nuisance in its own right as a hematophagous arthropod, it has been shown to cause tick bite paralysis and vectors the pathogen *Rickettsia parkeri*, similar to the more well-known Rocky Mountain spotted fever (Paddock *et al*. 2004; Espinoza-Gomez *et al*. 2011; Paddock and Goddard 2015).

Surveillance of tick populations, including *A. maculatum*, can provide information such as geographic distribution and spread, densities of populations and rates of infection with transmissible pathogens to susceptible hosts. In turn, data gained from tick surveillance can be used to implement management and control programs and to monitor effectiveness (Haemig *et al*. 2011). Tick surveillance techniques traditionally employ: baited traps, using host-associated chemicals such as carbon dioxide producing dry-ice; flagging or dragging fabric on substrates and vegetation; or the trapping and examination of wildlife for ticks. Carbon dioxide baited traps and flagging or dragging exploit the host-seeking and questing behaviors of ticks. Traps using CO_2 have successfully captured *A. maculatum* in Oklahoma, but in very low numbers despite reaching economic thresholds for livestock in the area (Semtner and Hair 1975).

Decades old information paired with the increased importance of *A. maculatum* interactions with humans and other animals has demonstrated a need to better understand its life history in Oklahoma. The low capture rates of *A. maculatum*, documented in the literature and further outlined in this study, indicate the need for a more effective and efficient surveillance method to collect and study the ecology of *A. maculatum* in the state. The objective of this study was to evaluate the attractiveness of chemicals of biological origin to *A. maculatum* in both a laboratory and field settings.

REVIEW OF THE LITERATURE

Distribution and Habitat of the Gulf Coast Tick

Distribution. *Amblyomma maculatum* Koch is a Neotropical-Nearctic species with a distribution comprised of USA, Mexico, Peru, Ecuador, Venezuela, Colombia, Costa Rica, Honduras, Belize, and Nicaragua (Estrada-Peña *et al*. 2005; Teel *et al*. 2010). In the United States, *A. maculatum* has a wide established distribution in the Gulf Coast states as well as populations in Oklahoma, Kansas, Kentucky, the Carolinas, and into the eastern states of Virginia (Fig. 1). An established population may also exist in Maryland and Delaware based off the number of immature ticks found on nesting birds and the number of adults captured pre- and post- winter (Florin *et al*. 2014). Incidental collections in the United States have occurred outside of the described range of established populations; ranging northeastward into New York and Maine and northcentral into Iowa (Wiedl 1981; Teel *et al*. 2010), westward to California (Estrada-Peña *et al*. 2005), as well as collections from migratory birds in Canada (Scott *et al*. 2001; Ogden *et al*. 2008). These incidental collections are not considered indicative of population establishment outside of the commonly accepted permanent range.

Cattle infested with *A. maculatum*, originating from the Gulf Coast region in the 1950s, are thought to have led to the establishment of populations in Oklahoma and parts of Kansas (Semtner and Hair 1973). The earliest account of *A. maculatum* in Oklahoma was in Pittsburg County in 1948 with established populations of *A. maculatum* reported in eighteen eastern Oklahoma counties by 1973 (Barker *et al*. 2004, Teel *et al.* 2010). Prior to 1973, *A. maculatum* had not reached pest levels in Oklahoma (Semtner and Hair 1973). By 2004, over forty Oklahoma counties were positive for *A. maculatum* (Fig. 2) (Barker *et al*. 2004). Teel *et al*. (2010) noted from the late 1960s to 1999, populations expanded from 25% to 65% of the counties in Oklahoma.

Figure 1. *Amblyomma maculatum* range in the United States (CDC)

Figure 2. Distribution of *Amblyomma maculatum* in Oklahoma with dates indicating population establishment year (Barker *et al*. 2004).

Habitat. Over time, the habitat preferences of the Gulf Coast tick have changed or were poorly understood in earlier years. In Neotropical regions, *A. maculatum* occur in the biogeographical Savannah and Pacific provinces (Estrada-Peña *et al*. 2005). The ideal habitat of Nearctic dwelling *A. maculatum* was originally thought to be in southern coastal habitats of the Gulf Coast, marked by high rainfall, high humidity, and high temperature (Bishopp and Hixson 1936). Contrary to these earlier reports, a review of more recent work demonstrates that *A. maculatum* tends to do well in coastal uplands and tall- grass prairies (Teel *et al*. 1998).

Texas populations of *A. maculatum* within their originally described distribution are predominately found in the Gulf Coast Prairies (Fleetwood 1985; Teel *et al*. 2010). Oklahoma and Kansas populations utilize a bounty of ecoregions that include Cross

Timbers, West Cross Timbers, Arkansas Valley and Ridges, Grand Prairie, Central Rolling Red Plains, and the Central Dissected Prairie (Teel *et al*. 2010). These regions consist of native grasslands often bordered by oak- and hickory- dominated wooded uplands. These vegetation communities are ideal for hosts commonly parasitized by this tick. Invasive plants such as honey mesquite and mixed brush species offer ideal habitat for *A. maculatum* and their hosts in Texas (Teel *et al*. 2010).

Biology of the Gulf Coast Tick

Biology, Ecology and Life History. The Gulf Coast tick is a three-host tick; each life stage utilizing a different host type (Teel *et al*. 2010). As with all tick species, *A. maculatum* is strictly hematophagous and must feed only on blood in order to molt from larva to nymph then on to adult. Adult male and female *A. maculatum* feed in order to mate, with females then producing viable eggs.

Sex pheromones are normally involved in mediating mating in hard ticks. *Amblyomma maculatum* males attach to a site on a host and then produce an attraction-aggregation-attachment (AAAP) pheromone which triggers females to attach to the site as well (Sonenshine *et al*. 1986). Adult female ticks, including *Amblyomma americanum*, commonly attract males by emitting sex pheromones, but adult female *A. maculatum* do not emit sex pheromones (Wexler 2005). Currently male-mediated host interactions have only been reported in three ixodid tick species: *A. maculatum*, *A. variegatum* and *A. hebraeum* (Rechav *et al*. 2000; Sleeba *et al* 2010). When given a choice to aggregate near attached females or attached males on a bovine host, both sexes, upon release, gathered near the attached feeding males (Gladney *et al*. 1974). AAAP production by *A.*

maculatum males requires a feeding period of four to six days (Kim 2004). One component of the AAAP, 2,6-dichlorophenol, is found in both male and female *A. maculatum*; the presence of this semiochemical in males is unusual (Sonenshine 1985). Additionally, *A. maculatum* lack the anterior reproductive tract pheromones common in other ixodid ticks, *Amblyomma americanum* and *Dermacentor variabilis* (Allan *et al*. 1991). Unlike other female tick species, Gladney (1971) noted female *A. maculatum* will not attach in the absence of males or when a fed *A. americanum* male is present.

Oviposition of *A. maculatum* is comparable to that of other hard ticks with females converting over 50% of their body weight to egg development and clutches averaging 3,568-18,218 eggs in a laboratory setting; field averages are around 8,000 eggs (Teel *et al*. 2010). Teel *et al*. (2010) summarized recorded days for engorgement, preoviposition and oviposition of adult female *A. maculatum:* engorgement periods were observed from eight days to 21 days, preoviposition observed for zero to 58 days, and oviposition lasting nine to 75 days. Oviposition occurs in the leaf litter, where the female waterproofs the eggs twice using the Gené's organ (Coburn 2009). Oviposition by females saw no significant affects when exposed various photoperiod ranges (Lohmeyer *et al*. 2009). When ticks were fed on non-preferential hosts, rabbits and dogs, egg vitality did not suffer, compared to the egg vitality of *Dermacentor variabilis* and *Rhipicephalus sanguineus* when fed on non-preferential rabbit hosts (Dipeolu 1991). However, *A. maculatum* fed on calcium-deficient rats were found to lay fewer eggs with lower success rates of hatching (Wanchinga and Sonenshine 1978). Temperature and microclimate greatly affect eclosion rates of eggs. Early studies found lower daily temperatures produced longer incubation periods of 19 to 28 days for eggs laid between May and early

September and 50 to 142 days for those deposited late September to November (Hooker *et al*. 1912; Bishopp and Hixson 1936; Hixson 1940). Longer incubation periods ranging from 62.6 to 43.9 days, were observed by Stacey (1971) from early April to early May, incubation periods from 39 to 32 days were seen in late May to early July, and mid-July saw the shortest incubation period of 27.8 days. Fleetwood (1985) detailed that a 1°C increase or decrease in microclimatic temperature resulted in an increase or decrease of egg development time by 1.8 days. Fleetwood (1985) also found the shortest developmental rates occurred between April and September, averaging 14- to 28 days of developmental time with eggs deposited in October requiring almost 40 days on average to develop. Habitat contributes to variation in eclosion rates and eclosion success, as well. Persimmon habitats had the highest rate of eclosion success as well as the longest eclosion rates from April to June when compared to sumac and meadow habitats (Stacey 1971). Hixson (1940) noted variations in vegetative cover may alter the temperature enough to cause changes in incubation periods. The egg stage is the stage most tolerant to desiccation (Yoder *et al*. 2008).

After eclosion, newly hatched *A. maculatum* larvae prefer to aggregate on vegetation's lower surfaces (Hixson 1940). Much like their effects on eclosion rates, temperature and microclimate play a large role in the longevity and survivorship of larvae. Longevity of larvae has been estimated to be 56 to 179 days (Teel *et al*. 2010). Fleetwood (1985) described larval survivorship and habitat relationship based on saturation deficit with shorter larval survival occurring in habitats with the highest cumulative saturation deficit. Fleetwood and Teel (1983) found newly hatched larvae were most active during the time of highest saturation deficit (mid- late hours of the afternoon). As the larvae aged, morning and evening hours became the main periods of activity. The larval stage is most sensitive to desiccation, with desiccation rapidly occurring at temperatures greater than 30°C to 35°C (Yoder and Tank 2006; Yoder *et al*. 2008). Though, Teel *et al*. (2010) found larvae of all ages responded to breath and touch stimuli even at the highest levels of saturation deficit. Length of engorgement of *A. maculatum* larvae is reportedly affected by host type with the engorgement period estimated to be between three days to more than sixteen days (Teel *et al*. 2010). Molting times of engorged larvae are affected by temperature but unaffected by duration of photoperiods (Lohmeyer *et al* 2009).

Information pertaining to the nymphal stage of *A. maculatum* is lacking the most. Once molted from the larval stage, nymphs seek shelter between grass blades and sheaths (Hixson 1940). Because most nymphal (and larval) collections are from trapped hosts, little is known regarding field behavior and location. Feeding and host preference studies appear to be the bulk of available literature. When fed on quail, both nymphs and larvae have high engorgement and molting success rates. Nymphs also have high success rates with engorgement and molting when fed on wood and cotton rats but larvae have lower success (Koch and Hair 1975). Nymphs reared on opossums and raccoons experience decreased molting and engorgement success (Koch and Hair 1975). Nymphal engorgement rates are significantly reduced when feeding on cotton rats versus quail (Moraru *et al.* 2012). Molting times, from nymph to adult, in a controlled 27°C and continuous relative humidity ranges from 21 days to 28 days and is independent of host type (Teel *et al.* 2010). Nymphs and adults are moderately tolerant to desiccation (Yoder *et al*. 2008). Under ideal laboratory conditions, unfed adults live two times as long as nymphs, and four times longer than larvae (Teel *et al*. 2010).

The Gulf Coast tick feeds on different hosts at each life stage and is not highly host restrictive during any stage. *Amblyomma maculatum* larvae, nymphs, and adults are found on a wide array of hosts. Teel *et al*. (2010) published an extensive list of seventy-one species of birds and mammals known to host the various life stages. Immatures, both larvae and nymphs, have a predilection for birds, especially ground dwelling species like bobwhite quail (*Colinus virginianus*) and meadowlarks (*Sturnella* spp.) (Semtner and Hair 1973; Teel *et al*. 1998, 2010). Both immature stages are also sometimes found on small mammals, such as rodents (Barker *et al.* 2004; Teel *et al*. 2010). Experimentally, Carolina anoles (*Anolis carolinensis*) have not been shown to be suitable hosts for immature *A. maculatum*. Paired with the lack of field observations of immatures feeding on reptiles, it is probable reptiles do not play a role in the Gulf Coast tick life cycle (Moraru *et al*. 2012). Noted attachment sites of immatures include the head and neck of preferred hosts (Koch and Hair 1975). When released experimentally onto cattle, immatures attach and feed on the tail-head, withers and midline, but little evidence has been found indicating a preference for feeding on cattle in the field (Ketchum *et al*. 2005). Adult *A. maculatum* prefer larger vertebrate hosts including ungulates: cattle, horses, feral swine, and white tail deer, in addition to various canids and felids (Smith *et al*. 1982; Teel *et al*. 2010; Duell *et al*. 2013; Sanders *et al*. 2013). Mobility of hosts such as birds, large litter size and uncontrolled movement of feral swine and the intense growth of white-tail deer populations may all contribute to the dispersal of *A. maculatum* and its associated pathogens within the environment (Paddock and Goddard 2015).

Seasonal Phenology. The seasonal activity of the Gulf Coast tick varies based on the area of the United States in which the population resides. *Amblyomma maculatum* of the Gulf Coast region are active about five months later than inland populations located throughout Texas, Oklahoma, and Kansas. Additionally, Oklahoma and Kansas populations are active five months sooner in the year than populations in Texas (Teel *et al*. 2010). In Texas, peak larval activity on birds is seen in December/January and nymphs are most abundant on hosts around February (Teel *et al*. 1988, 1998). Texas adults appear to peak in September according to recorded parasitism rates on cattle (Teel *et al*. 2010). Peak abundance of Oklahoma larval populations occurs in late June and early July (Semtner and Hair 1973). Nymphs have been observed to peak on birds twice, in late July and late August while late May and early June is the peak time for adult attachment on cattle (Semtner and Hair 1973). Unpublished data by Teel *et al* (2010) indicates cattle required treatment in March in order to protect them from infestations peaking in April and May. Ketchum *et al.* (2006) also reports populations of northern Oklahoma and Kansas are active as early as March with peak abundance occurring in April and May.

The seasonal phenology differences of the coastal *A. maculatum* populations and the inland populations of Oklahoma and Kansas have been linked with genetic differences (Teel *et al.* 2010). Seven different haplotypes were identified when the 16S mitochondrial rDNA genes of *A. maculatum* from cattle in Georgia, Oklahoma, Kansas and Texas were examined (Williams 2002). Oklahoma ticks shared two different haplotypes, one with Kansas, Oklahoma and Texas and the other shared by Kansas and Oklahoma (Williams 2002). Ketchum *et al*. (2006) described the reproductive compatibility of two distinct genetic *A. maculatum* populations from Kansas and Texas. Lostak (2008) subsequently reexamined the haplotype frequencies described by Williams (2002) and found a shift in dominant haplotypes in Texas and Oklahoma *A. maculatum* populations. This shift in dominant haplotype frequencies may suggest that host movement may facilitate dispersal into new areas (Lostak 2008).

Veterinary Importance of the Gulf Coast Tick

Amblyomma maculatum parasitizes companion animals, livestock, and wildlife. Infestation can lead to blood loss, irritation, and transmission of a range of pathogens. Additionally, conditions resulting from infestation can result in various levels of economic loss.

Physical and Economic Impacts. The Gulf Coast tick has been noted as a significant pest of livestock, especially cattle. It is considered the primary cause of a condition known as “gotch ear”, which can be seen in cattle as well as horses, mules, and goats (Bishopp and Trembley 1945; Edwards *et al*. 2011). Feeding by *A. maculatum* can cause tissue responses in the host ear such as thickening, and edema. Additionally, tissue and cartilage damage may become severe enough causing the middle of the ear to become permanently bent downward (Edwards 2011). In some calves, up to one-third of the ear may be lost due to necrosis (Mock 2000). Feeding damage to the ears can cause the animal to be less desirable and sell for less at market. Cattle breed may play a role in ear susceptibility to *A. maculatum* feeding activity. Fewer *A. maculatum* have been found on Brahman crosses compared with other cattle breeds (Semtner and Hair 1973).

Brahmans possess a natural immunity against the feeding activity of the tick as well as fewer successful attachments by *A. maculatum* (Stacey *et al*. 1978).

Infestation with *A. maculatum* can have physiological effects on weight gain and blood composition of cattle. Williams *et al*. (1977) found drylot Hereford steers infested with high levels *A. maculatum* (up to 225 ticks) were 24 kg lighter than control animals, and steers with light infestations (up to 125 ticks) were 14 kg lighter than controls. Stacey *et al*. (1978) also found high *A. maculatum* infestations (up to 400 ticks) resulted in reduced weight gains in Hereford steers but not in similarly infested Brahman steers. Blood compositional changes indicative of infection have been observed in cattle experimentally infested with *A. maculatum* (Williams *et al*. 1977; Stacey *et al*. 1978; Riley *et al*. 1995).

Before the eradication of the primary screwworm *Cochliomyia homnivorax* (Coquerel), from North America, *A. maculatum* had a significant economic impact through its facilitation of myiasis of the fly larvae on livestock (Bishopp and Hixson 1936; Spicer and Dove 1938). Lesions left by the tick on cattle ears provided a place for oviposition by primary screwworm and also offered suitable habitat for the larvae to burrow and feed (Gladney 1976). At one point, ranchers resorted to cutting off the ears of their cattle to control Gulf Coast tick and screwworm infestations. Hundreds of other animals naturally lost their ears due to the interaction of these two parasites (Gladney *et al*. 1977).

Pathogens and Resulting Diseases. Twelve *Amblyomma* species, many of them natives of Africa, are known vectors of *Ehrlichia ruminantium*, the causative agent for the often fatal disease heartwater (Walker 1987). Heartwater can infect a range of wildlife species including numerous African hoofstock: African buffalo (*Syncerus caffer),* blesbuck (*Damaliscus albifrons*), eland (*Taurotragus oryx*), springbuck (*Antidorcas marsupialis*), waterbuck (*Kobus ellipsiprymnus*), impala (*Aepyceros melampus*), African elephant (*Loxodonta africana*), and giraffe (*Giraffa camelopardalis*) (Deem 1998). *Ehrlichia ruminantium* infections in non-African ruminants have been documented in white-tailed deer (*Odocoileus virginianus*), Barbary sheep (*Ammotragus lervia*), and Rusa deer (*Cervus timorensis*) (Deem 1998).

Laboratory studies show *A. maculatum* can acquire *E. ruminantium* from feeding on infected animals. The pathogen can be passed transtadially to the subsequent life stages, with a transmission efficiency to that of the known natural vector, *Amblyomma variegatum* (F.) (Uilenberg 1982). *Amblyomma maculatum* has further been shown to be a similarly susceptible to several strains of heartwater to which the natural vectors, *A. variegatum* and *Amblyomma hebraeum*, are highly susceptible (Mahan *et al*. 2000). In the Caribbean, established populations of *A. variegatum* exist, most likely introduced to the area via the introduction of zebu cattle from Senegal in the 1830s (Uilenberg 1982; Uilenberg *et al*. 1984). Heartwater is present in Antigua and the French West Indies (Pegram and Eddy 2002). The occurrence of both heartwater and African *Amblyomma* species in the Caribbean islands poses a potential risk for introduction of the pathogen onto the American mainland (Uilenberg 1984; Barré *et al*. 1987). Cattle egrets (*Bubulcus ibis*) have played an important role in the dispersal of *A. variegatum* throughout the Caribbean islands (Pegram and Eddy 2002). The birds have posed a problem to eradication efforts of *A. variegatum* in the region due to their ability to transport immature ticks great distances (Pegram and Eddy 2002). Migrations of cattle egrets infested with *A. variegatum* nymphs and larvae occurs and has been recorded in Florida (Deem 1998). In addition to migrating cattle egrets, importation of exotic animals for wildlife ranching, zoological displays, and the pet trade can be possible corridors of introduction (Wilson and Richard 1984; Clark and Doten 1995; Deem 1998). In the event of importation of *E. ruminantium* via ticks, birds or exotic animals, white-tailed deer are especially susceptible and could provide a means for heartwater to spread in North America (Dardiri *et al*. 1987).

American canine hepatozoonosis is an emerging disease caused by the apicomplexan *Hepatozoon americanum* with *A. maculatum* as the invertebrate definitive host (Mathew *et al*. 1999; Ewing *et al*. 2002). Domestic and wild canids, especially coyotes, can become infected by this parasite and suffer severe and sometimes fatal infections (Kocan *et al*. 1999; Ewing *et al*. 2000; Potter and Macintire 2010). Canids become infected with *H. americanum* when they ingest infected *A. maculatum* during grooming. Predatory behavior and ingestion of prey items with infective immature *A. maculatum* stages may also be a factor (Ewing *et al*. 2002). Naïve *A. maculatum* become infected with the apicomplexan when they feed on canids exhibiting clinical signs of American canine hepatozoonosis and from canids that have recovered from the disease but remain carriers of the pathogen (Ewing *et al*. 2003).

Medical Importance of the Gulf Coast Tick

Domestic animals, livestock, and wildlife are not the only animals to which *A. maculatum* is attracted. They are known to readily feed on humans (Goddard 2002). They are ranked as one of the top four ixodid tick species o reported biting humans, though only about 1% to 3% of reported tick attachments are those of *A. maculatum* (Paddock and Goddard 2015). The bite and attachment of adult *A. maculatum* can cause a condition known as tick paralysis. Tick paralysis occurs when toxins in the tick saliva disrupt the motor neurons of humans, leading to paralysis of the respiratory muscles. Of the two occasions *A. maculatum* was shown to cause tick paralysis, removal of the ticks resulted in recovery from the neurological effects of the bite within 48 hours in both patients (Paffenbarger 1951; Espinoza-Gomez *et al*. 2011).

The bite of the Gulf Coast tick can potentially transmit the arthropod-borne bacterium, *Rickettsia parkeri*, a spotted fever group rickettsia. *R. parkeri* has been found in *A. maculatum* in the following states: Virginia (Fornadel *et al*. 2011; Wright *et al*. 2011), Mississippi (Ferrari *et al*. 2012), Kentucky, Tennessee (Pagac *et al*. 2014), Alabama, Florida, Georgia, Texas (Sumner *et al*. 2007), Arkansas, Louisiana, Delaware, Maryland, and North Carolina (Paddock and Goddard 2015). The bacterium was described and isolated from *A. maculatum* in the late 1930s (Parker *et al*. 1939). Fornadel *et al*. (2011) found 41.4% of *A. maculatum* tested positive for *R. parkeri* in one Virginia county. Additionally, 28% of the screened *A. maculatum* from Mississippi and Florida tested positive for *R. parkeri* (Paddock *et al*. 2010). The resulting infectious disease is known as *R. parkeri* rickettsiosis, Tidewater spotted fever and/or American Boutonneuse fever (Wright *et al*. 2011). *R. parkeri* in humans creates an infection that is similar to Rocky Mountain spotted fever (RMSF) with the distinction that it is usually less severe and a necrotic eschar is usually present at the inoculation site. Some incidences of RMSF may be misidentified as *R. parkeri* infections (Paddock *et al* 2004). Grasperge *et al*. (2014), using mouse models, showed *R. parkeri* proliferated at sites associated tick feeding by *A. maculatum* versus sites that were intradermally inoculated but lacked tick feeding. This may indicate the tick vector is more than just a depositor of the pathogen into the attachment site.

Various other pathogens have been identified in adult *A. maculatum,* including *R. felis,* the causative agent of flea-borne spotted fever and *Ehrlichia chaffeensis*, the causative agent of human monocytotropic ehrlichiosis; the role of the tick in the life history of these pathogens is not currently known (Williamson *et al*. 2010; Jiang *et al*. 2012; Paddock *et al*. 2015). Infected dog-to-tick-to-naïve dog experiments conducted by Ewing *et al*. (1997) demonstrated *A. maculatum* was incapable of transtadial transmission of another form of human ehrlichiosis, *E. ewingii*, the human granulocytotropic *Ehrlichia*.

Amblyomma maculatum have additional microbial endosymbionts, but their pathogenicity to humans and other animals are unknown at this point. '*Candidatus* Rickettsia andeanae' is one such endosymbiont. While isolated in *A. maculatum* from multiple states, the pathogenic status is unknown (Paddock and Goddard 2015). In Kansas and Oklahoma, 62% of field collected *A. maculatum* tested positive for '*Ca.* R. andeanae' (Paddock *et al*. 2015). The presence of '*Ca.* R. andeanae' in these populations may play a part in preventing *R. parkeri* infections in *A. maculatum* (Paddock *et al*. 2015). The successful lab culturing of '*Ca.* R. andeanae' may now allow for exploration of its role as a human pathogen (Luce-Fedrow *et al*. 2011). Furthermore, *R. amblyommii* has been identified in collected ticks and is thought to be a possible endosymbiont, but its pathogenicity to humans has yet to be determined (Yabsley *et al*. 2009; Trout *et al*. 2010; Paddock and Goddard 2015). In Oklahoma, *R. amblyommii* was identified via IFA in the blood of 52.3% of dogs tested displaced by tornadoes in 2013 and on 57.4% of dogs relinquished by owners, all dogs were naturally infected. One dog was identified by PCR with active infection. (Barrett and Little 2016).

Tick Surveillance Methods

Tick Surveillance. Tick surveillance occurs using passive or active surveillance methods or a combination of both. Passive surveillance often relies on public submission of ticks found attached to animals or people. Passive surveillance is advantageous due to its minimal labor and monetary requirements. It also can offer insight into the phenology of ticks through observation of when specimens are collected and submissions can be used to better understand the processes of invasion (Cortinas and Spomer 2013). Passive surveillance, though, has its shortcomings. The methods used can be incomplete, biased, or misjudge the distribution and population sizes of the ticks submitted and can over represent species of ticks utilizing domesticated animals and humans as hosts and under represent species infrequently found on these types of hosts (Johnson *et al*. 2004; Cortinas and Spomer 2014). Additionally, the reliance on the public can affect the results due to population densities, behaviors, demographics, education level, and attitudes toward the value of surveillance (Stone *et al*. 2005; Cortinas and Spomer 2014). Active surveillance uses techniques to actively seek out and collect ticks from the environment. Active surveillance can be costly, time consuming, and labor intensive. Three main active surveillance techniques exist: collection of ticks from sentinel animals or wild hosts (Lindenmayer *et al*. 1991; Barker *et al*. 2004); dragging or flagging vegetation with a cloth, simulating host movement (Kinzer *et al*. 1990; Carroll and Schmidtmann 1992);

and the use of CO_2, usually from dry ice, to attract ticks toward the point of gas release (Wilson *et al*. 1972; Semtner and Hair 1975; Guedes *et al*. 2012). The aforementioned active surveillance methods are known productive tick collection techniques.

Surveillance methods should take into account the species of tick being surveyed and the life stage being targeted, as each method has advantages and disadvantages (Carr 2011). Methods using sentinel animals or wild captured hosts can be costly, specialized equipment is often needed, labor intensive and require special permits to sedate and handle animals. Flagging and dragging methods do not require specialized equipment but are labor intensive. Carbon dioxide-baited traps require no special equipment, but require care in handling dry ice or CO_2 reaction kits. Access to dry ice is not always readily available and can be relatively expensive over time. These active surveillance techniques also pose issues with collection biases. Tick population distributions are not always known. Selected survey sites, whether using trapping, flagging, or sentinel animals, may not always have active tick populations. Even in areas with known targeted tick species, distribution patterns can be unknown and sampling area size will have an impact on active surveillance success. Biases emerge when trapping hosts if care is not taken to trap for the appropriate hosts in the appropriate habitats.

Issues with Gulf Coast Tick Collection. Collection efforts of the Gulf Coast tick using traditional trapping methods usually result in low capture success rate (Goddard and Paddock 2005; Goddard *et al*. 2011). Carbon dioxide traps have been successful in capturing *A. maculatum* in Oklahoma, but in very low numbers despite economic thresholds for livestock in the area being met (Semtner and Hair, 1975). A systematic literature study was completed to evaluate the differences between flagging/dragging and on-host collection methods for *A. maculatum*. In total, 75 studies were found, of which, 28 provided data on *A. maculatum* by flagging and dragging and 47 provided data on collections from various hosts. The data is summarized in Table 1 below. While difficult to analyze due to varying years of studies reported, it is notable that considerably less *A. maculatum*, in general, are collected than other tick species, especially when compared with *A. americanum*. This trend is true for both methods evaluated. The main conclusion from this study is that field collections using current techniques is time consuming and laborious and usually result in low capture success rate. As only field-collected ticks are useful for genetic studies, due to the issues that arise when involving host DNA, a novel method to improve the collection of *A. maculatum* in field settings would considerably reduce time and effort.

Table 1. Summary of systematic review of all studies which have collected *A. maculatum* using flagging/dragging or directly from hosts.

		Single year studies			**Multi-year studies**		
		No. studies	Range	Median	No. studies	Range	Median
Flagging/Dragging	*A maculatum*	11	2-356	49	17	1-707	108
	A americanum	2	204-4632	2418	7	113-16,431	1052
	Other species	5	24-2339	359	7	17-2349	93
On hosts	*A maculatum*	15	1-5,025	36	32	2-10,695	37
	A americanum	9	4-26,696	60	19	4-1079	315
	Other species	11	43-48,339	477	25	3-4389	387

Chemicals as Tick Attractants

The importance of this species as a pest and our relatively low level of understanding of its ecology in a pasture system makes it is necessary to develop new field collection methods to help improve monitoring and control programs. Due to the various disadvantages of known active surveillance techniques and the low capture rate of *A. maculatum* compared to other tick species, it may be advantageous to use conspecific chemicals mediating *A. maculatum* attraction, aggregation, and attachment behaviors. Furthermore, host-associated chemicals, alone or in conjunction with CO_2, may be used to improve trapping efficacy.

Pheromone mediated behavior is seen in many ixodid tick species. The attraction-aggregation-attachment pheromone (AAAP) is one such conspecific chemical of note. Cattle with actively feeding male *A. maculatum* attract more females than cattle without males (Sleeba *et al.* 2010). Free-living nymphs and adults of *A. hebraeum* in Africa were attracted to traps pairing AAAP and CO_2, as long as care was taken to place the trap in suitable habitats with known populations of the target species (Bryson *et al*. 2000). Additionally, Maranga *et al*. (2003) showed *A. variegatum* are significantly attracted to traps combining CO_2 and AAAP; carbon dioxide alone was found unattractive and the pheromone alone was found to be only slightly attractive. Kelly *et al*. (2014) has shown tail and collar tags impregnated with the acaricide deltamethrin AAAP reduced *A. variegatum* infestations on cattle from an average of 23.1 ticks on controls versus an average of 3.5 ticks on treated animals.

