Ticks are able to transmit tick-borne infectious agents to vertebrate hosts which cause major constraints to public and livestock health. The costs associated with mortality, relapse, treatments, and decreased production yields are economically significant. Ticks adapted to a hematophagous existence after the vertebrate hemostatic system evolved into a multi-layered defense system against foreign invasion (pathogens and ectoparasites), blood loss, and immune responses. Subsequently, ticks evolved by developing an ability to suppress the vertebrate host immune system with a devastating impact particularly for exotic and crossbred cattle. Host genetics defines the immune responsiveness against ticks and tick-borne pathogens. Pesticides are commonly regarded as substances used to control organisms such as insects, fungi, weeds, and microbes that destroy plants, particularly those for food production. EPF are a species of fungal pathogens for arthropods. They are considered cosmopolitan saprophytic organisms that live in diverse ecosystems and climates (e.g., tropical, temperate, arid and artic), where they interact with arthropods in many terrestrial and aquatic habitats.

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# Ticks and their control

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# Ticks and their control

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# Ticks and their control

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## Contents list

# INTRODUCTION

Ticks are able to transmit tick-borne infectious agents to vertebrate hosts which cause major constraints to public and livestock health. The costs associated with mortality, relapse, treatments, and decreased production yields are economically significant.

Ticks adapted to a hematophagous existence after the vertebrate hemostatic system evolved into a multi-layered defense system against foreign invasion (pathogens and ectoparasites), blood loss, and immune responses. Subsequently, ticks evolved by developing an ability to suppress the vertebrate host immune system with a devastating impact particularly for exotic and crossbred cattle.

Host genetics defines the immune responsiveness against ticks and tick-borne pathogens. To gain an insight into the naturally acquired resistant and susceptible cattle breed against ticks, studies have been conducted comparing the incidence of tick infestation on bovine hosts from divergent genetic backgrounds.

It is well-documented that purebred and crossbred *Bos taurus indicus* cattle are more resistant to ticks and tick-borne pathogens compared to purebred European *Bos taurus taurus* cattle. Genetic studies identifying Quantitative Trait Loci markers using microsatellites and SNPs have been inconsistent with very low percentages relating phenotypic variation with tick infestation.

Several skin gene expression and immunological studies have been undertaken using different breeds, different samples (peripheral blood, skin with tick feeding), infestation protocols and geographic environments. Susceptible breeds were commonly found to be associated with the increased expression of toll like receptors, MHC Class II, calcium binding proteins, and complement factors with an increased presence of neutrophils in the skin following tick feeding.

Resistant breeds had higher levels of T cells present in the skin prior to tick infestation and thus seem to respond to ticks more efficiently. The skin of resistant breeds also contained higher numbers of eosinophils, mast cells and basophils with up-regulated proteases, cathepsins, keratins, collagens and extracellular matrix proteins in response to feeding ticks.

Vector-borne pathogens cause diseases with a great impact on public and veterinary health and have accounted for 22% of emerging infections between 1940 and 2004 (Jones et al., 2008). As obligate hematophagous arthropod pests of vertebrates, ticks pose serious threats to beef and dairy cattle producers.

It has been estimated that 80% of the world's cattle population is at risk from tick and tick-borne diseases (TBDs) causing estimated annual losses of US$ 22–30 billion (Lew-Tabor and Rodriguez Valle, 2016).

The negative impact of ticks on cattle production is due to the direct effects of feeding, such as weight loss and damage of leather, and indirect effects, such as the transmission of tick-borne pathogens. The resulting diseases can potentially cause major production losses in livestock, thereby reducing farm incomes, increasing costs to consumers, and threatening trade between regions and/or world markets.

Since the establishment of extensive vector control programs, a steady decline in vector-borne diseases was observed last century, however recently the emergence and re-emergence of vector-borne diseases has been observed.

This re-emergence may be linked to new global trends associated with changes in animal husbandry, urbanization, animal transboundary transportation, and globalization (Ogden and Lindsay, 2016). In this scenario, various approaches for tick control are in practice around the world in accordance with local legislation, environmental conditions, price based selection, and geography.

Acaricide (synthetic pesticides) application is the most common component of tick control strategies, however the use of acaricides impose numerous limitations including the selective pressure for the development of more resistant ticks, environmental contamination, drug residues in food products, the expense of developing new acaricides, and the difficulty of producing tick-resistant cattle while maintaining desirable production characteristics (Willadsen, 2004; Abbas et al., 2014).

Anti-tick vaccines are a very promising alternative to acaricide usage, however are still insufficient to confer protection against multiple tick species in various geographical regions (de la Fuente and Contreras, 2015; de la Fuente et al., 2016; Schetters et al., 2016).

Anti-tick immunity has been described in guinea pigs, cattle and rabbits, and refers to the capacity of previously exposed hosts to interfere with tick feeding and reproductive fecundity (Nuttall, 1911; Trager, 1939; Hewetson, 1972).

A reduction in tick weight, duration of attachment, number of ticks feeding, egg mass, and molting success are some of the parameters measured to determine host anti-tick immunity (Trager, 1939). For the first time, Nuttall (1911) demonstrated host immunity to ticks as a phenomenon of natural immunity in humans. Experimentally, acquired resistance to tick infestation was observed by Trager (1939), who noticed that after repeated infestation of *Dermacentor variabilis* on guinea pigs, the host developed resistance to subsequent tick infestation, shown by the decreasing number of successfully feeding larvae.

Furthermore, it was found that as compared with larvae infesting a host with no previous exposure to ticks, larvae infesting resistant hosts weighed less. Several researchers continued to observe host resistance to tick feeding affecting each tick life stage (Gregson, 1941; Feldman-Muhsam, 1964; Wikel, 1996).

Various immunological determinants have been examined that influence host resistance to tick infestation including a high level of eosinophils, basophils, T cells, mast cells, specific immunoglobulins, histamine, and changes to gene transcription profiles (Kemp and Bourne, 1980; de Castro and Newson, 1993; Kashino et al., 2005; Veríssimo et al., 2008; Kongsuwan et al., 2010; Piper et al., 2010; Engracia Filho et al., 2017).

Bovines present contrasting, heritable phenotypes for infestation with *Rhipicephalus microplus* and related tick species as a consequence of co-evolution of resistant cattle with ticks and also decades of selective breeding. The *R. microplus* tick has a strong preference for *Bos taurus taurus* cattle over highly resistant *Bos taurus indicus* cattle (Wambura et al., 1998; Porto Neto et al., 2011b; Jonsson et al., 2014; Biegelmeyer et al., 2015).

In this article we review the tick:host physical interface, genetic and molecular studies, and immunological determinants of bovine host resistance to ticks.

Parasitic diseases are a global problem and considered as a major obstacle in the health and product performance of animals. These may be due to endo-parasites that live inside the body or ecto-parasites such as ticks, mites, flies, fleas, midges, etc., which attack the body surface. Among ecto-parasites, ticks are very important and harmful blood sucking external parasites of mammals, birds and reptiles throughout the world (Furman and Loomis, 1984).

The medical and economic importance of ticks had long been recognized due to their ability to transmit diseases to humans and animals. Ticks belong to phylum, Arthropoda and make up the largest collection of creatures in order Acarina.

Ticks are divided into two groups: soft bodied ticks (Argasidae) and hard bodied species (Ixodidae).

Hard ticks feed for extended periods of time on their hosts, varying from several days to weeks, depending on such factors as life stage, host type, and species of tick.

