ESSENTIAL LEAF OILS OF *JUNIPERUS COMMUNIS* AS A REPELLENT OR ATTRACTANT OF *ORNITHODOROS TARTAKOVSKYI*

By Chelsea J. Hughes

Soft ticks are vectors of multiple infectious agents, and some of these infectious agents can cause disease in humans and livestock. Therefore, there is a need for a repellent that will prevent soft ticks from feeding on these organisms and transmitting these infectious agents. I placed unfed nymphs in the center of a four-way arena to provide choices among carbon dioxide, *J. communis* essential leaf oil, *J. communis* essential leaf oil and carbon dioxide, and a control blank vial to examine the potential of *Juniperus communis* essential leaf oil as a repellent against the soft tick *Ornithodoros tartakovskyi*. I used a secondary attachment assay to determine if *J. communis* essential leaf oil prevented *O. tartakovskyi* from attaching to a membrane and feeding because a substance may prevent tick-host attachment but not be a repellent. Both assays indicated that *J. communis* essential leaf oil does not repel *O. tartakovskyi* but suggested attraction. Based on these results, I tested if *J. communis* essential leaf oil would attract *O. tartakovskyi* using a two-way olfactometer. After these trials, it appears that *J. communis* essential leaf oil does not influence the behavior of *O. tartakovskyi*, despite the findings of previous studies that demonstrate that such oils repel other species of ticks. The data also suggest that *O. tartakovskyi* may not behave naturally in a two-way olfactometer when given the choice between a blank control and carbon dioxide because the nymphs were not attracted to carbon dioxide, despite the reliance of ticks on this gas for finding hosts. This study has important implications for finding appropriate natural repellents.

ESSENTIAL LEAF OILS OF *JUNIPERUS COMMUNIS* AS A REPELLENT OR ATTRACTANT OF *ORNITHODOROS TARTAKOVSKYI*

by

Chelsea J. Hughes

A Thesis Submitted
In Partial Fulfillment of the Requirements
For the Degree of

Master of Science

Biology

at

The University of Wisconsin Oshkosh
Oshkosh WI 54901-8621

May 2018

COMMITTEE APPROVAL

Advisor

11 May 2018 Date Approved

Member

11 May 2018 Date Approved

Member

11-May-2018 Date Approved

PROVOST AND
VICE CHANCELLOR

June 1, 2018
Date Approved

FORMAT APPROVAL

6/1/2018
Date Approved

ACKNOWLEDGEMENTS

I would like to thank Greg Adler, Rob Mitchell, and Steve Bentivenga for serving as advisors on my thesis committee. Without your guidance and support, this project would never have come to fruition. I would also like to thank Greg Potratz and Tom Perzentka for providing me with supplies as well as helping me assemble various components of the olfactometers. Lastly, special thanks to Steve Schaar for his input and the Michalski lab for allowing me to borrow their equipment. I am extremely grateful for the aid of all mentioned contributors.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Introduction

A Review of Tick-borne Diseases

Humans have expanded beyond their ancestral homeland in the Rift Valley of East Africa and now occupy virtually all terrestrial habitats on earth (Maslin, 2014). In doing so, they have unwittingly entered the transmission cycles of an array of infectious agents, many of which cause diseases in not only humans but also domesticated animals. The transmission cycles of many agents require blood-feeding arthropods as vectors, including flies, heteropterans, fleas, and especially ticks (Leitner et al., 2015),(Klotz et al., 2010). A large number of infectious agents are transmitted by the 899 known tick species (Dantas-Torres et al., 2012), including both hard (Ixodidae) and soft (Argasidae) ticks (Table 1).

Table 1. *Review of tick-borne diseases*

Species	**Pathogen vectored**	**Hosts**	**References**
Soft ticks			
Argas africolumbae	*Borrelia anserina*, Pretoria virus	Birds	Labuda & Nuttall (2008), Gothe et al. (1981)
Argas arboreus	*Francisella [Wolbachia] persica*	Cattle, birds	Belozerov et al. (2003), Hoogstraal (1985), Mumcuoglu et al. (2005), Noda et al. (1997), Larson et al. (2016)
Argas cooleyi	Mono Lake virus, Sixgun virus, Sapphire II virus, Sunday Canyon virus	Humans	Hoogstraal (1985), Calisher et al. (1988), Vermeil et al. (1996), Labuda & Nuttall (2008),

			de la Fuente et al. (2008)
Argas hermanni	Chenuda virus, Abu Hammad virus, Royal Farm virus, West Nile virus, Grand Arbaud virus, Nyamanini virus, Quaranfil virus	Unknown	Hoogstraal (1985), Labuda & Nuttall (2008)
Argas monolakensis	Mono Lake virus	Humans	Schwan et al. (1992), Vermeil et al. (1996), de la Fuente et al. (2008)
Argas persicus	*Aegyptianella pullorum*, *Borrelia anserina*, *Rahnella aquatilis*, *Pseudomonas fluorescens*, *Enterobacter cloacae*, *Chryseomonas luteola*, *Chryseobacterium meningosepticum*	Poultry, other birds	Keirans et al. (2001), Ntiamoa-Baidu et al. (2004), Ghosh et al. (2007), Petney et al. (2004), Gothe et al. (1981), Montasser (2005)
Argas pusillus	Issyk-Kul fever virus	Bats, humans	Labuda & Nuttall (2008), Tahmasebi et al. (2010), de la Fuente et al. (2008)
Argas reflexus	*Aegyptianella pullorum*, Uukuniemi virus, CCHF virus	Domestic poultry	Hoogstraal (1985), Gavrilovskaya (2001), de la Fuente et al. (2008)
Argas vespertilionis	Issyk-Kul Fever virus, Sokuluk virus, *Borrelia burgdorferi*	Bats	Hubbard et al. (1998), Hoogstraal (1985), Gavrilovskaya (2001), de la Fuente et al. (2008)
Carios amblus	Mono Lake virus	Humans	Labuda & Nuttall (2008), Fuente et al. (2008)
Carios capensis	Hughes virus, Soldado virus, Quaranfil virus, Saumarez reef virus, Upolu virus, Nyaminini virus	Seabirds, humans	Converse et al. (1975), Hoogstraal (1985), Labuda & Nuttall (2004, 2008)
Carios kelleyi	*Borrelia johnsoni*, Two	Unknown	Schwan et al. (2009),

	undescribed *Rickettsia* spp., *Rickettsia felis*, *Borrelia lonestari*		Loftis et al. (2005), Reeves et al. (2006)
Carios maritimus	Chenuda virus, West Nile virus	Unknown	Labuda & Nuttall (2008)
Ornithodoros amblus	Huacho virus, Punta Salinas virus	Unknown	Hoogstraal (1985)
Ornithodoros asperus	*Borrelia caucasica*, *Borrelia microti*, *Borrelia baltazardi*	Humans, rodents	Assous & Wilamowski (2009), Parola & Raoult (2001)
Ornithodoros coniceps	Baku virus	Pigeons	Hoogstraal et al. (1979)
Ornithodoros coriaceus	African swine fever virus, Bluetongue virus	Pigs	Groocock et al. (1980), Stott et al. (1985), Kleiboeker et al. (1998), Labuda & Nuttall (2004)
Ornithodoros denmarki	Hughes virus, Soldado virus, Raza virus, Quaranfil group	Humans	Labuda & Nuttall (2004), de la Fuente et al. (2008)
Ornithodoros erraticus	Qalyub virus (QYB), African swine fever virus, *Borrelia microti*, *Borrelia hispanica*, *Borrelia crocidurae, Babesia meri*	Humans, pigs	Miller et al. (1985), Labuda & Nuttall (2004, 2008), Basto et al. (2006)
Ornithodoros graingeri	*Borrellia graingeri*	Humans	Parola & Raoult (2001)
Ornithodoros hermsi	*Borrelia hermsi*	Humans	Dana (2009), Schwan et al. (2007)
Ornithodoros kohlsi	Matucare virus	Unknown	Labuda & Nuttall (2008)
Ornithodoros lagophilus	Colorado tick fever group	Humans	Sonenshine et al. (2002)
Ornithodoros	CCHF virus	Cattle	Moemenbellah- Fard et al.

lahorensis			(2009), Ahmed et al. (2007), Ghosh et al. (2007), Hoogstraal (1985), Telmadarraiy et al. (2010)
Ornithodoros maritimus	Soldado virus	Seabirds	Labuda & Nuttall (2004), de la Fuente et al. (2008)
Ornithodoros moubata	African swine fever virus, West Nile virus, HIV, Hepatitis B virus, *Borrelia duttoni*	Humans, pigs	Cutler (2006), Lawrie et al. (2004), Shepherd et al. (1989), Durden et al. (1993), Humphery-Smith et al. (1993), Jupp et al. (1987), Haresnape & Wilkinson (1989), Labuda & Nuttall (2004, 2008)
Ornithodoros parkeri	African swine fever virus, Karshi and Langat virus, *Borrelia parkeri*	Humans	Kleiboeker & Scoles (2001), Turell et al. (1994, 2004), Dana (2009), Dworkin et al. (2002)
Ornithodoros porcinus	African swine fever virus, *Borrelia duttoni*	Humans, pigs	Haresnape & Wilkinson (1989), Labuda & Nuttall (2004, 2008), Lawrie et al. (2004), Shepherd et al. (1989), Durden et al. (1993), Humphery-Smith et al. (1993), Jupp et al. (1987)
Ornithodoros puertoricensis	African swine fever virus	Reptiles	Nava et al. (2007), Venzal et al. (2006, 2008), Bermúdez et al. (2010), Endris et al. (1989)
Ornithodoros rudis	*Borrelia venezuelensis*	Humans	Parola & Raoult (2001)
Ornithodoros savignyi	African swine fever virus, AHF virus, Bluetongue virus, *Borrelia crocidurae*	Camel, sheep, goats, cows, buffalo	Ghosh et al. (2007), Gaber et al. (1984), Helmy (2000), Shanbaky & Helmy (2000)