Ear Exudate and Rumen Fluid. Some tick species have on-host site preferences. Much like adult *Amblyomma maculatum*, the brown ear tick (*Rhipicephalus appendiculatus* Neumann) prefers to feed inside the ears of bovids, whereas the red-legged tick (*R. ecertsi* Neumann) predominately feeds around bovid anal regions (Wanzala *et al.* 2004). Ticks with site preferences on hosts can be attracted by compounds respective to their site preference. In one study, the brown ear tick was attracted to bovid ear volatiles and repelled by its anal secretions; the red-legged tick was found to be repelled by the collected ear volatiles and attracted to the anal secretions (Wanzala *et al*. 2004).

Adult hard ticks are frequently found parasitizing large ungulates, in particular, ruminants. In order to maintain chemical balance in the foregut and to relieve pressure build up ruminants often erupt gases. Ruminants eruct every two to three minutes and in one hour, converting half of the gases found in the rumen into breath (Donzé *et al*. 2004). These eructations may signal to hard ticks that a potential host may be present. Various ixodid tick species are attracted to odors produced by gut fermentation. Two species of *Amblyomma* ticks and three *Ixodes* species were found by Donzé *et al*. (2004) to be significantly attracted to rumen fluid odor in laboratory behavioral assays. All ticks used in the study originated from different continents: Asia, Europe, Africa and North America (*I. scapularis*).

1-octen-3-ol. The attractiveness of bovine emanations was discussed in the prior section. 1-octen-3-ol is a known compound of bovine emanations and often reported as an attractant to hematophagous insects. Multiple tick species are reportedly attracted to this semiochemical. *R. microplus* larvae are highly attracted to 1-octen-3-ol (Osterkamp *et al*. 1999; Ranju *et al*. 2012). Larvae of three other common ruminant ticks, *Hyalomma marginatum*, *Haemaphysalis bispinosa* and *R. haemaphysaloides*, also found the compound attractive (Ranju *et al*. 2012). Carr *et al*. (2013) reported 1-octen-3-ol was attractive to adult *A. americanum* at multiple concentrations.

Ammonium Hydroxide. Sweat and vertebrate urine are a mix of many different chemical components, including ammonia and ammonium hydroxide. Ticks may use ammonia in their host seeking behaviors. *R. sanguineus* even have ammonia-sensitive neurons located on the first tarsi (Haggart and Davis 1980). Adult *A. americanum* are attracted to varying concentrations of ammonium hydroxide in laboratory bioassays (Carr *et al*. 2013). It also is a known host odor attractant for the mosquito *Aedes aegypti* (Geier *et al*. 1999).

Squalene. One of the most abundant skin lipids of mammals is squalene. It is naturally occurring on the skin and in mammalian blood (Stewart 1992). Ticks from the genus *Dermacentor* secrete a waxy substance comprised of squalene when stimulated by pressure. It is assumed the secretion is a defense mechanism and is derived from the diet (Yoder *et al*. 1993; Yoder *et al.* 1998). All life stages of the lone star tick, *A. americanum*, are highly attracted to this lipid, in addition to adult American dog tick, *D. variabilis*. Squalene attraction studies identified adult females, nymphs, and larval *A. americanum*, were more attracted to squalene than to any other chemical tested (benzaldehyde, methyl salicylate, nonanoic acid, 2-nitrophenol) (Yoder *et al*. 1998). Additionally, squalene has been found to have long range attraction to *A. americanum* in laboratory and field settings. *A. americanum* were able to detect squalene from ¾ meter away, which is ¼ meter closer than other known tick attractants. Squalene had a response time of less than thirty minutes and 75% of the ticks found it attractive, compared to 0-43% of the ticks being attracted to AAAP active ingredients (Yoder *et al*. 1999).

2,6-dichlorophenol and *2-nitrophenol*. Attraction-aggregation-attachment-pheromones (AAAP) are chemicals used by several genera of ixodid ticks. They help mediate behaviors associate with mating, such as on-host mate attracting and encouraging aggregation and attachment. 2,6-dichlorophenol and 2-nitrophenol (also known as *o*-

nitrophenol) are known to be components of AAAP in many ticks including *A. maculatum* (Sonenshine 1985). 2,6-dichlorphenol has been isolated in at least twelve Ixodidae tick species, most frequently in males (Sonenshine 1985). Most tick sensory perception occurs using various pits and sensilla on the front legs. Several tick genera, including *Amblyomma*, have tarsal receptors capable of detecting 2,6-dichlorophenol and *A. variegatum* is able to sense 2-nitrophenol (Steullet and Guerin 1994). One experiment using 2,6-dichlorphenol extracted from mixed sex adult *A. maculatum* demonstrated male attraction when the chemical was placed on a rabbit host (Kellum and Berger 1977). The successful use of 2-nitrophenol to collect *A. variegatum* and *A. hebraeum* in a field setting has been demonstrated by Norval *et al*. (1991) and Barré *et al*. (1997). Additionally, a synthetic mix of 2,6-dichlorphenol and 2-nitrophenol and other pheromones was used in tags attached to a collar and the tail of cattle. *A. variegatum* aggregations on the body were highest at areas near the pheromone tags (Allan *et al*. 1998).

Chemicals associated with hosts and conspecifics have potential applications in attracting *A. maculatum*. Currently, only CO_2 and 2,6-dichlorophenol extracted from fed ticks have been found to be marginally attractive to *A. maculatum*. A lack of attractive chemicals, the low number of *A. maculatum* collected in comparison to other tick species, and the difficulty of collecting ticks off host, demonstrates a need to identify putative chemical attractants to enhance field collection rates.

METHODOLOGY

Y-Tube Olfactometer Selection Bioassays

Ticks. Adult *A. maculatum* and adult *A. americanum* were obtained from the Tick Rearing Facility, Oklahoma State University, Stillwater, Oklahoma. After receipt, ticks were held in a humidity chamber, using potassium sulfate to maintain humidity at the saturation point. The chamber was kept at room temperature under 15L: 9D light schedule. Ticks were acclimated to the laboratory environment for at least 48 hours after acquisition and prior to use in bioassays. All ticks were unfed for the entirety of the study.

Animal Safety Protocols: This project has approval from the Institutional Animal Care and Use Committee (IACUC) (Appendix A). All interactions with cattle in this study follow the protocols outlined in the Animal Care and Use Protocol (ACUP) No. AG-15-11.

Y-tube Olfactometer Assay Design. All tests took place within a fume hood equipped with fluorescent lights, at room temperature. Two-choice selection assays were conducted using a glass Y-tube olfactometer (Glassworks, Bartlesville, OK, USA) (Fig. 3) adapted from methods described by Carr *et al.* (2013). Filtered air was introduced into the Y-tube arms via the fume-hood's installed air delivery system, with an exception for CO_2 delivery (described later). Air from the fume hood ports was directed through filters using activated charcoal then further filtered using fine glass wool. After filtration, air flow rates were regulated using 150-mm correlated flowmeters (Cole-Parmer®). Flow rates per arm were adjusted symmetrically. Air from the flowmeters was directed into glass vacuum traps (Wilmad-LabGlass®), used as volatile holding chambers, which then allowed air to flow into the ports of the arms of the olfactometer. When required, CO_2 (3% in breathing quality air) was introduced into the system using a compressed gas tank (Stillwater Steel, Stillwater, OK, USA) and was not subjected to filtration. A designated 150-mm correlated flowmeter was used for CO_2 to reduce risk of contamination. Carbon dioxide was then introduced into the Y-tube through the glass vacuum trap and into a port of one Y-tube arm, as described for air introduction. To avoid positional bias, odorants were alternated between the two ports of the olfactometer. A vacuum integrated into the fume hood was used to remove gasses at the downwind end of the Y-tube equal to the rate at which air flowed into the system. To avoid contamination, all glassware was washed with Alconox® detergent and hot water and dried in an oven at 100°C between uses. Equipment incapable of being dried via the oven was washed with hot water and Alconox® detergent, rinsed with 95% ethanol and allowed to air-dry eight or more hours. Equipment was only handled while using gloves.

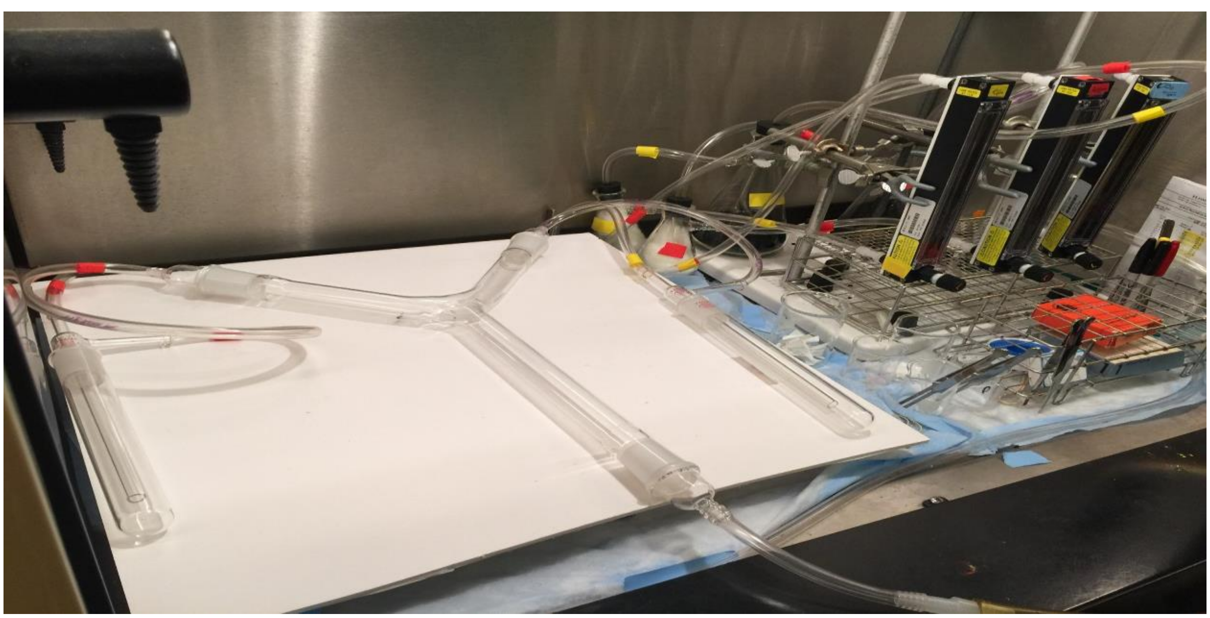


Figure 3. Y-tube olfactometer setup used in laboratory bioassays

Olfactometer Assay with Amblyomma americanum. Preliminary behavioral assays were conducted to establish that this Y-tube olfactometer system was an appropriate way to measure tick responses to odorants. Carbon dioxide is a known tick attractant and it has been shown to be attractive to *A. americanum*. Previously, *A. americanum* were shown to be significantly attracted to CO_2 (3% in breathing quality air) in a Y-tube olfactometer assay (Carr *et al.* 2013). Using an air flow rate of 100 ml/min and a trial time of five minutes, established by Carr *et al*. (2013), mixed-sex adult *A. americanum* response to CO_2 was determined. Additionally, tick responses to being marked with fluorescent powder (DayGlo ECO®, DayGlo Color Corp.) were also evaluated. Ticks evaluated for effect of powder marking were marked at least 24 hours before assays were conducted. All ticks were acclimated to the test setting at least 30 minutes prior to use. Each trial was conducted with five unfed mixed-sex adults, previously untested. Twelve replications were conducted, for a total of 60 ticks tested per assay. After placement at the starting point into the olfactometer (2.5-cm past the exhaust outlet), each trial ran for five minutes and positive responses were recorded along with the time it took to make a selection using a stop watch with multiple stop functions. Movement of 4-cm or more into one the arms of the olfactometer was recorded as a positive response. When two different arm treatments were used, the test substance and control were alternated between the arms to reduce positional bias.

As a form of assay control to ensure the system was responding in a 'normal' fashion as per results published by Carr *et al*. (2013), *A. americanum* were tested in the following assays: marked and unmarked ticks (60 per assay) to air only at a flow rate of 100 ml/min/port, and marked and unmarked ticks (60 per assay) to air and CO_2 at a flow rate of 100 ml/min/port. The air only assay consisted of air only flowing into both arms of the Y-tube olfactometer. The air only assay allowed behavioral observations to occur between the two treatment groups, ticks marked with fluorescent powder and ticks not marked with powder. The air and CO_2 assay consisted of air flowing through one arm of the Y-tube while CO_2 flowed through the opposing arm. This air and CO_2 experiment had a two-fold purpose, it was used to evaluate the behaviors between the marked and unmarked ticks as well as an evaluation of the attractiveness of CO_2.

Olfactometer Assay with Amblyomma maculatum. Each assay trial was conducted using five mixed-sex adult *A. maculatum*, *s*ix trial replicates were conducted, each time with previously untested ticks. Ticks were only handled while wearing gloves. The day of testing, ticks were acclimated to the experimental setting for at least 30 minutes prior to placement into the Y-tube olfactometer. Ticks were placed into the olfactometer at a starting point 2.5-cm past the exhaust opening. Each trial ran for ten minutes and selection times were recorded for the first positive response from individual ticks. Positive responses were recorded when a tick moved 4-cm into one of the arms of the olfactometer.

A standard air flow rate to be used for all trials had to be established. Using the previously described set up, trials were conducted using filtered air in both arms at correlated units of 0 (0 ml/min), 40 (24.8 ml/min), 70 (47.8 ml/min), 100 (76.7 ml/min), 120 (97.2 ml/min), 140 (122.1 ml/min), and 150 (138.3 ml/min). The flowmeters were correlated, scaled units found on the face were chosen for simplicity of adjustment. Additionally, responses to CO_2 and filtered air introduced into opposite arms were evaluated at the same correlated rates, 0, 40, 70, 100, 120, 140, 150. The volatile holding chambers remained empty for the flowrate determination trials.

Due to trials being conducted with five ticks at a time, differentiation of individuals was necessary. Ticks were marked with one of five different DayGlo ECO® pigments. Marking occurred at least 24 hours prior to testing. Using assay methods described, trials using no air, air in both arms, and air and CO_2 were conducted to ensure marking with fluorescent powder did not alter behavior. A correlated flow rate of 70, corresponding to 47.8 ml/min/port, was chosen due to responses observed in initial air and CO_2 flow rate assays. A no-air assay using 30 marked ticks and a no-air assay using 30 unmarked ticks was conducted and responses timed and recorded. An assay with only air entering through both arms of the Y-tube was similarly conducted, with 30 marked ticks and 30 unmarked ticks. Both the no-air assay and the air-only assay were used to determine the effect of marking on the behavior of *A. maculatum.* Finally, behavioral responses to CO_2 and air alternated between ports with 30 marked ticks and 30 unmarked ticks was also done. Much like the air and CO_2 assay conducted with *A. americanum,* this was done with *A. maculatum* to evaluate the effect of marking on tick behavior as well as the effect CO_2 has on their behavior.

Semiochemical Testing. The following chemicals (Sigma Aldrich) were tested: 2-nitrophenol (98%), 1-octen-3-ol (98%), 2,6-dichlorophenol (99%), squalene (≥ 98%, liquid), and ammonium hydroxide (28-30% NH_3 basis, ACS). Rumen fluid was obtained at two different time periods from a single donor cow by veterinary staff or technicians under the supervision of the Oklahoma State University Center for Veterinary Health Sciences. Rumen fluid was tested at three stages of freshness. Fresh rumen fluid was used within an hour of acquisition from the cow donor. Aged rumen fluid was created by storing fresh rumen fluid in a closed container in a refrigerator for two to six months. Ear exudate was obtained via rubbing the ear surfaces of cattle with clean flannel cloth with gloved hands per Oklahoma State University ACUP AG-15-11 (Appendix A). Flannel ear swabs were collected on three different occasions, with two swabs per collection being obtained for a total of six cloths. Swabs were immediately returned to the lab and tested for bioactivity within one hour of collection.

Potential attractants were tested using the Y-tube olfactometer methods described previously. An air flow rate of 47.8 ml/min/port was selected based off the airflow and CO_2 flow rate assays. Five different chemical dilutions (10%, 5%, 2.5%, 1% and 0.1%) were made using methanol for 2-nitrophenol; methanol was used as in the control. Five different dilutions (10%, 5%, 2.5%, 1% and 0.1%) were made using hexane for 1-octen-3-ol, squalene, and 2,6-dichlorphenol; hexane was used as the control for these chemicals. Five different dilutions (25%, 10%, 5%, 1% and 0.1%) were made using water for ammonium hydroxide with water acting as the control during testing. All dilutions were made, while wearing gloves, using a serial dilution method in 10mL volumetric flasks immediately before testing. With the exception of ear swabs, each putative attractant (25µl) was placed onto cellulose filter paper (2.5 cm circles) and immediately transferred into an odorant chamber with forceps. The appropriate control (25µl) was also placed onto cellulose filter paper (2.5 cm circles) and transferred into the opposite odorant chamber with new forceps to avoid contamination. Unaltered cellulose filter paper acted as the control for rumen fluid and was placed into the opposite odorant chamber via clean forceps. Additionally, two-month old rumen fluid was tested at volumes of 50µl and 100µl. This was done to evaluate if greater volume increased activity due to higher activity observed for this age of rumen fluid at 25µl compared to fresh and six-month old fluid. Individual cattle ear swabs were placed into one odorant chamber and a clean flannel piece was used as a control in the opposing chamber. Odor chambers containing flannel swabs were also submerged in a 38.6°C water bath, equivalent to the average body temperature of a cow. This was done to facilitate release of any odors collected from the ears on the flannel swabs. Rotation of controls and treatments were done between the two ports of the Y-tube arms to prevent positional bias. After placement of ticks into the olfactometer, a stopwatch with multiple stop capability was started and ran for ten minutes. All ticks walking at least 4 cm into either arm were recorded as making a choice and the time to selection was noted.

Mark, Release, Recapture Field Bioassay

Field Trials. Chemicals eliciting attraction in the laboratory assays were then field tested. Rumen fluid, aged two months, was tested against the controls: water and CO_2 (produced by dry ice). Field trials used a mark-release-recapture method to study efficacy of the traps. Lab-reared mixed sex adult *A. maculatum* were marked using the same methods described for use in the laboratory choice selection assays. Field trials were done at the Oklahoma State University North Range Research Station in Stillwater, OK, USA. Trials were conducted in May and June 2016, a total of three trials were conducted. Ambient temperatures ranged from 24°C and 27°C with relative humidity ranging from 37% to 47%. The field site was a pasture interspersed with Eastern red cedars and oak trees. Cattle were present in the pasture before and during the time period of testing. Field trials were conducted between 10:00-14:00 h. Each trial lasted two hours. Testing was not done if the wind speed was greater than 15 mph or if the ground cover was damp or wet.

Rumen fluid and water were dispersed by placing 20 ml of liquid into small glass dishes (60 x 15 mm) (PYREX®, Corning Glass Works). Rumen fluid was tested in both heated and unheated states; the water control was also heated. Heating was done to produce a more volatile state allowing for better air dispersal. Air-activated single use heat packets (HotHands® Warmers) were used as the heat source. Heat packets were activated when removed from the protective packaging and exposed to air 20 minutes prior to use to allow for optimal temperature (100°F to 180°F) to be reached, per manufacturer instructions. All test and control chemicals were placed into individual plastic storage containers with holes cut into the lower portions to direct released gases outwards into the test sites. These containers were then placed individually onto plywood boards (1m x 1m) (Lowes Home Improvement Inc.). Folded masking tape was placed on all four edges of the board, with the tape's sticky side facing the environment (Fig. 4). The taped boards and containers were then placed at the test locations.

Figure 4. Example of tick trap used to recapture marked *A. maculatum*.

A total of four boards with test or control chemicals were placed at the field site per trial (Fig. 5). The boards were placed ten meters apart, in a linear fashion. Twenty ticks were released per trap, each set of ticks marked with a different color fluorescent powder. Ten ticks were released two meters to either side of the trap (Fig. 6). Two hours after ticks were released, traps were examined for presence of recaptured ticks or wild caught *A. maculatum.*

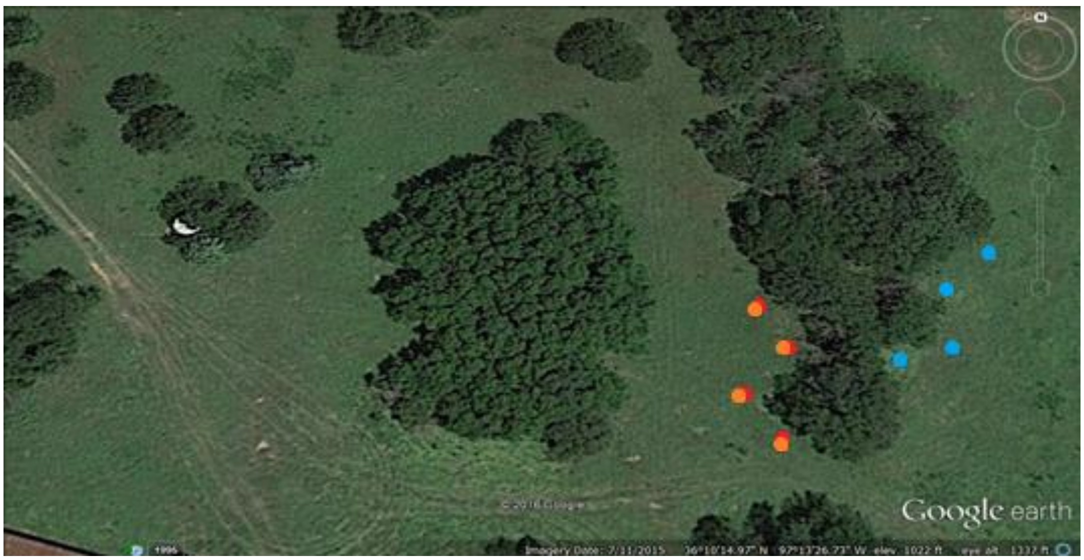


Figure 5. Image showing field site for mark-release-recapture trapping experiments. Each color denotes a separate trial.

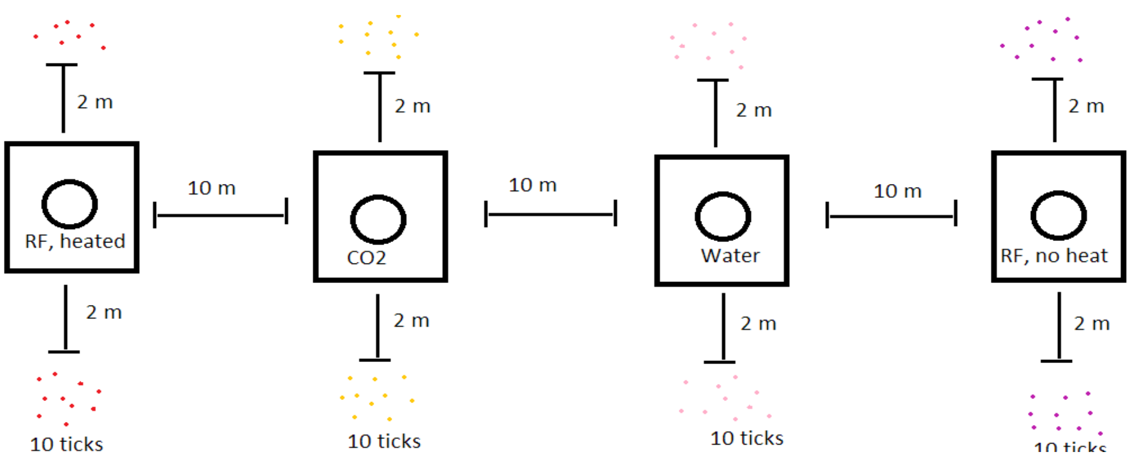


Figure 6. Diagram showing in-field trap placement and marked tick release points.

Statistical Analysis: All data from the olfactometer studies was analyzed using SAS (SAS 9.4, SAS Institute Inc., Cary, NC). Standard comparison tests using PROC FREQ were run to confirm that there was no bias and to test for significant differences between choices made by ticks.

RESULTS

Y-Tube Olfactometer Selection Bioassay

Lone Star Tick Assays. Effects of marking and carbon dioxide assay response rates are shown in Fig. 7. Adult *A. americanum* were attracted to CO_2 in the Y-tube olfactometer. The responses of marked and unmarked ticks were tested with *A. americanum* for two treatment types: air only from both arms, and air and CO_2 alternated between the arms of the olfactometer. With regard to marking ticks for identification in air-only assays, there was no bias (Chi-Square; df=1; p<0.8010). Additionally, marking in assays with the arms involving air or CO_2 showed no bias (Chi-Square; df=1; p<0.4862). The same CO_2 versus air assay used to evaluate marking behavior was also used to evaluate the overall effect of CO_2 on *A. americanum* behavior. Due to marking have no significant effect on behavior, the results of both marked (n=60) and unmarked (n=60) assays were pooled together. Using Chi-Square Goodness of Fit on the total ticks used, more adult *A. americanum* chose CO_2 (n=48) than air (n=23) (df=1; p<0.0044).

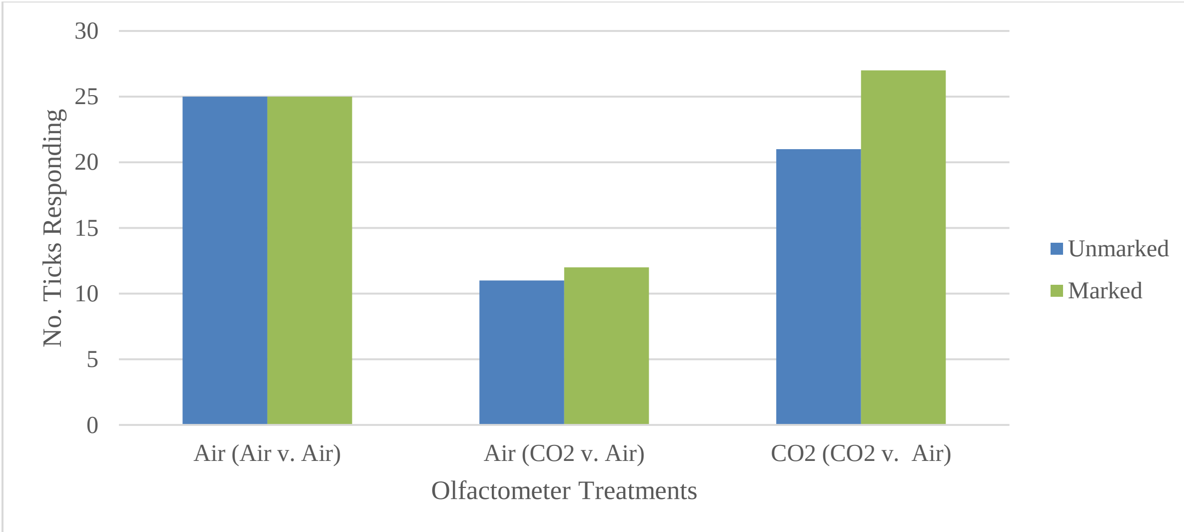


Figure 7. Effects of marking and carbon dioxide assay response on *Amblyomma americanum* adults in a Y-tube olfactometer. Marking effect is shown for two treatment types, air only (air v. air) and CO_2 and Air. The overall effect of CO_2 on behavior is also shown (CO_2 v. Air), with choices for the air and CO_2 arms shown separately. Marked and unmarked totals were combined due to no significant effect of marking.

Air Flow Rate Assays. Air flow rate assay using only air had fewer *A. maculatum* making selections (24.4%) than total overall selection, for or against, in the CO_2 assay (34.4%). *A. maculatum* exhibited attraction to CO_2 at the 40 (24.8 ml/min) and 70 (47.8 ml/min) correlated rates with 26.7% of all ticks selecting for CO_2 at both rates. Rates of 120 (97.2 ml/min), 140 (122.1 ml/min), and 150 (138.3 ml/min) had more ticks selecting for air in air-only assays than for CO_2 in that assay. Responses to all flow rates are shown in Fig. 8. Ticks placed in the olfactometer with no air flowing through the system made positive selections (for air or CO_2) 30% of the time. The correlated rate, 70, was chosen for subsequent tests due to the higher percentage of ticks making selections for CO_2 and air treatments overall, 21% selected versus selection rates of 17% (40 correlated flow rate), 18% (100 correlated flow rate), 13% (120 correlated flow rate), 19% (140 correlated flow rate) and 18% (150 correlated flow rate).

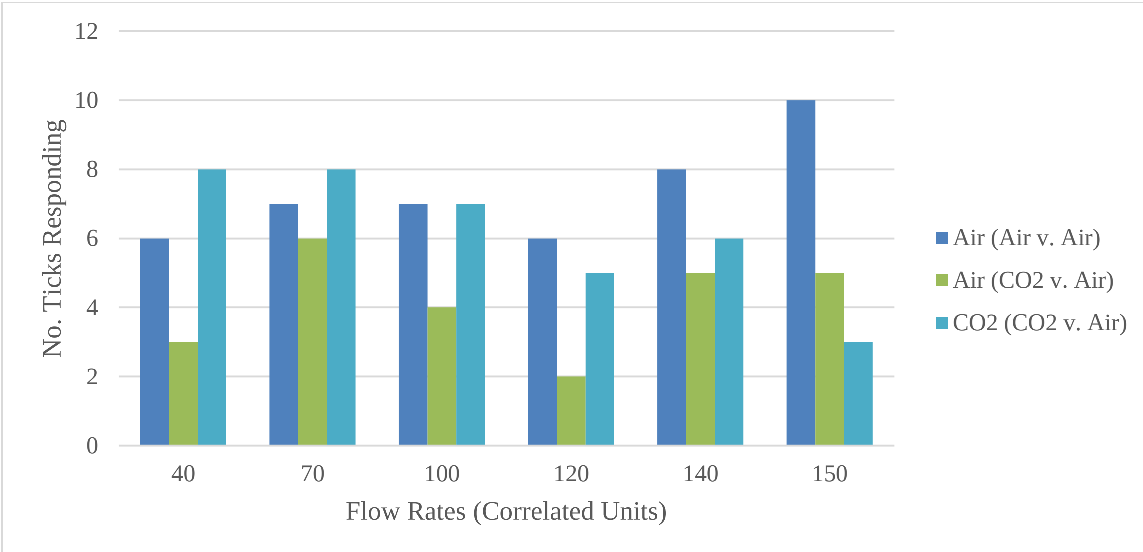


Figure 8. Total number of *Amblyomma maculatum* adults responding to filtered air in a Y-tube olfactometer compared to the number of ticks responding to 3% CO_2. The effect of the six air flow rates are shown using air only (air v. air) and CO_2 and Air. The effect of CO_2 at the varied rates is shown (CO_2 v. Air), with choices for the air and CO_2 arms shown separately.