The outside surface, or cuticle, of hard ticks actually grows to accommodate the large volume of blood ingested, which, in adult ticks, may be anywhere from 200 to 600 times their unfed body weight (Sonenshine, 1991).

Additionally, many soft ticks have an uncanny resistance to starvation, and can survive for many years without blood meal (Furman and Loomis, 1984).

The outside surface, or cuticle, of soft ticks expands, but does not grow to accommodate the large volume of blood ingested, which may be anywhere from 5 to 10 times their unfed body weight (Sonenshine, 1991).

Ticks cause great economic losses to livestock in the world and have adverse effect on livestock host in several ways (Snelson, 1975) and parasitize a wide range of vertebrate hosts, and transmit a wider variety of pathogenic agents than any other group of arthropods (Oliver, 1989).

There are 899 tick species those parasitize the vertebrates including Argasidae (185 species), Ixodidae (713 species) and Nuttalliellidae (1 specie) (Barker and Murrell, 2004).

Ticks are the most important ecto-parasites of livestock in tropical and sub-tropical areas, and are responsible for severe economic losses in livestock. The major losses, however, caused by ticks are due to their ability to transmit protozoan, rickettsial and viral diseases of livestock, which are of great economic importance world-wide.

Tick-borne protozoan diseases (e.g. Theileriosis and Babesiosis) and rickettsial diseases (e.g. Anaplasmosis) and cowdriosis and tick-associated dermatophilosis are major health and management problems of livestock in many developing countries.

The economically most important ixodid ticks of livestock in tropical regions belong to the genera of *Hyalomma*, *Boophilus*, *Rhipicephalus* and *Amblyomma* (Frans, 2000).

There are various ways to control ticks, but every method of tick control has certain shortcomings. Chemical control with acaricides was considered as one of the best methods, but it was shown recently that ticks have developed resistance against a range of acaricides (Martins et al., 1995).

However these chemicals are toxic and costly. Problems of acaricide resistance, chemical residues in food and the environment and the unsuitability of tick resistant cattle for all production systems make the current situation unsatisfactory, which is why there is debate on the development of an alternate and absolute control method, such as through vaccine.

Ticks have become an increasing problem to people and animals. Ticks are obligate blood-feeders that require an animal host to survive and reproduce.

They feed on a wide variety of mammals, birds, reptiles, and even amphibians. While most ticks feed on specific host animals and are not considered to be of medical or veterinary importance, several ticks have a wide host range and attack people, pets, or livestock.

Ticks can be a nuisance; their bites can cause irritation and, in the case of some ticks, paralysis. Severe infestations on animals can cause anemia, weight loss, and even death from the consumption of large quantities of blood. Ticks can also transmit many human and animal disease pathogens, which include viruses, bacteria, rickettsiae, and protozoa.

The association between ticks and disease was first demonstrated when Theobald Smith and Fred Kilbourne proved in 1893 that Texas cattle fever (cattle babesiosis) was caused by a protozoan transmitted by an infected tick.

In the late 1800s, Rocky Mountain spotted fever was the first human tick-borne disease identified in the United States, and for many years, was the major tick-associated disease in this country.

Although first recognized from the virulent cases in the Bitterroot Valley of Montana, it eventually became evident that most cases were distributed through the eastern United States.

Lyme disease was first recognized as a distinct clinical entity from a group of patients with arthritis in the area of Lyme, Connecticut, in 1975, although it became evident that this disease had an extensive history in Europe throughout the twentieth century. Today, Lyme disease is the leading arthropod associated disease in the United States with nearly 24,000 human cases reported to the Centers for Disease Control and Prevention (CDC) in 2005.

This may represent only about 10% of physician diagnosed cases. Surveys have found that up to a quarter of residents in Lyme disease endemic areas have been diagnosed with the disease and that many residents perceive the disease as a serious or very serious problem. Without an effective intervention strategy, the steadily increasing trend in Lyme disease case reports is likely to continue.

In the northeastern United States, the emergence of Lyme disease can be linked to changing landscape patterns. A Swedish naturalist named Pehr Kalm recorded in his journal of his travels in the United States in 1748-1750 that ticks wereabundant and annoying.

Over a century later in 1872, entomologist Asa Fitch noted that ticks were nearly or quite extinct along the route that Pehr Kalm had traveled. During this time, the land had been cleared for agriculture and white-tailed deer in many areas were drastically reduced or virtually eliminated due to habitat loss and unregulated hunting.

With the reestablishment of forested habitat and animal hosts through the latter half of the twentieth century, ticks that may have survived on islands off the southern New England coast were able to increase and spread. The blacklegged tick, Ixodes scapularis, which is commonly known as the “deer” tick, and the principal vector for Lyme disease or Lyme borreliosis, was present on Naushon Island, Massachusetts, in the 1920s and 1930s. Some I. scapularis from Montauk Point, Long Island, New York, that were collected in the late 1940s and early 1950s were found infected with Lyme disease bacteria.

The risk of human infection increased through the 1960s and 1970s until the recognition of the disease from the cluster of cases in Lyme, Connecticut, in 1975. Indeed, the disease was not new and cases had occurred in Europe through the 20th century under different names.

The rising incidence of Lyme disease is due to a number of factors including:

- Increased tick abundance
- Overabundant deer population
- Increased recognition of the disease
- Establishment of more residences in wooded areas
- Increased potential for contact with ticks.

With the steady increase in the incidence and geographic spread of Lyme disease, there is a need for homeowners, public health officials, and the pest control industry to learn how to manage or control the tick problem. The purpose of this handbook is to provide basic information on ticks and their biology, basic information on the diseases they carry, methods to reduce the risk of exposure to these parasites, and most importantly, information on how to reduce or manage tick populations, and therefore risk of disease, in the residential landscape.

Ticks are not insects but are arthropods more closely related to mites, spiders, scorpions, and harvestmen. There are about 80 species of ticks in the United States (~ 865 species worldwide). However, only about 12 or so in the U.S. are of major public health or veterinary importance with a few others that occasionally attack humans. The ticks discussed in this handbook belong to the family Ixodidae or hard ticks. The principal hard ticks recovered from humans in the mid-Atlantic and northeastern United States are the blacklegged (i.e., deer) tick, Ixodes scapularis, the American dog tick, Dermacentor variabilis, and the lone star tick, *Amblyomma americanum*.

Other tick species recorded as feeding on humans in the eastern U.S. include Ixodes cookei, Ixodes dentatus, and the brown dog tick, *Rhipicephalus sanguineus*.

The Argasidae or soft ticks form the other major group of ticks. Soft ticks are generally nest inhabitants that are associated with rodents, birds, or bats. Several species of soft ticks attack humans and can transmit disease organisms, mainly in western states, but are not the focus of this handbook. One species, Carios (Ornithodoros) kelleyi, a bat tick, has been recovered from states in the northeast, including Connecticut.

Scientific Names and a Few Terms

The scientific name of ticks, like other organisms, is given in two parts: genus (capitalized, often abbreviated by the first letter, e.g. I. scapularis) and species (not capitalized) sometimes followed by the name of the person who described the organism (given in parenthesis if the genus name is later changed).

The name Linneaus is abbreviated L. Common names like deer tick can vary regionally and some organisms may have no common name. The common names used in this guide follow those officially recognized by scientific societies. Several terms are used to define the cycles of animal, tick and pathogen

Pathogen:

The microorganism (i.e., virus, bacteria, rickettsia, protozoa, fungus) that may cause disease

- Parasite:

An animal that lives in or on a host for at least part of their life and benefits from the association at the expense of the host (from the Greek, literally para - beside and sitos - food).