Ornithodoros sonrai	African swine fever virus, Karshi and Langat virus, Bandia virus, *Borrelia crocidurae*, *Coxiella burnetii*	Pigs	Vial et al. (2006), Turell et al. (1994, 2004), Vial et al. (2007), Labuda & Nuttall (2008)
Ornithodoros talaje	*Borrelia mazzottii*	Rodents, humans, domestic animals	Parola & Raoult (2001)
Ornithodoros tadaridae	Estero Real virus		Málková et al. (1985), Labuda & Nuttall (2008)
Ornithodoros tartakovskyi	Karshi and Langat virus,Chim virus, *Borrelia latyschewii*	Humans, rodents	Londoño (1976), Parola & Raoult (2001), Rebaudet & Parola (2006), Turell et al. (2004)
Ornithodoros tholozani	Karshi and Langat virus, *Borrelia persica*	Humans	Labuda & Nuttall (2008), Sidi et al. (2005), Assous & Wilamowski (2009), Moemenbellah-Fard et al. (2009), Masoumi et al. (2009)
Ornithodoros turicata	African swine fever virus, *Borrelia turicatae*	Humans, pigs	Hess et al. (1987), Kleiboeker et al. (1998), Labuda & Nuttall (2004), Dana (2009)
Ornithodoros zumpti	*Borrelia tillae*	Humans	Rebaudet & Parola (2006)
Otobius lagophilus	Colorado tick fever viral group	Humans	Hoogstraal (1985)
Hard ticks			
Amblyomma americanum	*Borrelia lonstari*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Ehrlichia ruminatium*, *Rickettsia amblyommii*	Humans, dogs, white-tailed deer	de la Fuente et al. (2008), Carroll et al. (2011)
Amblyomma cajennense	*Rickettsia amblyommii*, *Rickettsia honei*, *Rickettsia rickettsii*	Humans, dogs	de la Fuente et al. (2008)

Amblyomma coelebs	*Rickettsia amblyommii*	Humans	de la Fuente et al. (2008)
Amblyomma neumanni	*Rickettsia amblyommii*	Humans	de la Fuente et al. (2008)
Amblyomma maculatum	*Hepatozoon americanum*, *Rickettsia parkeri*	Dogs, humans	de la Fuente et al. (2008)
Amblyomma triste	*Rickettsia parkeri*	Humans	de la Fuente et al. (2008)
Amblyomma variegatum	Crimean-Congo hemorrhagic fever virus, *Dermatophilus congolensis*, *Ehrlichia ruminatium*	Humans, ruminants	de la Fuente et al. (2008)
Boophilus spp.	*Babesia bigemina*, *Babesia bovis*, *Theileria equi*, *Borrelia theileri*	Cattle, buffalo, horses, mules, donkeys	de la Fuente et al. (2008)
Dermacentor spp.	Crimean-Congo hemorrhagic fever virus, *Babesia caballi*	Humans, horses, donkeys	de la Fuente et al. (2008)
Dermacentor andersoni	*Rickettsia rickettsii*	Humans, dogs	de la Fuente et al. (2008)
Dermacentor marginatus	*Babesia canis*, *Rickettsia sibirica sibirica*, *Rickettsia slovaca*	Humans, dogs	de la Fuente et al. (2008)
Dermacentor nuttalli	*Rickettsia sibirica sibirica*	Humans	de la Fuente et al. (2008)
Dermacentor reticulatus	*Babesia canis*, *Rickettsia slovaca*	Humans, dogs	de la Fuente et al. (2008)
Dermacentor silvarum	*Rickettsia sibirica sibirica*	Humans	de la Fuente et al. (2008)
Dermacentor sinicus	*Rickettsia sibirica sibirica*	Humans	de la Fuente et al. (2008)
Dermacentor	*Cytauxzoon felis*, *Ehrlichia*	Mammals	de la

variabilis	*chaffeensis*, *Rickettsia rickettsii*	(including humans)	Fuente et al. (2008)
Haemaphysalis spp.	*Babesia major*, *Babesia motasi*	Cattle	de la Fuente et al. (2008)
Haemaphysalis bispinosa	*Babesia gibsoni*	Dogs	de la Fuente et al. (2008)
Haemaphysalis leachi	*Babesia rossi*	Dogs	de la Fuente et al. (2008)
Haemaphysalis longicornis	*Babesia gibsoni*, *Rickettsia japonica,* Severe fever with thrombocytopenia syndrome virus (SFTSV)	Humans, dogs	de la Fuente et al. (2008), Zhuang et al. (2018)
Haemaphysalis novaeguineae	*Rickettsia marmionii*	Humans	de la Fuente et al. (2008)
Haemaphysalis punctata	Crimean-Congo hemorrhagic fever virus	Humans	de la Fuente et al. (2008)
Hyalomma spp.	*Babesia beliceri, Babesia occultans*, *Theileria annulata, Theileria lestoquardi, Theileria equi*, *Theileria ovis*	Cattle, sheep, goats, camels, humans, horses, mules, donkeys	de la Fuente et al. (2008)
Hyalomma marginatum	Crimean-Congo hemorrhagic fever virus	Humans	de la Fuente et al. (2008)
Ixodes spp.	*Babesia divergens*	Cattle, humans	de la Fuente et al. (2008)
Ixodes granulatus	Langat virus, *Rickettsia honei*	Humans	de la Fuente et al. (2008)
Ixodes hexagonus	*Anaplasma phagocytophilum*	Mammals (including humans)	de la Fuente et al. (2008)
Ixodes holocyclus	*Rickettsia australis*, *Rickettsia marmionii*	Humans	de la Fuente et al. (2008)
Ixodes ovatus	*Borrelia japonica*, *Rickettsia*	Humans	de la

	japonica		Fuente et al. (2008)
Ixodes scapularis	*Babesia odocoilei*, *Babesia microti, Anaplasma phagocytophilum*, *Borrelia burgdorferi*	Humans, rodents	de la Fuente et al. (2008), Ogden et al. (2006), Carroll et al. (2011)
Ixodes pacificus	*Anaplasma phagocytophilum*, *Borrelia burgdorferi, Ehrlichia equi*	Mammals (including humans)	de la Fuente et al. (2008), Richter et al. (1996)
Ixodes persulcatus	*Borrelia burgdorferi, Borrelia afzelii, Borrelia garinii*, *Borrelia miyamotoi*	Humans	de la Fuente et al. (2008), Kawabata et al. (1987)
Ixodes ricinis	*Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Borrelia afzelii*, *Borrelia garinii, Borrelia spielmani*, *Borrelia lusitaniae*, *Borrelia valaisiana*, *Rickettsia helvetica*, Eyach virus	Humans	de la Fuente et al. (2008)
Ixodes tasmani	*Rickettsia australis*	Humans	de la Fuente et al. (2008)
Ixodes vespertilionis	Issyk-Kul fever virus	Bats, humans	de la Fuente et al. (2008)
Rhipicephulus spp.	*Babesia bigemina*, Crimean-Congo hemorrhagic fever virus	Humans	de la Fuente et al. (2008)
Rhipicephulus appendiculatus	*Theileria parva*	Cattle	de la Fuente et al. (2008)
Rhipicephulus bursa	*Theileria ovis*	Sheep	de la Fuente et al. (2008)
Rhipicephulus evertsi	*Borrelia theileri*	Cattle	de la Fuente et al. (2008)
Rhipicephulus sanguineus	*Anaplasma platys*, *Babesia canis*, *Babesia gibsoni*, *Babesia vogeli*, *Ehrlichia canis*, *Rickettsia conorii conorii*, *Rickettsia conorii caspia*, *Rickettsia conorii indica*, *Rickettsia conorii*	Dogs, humans	de la Fuente et al. (2008)

	israelensis, *Rickettsia rickettsii*		
Rhipicephulus zambeziensis	*Theileria lawrencei*	Cattle	de la Fuente et al. (2008)

Tick-borne pathogens cause more than 100,000 cases of human illness each year. Mosquitos are the only organisms that vector more human pathogens than ticks (de la Fuente et al., 2008). The Centers for Disease Control recommend that repellents be applied to skin or clothing to prevent tick bites (CDC, 2002). Currently, permethrin is a pyrethroid pesticide with low mammalian toxicity that is commonly applied to clothing, but it should not be used on the skin (Schreck et al., 1982), and repeated use of such a toxicant can lead to resistance. Other acaricides, including arsenicals, chlorinated hydrocarbons, organophosphates, and carbamates, have similarly become less effective due to resistance (Foil et al., 2004). These acaricides may also contaminate animal products, including meat and milk (George et al., 2008). Accordingly, there is a profound need for natural tick repellents that are sufficiently safe to be applied to human skin and that do not promote resistance. Natural repellents are often biodegradable and are expected to cause less harm to mammals, including humans, and the environment (Carroll et al., 2011).

A potential natural repellent against ticks is an essential oil derived from the common juniper, *Juniperus communis* L. The major volatile components of the oil include isomers of linalool, α-pinene, β-pinene, α-terpineol, and limonene (Carroll et al., 2011). *Amblyomma americanum* nymphs, vectors of *Ehrlichia chaffeensis* (which causes human monocytic ehrlichiosis); (Armstrong et al., 2001), are repelled by racemates of α-terpineol and linalool and also by minor components of common juniper, including

carveol and carvone (Wheldon et al., 2011). *Juniper communis* essential leaf oil repels both *A. americanum* and *Ixodes scapularis* nymphs, the latter of which are the principal vectors of *Borrelia burgdorferi* (causes Lyme disease); (Spielman et al., 1985). It also repels the female mosquito (*Aedes aegypti*) that vectors the yellow fever virus from human volunteers with a minimum effective dose (MED) of 0.057 ± 0.013 mg/cm^2, which is nearly as effective as diethyltoluamide (DEET) (Carroll et al., 2011). Because *J. communis* essential leaf oil repels both hard ticks and mosquitos, it has the potential to prevent transmission of diseases through vector control of multiple species. However, the efficacy of common juniper essential oils as a repellent has not been tested on soft ticks.