Tick Marking Effect Assays. Marking ticks did not influence the behavior of *A. maculatum* in the olfactometer assays. Equal numbers of marked and unmarked ticks made selections in the no air trial (30%) and when selecting for CO_2 (26.7%) (Fig. 9).

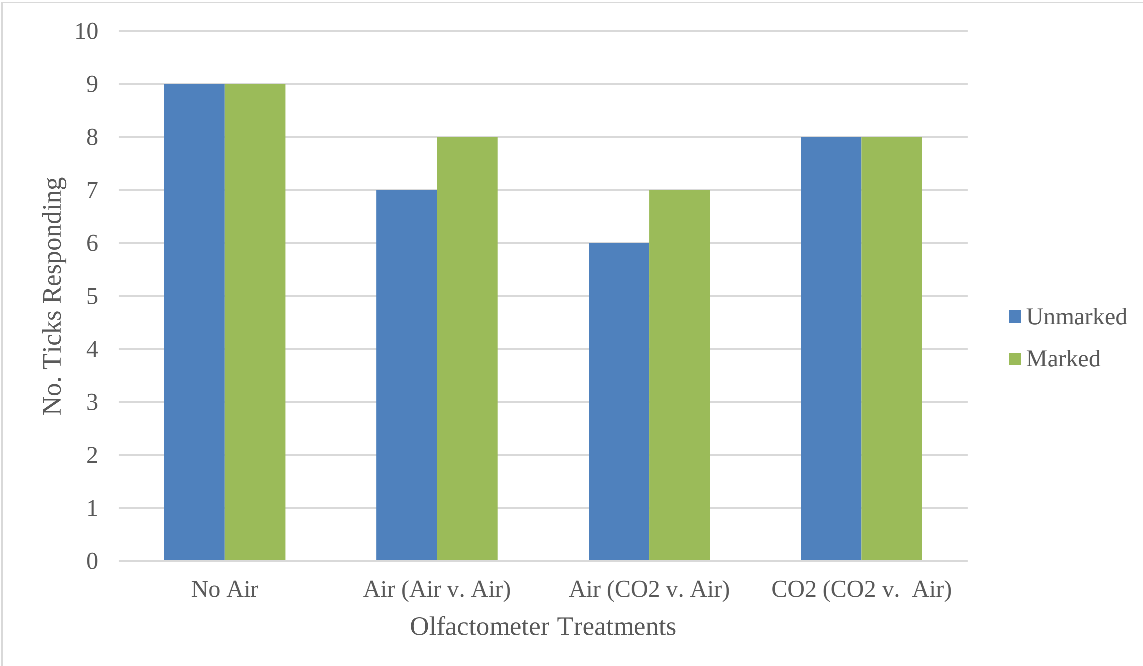


Figure 9. Total number responding *A. maculatum* in three different olfactometer treatments comparing marked versus unmarked ticks. Marking effect is shown for two treatment types, air only (air v. air) and CO_2 and Air. The overall effect of CO_2 on behavior is also shown (CO_2 v. Air), with choices for the air and CO_2 arms shown separately. Marked and unmarked totals were combined due to no significant effect of marking.

Responses to Putative Attractants in Olfactometer Assays. Of the seven chemicals tested, four had one or more concentrations that elicited a higher number of positive responses in *A. maculatum*: rumen fluid, 2-nitrophenol, squalene, and ammonium hydroxide. 2,6-dichlorophenol was the only chemical that had no responses or more selections for the control.

Response to 2-nitrophenol in Olfactometer Assays. Overall attractiveness of 2-nitrophenol is shown in Fig. 10. Only 4-12 ticks out of 30 responded to varying concentrations of 2-nitrophenol. Concentrations 10% and 0.1% were equally as attractive as the methanol control. The 5% concentration was slightly more attractive than all other concentrations and had the second highest (tied with squalene, 5% concentration) over all attraction rate compared to all of the chemicals. This over all attractiveness was still relatively low with 8 of 30 ticks positively responding.

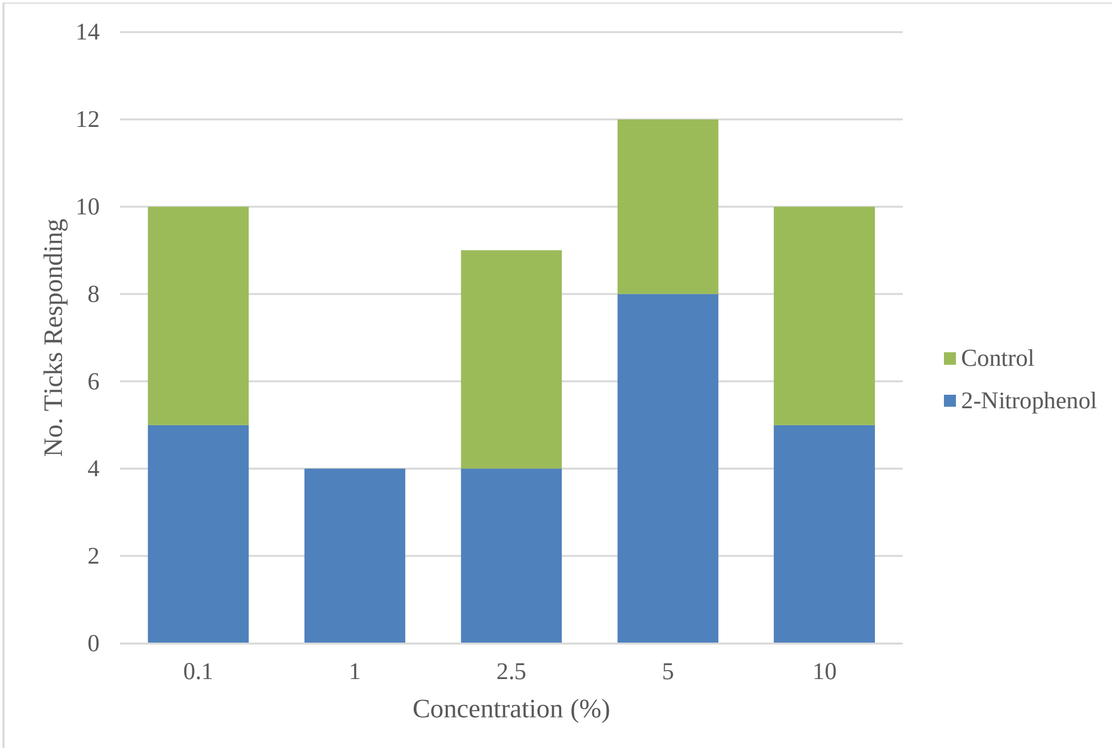


Figure 10. Total number adult *A. maculatum* Responding to 2-nitrophenol and Methanol Control in Laboratory Y-Tube Olfactometer.

Response to Squalene in Olfactometer Assays. Overall attractiveness of squalene is shown in Fig. 11. Differing slightly from the other chemicals, 8-12 ticks out of 30 responded to varying concentrations of squalene. Repellency was observed in the highest and lowest concentrations, with 0.1% repelling more ticks than the 10% concentration. The 0.1% concentration also repelled the highest number ticks (6, same number of ticks as ammonium hydroxide, 5% concentration) of all chemicals. Squalene at 5% attracted the second highest (same number of ticks as 2-nitrophenol, 5%) number of ticks of all chemicals. The 1% concentration did not repel any ticks.

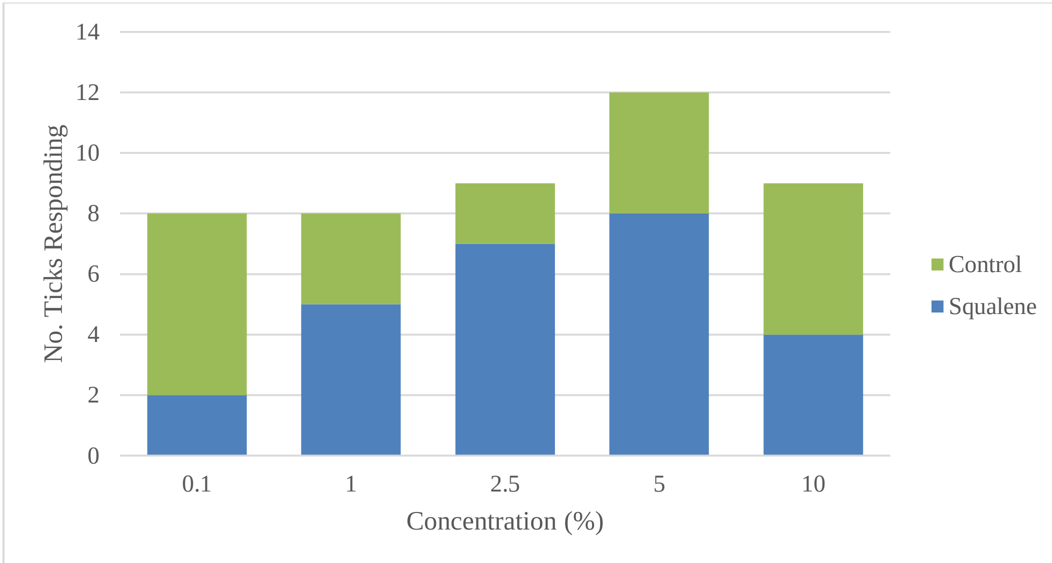


Figure 11. Total number adult *A. maculatum* Responding to Squalene and Hexane Control in Laboratory Y-Tube Olfactometer.

Response to Ammonium Hydroxide in Olfactometer Assays. Overall attractiveness of ammonium hydroxide is shown in Fig. 12. Only 4-8 ticks out of 30 responded to varying concentrations of ammonium hydroxide. More concentrations (1%, 5%, and 25%) of ammonium hydroxide were more repellent than they were attractive. The 10% concentration was equally attractive and repellent. The lowest concentration (0.1%) had some attractiveness. Tied with squalene (0.1% concentration), the 5% concentration of ammonium hydroxide repelled the most ticks (n=6) of all chemicals.

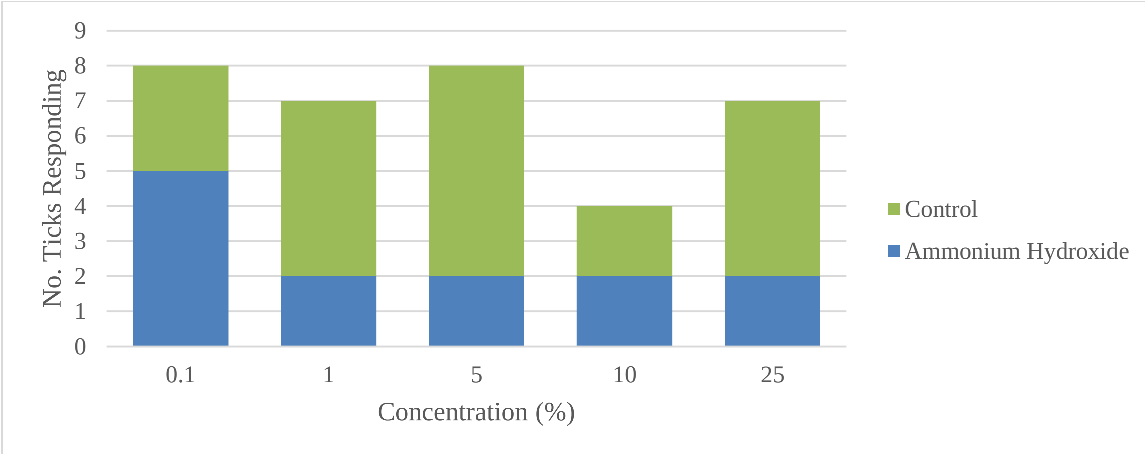


Figure 12. Total number adult *A. maculatum* Responding to Ammonium Hydroxide and Water Control in Laboratory Y-Tube Olfactometer.

Response to 1-octen-3-ol in Olfactometer Assays. Overall attractiveness of 1-octen-3-ol is shown in Fig. 13. Only 4-8 ticks out of 30 responded to varying concentrations of 1-octen-3-ol. Ticks did not select for 1-octen-3-ol more often than the hexane control. At concentrations of 2.5% and 5% equal numbers of ticks selected for either arm (n=4 and n=2, respectively). The highest number of ticks preferred the hexane control over the 0.1% concentration.

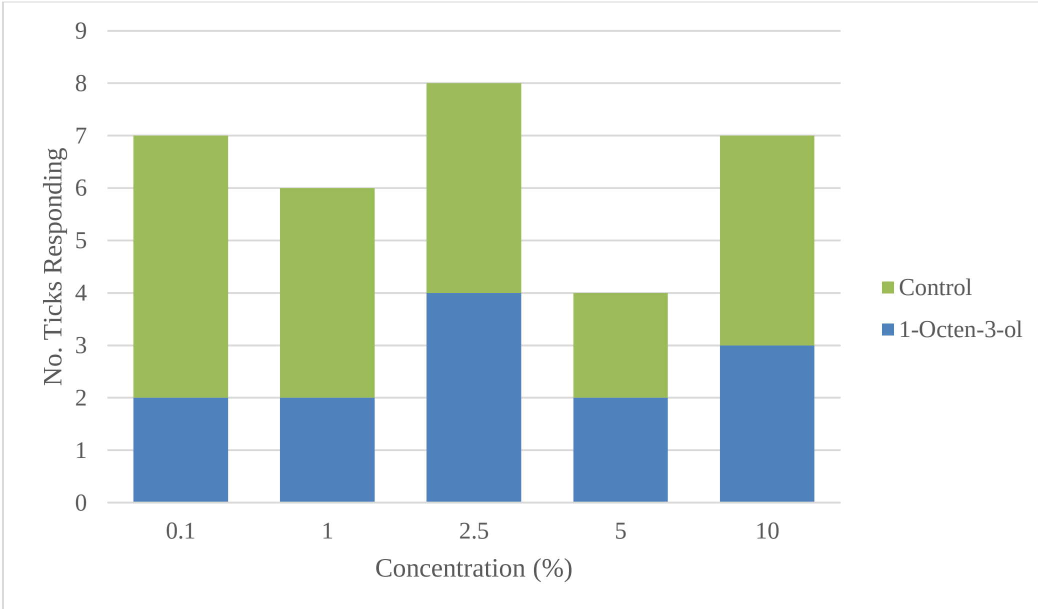


Figure 13. Total number adult *A. maculatum* Responding to 1-octen-3-ol and Hexane Control in Laboratory Y-Tube Olfactometer.

Response to 2,6-dichlorophenol in Olfactometer Assays. Overall attractiveness of 2,6-dichlorophenol is shown in Fig. 14. Lower than the result from the other assays, 7 ticks or less out of 30 responded to varying concentrations of 2,6-dichlorophenol. 2,6-dichlorophenol was the only chemical exhibiting more repellency than attractiveness. All concentrations repelled ticks, except for the 5% concentration that had zero responders. Aside from the 5% concentration, the 1% concentration tied with ear exudate for the fewest number of respondents (n=3) for or against the test substance.

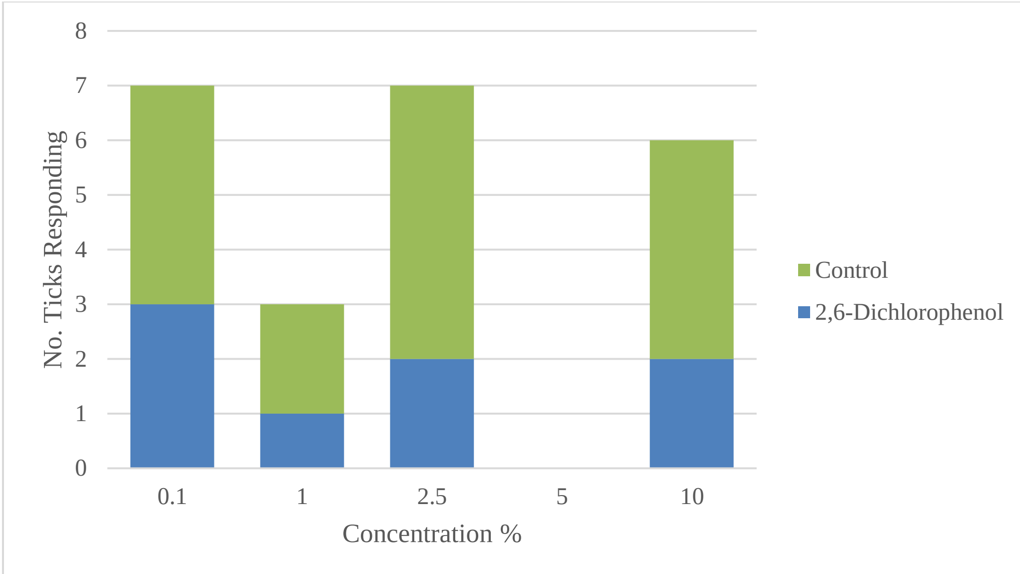


Figure 14. Total Number Adult *A. maculatum* Responding to 2,6-dichlorophenol and Hexane Control in Laboratory Y-Tube Olfactometer.

Response to Ear Exudate in Olfactometer Assays. *A. maculatum* was not attracted to ear exudate of cattle. Thirty ticks were evaluated and one selected for the arm with ear volatiles, while two selected for the control arm. Tied with 2,6-dichlorophenol (1% concentration), this was the lowest selection rate of all the chemicals tested.

Response to Rumen Fluid in Olfactometer Assays. Attractiveness of rumen fluid is shown in Fig. 15. Overall, many more ticks (7-17 of 30 ticks) responded to rumen fluid compared with the other chemicals tested. When compared to the control (air), significant preference was observed for rumen fluid (p=0.001) and no significant differences were observed by age (p=0.6052). Rumen fluid aged two months at three different volumes (25 µl, 50 µl and 100 µl) had the highest selection rate (p=0.0019) followed by fluid aged six months (p=0.0169). Fresh rumen fluid and fluid aged six months were just as attractive as squalene (2.5% concentration).

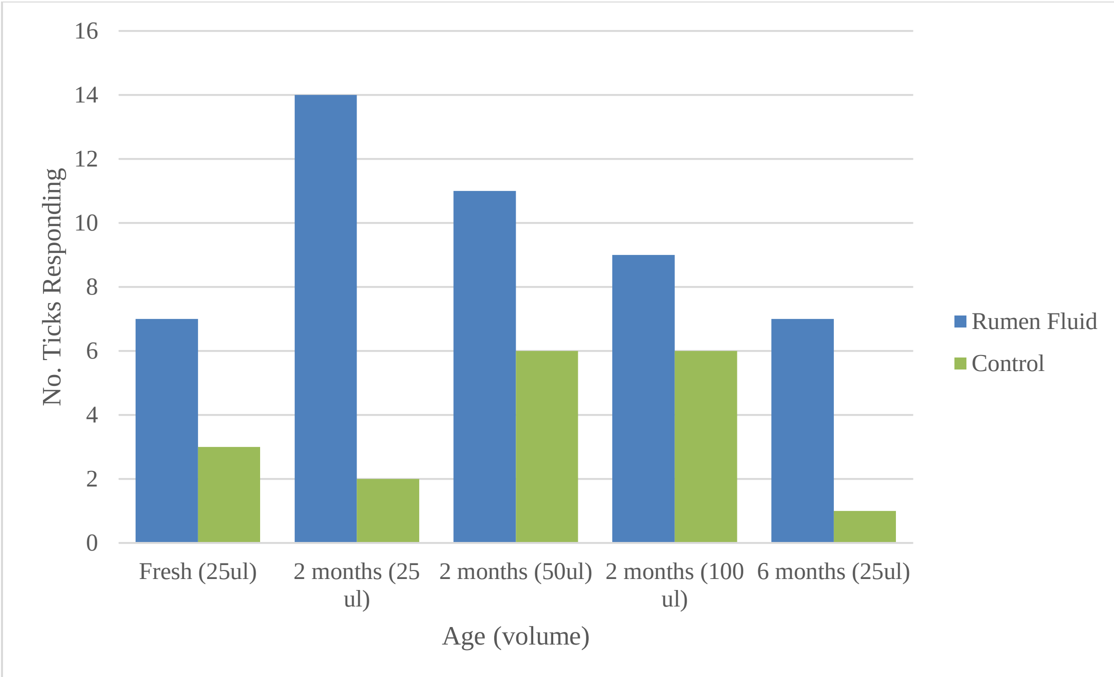


Figure 15. Total Number Adult *A. maculatum* Responding to Rumen Fluid and Air Control in Laboratory Y-Tube Olfactometer.

Field Collection of *Amblyomma maculatum*

Results from the laboratory olfactometer assays indicated that the most successful attractant, two-month old rumen fluid, should be tested further in a field setting. A total of 240 mixed-sex adult *A. maculatum* were marked and released during field attraction trials. Eleven marked ticks were recaptured during the assays and no wild *A. maculatum* were collected during that time (Fig. 16).

In the field, rumen fluid (heated and unheated) was tested in field trials against water and CO_2. Three trials were conducted, but one trial failed to recapture ticks. In the remaining trials, dry-ice baited CO_2 traps returned ten more ticks than water or unheated rumen fluid. One marked tick was collected on a trap using heated rumen fluid whereas the CO_2 trap in the same trial recaptured three marked ticks. Over the entirety of the 2 successful field studies conducted, CO_2 traps returned nine more marked ticks than heated rumen fluid traps. Traps baited with water or unheated rumen fluid recaptured zero ticks in each of the three field trials.

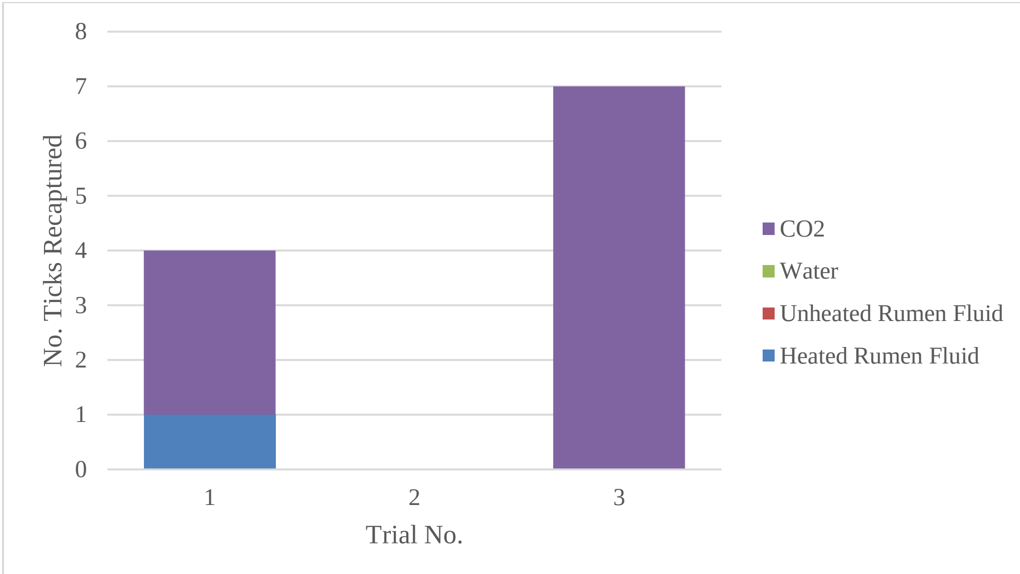


Figure 16. Number Recaptured *A. maculatum* During Three Different Field Assays Using a Mark-Release-Recapture Method.

DISCUSSION

For the first time, rumen fluid was shown to be attractive to adult *A. maculatum* in laboratory bioassays. Rumen fluid aged two months (25 µl) had the highest number of ticks selecting for it when compared to all other tested chemicals. The following chemicals were also found to be attractive to *A. maculatum* in the laboratory assays (listed from most ticks attracted to fewest): two-month old rumen fluid (50 µl and 100 µl); 2-nitrophenol (5% concentration) and squalene (5% concentration); fresh and six-month old rumen fluid, squalene (2.5%); ammonium hydroxide (1% concentration) and squalene (1% concentration); and 2-nitrophenol (1% concentration). The positive response of these chemicals to other tick species has been recorded in the literature.

While two-month old rumen fluid, at all volumes, was the most attractive to the total number of *A. maculatum* tested, rumen fluid at other ages was attractive as well. The finding that rumen fluid is attractive to an ixodid tick, *A. maculatum*, is in line with findings in previous studies. Donzé *et al.* (2004) found fresh rumen fluid exhibited significant attraction in laboratory assays to five other ixodid tick species, *Amblyomma variegatum; A. hebraeum; Ixodes ricinus; I. persulcatus; I. scapularis*.

Though not directly tested in this study, other studies have highlighted the potential mode of action that rumen fluid has on ticks. Fermentation in the rumen, facilitated by microflora, produces short-chain fatty acids described by Erwin *et al.* (1961) and were isolated using gas chromatography. Acetate, propionate, butyrate and valerate were isolated from the rumen fluid of a sheep fed alfalfa hay, molasses and minerals (Erwin *et al.* 1961). Though the Erwin study used rumen fluid from a sheep, other studies have found similar short chain fatty acids in cow rumen fluid (Donzé *et al.* 2004; Ranju *et al.* 2014). It is important to note several of the identified chemicals have shared chemical (NCBI) names: propionate is also known as propanoic acid (CID104745) and propionic acid (CID1032); acetate is also known as acetic acid (CID175); butyrate is known as butanoic acid (CID104775) and butyric acid (CID264).

Individual and combination chemostimulants identified in rumen fluid odor were tested by Donzé *et al.* (2004): butanoic acid, isobutanoic acid, 3-methylindole, 4-methyphenol, acetic acid, propanoic acid and methane. *A. variegatum* was attracted to 3-methylindole, 4-methylphenol, butanoic acid, and isobutanoic acid individually, but when combined 1:1:1:1 attraction was not induced (Donzé *et al.* 2004). The same chemicals were then combined in a proportion approximate to that of rumen fluid 100:10:1:1

(butanoic acid:isobutanoic acid:4-methylphenol:3-methylindole) which elicited attraction in *A. variegatum* and *I. scapularis* at half the rate of whole rumen fluid indicating proportion of volatiles is important to ticks (Donzé *et al.* 2004). Behavioral assays were conducted using butyric and propionic acid (rumen fluid odor components, individually and combined) with ticks known to parasitize ruminants (*Rhipicephalus microplus*, *R. haemaphysaloides*, *Haemaphysalis bispinosa*, and *H. marginatum*) as well as the brown dog tick, *R. sanguineus* (Ranju *et al.* 2014). This study found that ticks in the same genera do not always respond to stimulants in the same manner, nor do ticks respond the same to individual stimulants compared to combined components. No attraction was exhibited by *R. sanguineus* to any tested components; *H. marginatum* was attracted to individual and combined butyric acid and propionic acid but *H. bispinosa* was least attracted to the same chemicals (Ranju *et al.* 2014). Individually, butyric acid and propionic acid was attractive to *R. haemaphysaloides,* but not combined; *R. microplus* was attracted to the components combined but not attracted to propionic acid alone (Ranju *et al.* 2014). The reactivity differences between ticks from the same genus are more than likely what was observed by the differences in the current study.

The results of the current study support prior studies in regards to the attractiveness of rumen fluid to ixodid ticks, though unlike Donzé *et al.* (2004), older rumen fluid was more attractive than fresh. Several explanations for this finding may be plausible. Firstly, proportions and combinations of volatile components affect ticks differently, even ticks in the same genera. Aging rumen fluid may alter the volatiles of rumen fluid in a way that increases attractiveness to *A. maculatum*. As both fresh fluid and six-month old fluid attracted the same number of ticks, it is possible microflora in the fluid was not developed enough to attract *A. maculatum* early on and may have died by the time six-months elapsed. Finally, the host source of the fluid may influence behavior in the assays. In the current study and the study by Donzé *et al.* (2004), rumen fluid came from different cows, most likely fed on different forage bases and subjected to different husbandry protocols, thereby altering the rumen fluid composition. When evaluating the effect on rumen fluid microbial composition of N'Dama cattle and N'Dama x Jersey fed different diets, Nouala *et al.* (2009) found diet significantly altered the microbial community of the rumen. Additionally, cattle breed may also play a role in the differences in behavioral assays. The breed of the cow from which the rumen fluid was obtained is unknown, but in all likelihood, it was *Bos taurus*, the same species used by Donzé *et al.* (2004) in their rumen study. O'Kelly and Spiers (1992) found differences in rumen fluid between two breeds of cattle, Brahman and Hereford, fed the same diet. Brahman cattle had higher concentrations of propionic, butyric, isobutyric, and isovaleric acids than those in Hereford cattle (O'Kelly and Spiers 1992). The chemicals with altered concentrations described in the O'Kelly and Spiers (1992) are the same chemicals identified and previously tested for attractiveness with other ixodid ticks by Donzé *et al.* (2004) and Ranju *et al.* (2014).

While aged rumen fluid was attractive to *A. maculatum* in the laboratory, it did not perform in a similar fashion in the field. The observed effect may have been due to differences in rumen fluid between lab-fed and pasture-kept cattle. In an open system, with uncontrolled external inputs from the environment, ticks may need multiple cues to engage host seeking behaviors. A range of volatiles are released from ruminants approximately every two to three minutes, with most of the contents of the rumen being converted into breath every hour (Donzé *et al.* 2004). This range of volatiles paired with host skin odors, and physical cues such as vibrations and heat could trigger more aggressive host seeking in the field. Carbon dioxide acts synergistically with chemical lures for blood-feeding insects (Carr 2011) and may have the same effect with other host associated volatiles. While wild *A. maculatum* may be attracted to the rumen eructions of pasture-kept cattle (hence the infestations observed), they may not have been attracted to components of the rumen fluid from lab-fed cattle (which was attractive to lab-reared ticks). The donor cow was maintained by OSU-CVHS and would have more management inputs that would alter the rumen fluid composition when compared with pasture-kept cattle.