- Vector:

An insect or other arthropod, like a tick, that carries and transmits a disease pathogen. Diseases associated with pathogens transmitted by a vector are called vector borne diseases.

- Host:

An animal infected by a pathogen or infested with a parasite. • Reservoir: An animal host that is capable of maintaining a pathogen and serving as a source of infection.

- Zoonoses:

A disease caused by a pathogen that is maintained in vertebrate animals that can be transmitted naturally to humans or domestic animals by a vector or through other means (e.g. saliva, feces). • Endemic disease: A disease that is established and present more or less continuously in a community.

Pesticides are commonly regarded as substances used to control organisms such as insects, fungi, weeds, and microbes that destroy plants, particularly those for food production.

They are also used for many other purposes, from public health campaigns against vector insects that cause devastating diseases such as malaria, typhus and dengue, to urban fumigation for hygienic purposes or home spraying to eliminate annoying bugs.

Today, a huge number of substances are employed as pesticides. The earliest pesticides included mineral compounds and extracts of natural products such as tobacco, chrysanthemum flower, or derris root.

During the second half of the nineteenth century, new products were introduced such as copper fungicides (the most famous being the Bordeaux mixture of copper sulphate and calcium hydroxide) and arsenic compounds (Paris Green and calcium and lead arsenate), which maintained their predominance until the first third of the twentieth century.8 In a popular book written at the end of the nineteenth century, a horticulturalist of the New York State Agricultural Experiment.

No one knows for certain the extent of the damage done to wildlife from the use of pesticides. This is because; no detailed study has been carried out to determine the real damage to fauna. However, many species have been affected, especially animals (Mason, et al., 1986) at the top of the food chain, and according to (Bramble, 1989) the natural balance of predators and prey has been disrupted, particularly in the insect world (Murray, A., 1985).

Birds, too, have been a casualty from pesticide poisoning(Lundholm, 1987 ) According to (Urfi, 1994) cranes and storks have been affected due to green revolution agricultural toxins. It is believed that agricultural toxins have decimated water insects and invertebrates in the agricultural lands that constitute up to 75% of Uttar Pradesh.

As a result, Sarus cranes, the world's largest flying bird, begun to disappear due to the disappearance of their specialized food. Storks, too, have been affected.

The number of Black Necked Storks have shown a visible decline and among the many factors responsible, have been the thinning of the shells of their eggs due to pesticides (Urfi, 1994).

There is a paucity of studies conducted to determine the welfare estimates of environmental effects of pesticides both in developed and developing countries despite the damage done to local flora and fauna being high.

Antle et al., 1994, 1994 a, &1998, have conducted an analysis of economic, environmental and health tradeoffs in Ecuador but the welfare estimates of environmental damages are not presented.

Pingali and Rosegrant (1994), too, discuss the environmental consequences of the green revolution in Asia but no welfare estimates of environmental damage are presented.

In Britain, Marchant et al. (1990), Department of the Environment (1996), have estimated that nine species of British farmland birds have experienced a serious decline between 25%-60% during the 1980's and that pesticide usage has been a significant factor.

Foster and Mourato (1997) have used this analysis to form the basis of their contingent ranking16 methodology to value the various environmental consequences of pesticide use in bread production. In this study three product attributes are considered, namely the price of a standard loaf of bread, causes of human illness as a result of field exposure to pesticides during cultivation and a number of farmland bird species in a state of serious long-term decline as a result of pesticide use in arable farming.

Tradeoffs between money, human morbidity and bird biodiversity are then examined. The willingness to pay valuation is expressed in terms of pence per loaf for a unit reduction of one case of ill health each year and one less bird species facing serious long-term decline.

The willingness to pay results show that to protect a bird species is typically six times higher (at around six pence per loaf) than the willingness to pay toenvironmental degradation.

Their contingent valuation survey was carried out among farmers in the USA. The results show that about 66% of the farmers were willing to pay to avoid environmental risks. The mean environmental cost estimates were $ 12.54 to avoid high risks, $8.76 to avoid moderate risks, $5.79 to avoid low risks (n= 1,114).

# The cattle tick

The cattle tick, *Boophilus microplus,* is a significant ectoparasite of cattle and a vector for important diseases such as babesiosis and anaplasmosis.

*Boophilus microplus* (male)

Life cycle of cattle tick.

Stages de développement de la tique du bétail.

**Classification**

SPC List D disease

**Susceptible species**

*B. microplus* is primarily a parasite of cattle. However, heavy infestations can also occur on horses, sheep, deer and water buffalo. Rarely, ticks have been reported on marsupials, goats, dogs, cats and pigs.

**Distribution**

The cattle tick is widely distributed in Central and South America, parts of the southern USA, Africa, Asia, and northern Australia. The distribution of the cattle tick is largely determined by climatic factors. *B. microplus* requires high humidity and ambient temperatures of at least 15-20o C for egg laying and hatching.

## Clinical signs

Cattle tick infestation causes:

- Damage to hides
- Loss of production
- Anaemia and death
- Weakness leading to greater mortalities during droughts

## Post-mortem findings

Animals may be in poor condition and anaemic. Infestations will be obvious by the presence of engorged ticks attached to the animal's skin.

## Differential diagnosis

Several species of ticks may be found on cattle and it is important to differentiate these from *B. microplus*. Other ticks include:

- *Haemophysalis* spp
- *Ixodes holocyclus*

## Specimens required for diagnosis

Ticks should be collected and forwarded to an entomologist for confirmation of the diagnosis. *B. microplus* has pale legs, short mouth parts with transversely ridged palps, small eyes and lacks an anal groove.

## Transmission

The life cycle involves free-living stages. After feeding on cattle, engorged female ticks drop to the ground and lay eggs (up to 5000). After hatching, the larvae survive on pasture for several months.

The larvae then become quite active and climb up grass and transfer to animals as they brush past. The larvae attach and feed from the host. They moult to the nymphal stage and then undergo a further moult to the adult stage.

|  |  |
|---|---|
| Engorged female, dorsal. | Heavy parasistim by cattle ticks. |
| Femelle engorgée, vue dorsale. | Important parasitisme par des tiques du bétail. |

Photos Copyright FAO 1997

## Risk of introduction

Cattle ticks are most likely to be introduced with the importation of infested cattle. Cattle tick was introduced to New Caledonia with cattle imported from Australia in this way.

Transport of ticks on dogs is considered a potential risk for introducing the tick to new areas, although egg production is much reduced in this species and the risk is considered low.

## Control / vaccines

There are four methods available for controlling ticks:

- Treatment with acaricides
- Pasture spelling
- The use of resistant cattle

- Vaccination

Tick control by acaracide dipping has been widely used in endemic areas. Acaracides used for this purpose include various synthetic pyrethroids, amitraz, and some organophosphates. Dipping may have to be done as frequently as every 4-6 weeks in heavily infested areas. Many producers in tick endemic area have changed to *Bos indicus* type cattle because of their greater resistance to tick infection.

An anti-tick vaccine is also commercially available in Australia. The vaccine antigen, based on a tick gut protein and produced by recombinant technology, stimulates production of specific antibodies in cattle which damage the gut of engorging ticks, resulting in a fertility reduction of up to 70% in adult ticks, reducing tick population build-up. This vaccine is of limited use, but can be used as part of an integrated program for the control of ticks

Cattle ticks (*Rhipicephalus microplus*) are the most serious external parasite of cattle in northern Australia. They transmit tick fever and if uncontrolled, can cause serious losses to the cattle industry.