Soft Ticks

Soft ticks (Argasidae) include 193 species (Estrada-Peña et al., 2010) and are distributed nearly worldwide. These ticks, similar to hard ticks, are able to detect odors via a sensory organ on their front legs named the Haller's organ (Sonenshine, 2014). Unlike hard ticks, however, soft ticks do not usually have a scutum or a dorsal shield. They possess small spiracular plates and coxae without spurs. Their developmental stages include egg, larva, multiple nymphal stages, and adult. Embryogenesis in the eggs takes place on ground over 8-13 d. Their nymphal stages can include from two to eight instars. Nymphal molting occurs after 8-13 d (Vial, 2009). Soft ticks also differ behaviorally from hard ticks; hard ticks engage in a behavior called questing to search for hosts. Conversely, soft ticks inhabit the burrows and nests of their hosts. They pose a risk to humans when they act as endophilic parasites, colonizing the homes of human hosts.

Offspring are often nidicolous, remaining in the burrow of their birth (Manzano-Roman et al., 2012).

Most soft ticks can live for up to 20 y, and may survive several years without taking a blood meal (Sonenshine, 1992). They nevertheless will feed multiple times as nymphs and adults, contrary to hard ticks, which feed only once each during larval, nymphal, and adult stages (Turell, 2015). Adults complete feeding within an h, with some species completing their feeding within 10-30 min. Larvae typically must feed longer, requiring twelve h to several days. Because of this short feeding period, all life stages can take blood meals without human hosts noticing their presence (Vial, 2009). Female soft ticks usually lay eggs after each bloodmeal. Soft tick clutches can range from five to five hundred eggs, and females usually produce two to five clutches over their lifetime (Vial, 2009).

Soft ticks are important to global health, as they can transmit viral, bacterial, filarial, and protozoan species (Table 1), including pathogens that cause Q-fever, spotted fever, and tick-borne relapsing fever (TBRF). Relapsing fever is caused by multiple *Borrelia* spp. and is the most common bacterial disease transmitted by soft ticks. TBRF is classified as an emerging disease and considered a health risk to many countries as an exotic pathogen carried by travelers. Laboratory tests can be used to detect spirochetes in the blood, or PCR can be used to detect a flagellin gene (Manzano-Roman et al., 2012). Chemical control of soft ticks is also particularly difficult because they are nidicolous, meaning the nymphs remain in the burrow of their parents for a large portion of their lives, and it is therefore difficult to ensure that acaricides reach the ticks in their refuges (Astigarraga et al., 1995).

Ornithodoros tartakovskyi

Most *Ornithodoros* species feed indiscriminately on hosts, increasing the chance of transmitting zoonotic diseases such as TBRF to new hosts that could function as reservoirs (Vial, 2009). *Ornithodoros tartakovskyi* (Olenev 1931) is a soft tick found in Iran, central Asia, and China (Manzano-Roman et al., 2012). They prefer temperatures of under 26°C, limiting them to their burrow microhabitats (Vial, 2009). Nymphs of this species may also feed on engorged adults, allowing the adults to transmit human pathogens to the nymphs that feed on them (Londoño, 1976). The life cycle of this tick has never been fully described; however, in the breeding colony at the University of Wisconsin Oshkosh, the following stages have been observed: egg, one larval instar, three to four nymphal instars, and adult (S. Schaar, pers. comm.).

While these ticks are found in the burrows of numerous species of rodents, their primary host is the great gerbil, *Rhombomys opimus* (Balashov, 1972), a diurnal desert rodent that lives in family groups (Randall and Rogovin, 2002). These groups typically consist of an adult male, one to six females, and juveniles and pups, although solitary females have been observed. Wild populations face numerous predators and have evolved a complex series of vocalizations and "foot-drumming" behaviors that warn other group members of the presence and type of predator (Randall et al., 2000). *R. opimus* is of medical interest because it is a natural reservoir for zoonotic cutaneous leishmaniasis (ZCL), a disease caused by *Leishmania major* (Akhavan et al., 2010) that causes 20,000 to 40,000 deaths per y (Alvar et al. 2012).

While *Ornithodoros tartakovskyi*, primarily feeds on the great gerbil, this species feeds indiscriminately and have been observed feeding on terrapins, skinks, agamas,

geckos, sand snakes, toads, hedgehogs, dogs, mice, bee-eaters, rollers, and sparrows (Vial, 2009). *Ornithodoros* spp. are strongly attracted to carbon dioxide and certain species have been recorded leaving their burrows to reach the attractant (Adeyeye and Butler, 1991). Therefore, carbon dioxide is often used to trap and collect soft ticks (Nevill, 1964). Carbon dioxide attraction has never been specifically tested in *O. tartakovskyi*, but it is expected to similarly serve as an attractant to individuals of this species.

Ornithodoros tartakovskyi itself is of medical interest because it is a vector of Karshi virus (Flaviviridae), which is a member of the mammalian tick-borne flavivirus group (M-TBFV; previously the tick-borne encephalitis virus [TBEV] serocomplex) (Turell et al., 2008). Karshi virus does not typically cause severe disease in humans; however, in Uzbekistan, outbreaks of this virus are associated with febrile illness and occasional cases of encephalitis (Turell et al., 2004). Each year, at least 10,000 clinical causes of tick-borne encephalitis are due to viruses from the mammalian tick-borne flavivirus group (Turell, 2015). Female ticks of the *O. tartakovskyi* species can also transmit this virus vertically to progeny. *Ornithodoros* spp. are particularly effective vectors, with 90% of ticks transmitting the virus to murine hosts in laboratory settings (Turell et al., 2004).

Ornithodoros tartakovskyi are long-term reservoirs and may remain infective for nearly 8 y after being exposed to Karshi virus (Turell, 2015). The transmission cycle of Karshi virus can also involve ixodid ticks as vectors, including *Ixodes ricinus* in Europe and *I. persulcatus* in Asia. Thus, *O. tartakovskyi* nymphs acting as reservoirs can infect rodent hosts that are then parasitized by ixodid ticks. Ixodids questing for hosts are more

likely to infect human hosts, but *O. tartakovskyi* nymphs acting as long-term reservoirs are equally important participants in the transmission cycle. Rodents are the primary hosts of Karshi virus, but little is known about the replication of the virus. Four d after initial exposure, viral RNA concentrations in the brain rise rapidly, peaking the sixth day after exposure. For mice that can survive the infection, viral particles can persist in the brain for at least 28 d and in the blood for 4-8 d. However, in 2 d old laboratory mice, Karshi virus is fatal within 8-12 d, while in 9 d old laboratory mice, it was rarely fatal (Turell et al., 2008).

In addition, because of the conserved biology of flaviviruses, *O. tartakovskyi* may be able to vector other members of the mammalian tick-borne flavivirus group, such as Omsk hemorrhagic fever virus, Langat virus, Alkhurma hemorrhagic fever virus, Kyasanur Forest disease virus, Powassan virus, Royal Farm virus, Karshi virus, Russian spring-summer encephalitis virus, Central European encephalitis virus, Gadgets Gully virus, and Louping ill virus (Turell, 2015). Of these, Karshi virus is most closely related to Royal Farm virus, originally being considered a subtype of that virus until it was declared a new species in 2007 (Grard et al., 2007).

Chim virus, which is a potential pathogen of camels and humans, was isolated from *O. tartakovskyi* in Uzbekistan in 1971 (L'vov et al., 2014). Five strains of this RNA arbovirus were isolated from both ixodid and argasid ticks in great gerbil burrows (L'vov et al., 1979). *Ornithodoros tartakovskyi* is also able to transmit *Borrelia latyschewii*, which causes tick-borne relapsing fever (TBRF). TBRF symptoms include recurrent fever, headache, and myalgia, and it can be fatal if not treated with antibiotics. The disease occurs most commonly in humans, but animal cases also have been documented

and are likely underreported (Manzano-Roman et al., 2012). Therefore, a natural repellent could prevent transmission of several pathogens to both humans and livestock.

This tick may also vector *Acanthocheilonema* [*Dipetalonema*] *viteae*, a species of parasitic nematode commonly found in rodents that causes filariasis (Londoño, 1976). While *A. viteae* is not considered a human parasite, it is an important laboratory model for human microfilariae, including *Wuchereria bancrofti*, *Brugia malayi* (causative agent of lymphatic filariasis), and *Onchocerca volvulus* (causative agent of river blindness and onchodermatitis) (Dell et al., 1999). Because of its importance as a laboratory model, it is important to understand all of its hosts and potential vectors, including soft ticks.

Compounds Derived from Cupressaceae as a Potential Repellent

Members of the cypress family (Cupressaceae) are used with other aromatic plants in medicinal baths among the traditional Yao communities of Jinping County, Yunnan Province, SW China (Li et al., 2006). In particular, the three genera *Juniperus*, *Cupressus*, and *Cedrus* are used commercially for their cedarwood oils (Carroll et al., 2011). Oils from other members of the cypress family repel *I. scapularis* nymphs. For instance, essential oils from the heartwood of Alaska yellow cedar (*Chamaecyparis nootkatensis*) repelled nymphs four h after application, suggesting that multiple members of Cupressaceae may have anti-tick compounds, including the possible repellents nootkatone and valencene-13-ol (Dietrich et al., 2006).

Juniperus communis is the most widespread species of conifer in the world, and its range overlaps with the range of *O. tartakovskyi* in Uzbekistan, Iran, and Xinjian Province (China) (Farijon, 2013). *Juniperus communis* is also found in North Africa, temperate Eurasia, and North America, although in North America it is only found north

of Mexico. This tree is often planted as an ornamental, particularly in Europe (Farijon, 2013). Because of its aroma, essential oils from this tree are marketed for use in baths and oil diffusers and are widely available for purchase. If *J. communis* acts as a repellent against soft ticks, which have cosmopolitan distribution (de la Fuente et al., 2008), as it does against hard ticks, it could be an ideal natural repellent against ticks from multiple parts of the world.

Chapter 2

Experimental Process

Abstract

To examine the potential of *Juniperus communis* essential leaf oil as a repellent against *Ornithodoros tartakovskyi*, unfed nymphs were placed in the center of a four-way arena and given a choice among carbon dioxide, *J. communis* essential leaf oil, *J. communis* essential leaf oil and carbon dioxide, and a control blank vial. Because a substance may prevent ticks from attaching to hosts but not be a repellent, a secondary attachment assay was used to determine if *J. communis* essential leaf oil prevented *O. tartakovskyi* from attaching to a membrane and feeding. Unfed nymphs were placed in a two-way olfactometer to examine the potential of *J. communis* essential leaf oil as an attractant of *O. tartakovskyi*, giving nymphs a choice between carbon dioxide and *J. communis* essential leaf oil and carbon dioxide.