The selection of 2-nitrophenol (*o*-nitrophenol) as a putative attractant to *A. maculatum* in this study was due to its successful field recapture rates of *A. variegatum* and *A. hebraeum* (Norval *et al.* 1991; Barré *et al.* 1997) and its reported presence in bovine odor (Steullet and Guerin 1994). Barré *et al.* (1997) tested *o*-nitrophenol at low concentrations, production equivalent of attached male tick, and found it greatly increased trapping rates when paired with dry ice CO_2 baited traps. Norval *et al.* (1991) found 10% solutions of 2-nitrophenol were attractive to *A. variegatum* and *A. hebraeum*. In this study, 2-nitrophenol at concentrations of 1% and 5% returned double the amount of ticks as those that selected for the control arm. The attraction rates overall were not high in our study, and most concentrations (0.1%, 2.5%, 10%) attracted equal number of ticks as the control. The attractive concentrations fall between previously observed ranges, including Yoder *et al.* (1999) reporting 1% concentration being most effective in the laboratory with *A. americanum*. The overall low attraction may be due to the need for chemical pairing. As 2-nitrophenol is a component of attraction-aggregation-attachment pheromone, pairing with other components (such as CO_2) could have made it more attractive. As Barré *et al.* (1997) reported, CO_2 was necessary to see high recapture rates and may be necessary to elicit the same reaction rates in *A. maculatum*.

Some host associated odors may be cues ticks use in host seeking behaviors, but not all odors are attractive to all ticks equally. Squalene is common on the skin and in the blood of mammals (Stewart 1992). Yoder *et al.* (1999) found squalene to be an effective long range field attractant to *A. americanum*, of which all life stages are attracted to the semiochemical (Yoder *et al.* 1998). Yoder *et al.* (1999) found the 1% concentration of squalene was more attractive than higher concentrations (10%, 100%) to *A. americanum* in the lab with 100% concentrations acting as a repellent. This study also found less attraction by *A. americanum* to the 10% concentration. As seen with rumen fluid studies, it is possible not all tick species are attracted to the same chemicals. Though squalene works well for *A. americanum*, this study did not indicate *A. maculatum* exhibit the same behavior. Belonging to the same genera may contribute to some similarities while speciation affects many aspects including host attraction and host seeking cues.

Carr *et al.* (2013) reported that 1-octen-3-ol (2.5%) and ammonium hydroxide (1%, 5%, 10%, and 12.5%) were significantly attractive to adult *A. americanum*. In addition, ticks in the genera *Rhipicephalus* and *Haemaphysalis* are attracted to 1-octen-3-ol (Osterkamp *et al.* 1999; Ranju *et al.* 2012). Conversely, Carr *et al.* (2103) found adult *Dermacentor variabilis* were not significantly attracted to any semiochemicals tested. Significant repellency by *D. variabilis* was seen to 1-octen-3-ol (1% and 10%) and non-significant attraction was seen to ammonium hydroxide at all concentrations (Carr *et al.*

2013). Like both ticks in Carr *et al.* (2013), *A. maculatum* in the current study was attracted to ammonium hydroxide at the concentration of 1%, though all other concentrations were unattractive with the exception of 10% which had equal selection for both the control and ammonium hydroxide arm.

In addition to 1-octen-3-ol, it was expected that 2,6-dichlorphenol would be attractive to *A. maculatum*, though assays from the laboratory studies did not return those results. More *A. maculatum* selected for the control than for the arm with 2,6-dichlorophenol for all concentrations except 5%, which had zero ticks selecting for either arm. *A. maculatum* and several other genera of tick have receptors on their tarsi capable of detecting 2,6-dichlorphenol (Leahy and Booth 1978; Steullet and Guerin 1994). The presence of receptors indicates that *A. maculatum* uses the chemical to mediate behaviors. When 2,6-dichlorophenol was extracted from living ticks and applied to a rabbit host, it had attractive properties to *A. maculatum* (Kellum and Berger 1977). The chemical is a known component of sex pheromones (AAAP) in many ticks, including *A. maculatum* (Sonenshine 1985). Male *A. maculatum* emitted AAAP brings about an attraction-aggregation-attachment response in both females (Sleeba *et al*. 2010) and immature *A. maculatum* (Wexler 2005). In a related species, Bryson *et al.* (2000) recorded that *A. hebraeum* males emitting AAAP paired with CO_2, exhaled by the host, attracted unfed adult females and nymphs. Seeing as *A. maculatum* is a species in which the males also produce AAAP, these paired chemical cues may be needed as well. Such pairings have been observed with CO_2 exciting adult female *A. maculatum* but AAAP produced by attached males was needed to give females a questing direction (Dr. Pete Teel, personal communication).

Another reason why 2,6-dichlorophenol was not attractive to *A. maculatum* in the current study may have been due to the source of the chemical. In past studies that showed the attractiveness of 2,6-dichlorphenol, the semiochemical was extracted from ticks and then tested in the lab assay. In the current study, 2,6-dichlorophenol purchased from Sigma Aldrich was tested against *A. maculatum.* This difference in source may be why unexpected results were observed.

As with any assay, it is important to verify that the results being recorded are actually due to the chemicals being used and not due to something inherent in the set up. This study utilized a similar set up that was described by Carr *et al*. (2013). To verify the system was working, we tested the effects of CO_2 on *A. americanum*, the species identified as most responsive by Carr *et al*. (2013), and found a similar effect. This demonstrated the system was working as an appropriate medium for odorant behavioral assays with ticks and the results would provide an indication of a chemical's effect on the tick. Carbon dioxide, a commonly accepted tick attractant, was significantly attractive to *A. americanum* in the laboratory component of this study. *A. maculatum* were not as attracted to CO_2 in the Y-tube olfactometer demonstrating the uniqueness of this tick species and the need to find a more attractive chemical. Assays with *A. americanum* were conducted for five minutes each following methods used by Carr *et al.* (2013). To follow the Carr *et al*. (2013) protocol, *A. maculatum* assay times were initially conducted for five minutes, but little movement was observed with in the Y-tube, requiring an increase in time of 10 minutes. Similar slow reaction times were seen in a study by Holscher *et al.* (1980) where *A. maculatum* took longer to respond to CO_2 than *A. americanum* or *D. variabilis*.

A. maculatum in the lab behaved differently than *A. americanum*, appearing much slower and less responsive. It is possible that increasing lab trial times to an even longer period would result in more selections being made. After all, most field trials are for extended periods of time. The goal was not to find an attractant that quickly collected *A. maculatum*. The goal was to increase collection rates overall. Observations of *A. maculatum* in the lab revealed the ticks clumped together often when placed in the Y-tube olfactometer. Additionally, personal communication with the OSU tick rearing facility revealed they felt *A. maculatum* were "clumpy" ticks. Using an individual tick in the olfactometer per trial may reduce the time the ticks spend not moving or clumping together and could result in increased overall selection rates.

The attractiveness of rumen fluid as *A. maculatum* attractant in the field was tested for the first time. Rumen fluid had much higher selection rates in Y-tube olfactometer assays as has been reported by other studies. Additionally, rumen fluid from different sources may elicit different and stronger behavioral responses. It is possible pairing rumen fluid with CO_2 in a field setting might elicit a stronger response, as CO_2 has been shown to have a synergistic effect on chemical lures used to trap hematophagous insects (Carr 2011). The results from this study as well as previous studies demonstrate a need for further investigation into the role of chemical attractants for *A. maculatum*.

CHAPTER III

KNOWLEDGE, ATTITUDE AND PRACTICES SURVEY OF OKLAHOMA BEEF PRODUCERS IN RESPONSE TO TICKS, A SURVEY

ABSTRACT

Cow/calf production is a major commodity in the United States and in the state of Oklahoma. Tick parasitism is a concern for beef producers due to reductions in gains, cost of parasite control and risk to themselves. Farmers are a vulnerable population at increased risk for tick bites and tick-borne illnesses. The aim of this study was to determine the attitudes, knowledge and perceptions Oklahoma beef producers had in regard to ticks and the risks they pose to their cattle and themselves, the tick prevention methods, both personal and within the herd, and where they get information pertaining to cattle-tick-human interactions. A total of 183 KAP questionnaires were returned for analysis. Producers (68.9%) believed ticks were somewhat to moderately a problem for cattle. Whereas 42.1% of producers thought ticks were only somewhat of a problem, or less for themselves. Rocky Mountain spotted fever (78.7%) was of most concern for humans while only 9.3% indicated concern for ehrlichiosis. Respondents were more likely to check their body for ticks rather than wear protective clothing. Chemical control

methods were used most often to treat ticks on cattle and 30% used injectable dewormer to control ticks. Veterinarians were the main source of information for 3 of 4 regions in the state. Producers requested additional information about treatment, control, and prevention of ticks on their cattle. Ticks were perceived to be a greater risk for cattle than for humans, though ticks vector more pathogens to humans in Oklahoma. This study provides some data as to the use of extension services and information in regards to ticks and ticks-borne diseases by producers in Oklahoma. Areas of further focus have been highlighted by this study and could be helpful to extension specialists when developing educational tools.

INTRODUCTION

In the United States, cattle and calf sales generate nineteen percent of all annual agricultural revenue, amounting to $76.4 billion (USDA 2015). This industry incorporates the production and sale of beef cattle, feedlot cattle, dairy cattle, and others such as bulls and steers. Oklahoma has ranked in the top five cattle and calf production states between 2007 and 2012 (USDA 2015) with an inventory of 1.7 million cattle generating $1.6 billion in sales (USDA 2015). The economic success of the cattle industry relies on development and maintenance of healthy and productive animals. Ticks pose a threat to cattle in numerous ways. Tick parasitism has multiple physiological and physical effects causing stress and irritation, and conditions such as ‘gotch ear’. Tick infestations on cattle can lead to a reduction in weight gains and can require tick management programs which can quickly lead to economic losses (Williams *et al*. 1977;

Stacey *et al*. 1978). Around the world, ticks transmit bacterial, viral and protozoal disease agents which can result in severe illness and even death of livestock. In the United States, major pathogens, such as *Babesia bovis* and *Babesia bigemina* and their tick vectors, have been eradicated but reintroduction remains a threat (Pérez de León *et al.* 2010).

The importance of this industry implies the need to ensure that effective management systems are in place to prevent, control or eliminate ticks on cattle within the United States. To assist in this area, a multitude of tick management techniques have been developed including modern chemical acaricidal applications, use of tick resistant cattle breeds or crosses, ethno-veterinary approaches such as plant and herbal extracts and cultural control methods such as pasture rotation or burning. While the approaches are many, the use of tick management techniques by producers varies greatly from country to country as the rationale behind each control methods can widely differ as well. Lack of education, accessibility and availability of technologies, ease of use, effectiveness and cultural beliefs are all factors that can influence tick management decisions.

Conducting a survey of beef producers is an ideal way to discover what motivates producers to make their decisions. Knowledge, attitude and practice (KAP) surveys are used to better understand what people know, what they believe, and what they do in regards to a topic of interest (Kaliyaperumal 2004). KAP surveys pertaining to ticks, cattle, and producers are used outside of the United States. Surveys and questionnaires are more commonly used in the United States to gauge human perceptions about ticks and tick-borne illness. The few surveys that have been conducted in the U.S. are many years old and focused on a limited subject matter.

It is important to understand the needs and concerns of producers in order for extension services to best serve them. The objective of this study was survey Oklahoma beef producers to better understand the attitudes and knowledge they have in regard to ticks and the risks they pose to their cattle and themselves, their methods of prevention both personal and on their cattle, and where they get their information. Based on the information collected, it will be possible to highlight useful information for the creation of educational materials to be used by Extension specialists that are targeted toward issues producers feel are important.

REVIEW OF THE LITERATURE

Existing knowledge and beliefs form a foundation for many human behaviors. Agricultural systems were created, managed and modified; crops and animals were bred and domesticated, long before the existence of agricultural extension services and inputs from formal scientific community. Preexisting local knowledge is an ideal starting point when trying to introduce new knowledge into an area. To not take into account practices, attitudes and existing knowledge of a population is arrogant and runs the risk of introducing concepts that are already known or inappropriate. Assuming knowledge flow is one-way, from the scientific community and extension agents to producers, can lead to recommendations not working well or runs the risk of them being completely abandoned (Warburton and Martin 1999). Additionally, local knowledge can add to scientific knowledge.

Knowledge, attitude, and practice (KAP) surveys initially were developed as a method to evaluate the resistance to implementation of family planning programs and services (Cleland 1973; Ratcliffe 1976). These surveys have now been utilized in many fields and are a useful tool for data collection and evaluation. Data gathered through KAP surveys lends itself to providing insight into the efficacy of already implemented programs or help guide future directions of a program, such as identification of groups in need of further support and education or by showing areas in which modification is needed. Furthermore, the information gathered can be used to address attitudes that were previously unknown and a possible hindrance to program success.

Around the world, KAP surveys have been used successfully to develop and modify educational programs. Surveys and questionnaires are also able to identify the best mediums for education, be it through veterinarians, extension services, or through the media. Shabani *et al.* (2015) through surveys of communities in Tanzania, found producers had limited knowledge about Rift Valley Fever, its vectors, signs and symptoms, and preventative methods. The respondents indicated radio as their main source of information followed by friends, community members and animal health care professionals (Shabani *et al.* 2015). The study highlighted the importance of discovering the best mode of information dissemination. Furthermore, surveys were given to Kenyan cattle producers to better understand the perceptions about diseases encountered within their herds, especially trypanosomiasis. In response to the results obtained, educational materials were produced to improve the community’s understanding of trypanosomiasis transmission and its effective treatments (Machila *et al.* 2003, 2007). In the United States, KAP surveys are commonly used when evaluating human health risks in regards to disease-vectoring arthropods, like mosquitos. Two neighborhoods in an Upstate New York Community were surveyed using a KAP questionnaire paired with entomological surveys of the property of respondents. Tuiten *et al.* (2009) found respondents with the perception that West Nile Virus (WNV) was a risk to them or their family had fewer mosquito breeding habitats, like containers, on their property. The authors, however, found there was no relationship between the residents' knowledge about WNV groups at risk, vectors and their biology, and prevention methods did not reduce the number of breeding containers on properties. This demonstrates education programs imparting only knowledge are not wholly effective and there is importance in addressing attitudes and perceptions as well.

Worldwide KAP Survey Usage

A review of available publications indicates the use of KAP surveys and questionnaires in regards to cattle, ticks, and producers' perceptions occur more frequently in countries other than the United States. As highlighted in this review, surveys addressing issues faced by American beef producers, and in particular Oklahoma producers, are very few compared with other countries.

Africa. Surveys and questionnaires are frequently used to gather information from cattle producers in African countries. Evaluation of practices, attitudes and knowledge of cattle production ranges from health and safety of humans in regards to zoonotic disease risk to the understanding of cattle and tick interactions resulting in illness or disease. The information gathered often includes husbandry practices of the kept animals (feed types used, grazing and housing methods employed, and parasite control methods) as well as the knowledge and attitudes producers have in regards to tick infestations amongst their herds. Survey responses can be used to improve cattle production as well as improve the health and safety of humans, with it all especially benefitting small farms.

Both human and cattle health and safety concerns can be addressed through the use of KAP studies. Zoonotic diseases, such as rabies, anthrax, brucellosis, pose threat to both cattle and producers in Africa. A study evaluating KAP of rural producers in Zimbabwe found producers were aware of anthrax risks in their area, but consumption of anthrax-infected meat was still occurring due to factors such as the availability of treatments for the disease in humans, forgetting about anthrax, and fear of loss of income due to infected cattle (Chikerema *et al.* 2013) The study highlights that populations with high understanding of a disease will still have other influences which result in undesirable behaviors, such as selling and consuming infected meat. The authors suggested the introduction of meat inspection services could help reduce consumption of anthrax-infected meat (Chikerema *et al.* 2013).

Multiple surveys and questionnaires consistently found that producers feel as though ticks and their potential associated diseases are a major risk to their cattle (Catley and Aden 1996 (Somaliland); Mapiye *et al*. 2009; Nqeno *et al*. 2011; Chatikobo *et al*. 2013). Surveys revealed farmers in districts of Zimbabwe were most concerned about livestock diseases including those vectored by ticks (Chatikobo *et al.* 2013). Additionally, a never before reported skin ailment in cattle of the area was also described, demonstrating the value of such surveys (Chatikobo *et al.* 2013). Nqeno *et al* (2011) reported South African producers believed tick-borne diseases and poor health of cattle lead to decreased reproductive performance in cattle, exacerbated by a lack of veterinary support and training services in South Africa. The need for education and services encouraging use of indigenous cattle breeds to increase production on smallholder farms was demonstrated through questionnaires as these cattle are better suited to the environment and parasite burdens, such as ticks, of South Africa (Mapiye *et al.* 2009). In several Tanzanian communities, surveys distributed by Laisser *et al.* (2015) showed producers possessed large amounts of knowledge pertaining to ticks and their associated disease risks yet economic constraints and perception that their chosen cattle breed tolerates ticks and tick borne diseases led to control strategies not being employed. Tick control management is desired and needed with surveys given to Zimbabwe producers revealed that over fifteen ticks per head of cattle were of major concern to them (Cook 1991).

Tick and tick-borne disease control and management practices vary amongst producers and interviews in communities, especially in those with resource limited producers, allowing for further investigation into tick control methods commonly used. Such management practices included manual removal of ticks from infested livestock, the use of modern chemical acaricides, ethno-veterinary practices such as botanical compounds, biological control methods such as the use of birds to remove ticks on cattle, and even the application of engine oil to cattle (Cook 1991; Moyo and Masika 2009; Wanzala *et al*. 2012; Ndhlovu and Masika 2013). Surveying producers also helps understand their motivations behind the control practices they employ.

Frequently, traditional tick control acaricides are often too expensive for producers, especially those that raise indigenous breed cattle, so they resort to ethnoveterinary tick control and treatment methods and off label acaricidal usage

(Hlatshwayo and Mbati 2005; Mugabi *et al*. 2010). Off-label usage of chemicals and pesticides occur, possibly due to ease of access or due to lower costs. Carbyl, a common garden pesticide and Fenkill, an insecticide used in cotton farming, are reportedly used on some cattle in Zimbabwe; neither chemical is authorized to be used on cattle by the government (Ndhlovu and Masika 2013). Questionnaires given to farmers in Central Uganda revealed acaricidal usage often strayed from the directions given by veterinarians and manufacturers (Mugabi *et al.* 2010). Additionally, Mugabi *et al.* (2010) found farmers used mixtures of pesticides due to their perception that acaricides had different attributes, such as residual effect, killing power, or other insecticidal properties. Surveying producers offers insight into the reasons pesticides are used off label or against the advice of professionals, economical and perceived efficacy, and the information can be used to tailor pesticide recommendations taking into account the needs of those using them.

Additional alternative tick control methods are those based on ethno-veterinary knowledge. Ethno-veterinary medicine is the use of information and knowledge, methods, practices, tools and technologies, and beliefs of people to help care for animals, with the knowledge being developed and passed down through generations and within communities (McCorkle 1986). Ethno-veterinary medicinal knowledge varies within communities, between communities, and on larger regional scales. Ndhlovu and Masika (2013) reported farmers in Zhombe, Njelele and Shamrock (Zimbabwe) preferred ethno-veterinary methods due to their lower cost and ease of access.

This type of animal husbandry and care often uses herbs and plants to treat animal ailments. In western Kenya, livestock producers use over 150 different plants to prevent or control ticks with some of methods of use being pouring botanical suspensions on the body of animals; burning botanicals to produce smoke for fumigation; rubbing or dusting body parts of animals with plant products, and using pastures with vegetation with supposed anti-tick vegetation (Wanzala *et al.* 2012). Moyo and Masika (2009) found a small percentage (6.8%) of the South African producers surveyed used botanicals to treat ticks. Only 18.6% of the respondents were employed and alternative tick management methods could better serve the community due to their low cost and local availability (Moyo and Masika 2009). While cost of modern chemical controls is often cited as a leading factor for the use of ethnobotanicals, Nabukenya *et al.* (2014) reported 55% of farmers in two Ugandan districts treated their own animals due to the lack of veterinary services.

While many ethno-veterinary methods reported are of botanical origins, other strategies are reported. In the Free State province, South Africa, 70% of farmers of indicated using some type of tick control method on their cattle; alternative methods were used by 42% of the respondents while 28% used commercial acaricides (Hlatshwayo and Mbati 2005). One of the most common alternative tick control methods was the application of used engine oil rubbed onto cattle with tick infestations with an efficacy rate around 38.1% (Hlatshwayo and Mbati 2005). Farmers in the Eastern Cape Province, South Africa also applied used engine oil to their cattle to control ticks with affordability of engine oil versus conventional pesticides being cited as the reason for its continued use (Moyo and Maskia 2009). Kenyan farmers reported applying kerosene, soap, magadi soda (soda ash from Magadi, Kenya), and feeding salty soil to the animals to control tick infestations (Wanzala *et al.* 2012). Ndhlovu and Masika (2013 (Zimbabwe)) found producers also used ash and soap along with topical application of a paste made of cow dung. Removal of ticks from animals was practiced by producers in multiple regions. Ticks were often removed manually by hand, with scissors or blades, or removal was facilitated through chickens grooming the cattle (Dreyer *et al.* 1997 (South Africa); Masika *et al.* 1997 (South Africa); Chamboko *et al.* 1999 (Zimbabwe))

Europe and Asia. European KAP surveys exist, but very few pertain to cattle-producer-tick interactions. Two separate European studies used biological samples (Netherlands: milk from bulk tanks, Cyprus: placentas) paired with questionnaires answered by producers to determine risk factors associated with the Q fever, *Coxiella burnetii* (Cantas *et al.* 2011 (Netherlands); van Engelen *et al.* 2014 (Cyprus)). Both studies found an increased risk between tick infested cattle and PCR tests positive for Q fever, indicating a need for education stressing the need for stringent tick management practices in order to break the transmission cycle (Cantas *et al.* 2011; van Engelen *et al.* 2014).

The use of KAP surveys in Asian countries have revealed similar findings as those in Africa such as the use of off label chemical controls, ethno-botanicals to treat ectoparasites and associated diseases, other alternative methods. Sajid *et al.* (2009 (Pakistan)) revealed, through questionnaires, over 90% of farmers surveyed used the acaricides, ivermectin and cypermethrin, incorrectly possibly contributing to tick population resistance in the area. Ivermectin and cypermethrin is available over-the-counter and its accessibility may contribute to the high number of producers misusing the products. Many Pakistani livestock producers are poor and are unable to afford modern drugs used to treat livestock ailments, often relying on 28 different plants as part of ethno-veterinary care (ul Hassan *et al.* 2014 (Pakistan)). The majority (94.5%) of farmers surveyed by Farooq *et al.* (2008 (Pakistan)) were reliant and satisfied with ethno-veterinary medicine. Ethno-veterinary practices discovered included 49 plant-based remedies, and 28 using chemicals and other organic materials, like dairy products, kerosene or hot ash made from cow dung (Farooq *et al.* 2008).

The general Turkish population was surveyed regarding knowledge, beliefs, and practices in in regards to tick bites; farmers were identified as the population most at risk for bites (Arikan *et al*. 2010). Behaviors to protect against tick bites were also surveyed, with long sleeves and long pants being reported as the most commonly used method (65.1% respondents) and the application of insect repellents as the least commonly used method (3.3% respondents) (Arikan *et al.* 2010). Arikan *et al.* (2010) also found only 54% of the participants were aware of the association between Crimean-Congo hemorrhagic fever and tick bites. The study highlighted an insufficient level of knowledge regarding protection methods and risks associated with tick bites in the Turkish society (Arikan *et al*. 2010).

Oceania. Responses of cattle producers in Australia and Oceania to surveys and questionnaires evaluated similar subjects as previously discussed in other areas of the world. All surveys focused on control methods and risks associated with the cattle tick, *Rhipicephalus (Boophilus) microplus*. Acaricidal resistance is a major and ongoing issue that several surveys addressed (Bock *et al.* 1995 (Queensland); Jonsson *et al*. 2000 (Queensland); Bianchi *et al*. 2003 (New Caledonia)). Bock *et al.* (1995) found 65%-85% of the cattle in areas with *B. microplus* had 3/8 *Bos indicus* content affording resistance to cattle ticks and slight resistance to associated tick fever. Though Bianchi *et al.* (2003)

found crossbreeds were just as infested with ticks as others in New Caledonia. Even though most cattle have inferred cattle tick resistance, producers still relied on acaricidal treatments to prevent tick fever through control of ticks, often ignoring commonly prescribed schedules and strategies pairing dipping with the use of the tick fever vaccine (Bock *et al.* 1995; Jonsson and Matschoss 1998). The well-being of cattle and the risk of tick-borne illnesses were also addressed by a few of the other surveys (Bock *et al*. 1995, Jonsson and Matschoss 1998 (Queensland)). Though the vaccine for tick fever is available, over 66% of surveyed producers were not using the vaccine because they did not believe it was necessary (Bock *et al.* 1995; Jonsson and Matschoss 1998). The Oceanic KAP surveys revealed the need for better education to reduce resistance. Resistance occurs in ticks to commonly used acaricides and in the resistant cattle breeds; preventing the breeding of cattle not exhibiting the tick resistance trait can help ensure the longevity of that control method. The surveys also revealed producers did not perceive tick fever as an immediate threat, leading to the lax or non-existent vaccination regimens on farms.

North America and South America. Human behaviors can exacerbate the rate at which acaricide resistance develops. Multi-facet control programs have been suggested to reduce rate of resistance within target pest populations, including those of ticks. Rodriguez-Vivas *et al.* (2006 (Mexico)) found 79.6% of ranches used *Bos* spp. crossbreeds, as suggested in earlier studies, paired with acaricides on all ranches surveyed, with an additional 21.4% of ranches supplementing control strategies with pasture burning or macrocylic lactones. The survey results revealed that while most ranches used acaricides and another strategy, resistance was high within the regions, most likely due to the high acaricide frequency the ranches were using. The authors were able to determine risk factors associated with resistance.

When basic knowledge of tick biology, resistance development, and proper chemical usage is limited, tick management strategies will be less than successful. In Minas Gerais, a study of Brazilian milk producers found the majority had limited knowledge of the factors leading to successful tick control programs (Amaral *et al.* 2011). Producers of this Brazilian region noticed ticks on various species of animals, but were often unable to identify different tick species and 91% of those surveyed did not know the lifespan of ticks in the pasture, all of which reduced the success of tick control programs (Amaral *et al.* 2011). Acaricide applications were frequently applied incorrectly and application was motivated by visible infestations instead of using preventative strategies (Amaral *et al.* 2011). This Brazilian KAP study showed education levels were not correlated with the efficiency of tick management on farms and suggests a reevaluation of how producers acquire necessary knowledge (Amaral *et al.* 2011).

Much like European KAP studies, surveys from the United States infrequently focus on the relationship of producers, cattle, and ticks. In the realm of tick related human health, KAP studies were found often. Though tick-cattle interactions were not addressed, three studies utilized KAP surveys and questionnaires to better understand other risks experienced by U.S. dairy farmers. A multistate survey assessed the risks birds posed to commercial dairies in Wisconsin, New York and Pennsylvania (Shwiff *et al.* 2011). While birds commonly used cattle food and water sources, the bird abundance did not result in increased veterinary costs but the bird numbers were seen to coincide with more instances of *Salmonella* spp. and *Mycobacterium avium* spp. *paratuberculosis* being selfreported by producers (Shwiff *et al.* 2011). Additional surveys in Wisconsin and Minnesota were given to dairy farm producers to determine practices and beliefs related to animal well-being, the importance of stockmanship and its perceived role in cow comfort, perception of bio-security risks to dairies, and training practices on the farms to address the aforementioned concerns (Hoe and Ruegg 2006; Sorge *et al.* 2014). Hoe and Ruegg (2006) found many producers had opinions, such as the importance of potentially zoonotic Johne's disease and the belief that dehorning cattle causes pain, did not coincide with their actual practices, like enrollment in control programs for Johne's disease or administration of anesthetic during dehorning procedures (Hoe and Ruegg 2006).

Three studies addressing producers' knowledge of ticks and the effects on cattle were conducted in Oklahoma. Questionnaires provided by Wright *et al.* (1985) and Logan *et al.* (1985) specifically focused on the occurrence of anaplasmosis in the state. Cattle producers were surveyed in both studies and veterinarians were also included by Wright *et al.* (1985). Anaplasmosis had the highest reported occurrence in the eastern portion of Oklahoma and on farms with greater than 100 cattle (Logan *et al.*1985; Wright *et al.* 1985). Additionally, producers reporting ticks as a problem on their farms were 3.5 times more likely to have issues with anaplasmosis (Wright *et al.* 1985). One questionnaire found producers adopted control methods different than those suggested by veterinarians (Wright *et al.*1985). While previously discussed studies suggested non-compliance to be related to factors such as limited financial resources or limited access to veterinary or professional care or guidance, the respondents of one study reported receiving 59% of their anaplasmosis information from veterinarians (Wright *et al.* 1985).

A study focusing on cow-calf production practices, with a brief focus on tick control methods, have also been conducted in Oklahoma (Vestal *et al*. 2007). Producers in Oklahoma completed surveys distributed through the Oklahoma Cooperative Extensions offices, by request via Oklahoma State University's Master Cattleman website and at producer meetings (Vestal *et al.* 2007). The surveys found most producers (63% of respondents) controlled ticks using pesticide control methods such as, pour-ons, tags, or sprays, while pasture rotation (24% of respondents) and pasture burning (8% of respondents) were other methods used (Vestal *et al.* 2007). Producers with greater than 100 cows and 40% or more of household income were most likely to manage ticks using prescribed burns (Vestal *et al.* 2007). The study, however, did not address any understanding cattlemen have about tick biology or risk of tick borne diseases.