## HOSTS

Engorged female Adult female although cattle ticks favour cattle, infestations occur on buffaloes, deer, camels, horses and sheep. Cattle ticks may occasionally be seen on donkeys, goats, dogs and pigs. When cattle are heavily infested, ticks can be found anywhere on the body. On lightly infested animals, the main places to look for ticks are the escutcheon, tail butt, belly, shoulder, dewlap and ear.

## EFFECTS

Infested cattle lose condition due to 'tick worry' and loss of blood. Heavy infestations can kill calves and even adult cattle. Animals in poor condition are especially vulnerable. Previously unexposed cattle become heavily infested until they build up a degree of resistance.

Bos indicus (tropical breeds of cattle) and their crosses, develop a greater degree of resistance than do Bos taurus (British and European breeds of cattle).

Cattle ticks transmit the organisms that cause tick fever, which is a serious blood parasite disease of cattle. The disease can be lethal to susceptible animals. Others may suffer severe loss of condition. Hides of infested cattle are damaged by tick bites, reducing their value. In severe cases the hides may be unsaleable.

Horses also suffer from ‘tick worry’ and loss of blood from cattle tick infestations. They rub and bite affected areas, causing severe skin lesions. After a period, however, horses develop strong resistance to cattle ticks. Cattle ticks have little effect on other hosts.

## SEASONAL DISTRIBUTION

In the Northern Territory (NT), cattle ticks can be seen at any time of the year, but mainly occur during the wet season and early dry season.

## IDENTIFYING CATTLE TICKS

All three parasitic stages are generally present on infested cattle, but the easiest to identify is the adult stage. Cattle ticks are the only ticks with all legs that are a pale cream in colour.

## SPECIMENS

Larvae, nymphs or unfed adults should be put in a bottle with three parts methylated spirit and one part water. Engorged adult ticks should be put live on blotting paper, covered with moist cotton wool and put into a screw-top bottle with small holes for ventilation. Live adult females are required for testing for resistance to insecticides.

It is most important to provide the name and address of the property and the date the specimens were collected. As labels may become detached, lost, or illegible, identification notes should be written on a piece of paper in pencil, which is not affected by methylated spirit, and put in the specimen bottle or jar. Packages containing methylated spirit or live ticks must not be sent by post, air or bus.

## RESISTANT TICKS

Ticks can be killed by dipping or spraying cattle with an appropriate chemical (acaricide). Ticks can, however, develop resistance to acaricides. Larvae produced by engorged adult females are tested for acaricide resistance at a laboratory in

Brisbane. Contact your local Livestock Biosecurity Officer if you suspect acaricide resistance.

## CONTROL

Cattle ticks can be controlled by using resistant cattle, strategic treatment with chemicals, pasture spelling or a combination of these methods. In the NT, cattle ticks are usually controlled by using resistant cattle; treatment is usually limited to cattle awaiting export or moving to or through tick-free areas.

## LIFE CYCLE

There are four stages in the life cycle of cattle ticks. Cattle ticks are one host ticks, that is, the larva, nymph and adult remain on the same animal. The parasitic phase of the life cycle lasts about three weeks.

The life cycle consists of two parts, the parasitic part during which ticks feed on cattle, and the non-parasitic part during which they spend on the ground. © Northern Territory Government Page 2 of 4 Parasitic part of the life cycle When 'seed' ticks (larvae) infest a host they usually bite immediately and begin feeding.

However, during the first two days following infestation, feeding is intermittent and the larvae frequently detach to move about on the host. After five to six days, they ingest a large meal of tissue fluids and blood, and moult to become eight-legged nymphs. Nymphs also feed on the host's blood and moult to young adults after six to eight days.

Males usually moult first and can be found lying underneath engorged nymphs and female ticks. Males are much smaller and more active than females. The parasitic life of ticks is completed eight to 12 days after the nymphal moult.

The full life cycle is completed in 19 to 26 days. Fully engorged male ticks may either remain on the host or detach with the female. Males have been known to survive for 70 days, either on the host, or on vegetation, relying on dew or plant juices for their fluid needs.

Non-parasitic part of the life cycle This begins when the fully engorged female tick, in the stage during which it is most easily seen on infested cattle, falls to the ground and finds a suitable place to lay eggs.

The pre-egg laying period depends on environmental temperature and relative humidity, and can be as short as one to two days or as long as 40 days. The duration of egg-laying is also temperature-controlled and can range from two to 44 days. Each female tick may lay up to 3500 eggs.

During the wet season when both temperature and humidity are optimal, eggs hatch in approximately 18 to 21 days. © Northern Territory Government Page 3 of 4 The six-legged larvae which hatch from the eggs are known as seed ticks. They are extremely active in response to moving objects.

The close proximity of an animal is sufficient to activate them to climb to the tips of blades of grass, where they can attach more easily to a passing host. During the evening, seed ticks seek protection in the vegetation. The longevity of seed ticks is influenced by temperature and humidity.

They are extremely vulnerable to very low ambient temperatures and low humidity. In northern Australia the maximum longevity is two to four months depending on the season. The non-parasitic part of the life cycle ends when seed ticks find suitable hosts. These may not necessarily be cattle. Cattle ticks have been known to infest horses, sheep, dogs, buffalo, deer, pigs and hares, although cattle are the preferred host.

## Tick ecology

Chaka et al.(2001) studied determination of the physiological age of *Rhipicephalus appendiculatus*. The age structure of a population of vectors of disease pathogens is a most useful characteristic for epidemiological studies.

It has long been developed for insect vectors, where the success of an eradication programme can be plotted by the declining survival. Age grading of ticks requires theoretical adaptation because of the fundamentally different relationship between feeding and transmission opportunities of insect and ticks.

Estrada-Peña (2001) reported that seasonal dynamics exert a major influence on the dynamics of transmission of tick-born pathogen.

## Tick Biology and Behavior

Ticks, like many mite species, are obligate blood-feeders, requiring a host animal for food and development. Ticks have four stages in their life cycle: egg, the 6-legged larva (seed ticks), and 8-legged nymph and adult (male or female).

Larvae and nymphs change to the next stage after digesting a blood meal by molting or shedding the cuticle. Most of the ticks mentioned in this handbook have a 3-host life cycle, whereas each of the three active stages feed on a different individual host animal, taking a single blood meal. Larvae feed to repletion on one animal, drop to the ground and molt to a nymph.

The nymphs must find and attach to another animal, engorge, drop to ground and molt to an adult. The adult tick feeds on a third animal. A replete or engorged (blood filled) female tick will produce a single large batch of eggs and then die. Depending upon the species of tick, egg mass deposited can range roughly from 1,000 to 18,000 eggs.

3-host tick life cycle 8 Stafford The Connecticut Agricultural Experimentation Station Bulletin No. 1010 5 The larvae and nymphs generally feed on small to medium-sized hosts, while adult ticks feed on larger animals. Some ticks may have one-host (all stages staying and feeding on only one animal host before the female drops off) or other multi-host lifecycles. Depending upon the tick, the life cycle may be completed in 1, 2 or even 3 years, while a one-host tick may have more than one generation per year. Feeding for only a few days, the majority of the life of a tick is spent off the host in the environment either seeking a host, molting or simply passing through an inhospitable season (e.g., hot summers or cold winters).