Introduction

Ticks serve as vectors of numerous infectious agents that cause diseases in humans. Accordingly, there is a need for natural tick repellents that are sufficiently safe to be applied to human skin. Oils from the tree *Juniperus communis* repel species of hard ticks (Ixodidae), but they have not been tested against species of soft ticks (Argasidae). I selected the soft tick *Ornithodoros tartakovskyi* for this experiment due to the health threat that it poses. This tick is found in Iran, central Asia, and China and is a vector of infectious agents that may cause diseases in humans, including Chim virus, Karshi virus, *Borrelia latyschewii*, and *Acanthocheilonema viteae* (Table 1).

I tested the efficacy of *J. communis* essential leaf oil as a repellent against *O. tartakovskyi* by measuring the response of starved nymphs to the known host cue carbon dioxide, *J. communis* essential leaf oil, a combination of both, and a control blank vial. I hypothesized that undiluted *J. communis* essential leaf oil would repel *O. tartakovskyi* nymphs and that carbon dioxide would attract *O. tartakovskyi* nymphs. I also hypothesized that undiluted *J. communis* essential leaf oil would repel *O. tartakovskyi* nymphs even in the presence of carbon dioxide.

Materials and Methods

Resources.

All nymphs for the experiments were obtained from the breeding colony at the University of Wisconsin Oshkosh. The ticks were hatched and raised in incubators with a set day and night lights schedule (light conditions from 7AM-7PM and dark conditions from 7PM-7AM). Ticks were fed only defibrinated rabbit blood during their development. It is unknown which instar the nymphs were, but they had been fed three times prior to experimentation, meaning that they were between first and third instar (S. Schaar, pers. comm.).

Arena Preliminary Assay.

To test the efficacy of *J. communis* essential leaf oil as a repellent to O. *tartakovskyi*, I designed an experiment similar to that conducted by Carroll et al. (2011). The original experiment used a vertical filter paper bioassay, but this was not appropriate here because it relies on questing behavior, which is not exhibited in soft ticks.

Instead, I constructed a four-way arena. This arena had a flat surface, allowing *O. tartakovskyi* to move horizontally across the floor, as they would under natural conditions. I chose a four-way arena instead of a two-way olfactometer because repellent and attractant can mix in the central tubing of a two-way olfactometer; if the oil acted as a repellent, it would prevent the ticks from moving through the central tubing to make a choice. Using such an arena also allowed me to test for putative juniper repellency in the presence of the attractant carbon dioxide.

In the original experiment conducted by Carroll et al. (2011), hard ticks were tested in open-air environments, but changing air concentrations could have interfered with the experiment. Furthermore, no attractant was used in the original experiment. Adding carbon dioxide as an attractant to the experimental design allowed me to not only test whether *J. communis* essential leaf oil repelled ticks but if it could repel ticks even in the presence of an attractant.

Originally, I planned to use a four-way olfactometer, which would have prevented changing air concentrations because it is a closed system. Unfortunately, due to the design of the olfactometer, air could not be filtered out of the closed system. This design flaw caused carbon dioxide to accumulate in the olfactometer and rendered the nymphs unconscious. To prevent carbon dioxide from accumulating in the main chamber of the olfactometer, the experiment became open-air, meaning the arena was not entirely enclosed and therefore shared air with the external environment. Thus, the olfactometer became an arena test because it was not a closed system. However, changes in air concentrations were minimized by allowing only a single, stationary experimenter in the room during experimentation.

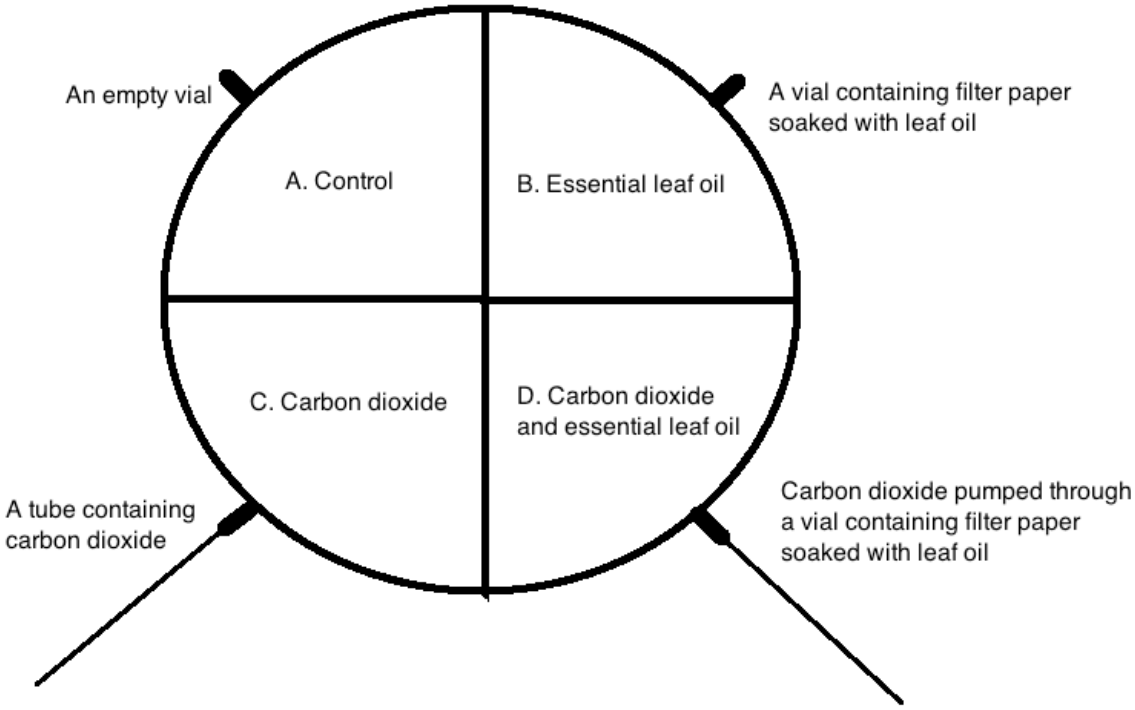

Figure 1. Arena setup

Undiluted *J. communis* essential leaf oil was used. In the original experiment conducted by Carroll et al., the concentration of leaf oil was weighed on filter paper (0.01 g oil/cm^2). In my experiment, however, the concentration of leaf oil was 0.1g/cm^2. In quadrant A, an empty vial was attached to the main chamber. This vial acted as a control. In quadrant B, a vial containing filter paper soaked with leaf oil was attached to the main chamber. In quadrant C, a tube containing carbon dioxide was attached to the main chamber. In quadrant D, carbon dioxide was pumped through a vial containing filter paper soaked with leaf oil. Quadrants A and B did not have an airstream passing through them. The four-way arena was surrounded by two double-sided tape barriers as a safety

precaution, thereby ensuring that nymphs could not escape from the arena during transfer to and from the main chamber. Carbon dioxide entered the chamber at 0.02 psi.

Third instar nymphs were starved from 26 October 2016 to March 2017 prior to experimentation to encourage them to actively seek food during the experiment. Nymphs were placed in the center of the main chamber and left unmolested for 15 min. After 15 min, their positions were recorded as center, quadrant A, B, C, or D. The central steel chamber of the olfactometer was washed with acetone between each trial to ensure that no residual phenols remained from the *J. communis* essential leaf oil. Three separate trials were conducted. Because so many nymphs chose quadrant D, I tested whether *J. communis* essential leaf oil was an attractant for *O. tartakovskyi.*

Attachment Preliminary Assay.

A repellent may not induce avoidance in its intended tick target, but it may prevent attachment to the surface when the tick attempts to feed. I developed the following attachment assay to test whether *J. communis* essential leaf oil inhibited the feeding behavior of *O. tartakovskyi.* Third instar nymphs were starved from December 2016 to April 2017 prior to experimentation to encourage them to attach and feed. To create a feeding membrane similar to mammal skin, the lid of a 5 mm x 10 mm sterile polystyrene Petri dish served as a feeding dish. Each lid was filled to the top with defibrinated rabbit blood and completely covered with parafilm. *J. communis* essential leaf oil was applied to the parafilm surface in 3 experimental trials testing 0%, 10%, and 30% concentrations diluted with acetone (pure oil could not be applied without destroying the membrane). Each trial was accompanied by controls with unaltered parafilm. Blood dishes were heated in an incubator at 37 °C for 30 min and subsequently

placed on a heated shaker. Ticks were placed on the surface of the parafilm and given 40 min to feed, after which I recorded the number of fed and unfed ticks based on visual appearance.

Two-Way Olfactometer Assay with *J. communis.*

Data from the arena assay suggested that *J. communis* essential leaf oil might attract nymphs. To test this hypothesis, I designed a two-way olfactometer assay. Air passed through a charcoal filter in a fume hood into two streams that were regulated by a flow meter to 0.5L/min. Each air stream then passed through an impinger that contained a cotton roll (control) or a cotton roll soaked in 0.1 g *J. communis* essential leaf oil. The airstreams then entered opposing arms of a glass olfactometer.