While KAP studies have been conducted in North America, opportunities exist to expand on producer knowledge and experiences in order to better understand the interactions between ticks, cattle, and humans in the United States and, more specifically, Oklahoma. Ticks have the ability to affect the health of both cattle and humans. Surveying populations can identify those most at risk, such as farmers in Turkey (Arikan *et al.* 2010). This information does not exist for cattle producers in the United States. To illustrate the point, the first cases of Heartland virus (Pastula *et al.* 2014) and Bourbon virus (Kosoy *et al*. 2015) in 2013 and 2014 respectively, were farmers in Missouri and Kansas, respectively. The Bourbon virus case resulted in a fatality (Kosoy *et al*. 2015). Yet, little attention has been given to this occupationally vulnerable group of professionals. Additionally, non-compliance of prescribed control methods exists in many areas reviewed, by understanding producer motivations, better system protocols can be developed to address needs. A questionnaire approach to data collection in order to modify or develop and enact tick control protocols with livestock producers has been shown to be common and successful around the world. KAP usage in the United States already exists in regards to ticks and human health and also is employed to improve cattle management practices in production systems like dairies, thus surveys to address ticks in cattle production systems should be done.

METHODOLOGY

Survey of KAP Literature in USA

The electronic databases, EBSCO Agricola and Web of Science, were searched for studies relating to cattle, ticks, and the use of KAP surveys and questionnaires in the United States. Publications dates included any and all dates available in the database until July 2015. Keywords used to narrow the search included various combinations of: "tick(s)" "questionnaire", "survey", "producer(s)", "knowledge", "attitude", "prevention", "cattle", "United States". Relevancy of a publication applied to those that included a survey or questionnaire administered to producers of cattle in regards to their knowledge, attitudes and/or prevention methods used in regards to ticks. KAP questionnaires and surveys pertaining to ticks in a non-cattle setting were excluded (i.e. ticks parasitizing non-cattle animals).

Oklahoma Beef Producers Questionnaire

Population. A questionnaire was administered to beef producers across the state of Oklahoma between August 2015 to April 2016. Nonprobability convenience sampling was used in this study so as to provide an opportunity to access information from as many producers as possible. Surveys were administered by county extension agents at extension meetings, Oklahoma State University directed ‘cow-calf boot camps’ and professional beef producer meetings. Producers who did not attend meetings and meeting participants under the age of 18 were excluded from the sample population.

Questionnaire. The questionnaire contained a total of 15 questions. The questions were multiple-choice (n=6), multiple-choice with at least one write-in option (n=4) and open-ended questions (n=5). The questions were designed to capture data such as location, production type, perception of ticks as a problem, perceived risks of ticks, tick bite preventative behaviors, tick biology, source of information, and follow-up opportunity. A pilot survey was conducted with 32 participants involved in cattle production. The pilot study was administered to identify possible deficiencies with question wording and to identify frequent responses to open-ended questions so future questions could be converted to multiple choice responses for ease of coding answers for analysis. The survey consisted of ten questions: three multiple-choice, three multiple-choice with at least one write-in option, and four open-ended questions. Topics covered were similar to those in the final survey.

Internal Validity of Study. Three questions in the survey had an answer indicating the respondent did not have ticks on their cattle. These questions focused on: the first month producers observed ticks attached to their cattle, the number of ticks producers observed on cattle in their herd, and the location on the body ticks most frequently were seen. If the respondents were consistent with answers throughout the survey, the answers indicating the lack of ticks should be consistent for the three questions.

Ethical Considerations. The protocol for this study was approved by the Oklahoma State University Institutional Review Board (Appendix B). Participation in the study was voluntary and informed consent was obtained from each participant.

Statistical Analysis. All data from producer surveys was transferred into spreadsheets created in Excel 13 (Microsoft Office, USA). Descriptive statistics, including chi-square analysis, from the producer survey was conducted using SPSS (version 21, IBM Corp., Armonk, NY, USA).

RESULTS

KAP Literature Search Results in the United States

A total of 779 abstracts were examined for relevancy to KAP usage in the United States to understand cattle producer and tick interactions and knowledge. Using the criteria "United States and Tick(s) and Cattle and KAP or K(nowledge) or A(ttitude) or P(revention) or Questionnaire(s) or Survey(s)" returned 14 results. Unfortunately, further examination of the articles found they were not relevant. Searches returned a number of relevant articles, meaning the search criteria covered a majority of keywords, primarily KAP-related and cattle and ticks, but were usually from regions outside of the United States. Relevancy of studies outside of the United States was noted for use in literature review.

Using EBSCO Agricola, keywords were paired in different combinations. Focusing on the United States key word combinations produced the following results: "Ticks cattle questionnaire United States" 0 results; "Ticks questionnaire United States"

0 results; “Tick questionnaire United States” 528 results, 7 with questionnaires, 0 from the United States; "Ticks surveys United States” 0 results; “Tick survey United States” 0 results; “Tick Cattle Producer United States” 0 results; “Tick cattle United States” 6 results, no questionnaires; “Tick knowledge cattle United States” 1 result, no surveys; “Tick knowledge cattle producer United States”, 0 results; “Tick attitude cattle United States”, 0 results.

Excluding the key word “United States”, additional inquiries using EBSCO Agircola retuned multiple hits: “Tick(s) survey cattle” 16 results, no results from USA (Africa, Turkey, Iraq, Japan, Spain, Australia, Morocco, Brazil, India); “Tick(s) cattle producer” 3 results (Australia); “Tick knowledge (cattle) producer”, 1 result, literature review; “Tick knowledge cattle” 33 results, 2 relevant (Africa); “Tick attitude cattle” 7 results, none from the United States; “Tick attitude producer” 5 results, none from the United States; “Tick prevention cattle questionnaire” 0 results; “Tick prevention cattle survey” 0 results; “Tick prevention cattle” 27 results, 1 relevant (Africa); “Tick prevention cattle producer” 1 result, not a survey; “Tick prevention producer survey” 0 results; “Tick prevention producer questionnaire” 0 results; “Tick prevention questionnaire” 2 results, 1 not relevant (United States), 1 relevant (Ethiopia);“Tick attitude questionnaire” 0 results; “Tick knowledge questionnaire”, 2 results, 1 relevant (Turkey).

Search results including “United States” as a key word were obtained using the Web of Science database. The following search results were found: “Tick cattle questionnaire United States”, 2 results, not relevant. “Tick questionnaire United States”, 16 results, not relevant. “Tick survey Cattle United States”, 7 results, 0 relevant; “Tick questionnaire cattle United States", 1 result, not relevant; "Tick prevention questionnaire cattle United States", 0 results; "Tick knowledge questionnaire cattle United States", 0 results; "Tick attitude cattle questionnaire United States", 0 results; "Tick attitude cattle United States", 1 result (Brazil); "Tick knowledge cattle United States", 1 result; "Tick prevention cattle United States", 1 result, not relevant; "Tick questionnaire cattle Oklahoma", 0 results.

Additional key words, excluding the phrase United States, entered into the Web of Science database, returned the following results: "Tick questionnaire cattle", 52 results; "Tick prevention questionnaire cattle", 3 results, none in the United States; "Tick knowledge questionnaire cattle", 5 results, none in the United States; "Tick attitude cattle questionnaire", 3 results, Australia and Africa; "Tick attitude cattle survey", 4 results, Africa and Australia; "Tick attitude producer survey", 3 results; "Tick knowledge producer survey", 1 result, Brazil; "Tick prevention producer survey", 0 results; "Tick prevention producer questionnaire", 0 results; "Tick knowledge producer questionnaire", 1 result, Brazil; "Tick attitude producer questionnaire", 3 results, Australia; "Tick attitude cattle", 12 results, Africa, Australia, Brazil; "Tick prevention cattle", 32 results, 2 relevant (Africa).

Oklahoma Beef Producer Surveys

A total of 198 surveys were returned over the study period. For analyses of the responses, the counties in the state were grouped into four different regions: Northeastern, Southeastern, Southwestern, and Northwestern (Fig. 17). Since results were evaluated based on state regions, surveys with missing county information (n=14) were excluded. One of the surveys excluded due to missing county information also reported being a chicken farm, not a beef producer. Another survey was excluded due to having only location data but the remainder of the survey was blank. After removal of these surveys, a total of 183 surveys were analyzed for this study.

Of the 183 surveys received, 51 (of a total of 77) counties were represented by a producer with cattle in that county. Thirty-seven producers stated they had cattle in pastures in two counties and three surveys indicated cattle in three counties. When breaking down the data into regions, only four of the 37 producer surveys with cattle in two counties had cattle in two regions while one with cattle in three counties were spread across two regions. To utilize the data from these producers in different regions, one region was selected randomly for analysis.

Sample Population Description. Respondents from regions SE (37.2%) and SW (33.9%) contributed more surveys to this study. Region NE returned 31 (16.9%) surveys with region NW returning the fewest surveys (n=22, 12%). The majority of the respondents ran cow/calf operations (95.6%) while others 15.3% ran stocker operations (15.3%), seed stock operations (3.8%) or 'other' operation types (5.5%). Other responses ranged from heifer development, livestock, and multispecies operations including sheep and equine.

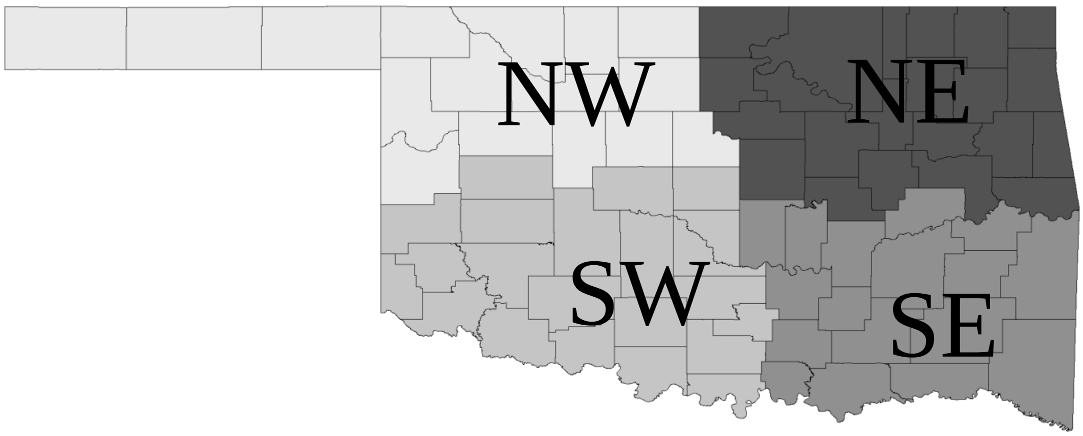


Figure 17. Counties of Oklahoma divided into four regions for analysis of beef producer survey responses. Region NE: Northeastern, Region SE: Southeastern, Region SW: Southwestern, Region NW: Northwestern.

Internal Validity of Study. Twenty-four surveys indicated the lack of ticks on cattle herds for question 8 while 22 and 25 surveys responded 'no ticks' for questions 9 and 10, respectively. This was a divergence range between four to eight percent.

Risk Perception of Ticks for Cattle. Producers were asked if ticks were a matter of concern for their cattle and were asked what most concerned them about tick infestations on their cattle. Results of these questions are shown in Table 2.

Table 2. Producer responses (% responding one answer by region) to questions about ticks as a perceived problem for cattle, most concerning aspect of ticks on their cattle, and where information about tick on cattle is obtained.

	Region				
	NE No. (%)	SE No. (%)	SW No. (%)	NW No. (%)	TOTAL No.
Do you believe ticks are a problem for CATTLE at your operation?					
Not a problem	3 (9.7)	15 (22.1)	13 (21)	2 (9.1)	33
Somewhat of a problem	12 (38.7)	29 (42.6)	17 (28.8)	10 (45.5)	68
Moderate problem	11 (35.5)	15 (22.1)	23 (40)	9 (41)	58
Serious problem	4 (12.9)	8 (11.8)	6 (10.2)	1 (4.5)	19
Never thought about it	1 (9.7)	1 (1.5)	3 (5.1)	0 (0)	5

In regards to your CATTLE, what concerns you most about tick infestation?					
Anaplasmosis	5 (25)	11 (26.2)	2 (6.7)	1 (7.1)	19
Disease or ticks	6 (30)	22 (52.4)	16 (53.3)	6 (42.9)	50
Herd health, gain	9 (45)	9 (21.4)	12 (40)	7 (50)	37
Where do you receive information about ticks infesting your cattle?					
Veterinarian	15 (40.5)	20 (30.8)	36 (46.2)	13 (50)	84
Extension specialist	13 (35.1)	33 (50.8)	25 (32.1)	9 (34.6)	80
Industry representative	0 (0)	4 (6.2)	8 (10.3)	1 (3.8)	13
Internet resource	9 (24.3)	8 (12.3)	9 (11.5)	3 (11.5)	29

The majority of survey participants (68.9%) considered ticks to be somewhat to a moderate problem for their cattle (Fig. 18). More producers in SE (42.6%) considered ticks somewhat a problem on their cattle while more in SW (37.1%) considered ticks a moderate problem. Interestingly, only 19 (10.4%) of producers across Oklahoma consider ticks to be a serious problem on their cattle, of which only 5% were from the northwest area of the state. The differences between regional responses, however, were not significant (Chi -square=12.035; df=12; p=.443). Interestingly, 3% (n=5) of respondents in three regions had never considered whether there were ticks on their cattle.

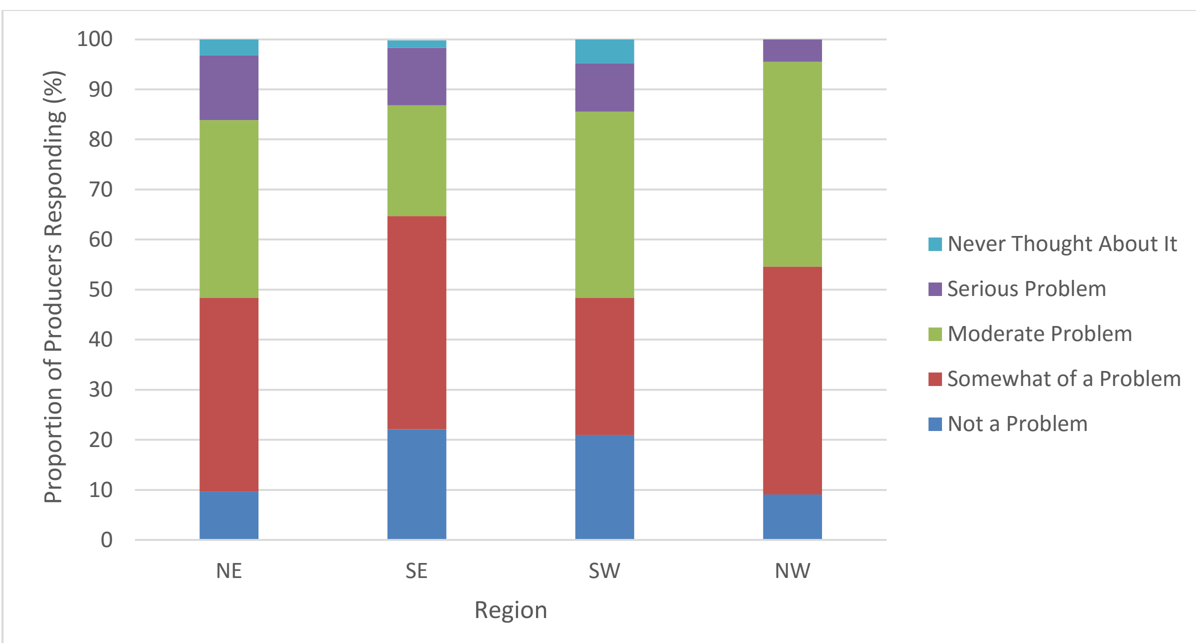


Figure 18. Regional producer responses when asked how concerning ticks are for their cattle.

Responding to an open-ended question exploring what concerns them most about tick infestations on their cattle, 106 respondents answered this question of which 50 (47%) were concerned about diseases while 37 (35%) were focused on some component of herd health (Table 2; Fig. 19). Interestingly, producers in the SE (52.4%) and SW (53%) were concerned about disease while a higher proportion in the NE (45%) and NW (50%) were concerned with herd health. When comparing responses between regions, more producers in region SE (58%) were worried about anaplasmosis than the other three regions 26% (NE), 10% (SW), and 3% (NW), but the differences in responses between regions were not significant (Chi-square=7.722; df=3; p= 0.051).

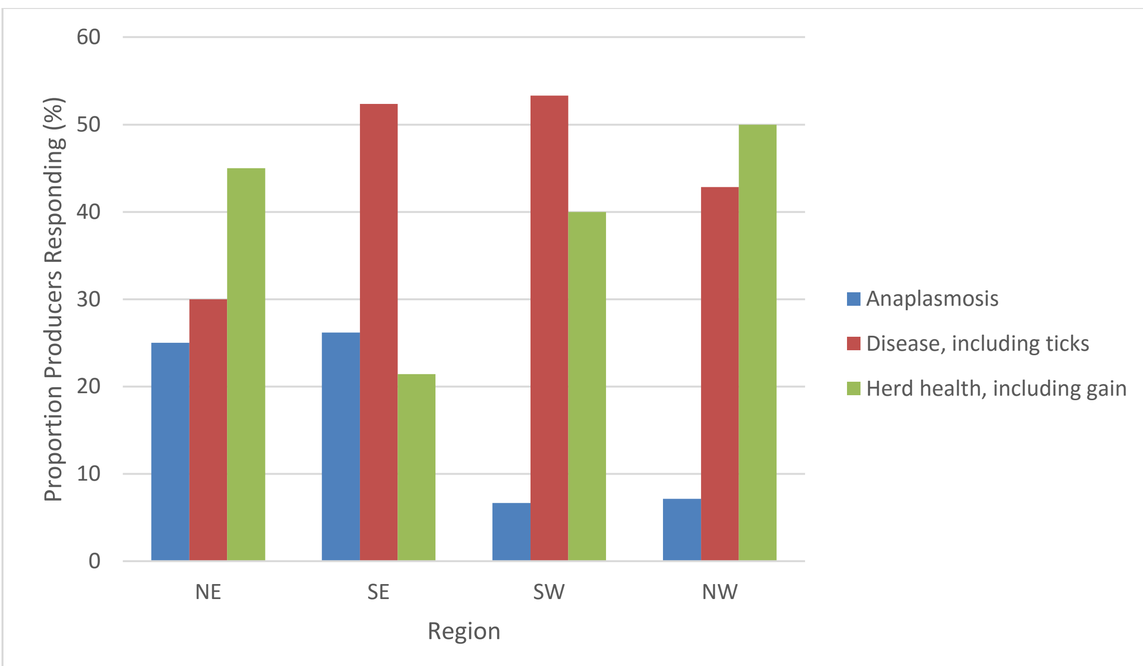


Figure 19. Reasons ticks are a concern to producers in regards to their cattle, by region.

Tick observations. Participants were asked to report which month ticks were first seen on cattle at their operations (Fig. 20). Almost half of the producers (48%; n=88) reported seeing ticks on their cattle for the first time in April and May. Twenty-four (13.1%) respondents stated they did not have ticks on their cattle, 54% of those seeing no ticks on cattle resided in region SW. Additionally, producers were asked to estimate the average number of blood fed ticks found within their herd. The majority (65.5%) reported seeing between 1 and 50 ticks. Greater than 50 ticks on average were seen by 16.9% of the producers and 22 (12%) producers did not have ticks on their cattle.

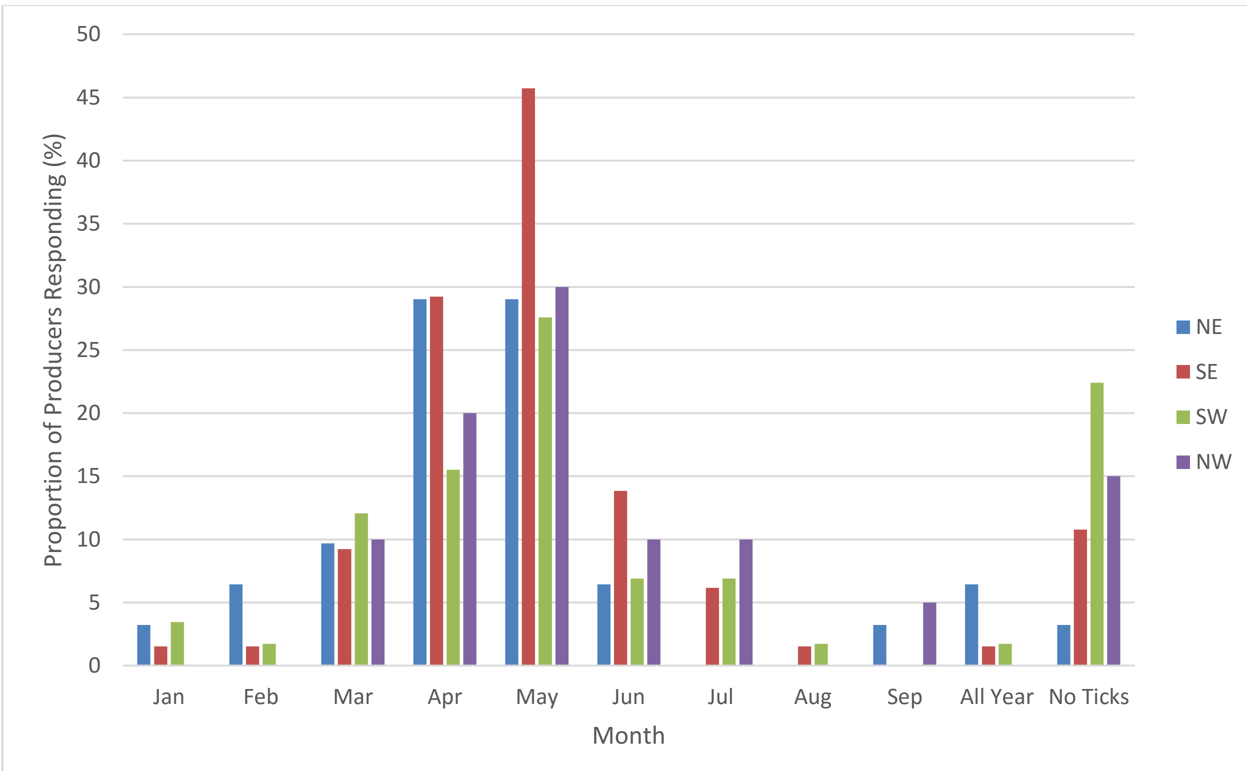


Figure 20. First observation of ticks by producers in cattle herds by month and by region within the state of Oklahoma.

Using a diagram of a cow, participants were asked to indicate the areas of the animal where they often see ticks, the total number of responses for each body region can be seen in Fig. 21. Ticks were observed most often in the inner ear and at the tailhead.

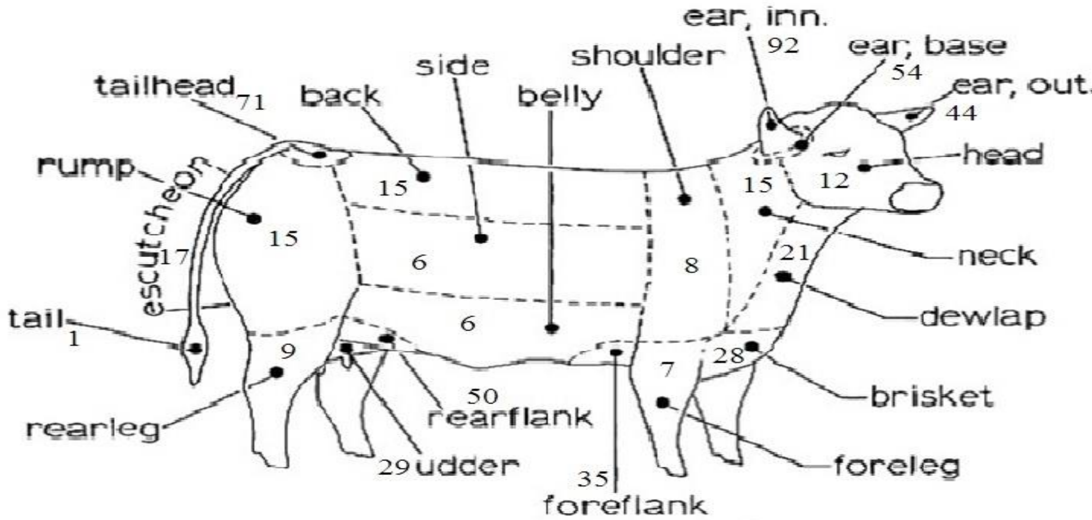


Figure 21. Beef producers in Oklahoma noted body regions of a cow where ticks are most frequently observed (modified from Barnard 1981).

Risk Perception of Ticks for Humans. Survey participants were asked if ticks were a matter of concern for their person, their family or their employees and what was most concerning about tick infestations. The majority of all Oklahoma beef producers (66.1%) believe ticks are either not a problem or only somewhat of a problem for themselves, their families, and their employees (Fig. 22). Twenty-four percent of producers in all regions believed ticks were not a problem. The SW region accounted for 43.2% of the "not a problem" responses as well as being the only region where respondents reported never thinking about the question before. There were no significant differences between responses from producers between regions to these questions.

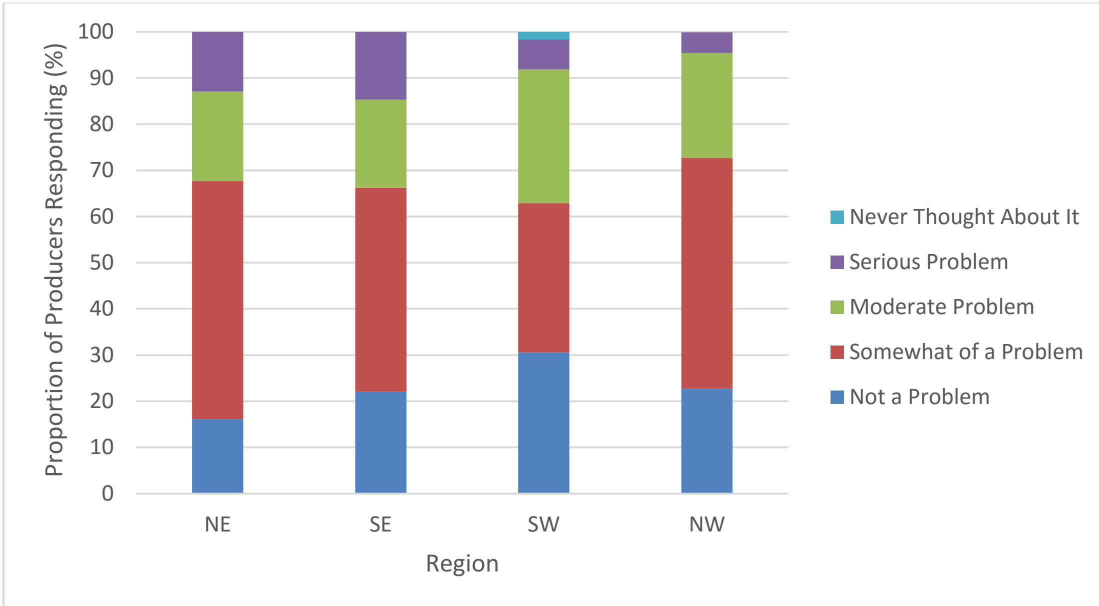


Figure 22. Regional producer responses when asked how concerning ticks are for themselves, their family, or their employees.

When asked what worried producers the most regarding ticks and their own (family) health, the majority (n=144; 78.7%) responded that Rocky Mountain spotted fever (RMSF) was the biggest concern (Fig. 23). Interestingly, 20.8% (n=38) and 24% (n=44) of the total responses reported concern about West Nile virus and anaplasmosis, respectively, while only 9.3% (n=17) of total surveys indicated concern about ehrlichiosis. When evaluating the responses by region, there were few notable differences regarding perception of disease risk, the lowest being the risk of Lyme disease. While slight, it is notable that more producers in the NE (6%) felt that Lyme disease is a concern followed by the SE and NW regions (4%). It is important to note Lyme disease was a write in answer in the 'Other' category; it was written-in frequently enough to report separately.

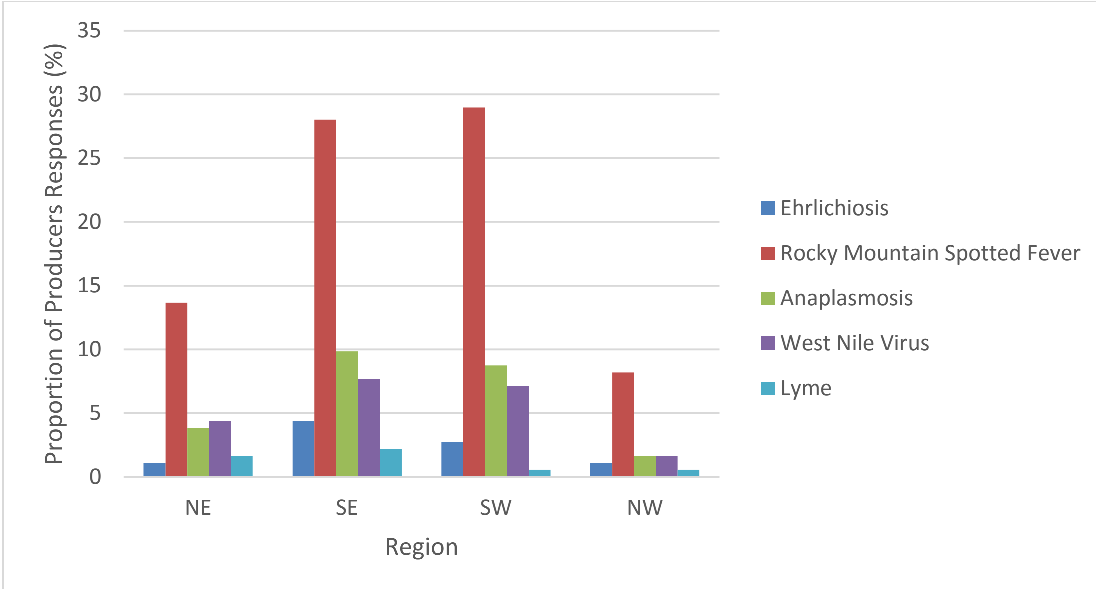


Figure 23. Human diseases of concern to producers in regards to exposure to ticks by region.

Preventative behaviors. Producers used at least one type of personal protective behavior (checking body for ticks, clothing barrier or chemical protection) (Fig. 24). The majority of respondents (67%) indicated they checked their body after leaving the field. A total of 28/183 (15.3%) respondents reported using no protection methods to protect their person against ticks. Of the 28 that indicated using no protection methods, 14 (50%) were from region SE, eight (29%) were from region SW, and six (21%) were from region NW (Chi-Square 9.775; df=3; p=.021). All respondents from region NE used at least one type of protective behavior. While a majority of producers (67%) use some form of protection by conducting a body check after being in the field, 50% use some form of chemical protection and only 1/3 use a physical barrier.