Soft ticks have a multi-host life cycle with multiple nymphal stages; each stage feeds briefly, and adults take multiple small blood meals, laying small egg batches after each feeding. As nest and cave dwellers, often with transient hosts, some argasid ticks may survive many years without a host.

However, most hard ticks do not successfully find a host and perish within months or a year or two at best. Larval ticks will be clustered on the egg mass after hatching and when ready to feed, ascend blades of grass or similar vegetation to await a host.

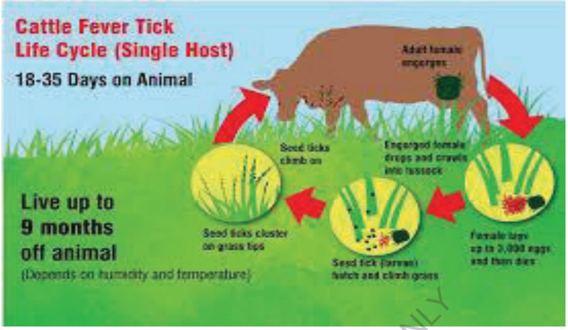
Ticks assume a questing position by clinging to the leaf litter or vegetation with the third and fourth pair of legs, and hold the first pair outstretched. Due to differences in susceptibility to desiccation and host preference, immature ticks generally remain in the low vegetation, while adult ticks may seek a host at a higher level in the vegetation.

Ticks detect their hosts through several host odors (including carbon dioxide, ammonia, lactic acid, and other specific body odors), body heat, moisture, vibrations, and for some, visual cues like a shadow.

When approached by a potential host, a tick becomes excited - waving the front legs in order to grab the passing host. Ticks cannot fly or jump; they must make direct contact with a host.

Once on a host a tick may attach quickly or wander over the host for some time. Some ticks attach only or principally on certain areas like the ear or thin-skinned areas, while other species may attach almost anywhere on the host. The ticks feed slowly, remaining on the host for several days, until engorged with blood (see following section on tick feeding).

Male ticks feed intermittently, take small blood meals, and may remain on a host for weeks. For most ticks mating occurs on the host, as the male tick also requires a blood meal. However, male Ixodes ticks do not need to feed prior to mating and mating may occur on or off the host.



Cattle fever ticks

## Tick Morphology

The body of a tick consists of a “false head” (the capitulum) and a thorax and abdomen fused into a single oval, flattened body. A larval tick has six legs, while nymphs and adults have eight legs present. The basal segment of the leg, the coxa, may have spurs that help in identification.

An adult tick will have a genital aperture on the ventral surface, located roughly between the second pair of legs. The respiratory system is evident by spiracular plates located ventrolaterally behind the fourth pair of legs in the nymphs and adults.

These plates may be oval, rounded, or comma-shaped. Hard ticks get their name from a tough dorsal shield or plate called the scutum present on all mobile stages of the tick. The scutum on the larva, nymph, and female tick covers the dorsal anterior third to half of the body. By contrast, the scutum on a male tick covers almost the entire dorsal surface and expansion during feeding is very limited.

The scutum differs in shape and others characteristics (i.e., presence or absence of simple eyes) between tick genera. In some ticks, ornate or patterned markings may be present that can aid in identification. A distinct semicircular anal groove curves around the front of the anal opening in Ixodes ticks.

In all other ticks, the anal groove is behind the anus or absent. Many ticks, but not Ixodes, have rectangular areas separated by grooves on the posterior margin of the tick body called festoons. Festoons, if present, may not be visible on fully engorged females. Argasid ticks are leathery, wrinkled and grayish in appearance.

The capitulum of soft ticks is located on the underside of the body and cannot be seen from above.

The capitulum in hard ticks is visible dorsally in all stages. The capitulum holds the mouthparts consisting of a base (basis capituli), two palps, paired chelicerae, and the median ventral hypostome, which is covered with denticles or recurved teeth.

The shape of the basis capituli, length of the palps, number of denticles, and other characteristics of the mouthparts are used to help identify tick genera and species. While the adults of some common ticks can be easily identified with a little training because of distinctive markings or color, the identification of most ticks and the immature stages requires the services of a trained entomologist and the use of keys developed by tick taxonomists.

These keys are designed to specifically identify adults, nymphs or larvae. Above right: Mouthparts of I. scapularis nymphs showing hypostome with rows of denticles (center) and two pair chelicerae (palps are partially visible).

## Tick Feeds

The term tick bite may be misleading as ticks do not bite and depart or feed rapidly like a mosquito. Ticks attach and feed gradually over a period of several to many days. Once a tick has found a suitable place to feed, it grasps the skin, tilts the body at a 45-60° angle, and begins to cut into the skin with the paired chelicerae.

The palps lay outwards on the skin surface. After the chelicerae and hypostome penetrate the skin, they become encased in "cement" secreted by the tick. The cement serves to hold the mouthparts in place while the tick feeds.

Mouthparts on larval and nymphal ticks are small with less penetration and produce a smaller host reaction. Adult Ixodes and Amblyomma ticks have long mouthparts that can reach the subdermal layer of skin, produce a larger reaction, and make the tick harder to remove. Insertion of the mouthparts often takes around 10-30 minutes, but can take longer (1-2 hours).

The reaction to a feeding tick may make the tick appear imbedded, but only the slender mouthparts actually penetrate the skin.

Physical and enzymatic rupture of tissue creates a lesion or cavity under the skin from which blood is imbibed. A variety of pharmacologically active compounds that aid the feeding process and possibly increase pathogen transmission are introduced in the tick's saliva (e.g., blood platelet aggregation inhibitors, anticoagulants, anti-inflammatory and immunosuppressive agents, enzymes, and vasodilators to increase blood flow).

Feeding is not continuous and most of the blood meal is taken up during the last 12-24 hours of feeding. The body weight of a feeding female tick can increase 80-120 times. Male ticks are intermittent feeders, take smaller amounts of blood, and do not change appreciably in size (male I. scapularis do not need to feed and are rarely found attached).

Ticks may attach and feed anywhere on the body, but there are differences depending upon exposure and species of tick. The distribution of the blacklegged tick is relatively uniform. However, over a third of I. scapularis were from the legs and arms and another third were from the back up through the shoulders, neck and head. By contrast, most American dog ticks are removed from the head and neck region.

The Blacklegged Tick or "Deer" Tick, Ixodes scapularis Say Blacklegged tick is the correct common name for the tick popularly known as the "deer" tick (the terms are not used together, it is not called the blacklegged deer tick).

Ixodes (pronounced x-zod-ease) scapularis transmits the causal agents of three diseases; Lyme disease, human babesiosis, and human granulocytic anaplasmosis (HGA). The northern range of the tick includes southern portions of Canada and coastal Maine through the mid-Atlantic states into Maryland, Delaware and northern parts of Virginia and in several north central states, particularly Wisconsin and Minnesota, extending down through Illinois and into Indiana.

This tick is also found throughout the southeastern United States west to southcentral Texas, Oklahoma, southern Missouri, and eastern Kansas. However, few I. scapularis in the southeast have been found infected with the bacterium that causes Lyme disease, the spirochete Borrelia burgdorferi.

Therefore, the risk for Lyme disease from this tick in the southeastern United States is considered relatively low. Unfed female I. scapularis have a reddish body and a dark brown dorsal scutum (plate) located behind the mouthparts.