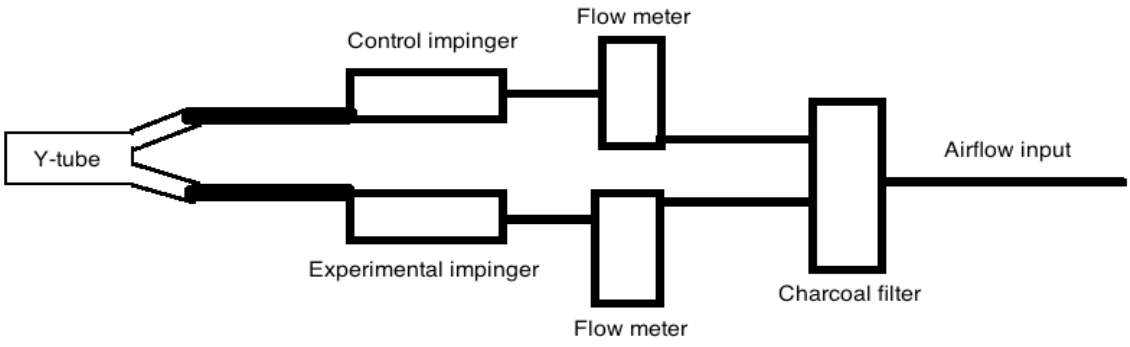

Figure 2. Two-way olfactometer setup

Nymphs were starved from 24 January to 8 August 2017 so that they would actively seek food. A single nymph was placed in the base of the olfactometer for each trial, with special care given to ensure that no tick was on its dorsum. The three openings of the Y tube were secured with mesh to prevent nymphs from exiting the tube. The entire two-way olfactometer was additionally surrounded by two-sided tape, which acted as a secondary containment barrier. Nymphs were given 5 min to make their choice. After every five trials, the olfactometer was flipped to avoid locational effects. The assay was repeated with 19 nymphs, and a Fisher's exact P-test was used to determine if the nymphs preferentially traveled toward the *J. communis* essential leaf oil.

Two-Way Olfactometer Assay with *J. communis* and Carbon Dioxide.

This assay was identical to the above assay except carbon dioxide was added to the control and experimental air streams by introducing a few grams of dry ice immediately downstream from the charcoal filter. Nymphs were starved from 24 January to 9 August 2017 to increase the probability that they would actively seek food.

Two-Way Olfactometer Assay with Carbon Dioxide Under Different Light Conditions.

This assay was identical to the above assay but at different times of day to compensate for the activity of the adult ticks, which may be nocturnal or negatively phototaxic (Sonenshine 2014). This assay was run between 6 PM-8PM. All other assays were run between 11 AM-5 PM.

Data Analysis.

I used loglinear analysis of categorical data to determine if the nymphs preferentially travelled toward a particular quadrant in the arena preliminary assay.

Loglinear analysis was used instead of Chi test because it is more statistically powerfully. I applied linear analysis of categorical data to determine if nymphal feeding differed based on the concentration of juniper essential leaf oil in the attachment preliminary assay. I constructed separate loglinear models for categorical data to test if nymphs preferentially traveled in the two-way olfactometer assay either toward or away from 1) *J. communis* essential leaf oil and 2) *J. communis* essential leaf oil and carbon dioxide compared to only carbon dioxide. I constructed a linear model for categorical data to determine if the concentration of juniper influenced nymphal behavior under light and dark conditions. SAS Version 9.2 (SAS Institute, Cary, NC) was used for all analyses. For all assays, each nymph was an experimental unit.

Results

Arena Preliminary Assay.

The loglinear analysis revealed that few ticks traveled to quadrants B and C, while most traveled toward quadrant D or remained in the center (Table 2; χ^2=42.76, P<0.0001, df=4).

Table 2. *The effect of J. communis essential leaf oil on O. tartakovskyi in a four-way arena*

	Quadrant A (Blank control)	Quadrant B (leaf oil)	Quadrant C (carbon dioxide)	Quadrant D (leaf oil and carbon dioxide)	Center
Total ticks	15	1	5	34	45

N=3. Significant.

Attachment Preliminary Assay.

Concentration had no effect on nymphal feeding behavior (Table 3; χ^2=4.34, P=0.1143, df=2).

Table 3. *The effect of J. communis essential leaf oil on O. tartakovskyi nymph feeding*

% Oil	Fed	Did Not Feed
0.00	27	7
10.00	31	2
30.00	31	2
Total ticks	89	11

N=3. Not significant.

Two-Way Olfactometer Assay with *J. communis.*

Most ticks demonstrated no response to the essential oil in the two-way olfactometer (Table 4; χ^2=10.16, P=0.0062, df=2).

Table 4. *The effect of J. communis essential leaf oil on O. tartakovskyi in a two-way olfactometer*

	Juniper	Control	Did Not Choose
Total	1	4	14

N=19. Significant.

Two-Way Olfactometer Assay with *J. communis* and Carbon Dioxide

Ticks again demonstrated no response to leaf oil when carbon dioxide was included (Table 5; χ^2=8.69, P=0.0032, df=2).

Table 5. *The effect of J. communis essential leaf oil and carbon dioxide on O. tartakovskyi in a two-way olfactometer*

	Juniper + Carbon Dioxide	Control	Did Not Choose
Total	2	0	18

N=20. Significant.

Two-Way Olfactometer Assay with Carbon Dioxide under Different Light Conditions.

Finally, the ambient light level did not influence tick choice (Table 6; χ^2=1.13, P=0.5681).

Table 6. *The effect of carbon dioxide on O. tartakovskyi in a two-way olfactometer under two ambient light conditions*

	Carbon Dioxide	Control	Did Not Choose
Light	5	3	17
Dark	3	5	12

N=25, 20. Not significant.

Discussion.

There is a profound need for natural tick repellents that are sufficiently safe to be applied to human skin. I tested *Juniperus communis* essential leaf oil as a repellent and as an attractant for *Ornithodoros tartakovskyi*. I also tested whether *J. communis* essential leaf oil inhibited *O. tartakovskyi* from feeding in an attachment assay. Based on the attachment assay, *J. communis* essential leaf oil does not inhibit *O. tartakovskyi* nymphs from feeding, thereby rendering this leaf oil an ineffective repellent. Although the data from the four-way arena preliminary assay suggested that *J. communis* essential leaf oil might be an attractant, the remaining assays did not support this observation. Therefore, my hypothesis that undiluted *J. communis* essential leaf oil would repel *O. tartakovskyi* nymphs even in the presence of carbon dioxide was not supported by my data. My

hypothesis that undiluted *J. communis* essential leaf oil would repel *O. tartakovskyi* nymphs and that carbon dioxide would attract *O. tartakovskyi* nymphs was also not supported by my data.

Chapter 3

Conclusion

Prior to this experiment, *J. communis* essential leaf oil was untested as a repellent against any *Ornithodoros* species. After these trials, it appears that *J. communis* essential leaf oil does not influence the behavior of *O. tartakovskyi*. However, these experiments also demonstrate that *O. tartakovskyi* may not behave naturally in a two-way olfactometer. In my experiments, when given the choice between a blank control and carbon dioxide, the nymphs were not attracted to the carbon dioxide. This was unexpected, as soft tick species are attracted to carbon dioxide (Sonenshine, 2014). While no documentation was found of carbon dioxide being tested directly on *O. tartakovskyi*, it is unlikely that this species would deviate from the behavior of all other soft ticks that have been tested. Furthermore, during handling, the ticks were drawn toward apparent human exudates while in the breeding colony at the University of Wisconsin Oshkosh and during experimentation, suggesting that they were attracted to human carbon dioxide exhalation. Thus, it is likely that these ticks do not behave normally in the artificial environment of the olfactometer.

The four-way arena experiment that I conducted should be repeated in the future in a four-way olfactometer instead of an open-air arena, in order to minimize changes in the concentration of odors. The experimental setup of the four-way arena assay had several additional shortcomings: quadrants were not alternated to avoid locational effects, and air streams were not regulated by flow meters. Since quadrants C and D had airflow, quadrants A and B should have had outside air pumped through them to ensure that all

odors reached the center chamber equally. Because the four-way arena assay was only a preliminary assay, it was not deemed necessary to redo the trials. However, any future arena or four-way olfactometer trials should be amended to correct these shortcomings.

Additionally, future arena or four-way olfactometer trials should be conducted over several h to determine the effect of *J. communis* essential leaf oil on *O. tartakovskyi* over time. A repellent is less marketable if the user must reapply it frequently. I originally planned to conduct the four-way arena assay over several h, but nymphs would quickly climb from the arena and become entangled in the containment tape.

The attachment assay that I conducted should be repeated in the future with a parafilm covered in only undiluted acetone. *J. communis* essential leaf oil was applied to the parafilm surface in 3 experimental trials testing 0%, 10%, and 30% concentrations diluted with acetone, but the effect of acetone itself on the feeding of *O. tartakovskyi* was not tested.

Ornithodoros tartakovskyi was chosen for this experiment due to accessibility of this tick species as well as the health threat that it poses. However, this experiment could be expanded to include other tick species within the family Argasidae. Many species of soft ticks vector pathogens that cause Q-fever, spotted fever, and TBRF (Manzano-Roman et al., 2012). In the future, a follow up study should be conducted to test whether any additional argasids or adult *O. tartakovskyi* are influenced by *J. communis* essential leaf oil. Other species besides *J. communis* within the family Cupressaceae also may have repellent properties. *Juniperus chinensis* and *Cupressus funebris* wood oils repelled nymphs of both *A. americanum* and *I. scapularis* (Carroll et al., 2011). These oils also should be tested against *O. tartakovskyi* and other argasids.

There is also some concern that *J. communis* essential leaf oil might irritate human skin. During experimentation, undiluted *J. communis* essential leaf oil destroyed the parafilm membrane, suggesting that it may be harmful to human skin if not diluted. Further testing needs to be conducted to determine if *J. communis* essential leaf oil is safe to use on human skin in the concentrations necessary to repel mosquitoes and hard ticks.

While it is true that *O. tartakovskyi* can be endophilic and can thereby directly transmit pathogens to humans, soft ticks are not the main vector of these pathogens. Hard ticks pose a greater direct risk to humans because of their questing behavior. Therefore, testing potential cupressaceous oil repellents against hard ticks would be more prudent in the future. From these trials, however, it is clear that using *J. communis* essential leaf oil as a repellent against hard ticks would not endanger the users by attracting soft ticks in areas where both types of ticks are syntopic. Finally, despite the potential shortcomings of the experimental design, improvements to the design are unlikely to alter my overall finding that juniper essential leaf oil has little value in repelling soft ticks.

References

Adeyeye, OA, and JF Butler. (1991). "Field Evaluation of Carbon Dioxide Baits for Sampling *Ornithodoros turicata* (Acari: Argasidae) in Gopher Tortoise Burrows." *Journal of Medical Entomology* 28.1: 45-48.