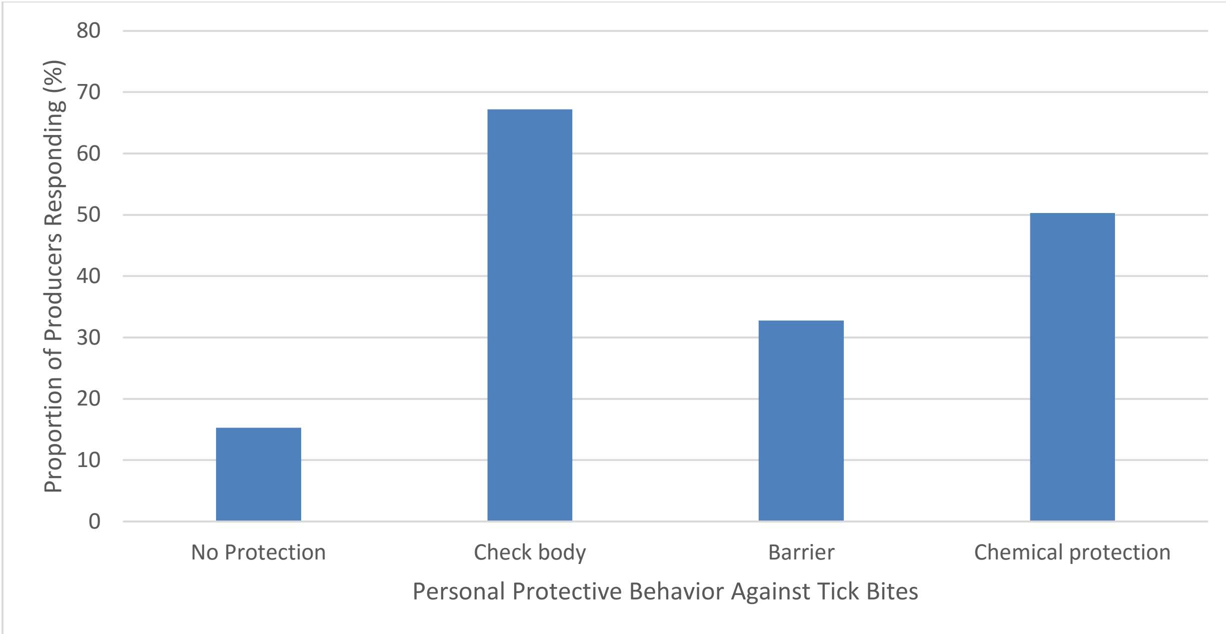


Figure 24. Personal protective behaviors producers from all regions reported using to prevent tick bites.

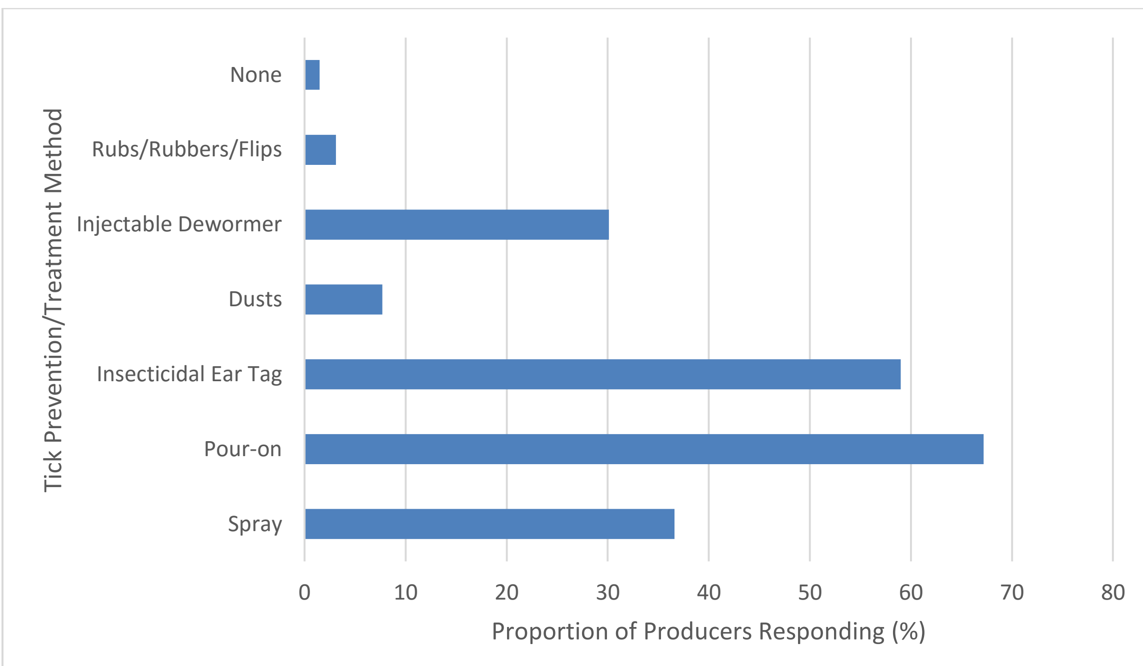


Figure 25. Methods producers in all regions use to prevent or reduce tick infestation on cattle.

Tick control methods in cattle herds used by producers are shown in Fig. 25. Overall, a majority of producers reported using pour-ons (67%) followed by insecticidal ear tags (59%) and sprays (40%). Thirty percent of the respondents reported using injectable dewormer for tick control while 3% use rubbers or ‘face flips’.

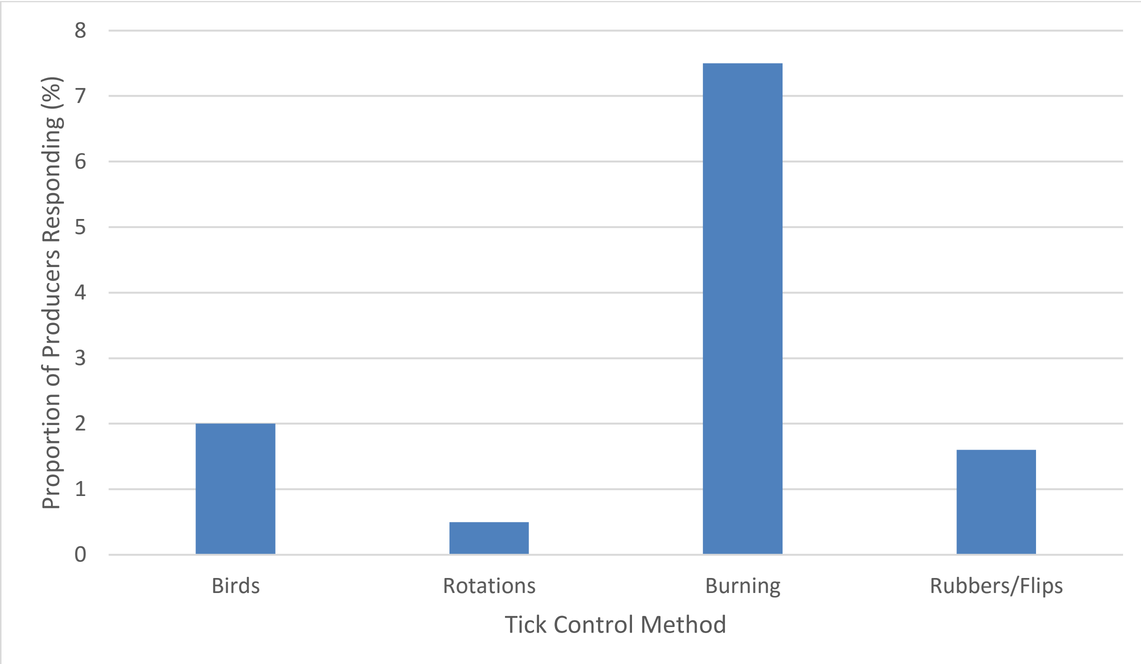


Figure 26. Non-traditional and alternative tick control methods used by producers in all regions. Methods included pasture burning, cattle rotations, and back rubbers and ‘face flips’.

Non-traditional tick control methods included modifications to the environment are shown in Fig. 26. Alternative control methods included pasture burning (7.5%) and the use of birds as tick control (2%). Some producers reported the use of back rubbers, as seen in the prior question. This question was open-ended and only 11.4% (n=21) producers answered the question.

Source of Information. Producers reported using extension specialists (43.7%) and veterinarians (45.9%) for information about ticks on their cattle (Fig. 27). While regions NE, SW, and NW utilized veterinarians most often (48%, 58%, 59% respectively), region SE utilized extension specialists (48.5%) more than veterinarians (29%). Region SW had the lowest utilization of extension specialists (40%) but the highest use of industry representatives (13%). Internet resources were used most frequently by region NE (29%) while the three other regions reported less usage of the internet (less than 14%). Other information sources mentioned in the open-ended component to the question included local farm or neighboring ranch (n=8), various publications/magazines (n=6), local feed store (n= 4), personal experience ('trial and error') (n=4), the Noble Foundation (n=2), OSU facts sheets (n=2), or the sale barn (n=2).

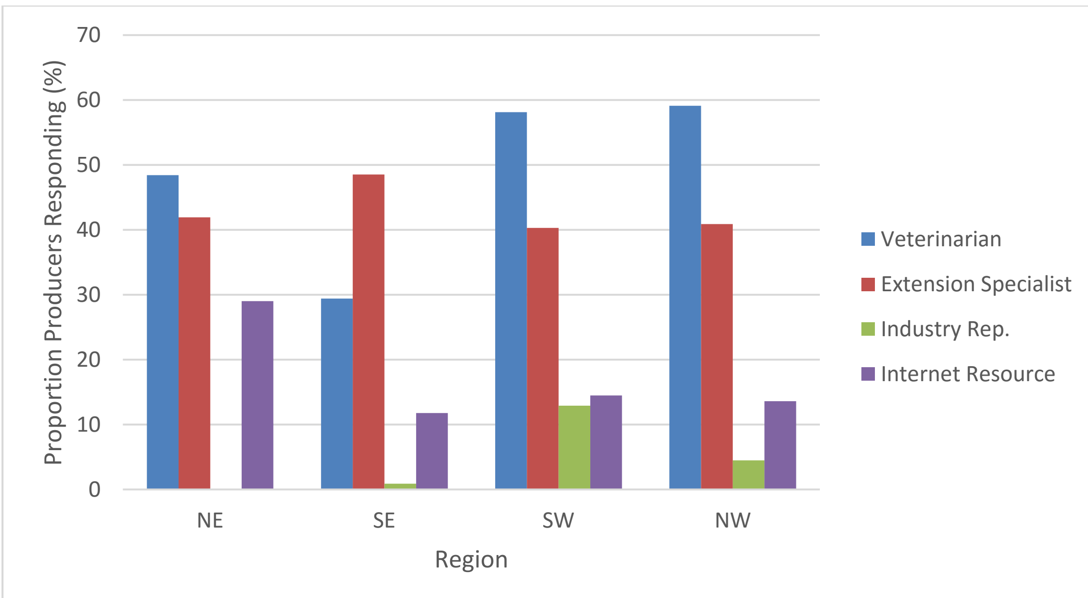


Figure 27. Sources that producers consult for information about tick and cattle interactions, by region

Respondents were asked in an open-ended question what information they would like to have regarding ticks and their impact on human and cattle health (Fig. 28). Responses were categorized into five types: Any information, Request for education materials, tick treatment and prevention methods, human health concerns, and disease and wellness. Any response that did not specifically state concern for human health or wellness was included in the disease and wellness portion. Of the 62 producers who responded to this questions, by far, the most requested information was for tick prevention, control and treatment methods (16.4%). While 16.4% wanted information on how to control ticks year-round, only 8% of the respondents requested information regarding disease and wellness. Those requesting educational materials (3.8%) included a range of fact sheets, pamphlets and information on the internet.

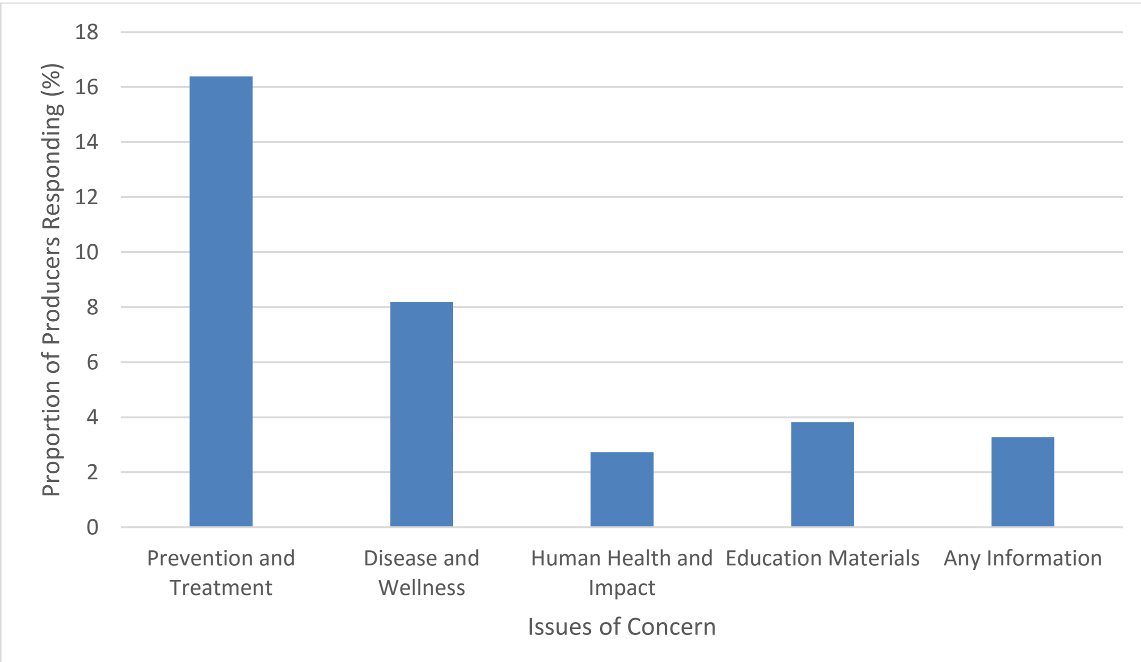


Figure 28. Information requested by producers from all regions regarding ticks on cattle or impact on human health.

DISCUSSION

A lack of knowledge exists in the United States evaluating the knowledge, attitudes, and practices in regards to ticks amongst beef producers. A systematic literature search pertaining to ticks, KAP, and US beef producers returned no relevant results whereas surveys have been used to solicit information from this important occupational group worldwide. Evaluation of practices such as preventative behaviors reducing tick exposure, attitudes pertaining to perceived risks of tick bites and associated pathogens, and understanding levels of knowledge possessed by US beef producers can help evaluate how extension programs can best serve the population. This study sought to fill in some of this missing information.

While differing between regions, beef producers in Oklahoma were aware of the presence of ticks on their cattle with most reporting first seeing ticks in the year between March and May. This coincides with seasonal observations made by Talley and Dubie (unpublished data) and Hoch *et al.* (1971). Producers also frequently reported seeing ticks on body regions of cattle consistent with observations made by Talley and Dubie (unpublished data) and Barnard *et al.* (1981, 1982).

Perceived risk of ticks varied between regions. Producers in Regions SE and SW were most concerned with disease and ticks, but only region SW perceived ticks to be more of a problem than region SE. This was an interesting finding, as region SE has some of the highest tick infestation rates in the state of Oklahoma (Barrett *et al*. 2015). It is possible region SE perceives ticks to only be somewhat of a problem because they have 'always' dealt with ticks in the area where as region SW is just now seeing an increase in tick populations. This new occurrence of ticks on their cattle may result in them perceiving them to be more of a problem, especially more than 'before'. In three regions (NE, SW, SW), 3% of the respondents had 'never thought about' the issues ticks for their cattle. These three regions have robust tick populations, and this response may be due in part to the respondent being new to the cattle industry as several survey opportunities were provided at meetings for new cattlemen. Herd health was of greatest concern to regions NE and NW. Region NE's concern with herd health was somewhat unexpected, when given the option to explicitly state that 'disease and ticks' were a concern. The northeastern part of the state has high tick populations as well as higher occurrence of disease (OADDL, unpublished data). One explanation for this could be that, in the minds of the producers involved, herd health and disease could be considered the same thing.

Anaplasmosis was mentioned frequently enough in the open-ended question that it was evaluated separately. Producers in the northeastern (region NE) and southeastern (region SE) regions of Oklahoma cited anaplasmosis as concern for their cattle more often than the remaining regions. It is not surprising these regions express a concern for the disease more frequently, as anaplasmosis in cattle had historically occurred mainly in the eastern part of the state (Logan *et al.* 1985; Wright *et al.* 1985). With region SE reporting 'disease and ticks' being of most concern paired with the higher anaplasmosis, it may indicate that anaplasmosis may be more of a problem than currently known. The southeastern region of the state may therefore be a good starting point for further study. It might also be prudent to evaluate further what "disease" concerns producers have in regards to ticks. It is possible producers associate other illnesses with ticks which in fact have other causative agents. That being an option, KAP studies with livestock producers have identified and described previously unknown tick-vectored ailments (Chatikobo *et al.* 2013).

When addressing ticks as a concern to the health of humans, more producers in all regions believed ticks to be no problem or only somewhat of a problem. This is a concerning perception, especially in Oklahoma where tick-borne illness rates have seen an upward trend (CDC 2016). Producers are in close association with cattle that harbor multiple tick species capable of vectoring pathogens to humans. Additionally, tick associated viruses, such as the Bourbon virus and Heartland virus, not only pose a threat in the United States, but the third known death from Heartland Virus (CBS News 2014; OSDH 2014) and the second case of Bourbon virus (KFOR News 2015) occurred in Oklahoma.

Producers in the southern part of the state (Region SE and Region SW) believed ticks were of no concern or only somewhat of a problem and were also more likely to report they neglected using any personal protective measures to reduce tick exposure. This behavior can put a person at increased risk for tick bites and their associated tick-borne illnesses. Farmers have been identified as a group with a higher risk for tick bites (Arikan *et al.* 2010; Kisomi *et al.* 2016). Overall, respondents were most likely to check their body for ticks after possible exposure than they were to wear protective clothing and/or wear chemical repellents. The low occurrence of clothing barriers to prevent tick exposure has also been seen in other studies. Beuajean *et al.* (2013) reported during a study of the general public in the Netherlands and their protective behaviors and perceptions regarding Lyme disease, very few respondents wore protective clothing, possibly due to perceptions that it was unnecessary and over protection or the climate influenced the behavior. Protective clothing was also one of the least reported tick bite prevention behaviors used by farmworkers in Malaysia (Kisomi *et al.* 2016).

Tick-borne illnesses are of real concern, especially in Oklahoma. In Oklahoma spotted fever group rickettsias, ehrlichiosis and emerging viruses are a public health concern (CDC 2016). This survey revealed that few producers acknowledge ehrlichiosis as a disease of concern, though Oklahoma has the highest number of cases of ehrlichiosis in the US and it is a reportable disease (CDC 2016). In fact, more producers were worried about West Nile virus being transmitted by ticks than they worried about ehrlichiosis and ticks. West Nile virus is a mosquito borne disease and is not vectored by ticks (Kramer *et al.* 2008). Multiple explanations may account for these answers. It is possible participants did not fully read the question. It specifically stated what diseases were of concern to the participant or their family and employees but they could have easily overlooked the human qualifier component of the question. Being as respondents are cattle producers, they are likely familiar with WNV and its impact on horses and could have interpreted the question to mean which diseases are of concern to everything, including animals. It is also possible the participants overlooked the tick-vector component of the question and interpreted it as asking what diseases are of concern to you (with no regard to its transmission cycle). Once again, participants may be familiar with WNV and its impact on human health as well. Furthermore, producers expressed high concern over anaplasmosis transmission by ticks. While human anaplasmosis does exist in Oklahoma at present, it is more commonly associated with livestock. It is possible the respondents misunderstood the question and marked it due to their concern about the disease in their cattle. The questions may have influenced responses to each other, they were placed one after the other and anaplasmosis was a multiple choice option for one. Seeing anaplasmosis as an option could have prompted it as a response in the write-in question. Finally, a small number of respondents worried about Lyme disease risks due to Oklahoma ticks and this it is important to note Lyme was a write-in response. Lyme disease, while an important disease with impact on human health, is not one of immediate concern in this state (Garvin *et al.* 2015).

Producers reported using some type of chemical control to manage tick infestations in their herds. Pour-ons, sprays and insecticidal ear tags are common methods used to control ticks on cattle. Interestingly, 30% said they use injectable dewormer to control for ticks. Injectable dewormer is used to control for *Rhipicephalus (Boophilus) microplus* with IVOMEC® Antiparasitic Injection for Cattle fact sheet indicating its use for control of the "cattle tick" (Merial 2013). In Oklahoma, *R. microplus* is not a problem as it has been eradicated from most of the United States minus a small region in Texas. It is possible producers are interpreting the common name "cattle tick" to mean "ticks that are found on cows" instead of one specific species. If producers are relying on this to be their main protection against other ticks parasitizing cattle, they may not be achieving proper control.

When asked what sources producers utilized when they sought information about ticks and cattle, most of the respondents reported using extension specialists and veterinarians. Regions NE, SE and NW used veterinarians most often while region SE sought the help of extension specialists most often. All four regions reported using internet resources as well, with the NE region using internet resources almost 3 times as often as region SE, SW, and NW. Industry representatives were used almost 3-12x times more often in the SW region than the SE and NW regions. The northeastern region did not report utilizing industry representatives for tick-cattle information. The results of the study may indicate an underutilization of extension specialists as it is possible that the extension agents in all regions of the state, particularly those focused on livestock, may not be able to address tick-related issues as much as other livestock concerns. Finally, the usage of veterinarians and industry representatives may point to a lack of interaction between extension agents and producers in the southwestern part of the state. This may occur due to a state-wide reduction in agents or due to a general lack of producer awareness in regards to services available to them through the extension service.

One particularly helpful component of the survey was to provide the producers with a forum in which to indicate their need for additional information in regards to ticks. New treatment methods and technologies, year-round control and efficacy of preventative methods were subjects most producers wanted addressed. Disease and wellness of cattle was also of concern. This is interesting, as some individual responses wanted more information about the disease risks that ticks pose and 'vector capacity'. As noted earlier, it is possible producers are associating illnesses in cattle with ticks, though they may be completely unrelated. Human health was one of the fewest requested topics, unsurprisingly considering prior risk perception responses.

This survey with Oklahoma beef producers has shed light on areas of further focus which could be helpful in the development of educational tools used by extension services:

- While this is probably being done already on some level, this study provides some data as to the use of extension services and information in regards to ticks and ticks-borne diseases by producers in Oklahoma. A special focus on the southwestern region of the state should occur as it is a region where producers utilize extension services the least in terms of ticks and turn to industry representatives almost three or more times higher than other regions.

- As producers from three of the four regions indicate using veterinarians as their go-to information source about tick and cattle interactions, extension specialists could work more with local veterinarians to reach more producers.
- Since most producers appear to worry about diseases and ticks and the effect of ticks on herd health, one way to increase the information flow is to promote online extension publications.
- Further evaluation of producers understanding of tick-cattle-disease risks should be done as producers may be misattributing cattle ailments to ticks.
- A focus on tick-borne illness and education and promotion of more involved personal protective behavior should occur. Producers are worried about human illnesses not associated with ticks but are not worried about ehrlichiosis, a major issue in the state.
- Producers requested that more information be made available regarding tick prevention, control, and treatment as well as diseases impact and effects on wellness ticks have on cattle.

No study is above certain limitations but efforts were made to keep the effects of the limitations to a minimum. One of the limiting factors for this survey was the use of convenience sampling to collect information from producers. This method of surveying could bring a certain bias into the interpretation of the results because the information was not gathered using a randomization of all producers within the state. One of the principle means to try and mitigate this issue was meet with producers at a wide variety of venues throughout the state. This enabled us to procure information from new and experienced producers as well as producers from all over the state. Additionally, by using local extension personnel for most of the surveys, we ensured that there was no direct bias that came into the data because of the presence of the study designers. All responses were part of a local meeting environment where producers could be comfortable and answer in their own way. Another issue when using extension services is that many producers do not know of them or do not know how to utilize services – hence one the reasons they were attending an OSU extension meeting. This survey does not reach that population, as most of the meetings where the surveys were distributed were a part of extension services and the producers answered prior to the meeting. Another limitation for the study was that multiple questions had possible issues with interpretation. It was not possible to consider all these interactions prior to colleting the data. Questions pertaining specifically to humans were placed near questions about cattle and participants could have assumed subsequent questions were cattle related as well. Additionally, one question focused on alternative tick control methods provided examples in the question to give producers an idea of what alternative methods were. It was clear from the responses that many were influenced by those examples. Another question asked producers to estimate the tick loads in their herds. The relatively low number of ticks reported per herd indicates producers believed the question was most likely per head or a much smaller scale than herd. As such, the answers to the question were really not usable. In all, we recognize there were some limitations regarding the design of the study and the structure of some of the questions. However, this study provides a baseline from which other studies can continue to dig deeper in our understanding of how Oklahoma producers really feel about tick risk to themselves/family and their cattle.

In conclusion, beef producers in Oklahoma are cognizant of ticks on their cattle and more producers perceive ticks to be a risk on some level. Educational initiatives with Oklahoma beef producers should address things such as personal protective behaviors and try to encourage a higher adoption rate. A lack of knowledge in regard to ticks as vectors of human diseases may play a large part in the lack of personal protections by some of the respondents. Perceived versus actual risk of tick-borne illnesses with an extra focus on ehrlichiosis education needs to occur, as the survey indicated a very low awareness of this tick-borne illness and an overinflated concern for an illness (West Nile virus) not associated with ticks. Addressing a knowledge deficit alone, though, may not be sufficient. A focus on where producers are getting their information and trying to work with veterinarians and local extension personnel to improve the kind of education being gathered would assist producers to have correct knowledge regarding ticks and tick-borne diseases and act accordingly with the right attention to prevention methods that work.

CHAPTER IV

OVERALL CONCLUSION

Humans, livestock and other animals are hosts for hard ticks (Ixodidae), many of which are capable vectors of pathogens responsible for debilitating and sometimes deadly diseases. It is important to understand how these parasites interact with their hosts and environment. The Gulf Coast tick (*Amblyomma maculatum* Koch) is a hard tick of medical, veterinary, and economic importance. Traditional monitoring methods (CO_2 baited traps, flagging and dragging) have limited capture success rates even though populations within pastures exist and are found within economic thresholds on livestock. The life history of *A. maculatum* in Oklahoma has not be been reevaluated for decades and is in need of updating to match current ecological changes of the species. In addition, little is known about human, cattle and tick interactions in the United States or in Oklahoma, even though farmers are a known group at risk for tick bites and associated tick-borne diseases (Arikan *et al.* 2010; Kisomi *et al.* 2016). In other parts of the world, these interactions are addressed through the use of knowledge, attitude and perception (KAP) surveys and questionnaires. This study's aim was to expand on the knowledge.

gaps of these two areas. The goal was to gain insight into what Oklahoma beef producers know about interactions between cattle, ticks and humans and to better understand Oklahoma *A. maculatum* populations.

Volatile compounds were assessed for attractiveness to *A. maculatum* using Y-tube olfactometer bioassays. Eight known tick attractants were tested: ammonium hydroxide, CO_2, 2,6-dichlorophenol, ear exudate from the ears of cattle, 2-nitrophenol, 1-octen-3-ol, rumen fluid from a cow, and squalene. Laboratory assays supported our hypothesis that rumen fluid would elicit the strongest positive response when compared to the other chemicals of biological origin. The lab assays found that *A. maculatum* exhibited some attraction to other chemicals, ammonium hydroxide (1% concentration), 2-nitrophenol (1 and 5% concentration) and squalene (1, 2.5, and 5% concentration) however, the overall attractiveness was much lower than that of rumen fluid and did not warrant testing in a field setting.

A mark-release-recapture study was conducted using rumen fluid as a field attractant. Field observations did not align with observations in the laboratory. Dry-ice baited CO_2 traps recaptured the highest number of released ticks. Further modification to both the field and lab assays should be done. Further evaluation of rumen fluid could lead to improvement in field capture rates of *A. maculatum* or could potentially offer insight into cues *A. maculatum* utilizes in the field to find its large ruminant hosts.

A fifteen question KAP survey in paper format was administered to Oklahoma beef producers. Questions were multiple-choice, multiple choice with write-in options and open-ended questions. The respondents were primarily cow/calf operators from the north- and south-eastern parts of the state. Producers believed ticks were more of a problem for their cattle than they were for themselves, family, or employees. Disease was the major concern for producers in both themselves and their cattle. Though, they perceived their cattle to be more at risk for disease than themselves. The study revealed producers have limited knowledge about the diseases transmitted by ticks (for both humans and cattle) and they did not perceive risk of infection to be high enough to engage in comprehensive personal protection behaviors to limit tick-bite exposure.

Beef producers are aware of ticks within their heard and ticks are perceived to be a risk to on some level. More often, producers used veterinarians as a resource instead of extension specialists. Extension specialists and veterinarians working together may be able to reach more producers. As already described, perceived risk of ticks is lower than the actual risks ticks pose to humans and implementation of a tick-borne disease education program could help alter this knowledge deficit. Changing the perception that ticks are less of a threat could in turn lead to better personal protections being used by producers. Producers responded that they wanted more information about new technologies available to control or prevent ticks, as well as the best way to manage infestations. The use of online extension publications can help reach more producers and increase the flow of information. Finally, producers express much concern about diseases in cattle due to ticks. Their perceived risk appears to be much more than the actual risk. The producers may be mistakenly associating ticks with unrelated disease and health issues. This questionnaire revealed areas of focus for further expansion of extension education with Oklahoma beef producers.

REFERENCES

Allan, S. A., N. Barré, D. E. Sonenshine and M. J. Burridge. 1998. Efficacy of tags impregnated with pheromone and acaricide for control of *Amblyomma variegatum*. Medical and Veterinary Entomology. 12:141-150.