Length of the female tick from the tip of the palpi to the end of the body is about 3 to 3.7 mm (about 1/10 of an inch). Male I. scapularis are smaller (2 – 2.7 mm) than the female and are completely dark brown. Nymphs are 1.3 to 1.7 mm in length, while larvae are only 0.7 to 0.8 mm. Female blacklegged ticks become fairly large when engorged with blood and, consequently, are sometimes confused with engorged female American dog ticks.

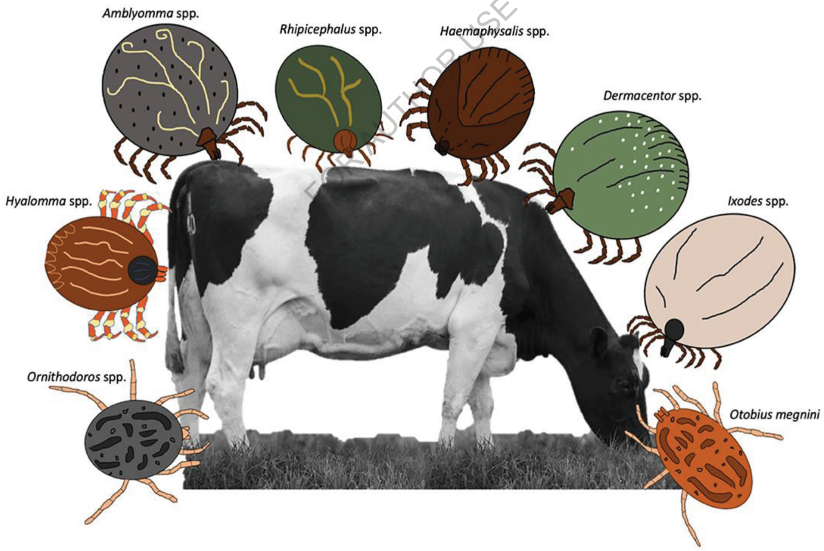
The Blacklegged Tick or "Deer" Tick, Ixodes scapularis Say Blacklegged ticks feed on a wide variety of mammals and birds, requiring 3-7 days to ingest the blood, depending on the stage of the tick. Larvae and nymphs of I. scapularis typically become infected with B. burgdorferi when they feed on a reservoir competent host. The white-footed mouse is the principal reservoir (source of infection) for *B. burgdorferi*, the protozoan agent of human babesisois, Babesia microti, and can serve as a reservoir for the agent of human granulocytic ehrlichiosis. Birds are also a major host for immature I. scapularis and have been implicated in the long-distance dispersal of ticks and *B. burgdorferi*.

White-tailed deer, Odocoileus virginianus (Zimmerman), are the principal host for the adult stage of the tick, which feeds on a variety of medium- to large-sized mammalian hosts. An engorged female tick may typically lay around 2,000-3,000 eggs.

The Lyme disease spirochete in northern states is maintained, in part, by the two-year life cycle of the tick. Eggs are laid by the female in May. Larvae hatch from those eggs in mid- to late July with August being the peak month for larval tick activity.

After feeding, the larvae drop from the host and molt to nymphs, which will appear the following year in late spring. May, June and July are peak months for nymphal tick activity in the northeast. Therefore, the nymphs precede larvae seasonally and can infect a new generation of animal hosts.

Larvae active later in the summer then become infected when feeding on reservoir host animals. The nymphal ticks will molt to adults after feeding and appear in the fall of the same year. Adult I. scapularis do not hibernate and may be active on warm winter days and the following spring. Adults of I. scapularis are more heavily infected with B. burgdorferi than the nymphs because the tick has had two opportunities to become infected, once as a larva and once as a nymph.



Entomopathogenic Fungi for Tick Control

The ileria or tick disease

## Tick Control

The main reasons for tick control are to protect hosts from irritation and production losses, formation of lesions that can become secondarily infested, damage to hides and udders, toxicosis, paralysis, and of greatest importance, infection with a wide variety of disease agents. Control also prevents the spread of tick species and the diseases they transmit to unaffected areas, regions, or continents.

### Cultural and Biologic Control

These measures can be directed against both the free-living and parasitic stages of ticks. The free-living stages of most tick species, both ixodid and argasid, have specific requirements in terms of microclimate and are restricted to particular microhabitats within the ecosystems inhabited by their hosts.

Destruction of these microhabitats reduces the abundance of ticks. Alteration of the environment by removal of certain types of vegetation has been used in the control of *Amblyomma americanum* in recreational areas in southeastern USA and in the control of *Ixodes rubicundus* in South Africa.

Control of argasid ticks such as *Argas persicus* and *A walkerae* in poultry can be achieved by eliminating cracks in walls and perches, which provide shelter to the free-living stages.

The abundance of tick species can also be reduced by removal of alternative hosts or hosts of a particular stage of the life cycle. This approach has occasionally been advocated for control of three-host ixodid ticks such as *Rhipicephalus* *appendiculatus*, *Amblyomma hebraeum*, and *Ixodes rubicundus* in Africa, and *Hyalomma* spp in southeastern Europe and Asia.

Rotation of pastures or pasture spelling has been used in control of the one-host ixodid tick *Rhipicephalus (Boophilus) microplus* in Australia. The method could also be applied to other one-host ticks, in which the duration of the spelling period is determined by the relatively short life span of the free-living larvae. However, it has minimal application to multihost ixodid ticks or argasid ticks because of the long survival periods of the unfed nymphs and adults.

Predators, including birds, rodents, shrews, ants, and spiders, play a role in some areas in reducing the numbers of free-living ticks. In the New World, fire ants (*Pheidole megacephala*) are noteworthy tick predators. Engorged ticks may also become parasitized by the larvae of some wasps (Hymenoptera), but these have not significantly reduced tick populations.

Zebu (*Bos indicus*) and Sanga (a *B taurus*, *B indicus* crossbreed) cattle, the indigenous breeds of Asia and Africa, usually become very resistant to ixodid ticks after initial exposure. In contrast, European (*B taurus*) breeds usually remain fairly susceptible.

The tick resistance of Zebu breeds and their crosses is being increasingly exploited as a means of control of the parasitic stages. The introduction of Zebu cattle to Australia has revolutionized the control of *R microplus* on that continent.

Use of resistant cattle as a means of tick control is also becoming important in Africa and the Americas. In Africa, infestations of ixodid ticks on livestock and wild ungulates may also be reduced by oxpeckers (*Buphagus* spp), which are birds that feed on attached ticks.

## Tick-Host Physical Interface

The cattle tick *R. microplus* co-evolved with Asian bovines (zebu breeds) and due to the global migration of *B. t. taurus* European breeds for dairy production during the eighteenth–nineteenth centuries, this tick spread across tropical and sub-tropical regions of the world (Frisch, 1999; Estrada-Peña et al., 2006; Barré and Uilenberg, 2010).

Currently *R. microplus* is considered to be a species complex, in which there are recognized geographic differences between the 5 clades including 3 clades of *R. microplus* (A, B, and C), as well as *R. australis* and *R. annulatus* (Burger et al., 2014; Low et al., 2015).

Each taxa transmits both anaplasmosis and babesiosis and each have a parasitic life cycle on cattle for ~21 days. They will be described collectively as *R. microplus* or simply as cattle ticks in this review. Cattle ticks are attracted to their hosts through stimuli such as carbon dioxide, temperature, vibrations, visual stimuli, and odor (Osterkamp et al., 1999).