Ahmed, J, H Alp, M Aksin, & U Seitzer. (2007). Current status of ticks in Asia. *Parasitology Research*, Vol. 101, No. 2, (September 2007), pp. 159-162, ISSN 0932-0113.

Akhavan, A, M Yaghoobi-Ershadi, A Khamesipour, et al. (2010). Dynamics of *Leishmania* infection rates in *Rhombomys opimus* (Rodentia: Gerbillinae) population of an endemic focus of zoonotic cutaneous leishmaniasis in Iran. *Bull. Soc. Pathol. Exot.*103: 84-89.

Alvar, J, et al. (2012)"Leishmaniasis Worldwide and Global Estimates of Its Incidence." *PLoS ONE.* 7(5): e35671

Armstrong, PM, LR Brunet, A Spielman, and SR Telford III. (2001). "Risk of Lyme disease: perceptions of residents of a lone star tick-infested community." *Bull. Wld. Hlth. Org.* 79: 916–925.

Assous, MV & A Wilamowski. (2009). Relapsing fever borreliosis in eurasia--forgotten, but certainly not gone! *Clinical Microbiology and Infection*, Vol. 15, No. 5, (May 2009), pp. 407-414, ISSN 1198-743X.

Astigarraga, A, A Oleaga-Pérez, R Pérez-Sánchez, & A Encinas-Grandes. (1995). A study of the vaccinal value of various extracts of concealed antigens and salivary gland extracts against Ornithodoros erraticus and Ornithodoros moubata. *Veterinary Parasitology*, Vol. 60, No. 1-2, (November 1995), pp. 133-147, ISSN 0304-4017.

Ataliba, AC, JS Resende, N Yoshinari, & MB Labruna.(2007). Isolation and molecular characterization of a Brazilian strain of *Borrelia anserina*, the agent of fowl spirochaetosis. *Research in Veterinary Science*, Vol. 83, No. 2, (October 2007), 145-149, ISSN 0034-5288.

Aydin, L & S Bakirci. (2007). Geographical distribution of ticks in Turkey. *Parasitology Research.* Vol. 101, No. 2, (September 2007), pp. 163-166, ISSN 0932-0113.

Balashov, YS (1972). "Geographic variability of *Ornithodoros tartakovskyi*. (Ixodoidea, Argasidae)." *Entomol Rev* 51: 439–448 Soft Ticks as Pathogen Vectors: Distribution,Surveillance and Control 147 patients with relapsing fever in Spain. *Lancet*, Vol. 348, No. 9021, (July 1996), pp.162-165, ISSN 0140-6736.

Baranda, JA, R Pérez-Sánchez, A Oleaga-Pérez, & A Encinas-Grandes. (1997). Antigens of interest for the diagnosis of parasitism in pigs by *Ornithodoros erraticus* and *Ornithodoros moubata*. *The Journal of Parasitology*. Vol. 83, No. 5, (October 1997), pp. 831-838, ISSN 0022-3395.

Baranda, JA, R Pérez-Sánchez, A Oleaga, R Manzano, & A Encinas-Grandes. (2000). Purification, N-terminal sequencing and diagnostic value of the major antigens of *Ornithodoros erraticus* and *O. moubata*. *Veterinary Parasitology*, Vol. 87, No. 1-2, (January 2000), pp. 193-206, ISSN 0304-4017.

Barbour, AG & SF Hayes. (1986). Biology of Borrelia species. *Microbiol rev*, Vol. 50, No. 4 (December 1986) pp. 381-400. ISSN 0146-0749

Barros-Battesti, DM, GA Landulfo, VC Onofrio, JL Faccini, A Marcili, FA Nieri-Bastos, JM Venzal, & MB Labruna. (2011). *Carios mimon* (Acari: Argasidae): description of adults and redescription of larva. *Exp Appl Acarol*. Vol. 54, No. 1, (May 2011), pp. 93-104, ISSN 0168-8162.

Basto, AP, RJ Nix, F Boinas, S Mendes, MJ Silva, C Cartaxeiro, RS Portugal, A Leitão, LK Dixon, & C Martins. (2006). Kinetics of African swine fever virus infection in *Ornithodoros erraticus* ticks. *Journal of General Virology.* Vol. 87, No. 7, (July 2006), pp. 1863-1871, ISSN 0022-1317.

Belozerov, VN, DJ Van Niekerk, & HJ Butler. (2003). Population structure of *Argas arboreus* (Acari: Argasidae) ticks associated with seasonally abandoned mixed heronries, dominated by cattle egrets (*Bubulcus ibis*), in South Africa. *Onderstepoort Journal of Veterinary Research*. Vol. 70, No. 4, (December 2003), pp. 325-30, ISSN 0030-2465.

Bermúdez, SE, RJ Miranda, & D Smith. (2010). Ticks species (Ixodida) in the summit municipal park and adjacent areas, Panama City, Panama. *Experimental Applied Acarology*, Vol. 52, No. 4, (December 2010), pp. 439-448, ISSN 0168-8162.

Calisher, CH, TG Schwan, JS Lazuick, RB Eads, & DB Francy. (1988). Isolation of Mono Lake virus (family Reoviridae, genus Orbivirus, Kemerovo serogroup) from *Argas cooleyi* (Acari: Argasidae) collected in Colorado. J*ournal of Medical Entomology* Vol. 25, No. 5, (September 1988), pp. 388-390, ISSN 0022-2585.

Carroll, JF, N Tabanca, M Kramer, NM Elejalde, DE Wedge, UR Bernier, M Coy, JJ Becnel, B Demirci, KHC Baser, J Zhang, and S Zhang. (2011). "Essential Oils of *Cupressus funebris*, *Juniperus communis*, and J. *chinensis* (Cupressaceae) as Repellents against Ticks (Acari: Ixodidae) and Mosquitoes (Diptera: Culicidae) and as Toxicants against Mosquitoes." *Journal of Vector Ecology* 36.2: 258-68.

CDC. (2002). Lyme disease. Department of Health and Human Services, Centers for Disease Control and Prevention, Ft. Collins, CO, 12 pp.

Converse, JD, H Hoogstraal, MI Moussa, CJ Feare, & MN Kaiser. (1975). Soldado virus (Hughes group) from O*rnithodoros (alectorobius) capensis* (Ixodoidea: Argasidae) infesting sooty tern colonies in the Seychelles, Indian Ocean. *American Journal of Tropical Medicine and Hygiene*, Vol. 24, No. 6(pt 1), (November 1975), pp. 1010-1018, ISSN 0002-9637.

Dana, AN. (2009). Diagnosis and treatment of tick infestation and tick-borne diseases with cutaneous manifestations. *Dermatologic Therapy*, Vol. 22, No.4, (July 2009), pp. 293- 326, ISSN 1396-0296.

Dantas-Torres F, BB Chomel, D Otranto. (2012) "Ticks and tick-borne diseases: a one health perspective." *Trends Parasitol*. 28:437–46

De la Fuente, J, A Estrada-Peña, JM Venzal, KM Kocan, & DE Sonenshine. (2008). Overview: ticks as vectors of pathogens that cause disease in humans and animals. Frontiers in Bioscience, Vol.1, No. 13, (May 2008), pp. 6938-6946, ISSN 1093-9946

Dell, et al. (1999). "Immunogenic Glycoconjugates Implicated in Parasitic Nematode Diseases." *Biochimica Et Biophysica Acta (BBA) - Molecular Basis of Disease*. 1455.2-3:353–362.

Dietrich, G, MC Dolan, J Peralta-Cruz, J Schmidt, J Piesman, RJ Eisen, and JJ Karchesy. (2006). "Repellent activity of fractioned compounds from *Chamaecyparis nootkatensis* essential oil against nymphal *Ixodes scapularis* (Acari: Ixodidae)." *J. Med. Entomol.* 43: 957–961.

Durden, LA, TM Logan, ML Wilson, & KJ Linthicum. (1993). Experimental vector incompetence of a soft tick, *Ornithodoros sonrai* (Acari: Argasidae), for Crimean-Congo hemorrhagic fever virus. *Journal of Medical Entomology*, Vol. 30, No. 2, (March 1993), pp. 493-496, ISSN 0022-2585

Dworkin, MS, TG Schwan, & DE Jr Anderson. (2002). Tick-borne relapsing fever in North America.The Medical Clinics of North America, Vol. 86, No. 2, (March 2002), pp. 417-33, ISSN 0025-7125

Endris, RG, JE Keirans, RG Robbins, & WR Hess. (1989). *Ornithodoros (Alectorobius) puertoricensis* (Acari: Argasidae): redescription by scanning electron microscopy. *Journal of Medical Entomology* Vol. 26, No. 3, (May 1989), pp. 146-154, ISSN 0022-2585.

Estrada-Peña, A, AJ Mangold, S Nava, JM Venzal, MB Labruna, and AA Guglielmone. (2010). A review of the systematics of the tick family arasidae (Ixodida). *Acarologia*, 50.3:317–333

Farjon, A. (2013). *Juniperus communis*. The IUCN Red List of Threatened Species 2013: e.T42229A2963096.

Foil, LD, P Coleman, M Eisler, H Fragoso-Sanchez, Z Garcia-Vazquez, FD Guerrero, NN Jonsson, IG Langstaff, AY Li, N Machila, RJ Miller, J Morton, JH Pruett, & S Torr. (2004). Factors that influence the prevalence of acaricide resistance and tick-borne diseases. Veterinary Parasitology, Vol. 125, No.1-2, (October 2004), pp. 163-181, ISSN 0304-4017.

Gaber, MS, GM Khalil, H Hoogstraal, & AE Aboul-Nasr. (1984). *Borrelia crocidurae* localization and transmission in *Ornithodoros erraticus* and *O. savignyi*. *Parasitology*, Vol. 88, No.3, (June 1984), pp. 403-413, ISSN 0031-1820.

Gavrilovskaya, IN. (2001). Issyk-Kul virus disease, In: The encyclopedia of arthropod-transmitted infections of man and domesticated animals. Cabi Publishing, pp. 231-234, ISBN-13:978-0851994734, NY.