Allan S. A., J. S. Phillips and D. E. Sonenshine. 1991. Role of genital sex pheromones in *Amblyomma americanum* and *A. maculatum* (Acari: Ixodidae). Experimental and Applied Acarology. 11:9-21.

Amaral, M. A. Z, C. M. B. M. Rocha, J. L. Faccini, J. Furlong, C. M. O. Monterio and M. C. A. Prata, 2011. Perception and attitudes among milk producers in Minas Gerais regarding cattle tick biology and control. Brazilian Journal of Veterinary Parasitology. 20(3):194-201.

Arikan, I., N. Kasifoglu, S. Metintas and C. Kalyoncu. 2010. Knowledge, beliefs, and practices regarding tick bites in the Turkish population in a rural area of the Middle Anatolian Region. Tropical Animal Health and Production. 42:669-675.

Barker, R. W., A. A. Kocan, S. A. Ewing, R. P. Wettemann and M. E. Payton. 2004. Occurrence of the Gulf Coast tick (Acari: Ixodidae) on wild and domestic mammals in North-Central Oklahoma. Journal of Medical Entomology. 41(2):170-178.

Barnard, D. R. 1981. Seasonal activity and preferred attachment sites of *Ixodes scapularis* (Acari: Ixodidae) on cattle in southeastern Oklahoma. Journal of the Kansas Entomological Society. 54(3):547-552.

Barnard, D. R., B. G. Jones and G. D. Rogers. 1982. Sites of attachment of *Amblyomma americanum* to cattle. Annals of the Entomological Society of America. 75(3):222-223.

Barré, N., G. I. Garris and O. Lorvelec. 1997. Field sampling of the tick *Amblyomma variegatum* (Acari: Ixodidae) on pastures in Guadeloupe; attraction of CO_2 and/or tick pheromones and conditions of use. Experimental and Applied Acarology. 21: 95-108.

Barré, N., G. Uilenberg, P. C. Morel and E. Camus. 1987. Danger of introducing heartwater onto the American mainland: Potential role of indigenous and exotic *Amblyomma* ticks. Onderstepoort Journal of Veterinary Research. 54:405-417.

Barret, A. W. and S. E. Little. 2016. Vector-borne infections in tornado-displaced and owner-relinquished dogs in Oklahoma, USA. Vector-Borne and Zoonotic Diseases. 20(20): 1-3.

Barrett, AW, B. H. Noden, J. M. Gruntmeir, T. Holland, J. R. Mitcham, J. E. Martin, E. M. Johnson, S. E. Little. 2015. County scale distribution of Amblyomma americanum in Oklahoma: addressing local deficits in tick maps based on passive reporting. Journal of Medical Entomology. 52(2):269–273.

Beaujean, D. J. M. A., M. Bults, J. E. van Steenbergen and H. A. C. M. Voeten. 2013. Study on public perceptions and protective behaviors regarding Lyme diseases among the general public in the Netherlands: implications for prevention programs. BMC Public Health. 13(225):1-11.

Bianchi, M.W., N. Barré and S. Messad. 2003. Factors related to cattle infestation level and resistance to acaricides in *Boophilus microplus* tick populations in New Caledonia. Veterinary Parasitology. 112:75-89

Bishopp, F. C. and H. Hixson. 1936. Biology and economic importance of the Gulf Coast tick. Journal of Economic Entomology. 20:1068-1076.

Bishopp, F. C. and H. L. Trembley. 1945. Distribution and hosts of certain North American ticks. Journal of Parasitology. 31:1-54.

Bock, R. E., G. W. Blight, T. G. Kingston and A. J. de Vos. 1995. A survey of cattle producers in the *Boophilus microplus* endemic area of Queensland to determine attitudes to the control of and vaccination against tick fever. Australian Veterinary Journal. 72(3):88-92.

Bryson, N. R., I. G. Horak, E. H. Venter and C. E. Yunker. 2000. Collection of free-living nymphs and adults of *Amblyomma hebraeum* (Acari: Ixodidae) with pheromone/carbon dioxide traps at 5 different ecological sites in heartwater endemic regions of South Africa. Experimental and Applied Acarology. 24:971-982.

Cantas, H., A. Muwonge, B. Sareyyupoglu, H. Yardimci and E. Skejerve. 2011. Q fever abortions in ruminants and associated on-farm risk factors in Northern Cyprus. BMC Veterinary Research. 7(13):1-7.

Carr, A. L. 2011. Response of the Ixodid ticks *Amblyomma americanum* and *Dermacentor variabilis* to hematophagous insect attractants in laboratory assays and field trials. M.S. thesis, North Carolina State University, Raleigh, North Carolina.

Carr, A. L., R. M. Roe, C. Arellano, D. E. Sonenshine, C. Schal and C. S. Apperson. 2013. Responses of *Amblyomma americanum* and *Dermacentor variabilis* to odorants that attract haematophagous insects. Medical and Veterinary Entomology. 27:86-95.

Carroll, J. F. and E. T. Schmidtmann. 1992. Tick sweep: Modification of the tick drag-flag method for sampling nymphs of the deer tick (Acari: Ixodidae). Journal of Medical Entomology. 29(2):352-355.

Catley, A. P. and A. Aden. 1996. Use of participatory rural appraisal (PRA) tools for investigating tick ecology and tick-borne disease in Somaliland. Tropical Animal Health and Production. 28:91-98.

CBS News. 2014. Rare Heartland Virus kills man in Oklahoma. Online: http://www.cbsnews.com/news/rare-heartland-virus-kills-man-in-oklahoma/. Accessed 5 February 2016.

[CDC] Center for Disease Control and Prevention. 2016. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever and other spotted fever group rickettsioses, ehrlichioses, and anaplasmosis – United States: a practical guide for health care and public health professionals. Morbidity and Mortality Weekly Report. 65(2).

Chamboko, T., A. W. Mukhebi, C. J. O. Callgen, T. F. Peter, R. L. Krusha, G. F. Medley, S. M. Mahan and B. D. Perry. 1999. The control of heartwater on large scale commercial and smallholder farms in Zimbabwe. Preventative Veterinary Medicine. 3:191-210.

Chatikobo, P., T. Choga, C. Ncube and J. Mutambara. 2013. Participatory diagnosis and prioritization of constraints to cattle production in some smallholder farming areas of Zimbabwe. Preventative Veterinary Medicine. 109:327-333.

Chikerema, S. K., G. Matope and D. M. Pfukenyi. 2013. Awareness and attitude toward zoonoses with particular reference to anthrax among cattle owners in selected rural communities of Zimbabwe. Vector-Borne and Zoonotic Diseases. 13(4):243-249.

Clark, L. G. and E. H. Doten. 1995. Ticks on imported reptiles into Miami international airport: November 1994 through January 1995. In Proceedings, 1995 Veterinary Epidemiology and Economics Symposium, 31 May-2 June 1995. College Station, TX. U.S. Department of Agriculture.

Cleland, J. 1973. A critique of KAP studies and some suggestions for their improvement. Studies in Family Planning. 4(2):42-47.

Coburn, J. M. 2009. Meteorological factors regulating the population expansion and contraction of *Amblyomma maculatum* (Acari: Ixodidae) in Texas. M. S. thesis, Texas A&M University, College Station. Accessed on 11 October 2014 at http://oaktrust.library.tamu.edu/bitstream/handle/1969.1/ETD-TAMU-2009-08-1830/COBURN-THESIS.pdf?sequence=2.

Cook, A. J. C. 1991. Communal farmers and tick control – A field study in Zimbabwe. Tropical Animal Health and Production. 23:161-166.

Cortinas, R. and S. Spomer. 2013. Lone star tick (Acari: Ixodidae) occurrence in Nebraska: Historical and current perspectives. Journal of Medical Entomology. 50(3):244-251.

Cortinas, R. and S. Spomer. 2014. Occurrence and county-level distribution of ticks (Acari: Ixodidae) in Nebraska using passive surveillance. Journal of Medical Entomology. 51(2):352-359.

Dardiri, A. H., L. L. Logan and C. A. Mebus. 1987. Susceptibility of white-tailed deer to experimental heartwater infections. Journal of Wildlife Disease Association. 23(2):215-219.

Deem, S. L. 1998. A review of heartwater and the threat of introduction of *Cowdria ruminantium* and *Amblyomma* spp. Ticks to the American mainland. Journal of Zoo and Wildlife Medicine. 29(2):109-113.

Dipeolu, O. 1991. Laboratory studies on the oviposition, egg-sizes and shapes and embryonic development of *Dermacentor variabilis, Rhipicephalus sanguineus* and *Amblyomma maculatum*. Acarologia. 32(3):233-244.

Donzé, G., C. McMahon and P. Guerin. 2004. Rumen metabolites serve ticks to exploit large mammals. The Journal of Experimental Biology. 207:4283-4289.**Dreyer, K., L. J. Fourie and D. J. Kok. 1997.** Predation of livestock ticks by chickens as a tick-control method in a resource-poor urban environment. Onderstepoort Journal of Veterinary Research. 64:273-276.

Dreyer, K., L. J. Fourie and D. J. Kok. 1997. Predation of livestock ticks by chickens as a tick-control method in a resource-poor urban environment. Onderstepoort Journal of Veterinary Research. 64:273-276.

Duell, J. R., R. Carmichael, B. H. Herrin, T. C. Holbrook, J. Talley and S. E. Little. 2013. Prevalence and species of ticks on horses in central Oklahoma. Journal of Medical Entomology. 50(6):1330-1333.

Edwards, K. T. 2011. Gotch ear: A poorly described, local, pathologic condition of livestock associated primarily with the Gulf Coast tick, *Amblyomma maculatum*. Veterinary Parasitology 183, 1-7.

Edwards, K. T., A. S. Varela-Stokes, C. D. Paddock, J. Goddard. 2011. Gotch ear in a goat: A case report. Vector-Borne Zoonotic Diseases. 8:1217-1219.

Erwin, E. S., G. J. Marco and E. M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. Journal of Dairy Science. 44(9):1768-1771.

Espinoza-Gomez, F., O. Newton-Sanchez, G. Flores-Cazares, M. De la Cruz-Ruiz, V. Melnikov, J. Austria-Tejeda and F. Rojas-Larios. 2011. Tick paralysis caused by *Amblyomma maculatum* on the Mexican Pacific Coast. Vector-Borne and Zoonotic Diseases. 11(7):945-946.

Estrada-Peña, A., J. M. Venzal, A. J. Mangold, M. M. Cafrune and A. A. Gugliemone. 2005. The *Amblyomma maculatum* Koch, 1844 (Acari: Ixodidae: Amblyominae) tick group: Diagnostic characters, description of the larva of *A. parvitarsum* Neumann, 1901, 16S rDNA sequences, distribution and hosts. Systematic Parasitology. 60:99-112.

Ewing, S. A., J. E. Dawson, J. S. Mathew, R. W. Baker, K. W. Pratt and S. R. Telford III. 1997. Attempted transmission of human granulocytotropic *Ehrlichia* (HE) by *Amblyomma americanum* and *Amblyomma maculatum*. Veterinary Parasitology. 70:183-190.

Ewing, S. A., J. G. DuBois, J. S. Mathew and R. J. Panciera. 2002. Larval Gulf Coast ticks (*Amblyomma maculatum*) [Acari: Ixodidae] as host for *Hepatozoon americanum* [Apicomplexa: Adeleorina]. Veterinary Parasitology. 103:43-51.

Ewing, S. A., R. J. Panciera, J. S. Mathew, C. A. Cummings and A. A. Kocan. 2000. American canine hepatozoonosis. An emerging disease in the New World. Annals of New York Academy of Sciences. 916:81-92.

Ewing, S. A., R. J. Panciera and J. S. Mathew. 2003. Persistence of *Hepatozoon americanum* (Apicomplexa: Adeleorina) in a naturally infected dog. The Journal of Parasitology. 89(3):611-613.

Farooq, Z., Z. Iqbal, S. Mushtaq, G. Muhammad, M. Z. Iqbal and M. Arshad. 2008. Ethnoveterinary practices for the treatment of parasitic diseases in livestock in Cholistan desert (Pakistan). Journal of Ethnopharmacology. 118:213-219.

Ferrari, F. A. G., J. Goddard, C. D. Paddock and A. S. Varela-Stokes. 2012. *Rickettsia parkeri* and *Candidatus* Rickettsia andeanae in Gulf Coast ticks, Mississippi, USA. Emerging Infectious Diseases. 18(10):1705-1706.

Fleetwood, S. C., 1985. The environmental influences in selected vegetation microhabitats on the various life stages of *Amblyomma maculatum* Koch (Acari: Ixodidae). Ph.D. dissertation, Texas A and M University, College Station.

Fleetwood, S.C. and P.D. Teel. 1983. Variation in activity of ageing *Amblyomma maculatum* Koch (Acarina: Ixodidae) larvae in relation to vapor pressure deficits in pasture vegetation complexes. Protection Ecology. 5:343-352.

Florin, D. A., R. J. Brinkerhoff, H. Gaff, J. Jiang, R. G., Robbins, W. Eickmyer, J. Butler, D. Nielsen, C. Wright, A. White, M. E. Gimpel and A. L. Richards. 2014. Additional U.S. collections of the Gulf Coast tick, *Amblyomma maculatum* (Acari: Ixodidae), from the State of Delaware, the first reported field collections of adult specimens from the State of Maryland, and data regarding this tick from surveillance of migratory songbirds in Maryland. Systematic and Applied Acarology. 19(3):257-262.

Fornadel, C. M., X. Zhang, J. D. Smith, C. D. Paddock, J. R. Arias and D. E. Norris. 2011. High rates of *Rickettsia parkeri* infection in Gulf Coast ticks (*Amblyomma maculatum*) and identification of '*Candidatus* Rickettsia andeanae' from Fairfax County, Virginia. Vector-Borne and Zoonotic Diseases. 11(12):1535-1539.

Garvin, S. D., B. H. Noden, J. W. Dillwith, S. F. Fox, M. E. Payton and R. W. Barker. 2015. Sylvatic infestation of Oklahoma reptiles with immature *Ixodes* scapularis (Acari: Ixodidade). Journal of Medical Entomology. 52(2):873-878.

Geier, M., O. J. Bosch and J. Boeckh. 1999. Ammonia as an attractive component of host odour for the yellow fever mosquito, *Aedes aegypti*. Chemical Senses. 24:1-7.

Gladney, W. J. 1971. Mate-seeking by female *Amblyomma maculatum* (Acarina: Ixodidae) on a bovine. Nature. 232:401-402.

Gladney, W. J. 1976. Field trials of insecticides in controlled-release devices for control of the Gulf Coast tick and prevention of screwworm in cattle. Journal of Economic Entomology. 69:757-760.

Gladney, W. J., S. E. Ernst and R. R. Grabbe. 1974. The aggregation response of the Gulf Coast tick on cattle. Annals of the Entomological Society of America. 67(5):750-752.

Gladney, W. J., M. A. Price and O. H. Graham. 1977. Field tests of insecticides for control of the Gulf Coast tick on cattle. Journal of Medical Entomology. 13(4-5):579-586.

Goddard, J. 2002. A ten-year study of tick biting in Mississippi: Implications for human disease transmission. Journal of Agromedicine. 8(2):25-32.

Goddard, J. and C. D. Paddock. 2005. Observations on distribution and seasonal activity of the Gulf Coast tick in Mississippi. Entomological Society of America, 42(2):176-179.

Goddard, J., A. Varela-Stokes and J. C. Schneider. 2011. Observations on questing activity of adult Gulf Coast ticks, *Amblyomma maculatum* Koch (Acari: Ixodidae), in Mississippi, U.S.A. Systematic and Applied Acarology. 16:195-200.

Grasperge, B. J., T. W. Morgan, C. D. Paddock, K. E. Peterson and K. R. Macaluso. 2014. Feeding by *Amblyomma maculatum* (Acari: Ixodidae) enhances *Rickettsia parkeri* (Rickettsiales: Rickettsiaceae) infection in the skin. Journal of Medical Entomology. 51(4):855-863.

Guedes, E., M. C. de Azevedo Prata, E. S. dos Reis, P. H. D. Conçado, R. C. Leite. 2012. Comparative efficiency of two models of CO_2 traps in the collection of free-living stages of ixodides. Parasitology Research. 111:2325-2328.

Haemig, P. D., S. Sjötedt de Luna, A. Grafström, S. Lithner, Ä. Lundkvist, J. Waldenström, J. Kindberg, J. Stedt, B. Olsén. 2011. Forecasting risk of tick-borne encephalitis (TBE): Using data from wildlife and climate to predict next year's number of human victims. Scandinavian Journal of Infectious Diseases. 43:366–372.

Haggart, D. A. and E. E. Davis. 1980. Ammonia-sensitive neurons on the first tarsi of the tick, *Rhipicephalus sanguineus*. Journal of Insect Physiology. 26:517-523.

Hixson, H. 1940. Field biology and environmental relationships of the Gulf Coast tick in southern Georgia. Journal of Economic Entomology. 33:179-189.

Hlatshwayo, M. and P. A. Mbati. 2005. A survey of tick control methods used by resource-poor farmers in the Qwa-Qwa area of the eastern Free State Province, South Africa. Onderstepoort Journal of Veterinary Research. 72:245-249.

Hoch, A. L., R. W. Barker and J. A. Hair. 1971. Further observation on the control of lone star ticks (Acarina: Ixodidae) through integrated control procedures. Journal of Medical Entomology. 8(6):731-734.

Hoe, F. G. H. and P. L. Ruegg. 2006. Opinions and practices of Wisconsin dairy producers about biosecurity and animal well-being. Journal of Dairy Science. 89:2297-2308.

Holscher, K. H., H. L. Gearhart and R. W. Barker. 1980. Electrophysical responses of three tick species to carbon dioxide in the laboratory and field. Annals of the Entomological Society of America. 73(3):288-292.

Hooker, W. A., F. C. Bishopp and H. P. Wood. 1912. The life history and bionomics of some North American ticks. U.S. Department of Agriculture, Bureau of Entomology Bulletin. 106.

Jiang, J., E. Y. Stromdahl and A. L. Richards. 2012. Detection of *Rickettsia parkeri* and *Candidatus* Rickettsia andeanae in *Amblyomma maculatum* Gulf Coast ticks collected from humans in the United States. Vector-Borne and Zoonotic Diseases. 12(3):175-182.

Johnson, J. L., H. S. Ginsberg, E. Zhioua, U. G. Whitworth, Jr., D. Markowski, K. E. Hyland and R. Hu. 2004. Passive tick surveillance, dog seropositivity, and incidence of human Lyme disease. Vector-Borne and Zoonotic Diseases. 4(2):137-142.

Jonsson, N. N. and Matschoss, A. L., 1998. Attitudes and practices of Queensland dairy farmers to the control of the cattle tick, *Boophilus microplus*. Australian Veterinary Journal. 75(11):746-751.

Jonsson, N. N., D. G. Mayer and P. E. Green. 2000. Possible risk factors on Queensland dairy farms for acaricide resistance in cattle tick (*Boophilus microplus*). Veterinary Parasitology. 88:79-92.

Kaliyaperumal, K. 2004. Guideline for Conducting a knowledge, attitude, and practice (KAP) study. Community Ophthalmology. 4(1):7-9.

Ketchum, H. R., P. D. Teel, O. F. Strey and M. T. Longnecker. 2006. Mating success of two geographically distinct populations of Gulf Coast ticks, *Amblyomma maculatum* Koch. Veterinary Parasitology. 140:143-147.

Ketchum, H. R., P. D. Teel, O. F. Strey and M. T. Longnecker. 2005. Feeding predilection of Gulf Coast tick, *Amblyomma maculatum* Koch, nymphs on cattle. Veterinary Parasitology. 133:349-356.

Kellum, D. and R. S. Berger. 1977. Relationship of the occurrence and function of 2,6-Dichlorophenol in two species of *Amblyomma* (Acari: Ixodidae). Journal of Medical Entomology. 13(6):701-705.

Kelly, P. J., H. M. Lucas, C. M. Randolph, K. Ackerson, J. K. Blackburn and M. J. Dark. 2014. Efficacy of slow-release tags impregnated with aggregation-attachment pheromone and deltamethrin for control of *Amblyomma variegatum* on St. Kitts, West Indies. Parasites and Vectors. 7(182):1-8.

KFOR News. 2015. Rare illness caused by a tick diagnosed found in Oklahoma, only second case in U.S. Online: http://kfor.com/2015/05/27/rare-illness-caused-by-a-tick-diagnosed-found-in-oklahoma-only-second-case-in-u-s/. Accessed 6 March 2016.

Kim, H. J. 2004. Potential application of a Gulf Coast tick, *Amblyomma maculatum* Koch, aggregation-attachment-pheromone for surveillance of free-living adults. M. S. thesis, Texas A&M, College Station. Accessed 11 October 2014 at http://oaktrust.library.tamu.edu/bitstream/handle/1969.1/1488/etd-tamu-2004C-ENTO-Kim.pdf?sequence=1.

Kinzer, D. R., S. M. Presley and J. A. Hair, 1990. Comparative efficiency of flagging and carbon dioxide-baited sticky traps for collecting lone star tick, *Amblyomma americanum* (Acarina: Ixodidae). Journal of Medical Entomology. 27(5):750-755.

Kisomi, M. G., L. P. Wong, S. T. Tay, A. Bulgiba, K. Zandi, K. L. Kho, F. X. Koh, B. L. Ong, T. Jaafar and Q. N. H. Nizam. 2016. Factors associated with tick bite preventive practices among farmworkers in Malaysia. PLoS ONE. 11(6):1-17.

Kocan, A. A., M. Breshears, C. Cummings, R. J. Panciera, S. A. Ewing and R. W. Barker. 1999. Naturally occurring hepatozoonosis in coyotes from Oklahoma. Journal of Wildlife Diseases. 35(1):86-89.

Koch, H. G. and J. A. Hair. 1975. The effect of host species on the engorgement, molting success, and molted weight of the Gulf Coast tick, *Amblyomma maculatum* Koch (Acarina: Ixodidae). Journal of Medical Entomology. 12(2):213-219.

Kosoy, O. I., A. J. Lambert, D. J. Hawkinson, D. M. Pastula, C. S. Goldsmith, D. C. Hunt and J. E. Staples. 2015. Novel Thogotovirus associated with fibrile illness and death, United States, 2014. Emerging Infectious Diseases. 21(5):760-764.

Kramer, L. D., L. M. Styer and G. D. Ebel. 2008. A global perspective on the epidemiology of West Nile virus. Annual Review of Entomology. 53:61–81.

Laisser, E. L. K., S. W. Chenyambuga, G. Msalya, M. J. Kipanyula, R. H. Mdelgela, E. D. Karimuribo, A. J. Mwilawa and L. J. M. Kusiluka. 2015. Knowledge and perception on ticks, tick-borne diseases and indigenous cattle tolerance to East Coast fever in agro-pastoral communities of Lake Zone in Tanzania. Livestock Research for Rural Development. 27(4).

Leahy, M. G. and K. S. Booth. 1978. Perception of a sex pheromone, 2,6-dichlorophenol, in hard ticks. Tick-borne Diseases and Their Vectors, Centre for Tropical Veterinary Medicine, Edinburgh University Press, Scotland. 88-94

Lindenmayer, J. M., D. Marshall and A. B. Onderdonk. 1991. Dogs as sentinels for Lyme disease in Massachusetts. American Journal of Public Health. 81(11):1448-1455.

Logan, T. M., J. C. Fox, J. A. Hair, K. M. Kocan, B. H. Espe and P. D. Woodson. 1985. The prevalence and distribution of anaplasmosis in Oklahoma. Oklahoma Veterinary Medical Association. 37(4):96-99.

Lohmeyer, K. H., J. M. Pound and J. E. George. 2009. Effects of photoperiod on reproduction, nymphal developmental timing, and diapause in *Amblyomma maculatum* (Acari: Ixodidae). Journal of Medical Entomology. 46(6):1299-1302.

Lostak, T. K. 2008. Genetic variation in the 16S mitochondrial rDNA gene from Texas and Oklahoma populations of *Amblyomm maculatum*. M. S. thesis, Texas A&M Univesity, College Station.

Luce-Fedrow, A., C. Wright, H. D. Graff, D. E. Sonenshine, W. L. Hynes and A. L. Richards. 2011. *In vitro* propagation of *Candidatus* Rickettsia andeanae isolated from *Amblyomma maculatum*. FEMS Immunology and Medical Microbiology. 64:74-81.

Machila, N., R. Emongor, A.P. Shaw, S. C. Welburn, J. McDermott, I. Maudlin and M. C. Eisler. 2007. A community education intervention to improve bovine trypanosomiasis knowledge and appropriate used of trypanocidal drugs on smallholder farms in Kenya. Agricultural Systems. 94:261-**272.**

Machila, N., S. W. Wanyangu, J. McDermott, S. C. Welburn, I. Maudlin and M. C. Eisler. 2002. Cattle owners' perceptions of African bovine trypanosomiasis and its control in Busia and Kwale Districts of Kenya. Acta Tropica. 86:25-34.

Mahan, S. M., T. F. Peter, B. H. Simbi, K. Kocan, E. Camus, A. F. Barbet and M. J. Burridge. 2000. Comparison of efficacy of American and African *Amblyomma* ticks as vectors of heartwater (*Cowdria ruminantium*) infection by molecular analyses and transmission trials. The Journal of Parasitology. 86 (1):44-49.

Masika, P. J., A. Sonandi and W. van Averbeke. 1997. Tick control by small-scale cattle farmers in the central Eastern Cape Province, South Africa. Journal of the South African Veterinary Association. 68(2):45-48.

Mathew, J. S., S. A. Ewing, R. J. Panciera and K. M. Kocan. 1999. Sporogonic development of *Hepatozoon americanum* (Apicomplexa) in its definitive host, *Amblyomma maculatum* (Acarina). The Journal of Parasitology. 85(6):1023-1031.

Mapiye, C., M. Chimonyo, K. Dzama, J. G. Raats and M. Mapekula. 2009. Opportunities for improving Nguni cattle production in the smallholder farming systems of South Africa. Livestock Science 124:196-204.

Maranga, R. O., A. Hassanali, G. P. Kaaya and J. M. Mueke. 2003. Attraction of *Amblyomma variegatum* (ticks) to the attraction-aggregation-attachment-pheromone with or without carbon dioxide. Experimental and Applied Acarology. 29:121-130.

Merial. 2013. Fact IVOMEC Antiparasitic Injection for Cattle product fact sheet. Merial Australia. Accessed on 5 July 2016 at http://www.merial.com.au/SiteCollectionDocuments/IvomecInjection_FactSheet_LR.pdf.

McCorkle, C. M. 1986. An introduction to ethnoveterinary research and development. Journal of Ethnobiology. 6(1):129-149.

Mock, D. E. 2000. Cattle and horse owners in the entire eastern half of Kansas should be alert for Gulf Coast ticks in ears of livestock. Kansas Insect Newsletter, Kansas State University, Manhattan, KS. 1:5. Accessed on October 15, 2014 at http://entomology.k-state.edu/doc/extension-newsletters/kin100.pdf.

Moraru, G. M., J. Goddard and A. S. Varela-Stokes. 2012. Observations on host preference and feeding success of immature *Amblyomma maculatum* (Acari: Ixodidae). Journal of Entomological Science. 47(3):221-226.

Moyo, B. and P. J. Masika. 2009. Tick control methods used by resource-limited farmers and the effect of ticks on cattle in rural areas of the Eastern Cape Province, South Africa. Tropical Animal Health and Production. 41:517-523.

Mugabi, K. N., A. Mugisha and M. Ocaido. 2010. Socio-economic factors influencing the use of acaricides on livestock: A case study of the pastoralist communities of Nakasongola District, Central Uganda. Tropical Animal Health and Production. 42:131-136.

National Center for Biotechnology Information. PubChem compound database: Acetate, CID=175. Accessed on 5 July 2016 at https://pubchem.ncbi.nlm.nih.gov/compound/175.

National Center for Biotechnology Information. PubChem compound database: Butyric Acid, CID=264. Accessed on 5 July 2016 at https://pubchem.ncbi.nlm.nih.gov/compound/264.

National Center for Biotechnology Information. PubChem compound database: Propionic Acid, CID=1032. Accessed on 5 July 2016 at https://pubchem.ncbi.nlm.nih.gov/compound/1032.

National Center for Biotechnology Information. PubChem compound database: Propanoate, CID=104745. Accessed on 5 July 2016 at https://pubchem.ncbi.nlm.nih.gov/compound/104745.

National Center for Biotechnology Information. PubChem compound database: Butanoate, CID=104775. Accessed on 5 July 2016 at https://pubchem.ncbi.nlm.nih.gov/compound/104775.

Nabukenta, I., C. Rubaire-Akiiki, D. Olila, K. Ikwap and J. Höglund. 2014. Ethnopharmacological practices by livestock farmers in Uganda: Survey experiences from Mpigi and Gulu districts. Journal of Ethnobiology and Ethnomedicine. 10(9):1-13.

Ndhlovu, D. N. and P. J. Masika. 2013. Ethno-veterinary control of bovine dermatophilosis and ticks in Zhombe, Njelele and Shamrock resettlement in Zimbabwe. Tropical Animal Health and Production. 45:525-532.

Norval, R. A. I., T. Peter, C. E. Yunker, D. E. Sonenshine and M. J. Burridge. 1991. Responses of the ticks *Amblyomma hebraeum* and *A. variegatum* to known or potential components of the aggregation-attachment pheromone. I. Long-range attraction. Experimental and Applied Acarology. 13:11-18.

Nouala, F. S., S. Muetzel, E. Hoffman and K. Becker. 2009. Comparative digestive ability and rumen microbial community of N'Dama and N'Dama x Jersey cattle fed different diets. South African Journal of Animal Science. 39(3):243-251.

Nqeno, N., M. Chimonyo and C. Mapiye. 2011. Farmers' perceptions of the causes of low reproductive performance in cows kept under low-input communal production systems in South Africa. Tropical Animal Health and Production. 43:315-321.

Ogden, N. H., L. R. Lindsat, K. Hanincoy, I. K. Barker, M. Bigras-Poulin, D. F. Charron, A. Heagy, C. M. Francis, C. J. O'Callaghan, L. Schwartz and R. A. Thompson 2008. Role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. Applied and Environment Microbiology. 74:1780-1790.

O'Kelly, J. C. and W. G. Spiers. 1992. Possible contribution of protozoa to differences in rumen metabolism between cattle breeds. Australian Journal of Agricultural Research. 43(8):1795-1808.