The susceptible European (*B. t. taurus*) breeds which were introduced into regions in which *R. microplus* is endemic failed to resist tick infestation to the same extent as tropical *B. t. indicus* breeds, which have developed an effective anti-tick immune response (Frisch, 1999).

The immune response varies among newly introduced European cattle (Taurine breeds, susceptible hosts) whereas Asian bovines (zebuine breeds or resistant hosts) co-evolved with ticks (Utech et al., 1978).

Physical barriers that affect tick resistance include density of the fur coat, skin thickness, skin pigmentation (light or dark), skin vibration and/or self-cleaning ability, tongue papillae, and odor (de Castro et al., 1985; Spickett et al., 1989; Veríssimo et al., 2002, 2015; Martinez et al., 2006; Gasparin et al., 2007).

In addition to physical differences between resistant and susceptible hosts, their behavior also affects the *R. microplus* parasitic load. Self-grooming is widely used by cattle as an important defense mechanism against ticks (Riek, 1956; Snowball, 1956; Bennett, 1969) and the level of resistance may possibly be associated with tongue morphology.

For example the papillae from tick-resistant breeds have smaller spacing, which is more effective in removing *R. microplus* larvae from the skin (Veríssimo et al., 2015).

However, there is also conjecture that resistant breeds simply groom more often (Kemp et al., 1976). It has been suggested that innate characteristics such as thinner coats and lower fur density have direct impacts in decreasing tick preferential attachment and infestation (Spickett et al., 1989; Veríssimo et al., 2002; Gasparin et al., 2007; Marufu et al., 2011).

However, other studies have shown that skin features have no influence on tick infestation (Wagland, 1978; Doube and Wharton, 1980). Evidence for resistance of cattle to ticks due to physical parameters is scarce and further studies to examine the mechanisms that govern these physical phenomena are still needed.

## Immunological Determinants Associated with Host Resistance: Host Counter Attack

It is well-established that cattle have three subclasses of IgG (IgG1, IgG2, and IgG3), and during blood meals ticks ingest a substantial amount of IgG (Knight et al., 1988; Symons et al., 1989; Kacskovics and Butler, 1996; Rabbani et al., 1997; Gudderra et al., 2002; Saini et al., 2007).

Host IgGs can be found in the tick hemolymph and are potentially biologically active against specific tick proteins (Ben-Yakir et al., 1987). Furthermore, specific host antibodies neutralize the tick salivary pharmacopeia and can damage the tick by binding to tick internal organs such as salivary glands, midgut, or ovaries (Ackerman et al., 1981; Willadsen and Kemp, 1988; Tellam et al., 1992).

In other tick-host systems (*Dermacentor andersoni* and guinea pigs), antibodies have been shown to mediate inflammatory reactions by triggering effector-cell recruitment and cellular immune response as a consequence of both Fc receptor activation of leukocytes and complement activation that are harmful to the tick, also an immune mechanism in human auto-immune disease syndromes (Wikel and Whelen, 1986; Hogarth, 2002).

It was documented previously that the passive transfer of plasma from genetically immune resistant animals to naïve hosts, increases resistance to tick challenge and this response was believed to be mediated by antibodies (Roberts and Kerr, 1976; Shapiro et al., 1986).

The pattern of antibody responses to immunogens from tick salivary glands and guts have been reviewed by different research groups (Willadsen, 1980; Wikel, 1982; Kaufman, 1989; Kashino et al., 2005; Cruz et al., 2008; Piper et al., 2009, 2010; Garcia et al., 2017).

Some studies have shown that during laboratory animal infestations, such as guinea pigs, rabbits and mice, reactive antibody titers to tick salivary antigens increased (Allen and Humphreys, 1979; Allen, 1989).

The densities of *Amblyomma hebraeum, Rhipicephalus appendiculatus*, and *Rhipicephalus evertsi evertsi* ticks was higher on the susceptible breed (*B. t. taurus* Hereford) as compared to resistant cattle (*B. t. indicus* Brahman) with a positive correlation between the level of tick infestation and the level of IgG in susceptible hosts (Rechav, 1987).

Piper and colleagues confirmed this correlation noting that susceptible cattle (Holstein-Friesian) have higher levels of tick specific IgGs compared to Brahmans suggesting that these antibodies do not confer immunity to ticks (Piper et al., 2008, 2009, 2010).

As with other immune parameters in high and low resistance animals, the interpretation of data can be problematic as a susceptible animal will have more ticks feeding at any time, which would in turn be expected to result in a higher antigenic challenge.

However, the negative relationship between IgG levels and host resistance was later confirmed and shown to be independent of the number of feeding ticks, using Santa Gertrudis cattle [a stable composite breed of *B. t. taurus* (5/8) and *B. t. indicus* (3/8)], in which there is wide variation in host resistance to ticks (Piper et al., 2017).

Susceptible animals had significantly higher tick-specific IgG1 antibody titres (to several tick antigens including adult female salivary glands and guts, and whole larvae) compared to tick resistant cattle (Piper et al., 2017). In contrast, Kashino et al reported that tick saliva-specific IgG1 and IgG2 antibodies decreased in susceptible (Holstein) compared to resistant (Nelore) cattle where the IgG levels remained the same, however, only IgG levels to tick salivary antigens were examined (Kashino et al., 2005). Previous studies have surmised that there are genetic differences in the bovine host's ability to elicit antibody responses to antigens in *R. microplus* and *D. andersoni* tick saliva (Whelen et al., 1986; Opdebeeck and Daly, 1990). Despite the fact that differences in the IgG levels against tick antigens between heavy or light infestations have been reported, there is individual variation in the same bovine breed with respect to humoral immune responses to tick antigenic molecules (Cruz et al., 2008). In addition, despite most studies reporting increased total IgG production against wide ranging tick antigens in susceptible breeds compared to resistant, IgG responses to salivary proteins were significantly higher in tick naïve resistant hosts (Nelore) at the first larval challenge (Garcia et al., 2017).

Variation in IgG2 allotypes has been associated with variation in immune responses to pathogens. When two allotypes IgG2a and IgG2b were found to differ in sequence at the CH1–CH3 regions it was reported that IgG2b was more able to initiate the bovine complement cascade while animals with the IgG2a allotype were more susceptible to extracellular pyogenic pathogens (Heyermann and Butler, 1987; Bastida-Corcuera et al., 1999). Other studies have shown that the distribution and presence of IgG2 allotypes differed significantly between taurine and indicine breeds (Butler et al., 1994; Carvalho et al., 2011). Blakeslee et al. (1971) described that ~80% of susceptible bovines (Holstein) have the IgG2a allotype and that the IgGγ2b was rare in these animals (Blakeslee et al., 1971). Recently, it was shown that the IgGγ2a allotype was significantly more frequent in taurine hosts (tick susceptible) and IgGγ2b was significantly frequent in indicine cattle (tick resistant) (Carvalho et al., 2011). Male tick saliva contains IgG-binding proteins (IGBPs) secreted into the host which assists the female tick to evade the host immune response (Wang and Nuttall, 1999; Santos et al., 2004; Gong et al., 2014). Carvalho et al. (2011) suggested that certain IgG2 allotypes may hinder the function of these tick IGBPs.

Aside from IGBPs, other tick specific proteins have been examined in terms of their immune recognition by tick resistant and susceptible cattle. A *R. microplus* recombinant serine protease inhibitor (Serpin- rRMS-3) was recognized by resistant bovines and not susceptible, suggesting that RMS-3 could be a protective antigen (Rodriguez-Valle et al., 2012).