George, JE, JM Pound & RB Davey. (2008). Acaricides for controlling ticks on cattle and the problem of acaricides resistance. In: Ticks: biology, disease and control. Alan S.Bowman & Pat Nuttall (Eds.), pp. 408-423,Cambridge University Press, ISBN 978-0-521-86761-0, Cambridge, UK.

Ghosh, S, GC Bansal, SC Gupta, D Ray, MQ Khan, H Irshad, M Shahiduzzaman, U Seitzer, & JS Ahmed. (2007). Status of tick distribution in Bangladesh, India and Pakistan. Parasitology Research Vol. 101, No. 2, (September 2007), pp. 207-216, ISSN 0932-0113

Gothe, R, C Buchheim & W Schrecke. (1981). *Argas (Persicargas) persicus* and *Argas (Argas) africolumbae* as natural vectors of *Borrelia anserina* and *Aegyptianella pullorum* in upper Volta. *Berliner und Munchener Tierarztliche Wochenschrift*, Vol. 94, No. 14, (July 1981), pp. 280-285, ISSN 0005-9366.

Grard G, G Moureau, RN Charrel, JJ Lemasson, JP Gonzalez, P Gallian, TS Gritsun, EC Holmes, EA Gould, X de Lamballerie. (2007). Genetic characterization of tick-borne flaviviruses: new insights into evolution, pathogenetic determinants and taxonomy. *Virology* 361:80–92.

Groocock, CM, WR Hess & WJ Gladney. (1980). Experimental transmission of African swine fever virus by *Ornithodoros coriaceus*, an argasid tick indigenous to the United States. *American Journal of Veterinary Research*, Vol.41, No. 4, (April 1980), pp. 591-594, ISSN 0002-9645.

Haresnape, JM & PJ Wilkinson. (1989). A study of African swine fever virus infected ticks (*Ornithodoros moubata*) collected from three villages in the asf enzootic area of Malawi following an outbreak of the disease in domestic pigs. *Epidemiology and Infection*, Vol. 102, No. 3, (June 1989), pp. 507-522, ISSN 0950-2688.

Helmy, N. (2000). Seasonal abundance of *Ornithodoros (O.) savigny*i and prevalence of infection with *Borrelia* spirochetes in Egypt. *Journal of the Egyptian Society of Parasitology*, Vol. 30, No. 2, (August 2000), pp. 607-619, ISSN 0253-5890.

Hess, WR, RG Endris, TM Haslett, MJ Monahan, & JP Mccoy. (1987). Potential arthropod vectors of African swine fever virus in North America and the Caribbean basin. *Veterinary Parasitology*, Vol. 26, No. 1-2, (December 1987), pp. 145-155, ISSN 0304-4017.

Hoogstraal, H. (1985). Argasid and Nuttalliellid ticks as parasites and vectors. *Advances in Parasitology*, Vol. 24, pp. 135-238, ISSN 0065-308X.

Hubbard, MJ, AS Baker, & KJ Cann. (1998). Distribution of Borrelia burgdorferi s.l. spirochaete DNA in British ticks (Argasidae and Ixodidae) since the 19th century, assessed by PCR. *Medical and Veterinary Entomology*, Vol. 12, No. 1, (January 1998), pp. 89-97, ISSN 0269-283X

Humphery-Smith, I, G Donker, A Turzo, C Chastel, & H Schmidt-Mayerova. (1993). Evaluation of mechanical transmission of HIV by the African soft tick, *Ornithodoros moubata*. *AIDS*. Vol. 7, No. 3, (March 1993), pp. 341-347, ISSN 0269-9370.

Jupp, PG, JJ Joubert, AJ Cornel, C Swanevelder, & OW Prozesky. (1987). An experimental assessment of the tampan tick *Ornithodoros moubata* as vector of hepatitis B virus. *Medical Veterinary Entomology*, Vol. 1, No. 4, (October 1987), pp. 361-368, ISSN 0269-283X.

Kawabata, M, et al. (1987) "Lyme Disease in Japan and Its Possible Incriminated Tick Vector, Ixodes Persulcatus." *Journal of Infectious Diseases*. 156(5): 854–854

Keirans, JE & LA Durden. (2001). Invasion: exotic ticks (Acari: Argasidae, Ixodidae) imported into the United States. A review and new records. Journal of Medical Entomology, Vol. 38, No. 6, (November 2001), pp. 850-861, ISSN 0022-2585.

Kleiboeker, SB, TG Burrage, GA Scoles, D Fish, & DL Rock. (1998). African swine fever virus infection in the argasid host, *Ornithodoros porcinus porcinus*.*Journal of Virology*,Vol. 72, No. 3, (March 1998), pp. 1711-1724, ISSN 0022-538X

Kleiboeker, SB & GA Scoles. (2001). Pathogenesis of African swine fever virus in Ornithodoros ticks. *Animal Health Research Reviews*, Vol. 2, No.2, (December 2001), pp. 121-128, ISSN 1466-2523.

Klotz, JH, et al. (2010) "Kissing Bugs: Potential Disease Vectors and Cause of Anaphylaxis." *Clinical Infectious Diseases*. 50(12):1629–1634

Labuda, M & PA Nuttall. (2004). Tick-borne viruses. Parasitology. Vol. 129, pp. 221-245, ISSN 0031-1820.

Labuda, M & PA Nuttall. (2008). Viruses transmitted by ticks. In:Ticks: biology, disease and control. Alan S. Bowman & Pat Nuttall (Eds.), pp. 253-280, Cambridge University Press, ISBN 978-0-521-86761-0,Cambridge. UK.

Larson, MA, et al. (2016). "Reclassification of Wolbachia Persica as Francisella Persica Comb. Nov. and Emended Description of the Family Francisellaceae." *International Journal of Systematic and Evolutionary Microbiology*, vol. 66, no. 3 pp. 1200–1205

Lawrie, CH, NY Uzcátegui, EA Gould, & PA Nuttall. (2004). Ixodid and Argasid tick species and West Nile Virus. *Emerging Infectious Diseases*, Vol. 10, No. 4, (April 2004), pp. 653-657, ISSN 1080-6040.

Leitner, WW, et al. (2015) "Arthropod Vectors and Disease Transmission: Translational Aspects." *PLOS Neglected Tropical Diseases*. 9:11

Li, S, C Long, F Liu, S Lee, Q Guo, R Li, and Y Liu. (2006). "Herbs for medicinal baths among the traditional Yao communities of China." *J. Ethnopharmacol.* 108: 59–67.

Loftis, AD, JS Gill, ME Schriefer, ML Levin, ME Eremeeva, MJ Gilchrist, & GA Dasch. (2005). Detection of *Rickettsia*, *Borrelia*, and *Bartonella* in *Carios kelleyi* (Acari:Argasidae). J*ournal of Medical Entomology*, Vol. 42, No. 2, (May 2005), pp. 473-480, ISSSN 0022-2585.

Londoño, I. (1976). "Transmission of microfilariae and infective larvae of *Dipetalonema viteae* (Filarioidea) among vector ticks, *Ornithodoros tartakowskyi* (Argasidae), and loss of microfilariae in coxal fluid." *Journal of Parasitology* 62.5:786-788.

L'vov, DK, SV Al'khovskii , MIu Shchelkanov, AM Shchetinin, VA Aristova, TN Morozova, AK Gitel'man, PG Deriabin, AG Botikov. (2014). Taxonomic status of the Chim virus (CHIMV) (Bunyaviridae, Nairo-virus, Qalyub group) isolated from the Ixodidae and Argasidae ticks collected in the great gerbil (*Rhombomys opimus* Lichtenstein, 1823) (Muridae, Gerbillinae) burrows in Uzbekistan and Kazakhstan. Vopr Virusol. 59: 18–23.

L'vov, DK, GA Sidorova, VL Gromashevskiĭ, TM Skvortsova, LK Berezina. (1979). Chim virus, a new arbovirus isolated from ixodid and argasid ticks collected in the burrows of great gerbils on the territory of the Uzbek SSR. Vopr Virusol. 3:286-9

Málková, D, J Holubová, V Cerný, M Daniel, A Fernández, J de la Cruz, M Herrera & CH Calisher. (1985). Estero real virus: a new virus isolated from argasid ticks *Ornithodoros tadaridae* in Cuba. *Acta Virologica*, Vol. 29, No. 3, (May 1985), pp. 247-250, ISSN 001-723X.

Manzano-Roman, R, V Diaz-Martin, J De La Fuente, and R Perez-Sanchez. (2012). "Soft Ticks as Pathogen Vectors: Distribution, Surveillance and Control." *Parasitology*

Maslin, MA, et al. (2014) “East African Climate Pulses and Early Human Evolution.” *Quaternary Science Reviews*. 101: 1–17

Masoumi, Asl H, MM Goya, H Vatandoost, SM Zahraei, M Mafi, M Asmar, N Piazak & Z Aghighi. (2009). The epidemiology of tick-borne relapsing fever in Iran during 1997-2006. *Travel Medicine and Infectious Disease*, Vol. 7, No. 3, (May 2009), pp. 160-164, ISSN 1477-8939

Miller, BR, R Loomis, A Dejean & H Hoogstraal. (1985). Experimental studies on the replication and dissemination of qalyub virus (Bunyaviridae: Nairovirus) in the putative tick vector, *Ornithodoros (Pavlovskyella) erraticus. The American Journal of Tropical Medicine and Hygiene* Vol. 34, No.1, (January 1985), pp. 180-187, ISSN 0002-9637.

Moemenbellah-Fard, MD, O Benafshi, J Rafinejad & H Ashraf. (2009). Tick-borne relapsing fever in a new highland endemic focus of western Iran. *Annals of Tropical Medicine and Parasitology* Vol. 103, No. 6, (September 2009), pp. 529-537, ISSN 0003-4983.

Montasser, AA. (2005). Gram-negative bacteria from the camel tick *Hyalomma dromedarii* (Ixodidae) and the chicken tick *Argas persicus* (Argasidae) and their antibiotic sensitivities. *Journal of the Egyptian Society of Parasitology*, Vol. 35, No. 1, (April 2005), pp. 95-106, ISSN 0253-5890.