[OSDH] Oklahoma State Department of Health. 2014. Oklahoma State Department of Health confirms first case and death of Heartland virus. Online: https://www.ok.gov/health/Organization/Office_of_Communications/News_Releases/2014_News_Releases/Oklahoma_State_Health_Department_Confirms_First_Case_and_Death_of_Heartland_Virus.html. Accessed 21 July 2016.

Osterkamp, J., U. Wahl, G. Schmalfuss and W. Haas. 1999. Host-odour recognition in two tick species is coded in a blend of vertebrate volatiles. Journal of Comparative Physiology A. 185:59-67.

Paddock, C. D., A. M. Denison, M. W. Dryden, B. H. Noden, R. R. Lash, S. S. Abdelghani, A. E. Evans, A. R. Kelly, J. A. Hecht, S. E. Karpathy, R. R. Ganta and S. E. Little. 2015. High prevalence of '*Candidatus* Rickettsia andeanae' and apparent exclusion of *Rickettsia parkeri* in adult *Amblyomma maculatum* (Acari: Ixodidae) from Kansas and Oklahoma. Ticks and Tick-borne Diseases. 6:297-302.

Paddock, C. D., P. Fournier, J. W. Sumner, J. Goddard, Y. Elshenawy, M. G. Metcalfe, A. D. Loftis and A. Varela-Stokes. 2010. Isolation of *Rickettsia parkeri* and identification of a novel spotted fever group *Rickettsia* sp. from Gulf Coast ticks (*Amblyomma maculatum*) in the United States. Applied Environmental Microbiology. 76(9):2689-2696.

Paddock, C. D. and J. Goddard. 2015. The evolving medical and veterinary importance of the Gulf Coast tick (Acari: Ixodidae). Journal of Medical Entomology. 52(2):230-252.

Paddock, C. D., J. W. Sumner, J. A. Comer, S. R. Zaki, C. S. Goldsmith, J. Goddard, S. L. McLellan, C. L. Tamminga and C. A. Ohl. 2004. *Rickettsia parkeri*: A newly recognized cause of spotted fever rickettsiosis in the United States. Clinical Infectious Disease. 18:805-811.

Paffenbarger, R. S. 1951. Tick paralysis: Implicating *Amblyomma maculatum*. New Orleans Medical and Surgical Journal. 103(8):329-333.

Pagac, B. B., M. K. Miller, M. C. Mazzei, D. H. Nielsen, J. Jiang and A. L. Richards. 2014. *Rickettsia parkeri* and *Rickettsia montanensis*, Kentucky and Tennessee, USA. Emerging Infectious Diseases. 20(10):1750-1752.

Parker, R. R, G. M. Kohls, G. W. Cox and E. D. Gordon. 1939. Observation on an Infectious Agent from *Amblyomma maculatum*. Public Health Reports (1896-1970). 54(32):1482-1484.

Pastula, D. M., G. Turabelidze, K. F. Yates, T. F. Jones, A. J. Lambert, A. J. Panella, O. I. Kosoy, J. O. Velez, M. Fischer and E. Staples. 2014. Notes from the field: Heartland virus diseases – United States, 2012-2013. Morbidity and Mortality Weekly Report. 63(12):270-271

Pegram, R. G. and C. Eddy. 2002. Progress towards the eradication *Amblyomma variegatum* from the Caribbean. Experimental and Applied Acarology. 28:273-281.

Pérez de León, A. A., D. A. Strickman, D. P. Knowles, D. Fish, E. Thacker, J. De La Fuente, P. J. Krause, S. K. Wikel, R. S. Miller, G. G. Wagner, C. Almazán, R. Hillman, M. T. Messenger, P. I. Ugstad, R. A. Duhaime, P. D. Teel, A. Ortega-Santos, D. G. Hewitt, E. J. Bowers, S. J. Bent, M. H. Cochran, T. F. McElwain, G. A. Scoles, C. E. Suarez, R. Davey, J. M. H. Feeman, K. Lohymeyer, A. Y. Li, F. D. Guerrero, D. M. Kammlah, P. Philips, J. M. Pound. 2010. One Health approach to identify research needs in bovine and human naesioses: workshop report. Parasites & vectors. 3:36-47.

Potter, T. M. and D. K. Macintire. 2010. *Hepatozoon americanum*: an emerging disease in the south-central/southeastern United States. Journal of Veterinary Emergency and Critical Care. 20:70-76.

Ranju, R. S., B. R. Latha, V. Leela and S. A. Basith. 2012. Effect of 1-octen-3-ol, the ruminant kairomone on ixodid tick larvae. Veterinarski Arhiv. 82(6):609-615.

Ranju, R. S., B. R. Latha, V. Leela and S. A. Basith. 2014. Effect of ruminant kairomone volatile fatty acids on larval stages of five ixodid tick species. The Journal of Zoology Studies. 1(3):14-18.

Ratcliffe, J. W. 1976. Analyst biases in KAP surveys: A cross-cultural comparison. Studies in Family Planning. 7(11):322-330.

Rechav, Y. C. Drey, L. J. Fielden and M. Goldberg. 2000. Production of pheromones by artificially fed males of the tick *Amblyomma maculatum* (Acari: Ixodidae). Journal of Medical Entomology. 37(5):761-765

Riley, P. J., R. L. Byford, D. M. Hallford, J. W. Campbell and E. Perez-Eguia. 1995. Physiological responses of beef cattle to Gulf Coast tick (Acari: Ixodidae) infestations. Journal of Economic Entomology. 88(2):320-325.

Rodriguez-Vivas, R. I., M. A. Alonso-Diaz, F. Rodriguez-Arvelo, H. Fragoso-Sanchez, V. M. Santamaria and R. Rosario-Cruz. 2006. Prevalence and potential risk factors for organophosphate and pyrethroid resistance in *Boophilus microplus* ticks on cattle ranches from the State of Yucatan, Mexico. Veterinary Parasitology. 136:335-342.

Sajid, M. S., Z. Iqbal, M. N. Khan and G. Muhammad. 2009. In vitro and in vivo efficacies of ivermectin and cypermethrin against the cattle tick *Hyalomma anatolicum anatolicum* (Acari: Ixodidae). Parasitology Research. 105:1133-1138.

Sanders, D. M., A. L. Schuster, P. W. McCardle, O. F. Strey, T. L. Blankenship and P. D. Teel. 2013. Ixodid ticks associated with feral swine in Texas. Journal of Vector Ecology. 38(2):361-373.

Scott, J. D., K. Fernando, S. N. Banerjee, L. A. Durden, S. K. Byrne, M. Banerjee, R. B. Mann and M. G. Morshed. 2001. Birds disperse Ixodid (Acari: Ixodidae) and *Borrelia burgdorferi*-infected ticks in Canada. Journal of Medical Entomology. 38:493-500.

Semtner, P. J. and J. A. Hair. 1973. Distribution, seasonal abundance, and hosts of the Gulf Coast tick in Oklahoma. Annals of the Entomological Society of America. 66(6):1264-1268.

Semtner, P. J. and J. A. Hair. 1975. Evaluation of CO_2-batied traps for survey of *Amblyomma maculatum* Koch and *Dermacentor variabilis* Say. Journal of Medical Entomology. 12 (1):137-138.

Sleeba, S. B., P. D. Teel, M. T. Longnecker and O. F. Strey. 2010. Host selection by questing female *Amblyomma maculatum* Koch, to cattle with feeding male ticks in southern Texas. Veterinary Parasitology. 172:105-108.

Shabani, S. S., M. J. Ezekiel, M. Mohamed and C. S. Moshiro. 2015. Knowledge, attitudes and practices on Rift Valley fever among agro pastoral communities in Kongwa and Kilombero districts, Tanzania. BMC Infectious Diseases. 15(363):1-9.

Shwiff, S. A., J. C. Carlson, J. H. Glass, J. Suckow, M. S. Lowney, K. M. Moxcey, B. Larson and G. M. Linz. 2011. Producer survey of bird-livestock interactions in commercial dairies. Journal of Dairy Science. 95(11):6820-6829.

Smith, H. M., W. R. Davidson, V. F. Nettles, R. R. Gerrish. 1982. Parasitisms among wild swine in the southeastern United States. Journal of the American Veterinary Medical Association. 181(11):1281-1284.

Sonenshine, D. E. 1985. Pheromones and other semiochemicals of the acari. Annual Review of Entomology. 30:1-28.

Sonenshine, D. E., D. Taylor and K. Carson. 1986. Chemically mediated behavior in acari: Adaptations for finding hosts and mates. Journal of Chemical Ecology. 12(5):1091-1108.

Sorge, U. S., C. Cherry and J. B. Bender. 2014. Perception of the importance of human-animal interactions on cattle flow and worker safety on Minnesota dairy farms. Journal of Dairy Science. 9(7):4632-4638.

Spicer, W. J. and W. E. Dove. 1938. The screwworm and the Gulf Coast tick in Texas. Journal of Economic Entomology. 31:642-646.

Stacey, B. R. 1971. Season and habitat effect on Gulf Coast tick biology in Central Oklahoma. M.S. thesis, Oklahoma State University, Stillwater. Accessed on June 14, 2016 at https://shareok.org/bitstream/handle/11244/19201/Thesis-1977-S775s.pdf?sequence=1&isAllowed=y.

Stacey, B. R., R. E. Williams, R. G. Buckner and J. A. Hair. 1978. Changes in weight and blood composition of Hereford and Brahman steers in drylot and infested with adult Gulf Coast ticks. Journal of Economic Entomology. 71(6):967-970.

Steullet, P. and P. M. Guerin. 1994. Identification of vertebrate volatiles stimulating olfactory receptors on tarsus I of the tick *Amblyomma variegatum* Fabricius (Ixodidae) II. Receptors outside the Haller's organ capsule. Journal of Comparative Physiology A. 174:39-47.

Stewart, M. E. 1992. Sebaceous gland lipids. Seminars in Dermatology. 11:100-105.

Stone, E. G., E. H. Lacombe and P. W. Rand. 2005. Antibody testing and Lyme disease risk. Emerging Infectious Diseases. 11(5):722-724.

Sumner, J. W., L. A. Durden, J. Goddard, E. Y. Stromdahl, K. L. Clark, W. K. Reeves and C. D. Paddock. 2007. Gulf Coast ticks (*Amblyomma maculatum*) and *Rickettsia parkeri*, United States. Emerging Infectious Diseases. 13(5):751-753.

Teel, P. D., S. C. Fleetwood, S. W. Hopkins and D. Cruz. 1998. Population dynamic of immature *Amblyomma maculatum* (Acari: Ixodidae) and other ectoparasites on meadowlarks and northern bobwhite quail resident to the coastal prairie of Texas. Journal of Medical Entomology. 35 (4):483-488.

Teel, P. D., S. C. Fleetwood, S. W. Hopkins and D. Cruz. 1988. Ectoparasites of Eastern and Western meadowlarks from the Rio Grande Plains of South Texas. Journal of Medical Entomology. 25 (1):32-38.

Teel, P. D., H. R. Ketchum, D. E. Mock, R. E. Wright and O. F. Strey. 2010. The Gulf Coast tick: A review of the life history, ecology, distribution, and emergence as an arthropod of medical and veterinary importance. Journal of Medical Entomology. 47:707-722.

Trout, R., C. D. Steelman, A. L. Szalanski and P. C. Williamson. 2010. Rickettsia in Gulf Coast ticks, Arkansas, USA. Emerging Infections Diseases. 16(5):830-832.

Tuiten, W., C. J. M. Koenraadt, K. McComas and L. C. Harrington. 2009. The effect of West Nile Virus perceptions and knowledge on protective behavior and mosquito breeding in residential yards in Upstate New York. EcoHealth. 6:42-51.

Uilenberg, G. H. 1982. Experimental transmission of *Cowdria ruminantium* by the Gulf Coast tick, *Amblyomma maculatum*: danger of introducing heartwater and benign African theileriasis onto the American mainland. Journal of Veterinary Research. 43:1279-1282.

Uilenberg, G., N. Barré, E. Camus, M. J. Burride and G. I. Garris. 1984. Heartwater in the Caribbean. Preventative Veterinary Medicine. 2:255-267.

Ul Hassan, H., W. Murad, A. Tariq and A. Ahmad. 2014. Ethnoveterinary study of medicinal plants in Malakand Valley, District Dir (Lower), Khyber Pakhtunkhwa, Pakistan. Irish Veterinary Journal. 67(6):1-6.

[USDA] U.S. Department of Agriculture, National Agricultural Statistics Service. 2015. 2012 Census of agriculture: Cattle industry highlights. ACH12-20. 1-4. Accessed on June 25, 2016 at https://www.agcensus.usda.gov/Publications/2012/Online_Resources/Highlights/Cattle/Cattle_Highlights.pdf.

Van Engelen, E., N. Schotten, B. Schimmer, J. L. A. Hautvast, G. van Schaik and Y. T. H. P. van Duijnhoven. 2014. Prevalence and risk factors for *Coxiella burnetti* (Q fever) in Dutch dairy cattle herds based on bulk milk testing. Preventative Medicine. 117:103-109.

Vestal, M., C. Ward, D. Doye and D. Lalman. 2007. Cow-Calf production practices in Oklahoma-Part 2. Oklahoma Cooperative Extension Service, AGEC-246, 1-7. Accessed on December 1, 2015 at http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Document-4572/AGEC-246web.pdf.

Walker, J. B. 1987. The tick vectors of *Cowdria ruminantium* (Ixodoidea, Ixodidae, Genus *Amblyomma*) and their distribution. Onderstepoort Journal of Veterinary Research. 54:353-379.

Wanchinga, D. M. and D. E. Sonenshine. 1978. Observations on the feeding and development of *Amblyomma maculatum* Koch 1844 (Acarina: Ixodidae): effects of vitamin and mineral deficiencies in host diets. Zambia Journal of Science and Technology. 3(2):25-30.

Wanzala, W., N. F. K. Sika, S. Gule and A. Hassanali. 2004. Attractive and repellent host odours guide ticks to their respective feeding sites. Chemoecology. 14:229-232.

Wanzala, W., W. Takken, W. R. Mukabana, A. O. Pala and A. Hassanali. 2012. Ethnoknowledge of Bukusu community on livestock tick prevention and control. Journal of Ethnopharmacology. 140:298-324.

Warburton, H. and A. Martin. 1999. Local people's knowledge in natural resources research. Socio-Economic Methodologies for Natural Resources Research. Chatam, UK: Natural Resources Institute.

Wexler, A. 2005. Interaction of Gulf Coast Tick, *Amblyomma maculatum* Koch, nymphs on cattle. M. S. thesis, Texas A&M, College Station. Accessed on 3 July 2016 at http://oaktrust.library.tamu.edu/bitstream/handle/1969.1/4413/etd-tamu-2005B-ENTO-Wexler.pdf?sequence=1.

Wiedl, S. C. 1981. A new distribution record for *Amblyomma maculatum* (Acari: Ixodidae). Journal of Medical Entomology. 18(2):170.

Williams, H. 2002. The biology and zoogeography of the Gulf Coast tick, *Amblyomma maculatum* Koch, the potential vector of *Ehrlichia ruminantium* (Dumler) in the United States. Ph. D. dissertation, Texas A&M University, College Station.

Williams, R. E., J. A. Hair and R. G. Buckner. 1977. Effects of the Gulf Coast tick on blood composition and weights of drylot Hereford steers. Journal of Economic Entomology. 70(2):229-233.

Williamson, P. C., P. M. Billingsley, G. J. Teltow, J. P. Seals, M. A. Turnbough and S. F. Atkinson. 2010. *Borrelia*, *Ehrlichia*, and *Rickettsia* spp. in ticks removed from persons, Texas, USA. Emerging Infectious Diseases. 16(3):441-446.

Wilson, D. D. and R. D. Richard. 1984. Interception of a vector of heartwater *Amblyomma hebraeum* Koch (Acari: Ixodidae) on black rhinoceroses imported into the United States. Proceedings, Annual Meeting of the United States Animal Health Association. 88:303-311.

Wilson, J. G., D. R. Kinzer, J. R. Sauer and J. A. Hair. 1972. Chemo-attraction in the lone star tick (Acarina: Ixodidae): I. Response of different developmental stages to carbon dioxide administered via traps. Journal of Medical Entomology. 9(3):245-252.

Wright, C. L., R. M. Nadolny, J. Jiang, A. L. Richards, D. E. Sonenshine, H. D. Gaff and W. L. Hynes. 2011. *Rickettsia parkeri* in Gulf Coast ticks, Southeastern Virginia, USA. Emerging Infections Diseases. 17(5):896-898.

Wright, J. C., K. M. Kocan, S. A. Ewing and B. H. Espe. 1985. Oklahoma Anaplasmosis survey. Oklahoma Veterinary Medical Association. 37(4):130-132.

Yabsley, M. J., T. N. Nims, M. Y. Savage and L. A. Durden. 2009. Ticks and tick-borne pathogens and putative symbionts of black bears (*Ursus americanus floridanus*) from Georgia to Florida. Journal of Parasitology. 95(5):1125-1128.

Yoder, J. A., A. D. Atwood and B. W. Stevens. 1998. Attraction to squalene by ticks (Acari: Ixodidae): First demonstration of a host-derived attractant. International Journal of Acarology. 24(2), 143-147.

Yoder, J. A., B. S. Christensen, T. J. Croxall, L. K. Schumaer and J. L. Tank. 2008. Moisture requirements for activity/survival of the Gulf Coast tick, *Amblyomma maculatum* Koch (Acari: Ixodidae), based on a water balance study of all life cycle stages. International Journal of Acarology. 34(3):285-292.

Yoder, J. A., R. J. Pollack, A. Spielman, D. E. Sonenshine and D. E. Johnston. 1993. Secretion of squalene by ticks. Journal of Insect Physiology. 39(4):291-296.

Yoder, J. A., B. W. Stevens and K. C. Crouch. 1999. Squalene: A naturally abundant mammalian skin secretion and long distance tick-attractant (Acari: Ixodidae). Journal of Medical Entomology. 36(4), 526-529.

Yoder, J. A. and J. L. Tank. 2006. Similarity in critical transition temperature for ticks adapted for different environments: studies on the water balance of unfed ixodid larvae. International Journal of Acarology. 32(3):323-329.

APPENDICES

Appendix A.

From: IACUC
To: Noden, Bruce
Cc: Dubie, Trisha
Subject: ACUP AG-15-11 (Noden) Approved
Date: Wednesday, July 01, 2015 8:40:42 AM

Oklahoma State University
Institutional Animal Care and Use Committee (IACUC)

Protocol expires: 6/30/2018

Date: Wednesday, July 01, 2015 Animal Care and Use Protocol (ACUP) No AG-15-11

Proposal: Olfactory analysis of compounds collected from the ears of cattle and their attractiveness to *Amblyomma maculatum*

Principal Investigator:

Bruce Noden
Entomology and Plant Pathology

Campus

Reviewed and Processed as: Full Committee

Approval Status Recommended by Reviewer(s) : Approved

The revised protocol is approved. You are approved to use a maximum of two cattle for the next three years.

Signatures

Karen McBee, IACUC Chair

Wednesday, July 01, 2015
Date

cc: Department Head, Entomology and Plant Pathology
Director, Animal Resources

Approvals are valid for three calendar years, after which time a request for renewal must be submitted. Any modifications to the research project, course, or testing procedure must be submitted for review and approval by the IACUC, prior to initiating any changes. Modifications do not affect the original approval period. Approved projects are subject to monitoring by the IACUC. OSU is a USDA registered research facility and maintains an Animal Welfare Assurance document with the Public Health Service Office of Laboratory Animal Welfare, Assurance number AA3722-01.

Appendix B.

Oklahoma State University Institutional Review Board

Date:	Monday, August 03, 2015
IRB Application No	AG1535
Proposal Title:	Knowledge, attitude and prevention survey of ticks with Oklahoma Beef Producers
Reviewed and Processed as:	Exempt

Status Recommended by Reviewer(s): Approved   Protocol Expires: 8/2/2018

Principal Investigator(s):

Bruce Noden	Justin Talley
Stillwater, OK 74078	Stillwater, OK 74078

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval. Protocol modifications requiring approval may include changes to the title, PI advisor, funding status or sponsor, subject population composition or size, recruitment, inclusion/exclusion criteria, research site, research procedures and consent/assent process or forms.
2. Submit a request for continuation if the study extends beyond the approval period. This continuation must receive IRB review and approval before the research can continue.
3. Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of the research; and
4. Notify the IRB office in writing when your research project is complete.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact Dawnett Watkins 219 Scott Hall (phone: 405-744-5700, dawnett.watkins@okstate.edu).

Sincerely,

Hugh Crethar, Chair
Institutional Review Board

PARTICIPANT INFORMATION

OKLAHOMA STATE UNIVERSITY

Title: Knowledge, Attitude, and Prevention Survey of Ticks with Oklahoma Beef Producers

Investigator(s): Bruce Noden, Ph.D., Principal Investigator. Justin Talley, Ph.D., Principal Investigator. Trisha Dubie, Ph.D., Postdoctoral Fellow. Krista Pike, B.S., Graduate Research Assistant.

Purpose: The purpose of the proposed study is to better understand the attitudes and knowledge that Oklahoma beef producers have in regard to ticks and the risks they pose to their cattle and themselves, and to understand the methods used to prevent infestations of ticks on cattle and themselves, employees, and family. Limited research has been conducted in the United States on this subject. The information gained will be used to create educational materials targeted at issues producers feel are important. These materials will be used by Extension specialists.

What to Expect: This research study is administered as a paper survey. Participation in this research will involve completion of one questionnaire. Questions will be multiple choice and fill-in-the-blank. The questionnaire will ask for information regarding the type of cattle operation you have and the county location(s) of your operation. Questions will address any risks you believe ticks pose to you, employees, family, and cattle at your operation. Questions will also ask you about the frequency of ticks you see on your cattle, the locations on the body of the cattle, and any treatment methods used to control infestations. You may skip any questions that you do not wish to answer. You will be expected to complete the questionnaire once. It should take you about 30 minutes to complete.

If you would like to allow an investigator to contact you for clarification of answers given on the survey, you may provide a contact phone number. The follow-up is optional. It should take 15 minutes or less.

Risks: There are no risks associated with this project which are expected to be greater than those ordinarily encountered in daily life.

Benefits: This survey will allow for the creation of educational materials that address the concerns and needs cattle producers have in regards to tick infestation on their livestock. This information and the creation of the educational materials may benefit the participants of this study as well as producers across the state that did not participate in this study.

Your Rights and Confidentiality: Your participation in this research is voluntary. There is no penalty for refusal to participate, and you are free to withdraw your consent and participation in this project at any time.

Confidentiality: Do NOT provide any information other than what is indicated on the survey, this includes writing your name on the survey. The records of this study will be kept private. Any written results will discuss group findings and will not include information that will identify you. Research records will be stored on a password protected computer in a locked office and only researchers and individuals responsible for research oversight will have access to the records. Data will be destroyed three years after the study has been completed.

Contacts: You may contact any of the researchers at the following addresses and phone numbers, should you desire to discuss your participation in the study and/or request information about the results of the study:

- Bruce Noden, Ph.D., 127 Noble Research Center, Dept. of Entomology and Plant Pathology Oklahoma State University, Stillwater, OK 74078, 405-744-3225.
- Justin Talley, Ph.D., 127 Noble Research Center, Dept. of Entomology and Plant Pathology Oklahoma State University, Stillwater, OK 74078, 405-744-9420.
- Trisha Dubie, Ph.D., 127 Noble Research Center, Dept. of Entomology and Plant Pathology Oklahoma State University, Stillwater, OK 74078, trishad@okstate.edu.
- Krista Pike, B.S., 127 Noble Research Center, Dept. of Entomology and Plant Pathology Oklahoma State University, Stillwater, OK 74078, krista.pike@okstate.edu.

If you have questions about your rights as a research volunteer, you may contact the IRB Office at 223 Scott Hall, Stillwater, OK 74078, 405-744-3377 or irb@okstate.edu

If you choose to participate: Returning your completed survey to the survey administrator indicates your willingness to participate in this research study.

The purpose of this survey is to better understand your experiences with ticks at your operation and to address your concerns about ticks and their impact on your cattle. Information obtained from the survey will be used to create educational materials that address the concerns of Oklahoma beef producers in regards to tick infestations.

Participation in this survey is completely voluntary. You may answer as many or as few questions as you desire, and you may discontinue this survey at any point. Your responses will remain confidential.

1. In what county or counties do you keep your cattle? (Fill in response)

2. What type of operation(s) do you run? (Fill in response)
   a. Cow/calf
   b. Stocker
   c. Seed stock
   d. Other: ___________________

3. Do you believe ticks are a problem for CATTLE at your operation? (Circle One)
   a. Not a Problem
   b. Somewhat of a Problem
   c. A Moderate Problem
   d. Serious Problem
   e. Never Thought About It

4. Are ticks an issue of concern for YOURSELF, FAMILY, or EMPLOYEES at your operation? (Circle One)
   a. Not a Problem
   b. Somewhat of a Problem
   c. A Moderate Problem
   d. Serious Problem
   e. Never Thought About It

5. What methods do you use to protect YOURSELF from ticks? (Circle All That Apply)
   a. No protection method
   b. Check body for ticks after leaving field
   c. Clothing barrier, i.e. tucking of pant legs into shoes, wearing long sleeves/pants
   d. Chemical protection, i.e. bug spray

6. In regards to your CATTLE, what concerns you most about tick infestation? (Fill in response)

7. In regards to YOURSELF, what concerns you most about tick infestation? (Circle all that apply)
   a. Ehrlichiosis
   b. Rocky Mountain Spotted Fever
   c. Anaplasmosis
   d. West Nile Virus
   e. Other____________________________
   ____
   ____
   ____

8. In what month do you FIRST notice ticks attached to your cattle? (Circle One)

   Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sep. Oct. Nov. Dec.

   I do not have ticks on my cattle

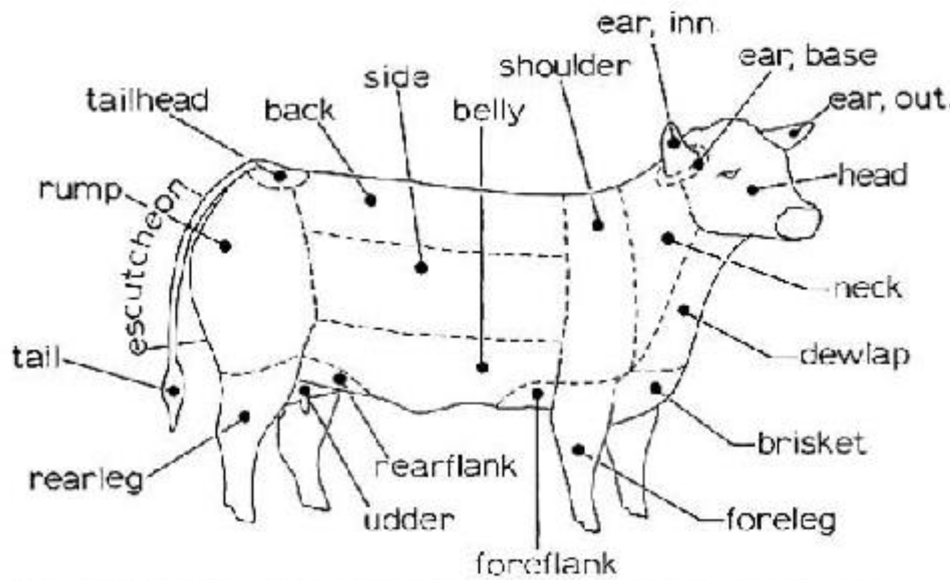
9. When ticks are at their worst, estimate how many blood fed ticks could be found on average within your herd. Picture shows unfed tick versus fed ticks. (Circle One)
   a. 1-10

4

b. 11-50
c. 51-100
d. >100
e. None, I do not have ticks on my cattle

10. Where on the body of your cattle are ticks most often seen? (Circle All That Apply)

I do not have ticks on my cattle



11. What treatment methods do you use to prevent or reduce tick infestation on your cattle?

a. Spray
b. Pour-on
c. Insecticidal Ear Tags
d. Dusts
e. Injectable Dewormer
f. Other: ________________________________ (Fill in response)

12. If a non-traditional method of tick control is utilized could you please specify what has been used in the

13. Where do you receive information about ticks infecting your cattle? (Circle Once)

a. Veterinarian
b. Extension Specialist
c. Industry Representative
d. Internet Resource
e. Other, please specify: _________________
____

14. What information would you prefer to have regarding ticks on cattle or the impact they have on human health?

15. If you would welcome the opportunity to help clarify answers to any of the questions at a later date, please provide a phone number where you can be reached. Please include Area Code.

____

VITA

Krista Danielle Pike

Candidate for the Degree of

Master of Science

Thesis: RESPONSES OF THE GULF COAST TICKS TO ODORANTS TO ENHANCE FIELD COLLECTION AND A KNOWLEDGE, ATTITUDE AND PRACTICE SURVEY OF TICKS WITH OKLAHOMA BEEF PRODUCERS

Major Field: Entomology and Plant Pathology

Education:
- Bachelor of Science in Agricultural Science and Natural Resources, Oklahoma State University, Stillwater, OK. 2014.
- Completed the requirements for the Master of Science in Entomology and Plant Pathology at Oklahoma State University, Stillwater, Oklahoma in July, 2016.

Experience:
- Teaching Assistant. Spring 2015 & Spring 2016, Oklahoma State University.
  Courses: ENTO 3044/5044 "Insect Physiology" (Spring 2015)
  ENTO 2993 "Introduction to Entomology" (Spring 2016)
- Insectary Assistant, Public Outreach, and Invited Speaker. Insect Adventure, OSU. 2011-present.
- Lab Technician. Dr. Kristen Baum, Department of Integrated Biology, OSU. 2014.

Professional Memberships: Entomological Society of America, Southwestern Branch.

Presentations (underline=presenter)**:**
- Pike, K. Noden, Responses of the Gulf Coast tick to odorants to enhance field collection. Presentation at Annual Southwest Branch ESA, Tyler, TX (Feb 2016).
- Pike, K. Noden, Responses of the Gulf Coast tick to odorants to enhance field collection. Presentation at Annual ESA, Minneapolis, MN (Nov 2015).

Awards:
- 1st place M.S. student oral competition, Southwestern Branch of the ESA (2016)