Mumcuoglu, KY, C Banet-Noach, M Malkinson, U Shalom & R Galun. (2005). Argasid ticks as possible vectors of west Nile virus in Israel. *Vector Borne Zoonotic Diseases*.Vol. 5, No. 1, pp. 65-71, ISSN 1530-3667.

Nava, S, M Lareschi, C Rebollo, C Benítez Usher, L Beati, RG Robbins, LA Durden, AJ Mangold, & AA Guglielmone. (2007). The ticks (Acari: Ixodida: Argasidae, Ixodidae) of Paraguay. *Annals of Tropical Medicine and Parasitology* Vol. 101, No. 3, (April 2007), pp. 255-70, ISSN 0003-4983.

Nevill EM. (1964). "The role of carbon dioxide as stimulant and attractant to the sand tampan*, Ornithodoros savignyi* (Audouin)." *Onderstepoort J. Vet. Res.* 31,59–68.

Noda, H, UG Munderloh, & TJ Kurtti. (1997). Endosymbionts of ticks and their relationship to Wolbachia spp. and tick-borne pathogens of humans and animals. *Applied and Environmental Microbiology*, Vol. 63, No. 10, (October 1997), pp. 3926-3932, ISSN 0099-2240.

Ntiamoa-Baidu, Y, C Carr-Saunders, BE Matthews, PM Preston, & AR Walker. (2004). An updated list of the ticks of Ghana and an assessment of the distribution of the ticks of Ghanaian wild mammals in different vegetation zones. *Bulletin of Entomological Research,* Vol. 94, No.3, (June 2004), pp. 245-60, ISSN 0007-4853.

Ogden, Nh, et al. (2006) "Climate Change and the Potential for Range Expansion of the Lyme Disease Vector *Ixodes scapularis* in Canada." *International Journal for Parasitology*. 36(1):63–70

Parola, P & D Raoult. (2001). Ticks and tickborne bacterial disease in humans: an emerging infection threat. *Clinical Infectious Diseases*, Vol. 32, pp. 897-928, ISSN 1058-4838.

Petney, TN, RH Andrews, LA Mcdiarmid, & BR Dixon. (2004). *Argas persicus* sensu stricto does occur in Australia. *Parasitology Research*, Vol. 93, No.4, (July 2004), pp. 296-299, ISSN 0932-0113.

Randall, JA, KA Rogovin, and D Shier. (2000). "Antipredator behavior of a social desert rodent: footdrumming and alarm calling in the great gerbil, *Rhombomys opiums.*" *Behavioral Ecology and Sociobiology* 48: 110-118.

Randall, JA and KA Rogovin. (2002). Variation in and Meaning of Alarm Calls in a Social Desert Rodent *Rhombomys opimus*. Ethology, 108: 513–527.

Rebaudet, S & P Parola. (2006). Epidemiology of relapsing fever borreliosis in Europe. FEMS Immunology and Medical Microbiology, Vol. 48, No.1, (October 2006), pp. 11-15, ISSN 0928-8244.

Reeves, WK, AD Loftis, F Sanders, MD Spinks, W Wills, AM Denison, & GA Dasch. (2006). Borrelia, Coxiella, and Rickettsia in *Carios capensis* (Acari: Argasidae) from a brown pelican (*Pelecanus occidentalis*) rookery in South Carolina, USA. *Experimental Applied Acarology*, Vol. 39, No. 3-4, pp. 321-329, ISSN 0168-8162.

Richter, PJ, et al. (1996) "*Ixodes pacificus* (Acari: Ixodidae) as a Vector of *Ehrlichia equi* (Rickettsiales: Ehrlichieae)." *Journal of Medical Entomology*. 33(1): 1–5

Shepherd, AJ, R Swanepoel, AJ Cornel, O & Mathee. (1989). Experimental studies on the replication and transmission of crimean-congo hemorrhagic fever virus in some African tick species. *American Journal of Tropical Medicine and Hygiene* Vol. 40, No. 3, (March 1989), pp. 326-331, ISSN 0002-9637.

Schreck, CE, GA Mount, and DA Carlson. (1982). "Wear and wash persistence of permethrin used as a clothing treatment for personal protection against the lone star tick (Acari: Ixodidae)." *J. Med. Entomol*. 19*:* 143–146.

Schwan, TG, SJ Raffel, ME Schrumpf, JS Gill & J Piesman. (2009). Characterization of a novel relapsing fever spirochete in the midgut, coxal fluid, and salivary glands of the bat tick *Carios kelleyi*.Vector Borne and Zoonotic Diseases, Vol. 9, No. 6, (December 2009), pp. 643-647, ISSN 1530-3667.

Shanbaky, NM & N Helmy. (2000). First record of natural infection with *Borrelia* in *Ornithodoros (Ornithodoros) savignyi*. Reservoir potential and specificity of the tick to *Borrelia*. *Journal of the Egyptian Society of Parasitology*, Vol. 30, No. 3, (December 2000), pp. 765-780, ISSN 0253-5890.

Sidi, G, N Davidovitch, RD Balicer, E Anis, I Grotto, & E Schwartz. (2005). Tick-borne relapsing fever in Israel. *Emerging Infectious Diseases*, Vol. 11, No. 11, (November 2005), pp. 1784-1786, ISSN 1080-6040.

Sonenshine, DE, RS Lane, and WL Nicholson. (2002). Ticks (Ixodida). In: Medical and Veterinary Entomology, G. Mullen and L. Durden (Eds). pp. 517-558. Academic Press, ISBN: 978-0-12-372500-4, Boston, USA.

Sonenshine, DE, and RM Roe. (2014). "Ecology of Nidicolous Ticks." *Biology of Ticks*, Oxford University Press. pp. 43.

Spielman, A, ML Wilson, JF Levine, and J Piesman. (1985). "Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease." *Annu. Rev. Entomol.* 30: 439–460.

Stott, JL, BI Osburn, & L Alexander. (1985). *Ornithodoros coriaceus* (Pajaroello tick) as a vector of bluetongue virus. *American Journal of Veterinary Research* Vol. 46, No. 5, (May 1985), pp. 1197-1199, ISSN 0002-9645.

Tahmasebi, F, SM Ghiasi, E Mostafavi, M Moradi, N Piazak, A Mozafari, A Haeri, AR Fooks & S Chinikar. (2010). Molecular epidemiology of Crimean- Congo hemorrhagic fever virus genome isolated from ticks of Hamadan province of Iran. *Journal of Vector Borne Diseases*, Vol. 47, No. 4, (September 2010), pp. 211-216, ISSN 0972-9062.

Telmadarraiy, Z, SM Ghiasi, M Moradi, H Vatandoost, MR Eshraghian, F Faghihi, Z Zarei, A Haeri, & S Chinikar. (2010). A survey of crimean-congo haemorrhagic fever in livestock and ticks in Ardabil province, Iran during 2004-2005. *Scandinavian Journal of Infectious Diseases*, Vol. 42, No. 2, pp. 137-141, ISSN 0036-5548.

Turell, MJ, CN Mores, JS Lee, JJ Paragas, D Shermuhemedova, TP Endy, and S Khodjaev. (2004). "Experimental Transmission of Karshi and Langat (Tick-Borne Encephalitis Virus Complex) Viruses By Ornithodoros Ticks (Acari: Argasidae)." *Journal of Medical Entomology* 41.5:973-77.

Turell, MJ, et al. (2008). Assay for and Replication of Karshi (Mammalian Tick-Borne Flavivirus Group) Virus in Mice. *The American Journal of Tropical Medicine and Hygiene.* 78(2): 344–347

Turell, MJ. (2015). "Experimental Transmission of Karshi (Mammalian Tick-Borne Flavivirus Group) Virus by Ornithodoros Ticks 2,900 Days after Initial Virus Exposure Supports the Role of Soft Ticks as a Long-Term Maintenance Mechanism for Certain Flaviviruses." *PLOS Neglected Tropical Diseases* 9.8

Venzal, JM & A Estrada-Peña. (2006). Larval feeding performance of two neotropical *Ornithodoros* ticks (Acari: Argasidae) on reptiles. *Experimental Applied Acarology*, Vol. 39, No. 3-4, pp. 315-20, ISSN 0168-8162.

Venzal, JM, A Estrada-Peña, AJ Mangold, D González-Acuña & AA Guglielmone. (2008). The *Ornithodoros* (*Alectorobius*) *talaje* species group (Acari: Ixodida: Argasidae): description of *Ornithodoros* (Alectorobius) rioplatensis n. sp. from southern South America. *Journal of Medical Entomology*, Vol. 45, No. 5, (September 2008), pp. 832-40, ISSN 0022-2585.

Vermeil, C, M Marjolet, & C Chastel. (1996). Argas et arbovirus. Actualités. *Bulletin de la Societé de Pathologie Exotique*, Vol. 89, pp. 363-365, ISSN 0037-9085.

Vial, L, P Durand, C Arnathau, L Halos, G Diatta, JF Trape, & F Renaud. (2006). Molecular divergences of the Ornithodoros sonrai soft tick species, a vector of human relapsing fever in West Africa. *Microbes and Infection*, Vol. 8, No. 11, (September 2006), pp. 2605-2611, ISSN 1286-4579.

Vial, L, B Wieland, F Jori, E Etter, L Dixon, & F Roger. (2007). African swine fever virus DNA in soft ticks, Senegal. *Emerging Infectious Diseases*, Vol. 13, No. 12, (December 2007), pp. 1928-1931, ISSN 1080-6040.

Vial, L. (2009). Biological and ecological characteristics of soft ticks (Ixodida: Argasidae) and their impact for predicting tick and associated disease distribution. Parasite, Vol. 16, No. 3, pp. 191-202, ISSN 1252-607X.

Weldon, PJ, JF Carroll, M Kramer, RH Bedoukian, RE Coleman, and UR Bernier. (2011). Anointing chemicals and hematophagous arthropods: Responses by ticks and mosquitoes to Citrus (Rutaceae) peel exudates and monoterpene components. *J. Chem. Ecol*. 37*:* 348–359.

Zhuang, L, et al. (2018) “Transmission of Severe Fever with Thrombocytopenia Syndrome Virus by *Haemaphysalis longicornis* Ticks, China.” *Emerging Infectious Diseases*. 24(5)