Another study by the same group demonstrated that host responses to six *R. microplus* lipocalins (LRMs which include tick histamine binding proteins) were higher in resistant cattle (Rodriguez-Valle et al., 2013).

Both RMS-3 and the LRM proteins were identified based on the *in silico* identification of B cell binding epitopes. In addition, predicted T cell epitopes from 3 LRMs stimulated the generation of a significantly higher number of interferon gamma (IFN-γ) secreting cells (consistent with a Th1 response) in tick-susceptible Holstein–Friesians compared with tick-resistant Brahman cattle. In contrast, expression of the Th2-associated cytokine interleukin-4 (IL4) was lower in Holstein–Friesian (susceptible) cattle when compared with Brahman (resistant) cattle (Rodriguez-Valle et al., 2013).

IL4 is known to decrease the production of Th1 cells and IFN-γ, and is thus a key regulator of both the humoral and adaptive immune responses.

The immunological parameters of tick resistance have been shown to differ between tick susceptible and tick resistant breeds as well as within the same breeds. The studies reported differ in the parameters of trials and tick infestations, for example, the use of tick naïve cattle artificially infested or the use of cattle naturally exposed to ticks (Kashino et al., 2005; Piper et al., 2008, 2009). The study undertaken by Kashino et al. (2005) used susceptible cattle that had been treated with acaricides when high tick numbers were observed and the cattle had been vaccinated with GAVAC (Bm86 based tick vaccine), introducing additional variables to the study. Further studies examining specific tick proteins to compare divergent host immune responses are still warranted.

## Molecular Genetic Variants Associated with Host Resistance

Numerous studies have attempted to identify genetic markers for host resistance to tick infestation and they are summarized and discussed by Porto Neto et al. (2011b) and Mapholi et al. (2014).

Approaches have included immunological methods (Stear et al., 1984, 1989, 1990); protein-based analyses (Ashton et al., 1968; Carvalho et al., 2008); candidate gene sequence or genotype (Acosta-Rodriguez et al., 2005; Martinez et al., 2006; Untalan et al., 2007); genomic detection of quantitative trait loci (QTL) using SNPs or microsatellites, with or without fine mapping (Barendse, 2007; Gasparin et al., 2007; Regitano et al., 2008; Prayaga et al., 2009; Machado et al., 2010; Porto Neto et al., 2010a, 2011a; Turner et al., 2010; Cardoso et al., 2015; Mapholi et al., 2016; Sollero et al., 2017).

There is one example of a meta-analysis of genomic association with transcriptome in tick infestation (Porto Neto et al., 2010b). Although this appears to represent a large body of science, it has generated relatively little data which can be used for improved genetic selection. It can be concluded from the studies on the major histocompatibility complex (MHC, also referred to as the bovine lymphocyte antigen (BoLA) system) that the MHC makes a contribution to variation in resistance however there is no single, consistent genotype of any gene in the MHC that is associated with high or low resistance to ticks across breeds and production systems.

A number of QTL markers have been identified using microsatellites and SNPs, however these have mostly been inconsistent and the loci have had relatively weak effects. The research of Barendse (2007) and Turner et al. (2010) found several significant loci but most of them had effects in the order of

1% of the phenotypic variation in tick infestation. The lack of consistent and strong findings is not surprising. Counting ticks is difficult and time consuming thus studies resort to scoring systems, which are less precise than counts, and this can have an effect on heritability.

Alternatively, the numbers used tend to be relatively small and studies are underpowered. The most robust report is that by Turner et al. (2010), who reported on a study in which ticks were counted and heritability was a respectable 37%, and which used 1,960 cattle. In contrast, Prayaga et al. (2009) used a scoring system, 900 animals and estimated heritability of tick score to be 9%.

Furthermore, a study examining the genomic prediction for tick resistance in Braford (Brahman x Hereford/tick resistant x tick susceptible breed, respectively) and Hereford cattle in Brazil showed that genomic selection for tick resistant Braford cattle may be achievable (Cardoso et al., 2015).

A recent trait tag-SNP approach by the same group reported 914 SNPs explaining more than 20% of the estimated genetic variance for tick resistance (Sollero et al., 2017).

Despite the challenges of the genomic approach to identifying either mechanisms or markers for host resistance to ticks in cattle, they have enabled the identification of allelic variation in genes that are very likely to influence the trait. The *ELTD1* gene (EGF, latrophilin, and seven transmembrane domain containing 1) was identified from GWAS in dairy and beef cattle (Prayaga et al., 2009; Turner et al., 2010).

Its association with the host resistance phenotype was confirmed but its effect was limited to <1% of the total phenotypic variation in the trait (Porto Neto et al., 2011a).

Similarly, haplotypes that included the *ITGA11* gene (integrin alpha 11) were significantly associated with tick burden and explained about 1.5% of the variation in the trait. Finally, the potential functional role of allelic variation in a gene identified by the same GWAS studies (Prayaga et al., 2009; Turner et al., 2010) *RIPK2* (serine-threonine kinase 2) was further examined using knock out mice (Porto Neto et al., 2012).

This gene is known to play an essential role in the modulation of innate and adaptive immune responses and it was found that it influenced the recognition of tick salivary antigens by mice.

Limited association of tick burden or phenotype to the genotype is currently available and large bovine genomic meta-analyses may contribute to the identification of within breed markers for tick resistance in the future.

## Variation in Gene Expression among Resistant and Susceptible Hosts and Relationship with Immune Responses

Bioactive molecules secreted by *R. microplus* ticks into the skin of the host during attachment and blood feeding stimulate host effective responses. The variation in the mechanisms by which each host breed responds to each of these bioactive molecules likely results in different levels of resistance.

It is well-established that the resistance to tick infestation is due to a complex set of responses, however the specific mechanisms and their relative importance continues to be the subject of debate.

Table 1 summarizes selected up-regulated genes including those that are potentially associated with immune responsiveness, blood coagulation, calcium regulation, and/or wound healing from several studies undertaken to date.

The parameters of all of the studies differ from each other including: the number of biological replicates, the number of larvae used in infestations, the breeds and subspecies used, their prior exposure, the methodology and platform used to measure host responses (immunohistochemistry, microarray platforms, qPCR), the timing of sample collection, and the samples analyzed (skin or blood). Without undertaking a formal meta-analysis of the original data, we have attempted to summarize differences and similarities in relation to susceptible vs. resistant animals among reports.

Studies on PBL suggest that resistant hosts are more likely to develop a stable T-cell-mediated response against *R. microplus*, while susceptible cattle demonstrated cellular and gene expression profiles consistent with innate and inflammatory responses to tick infestation (Kashino et al., 2005; Piper et al., 2009).

The up-regulation of genes in tick susceptible cattle involved in inflammatory and other important immunological responses mediate a greater natural potential to develop higher pro-inflammatory responses in comparison to tick resistant animals.

Gene expression studies on skin taken from larval attachment sites have demonstrated that cytokines, chemokines, and complement factors were differentially expressed between naïve-skin and infested skin in susceptible Holstein–Friesian cattle. It was also found that immunoglobulin transcripts were differentially expressed in infested skin from Holstein-Friesian compared to resistant Brahman cattle. Therefore, the chronic pathology established in *B. t. taurus* cattle might facilitate the tick feeding process (Piper et al., 2010).

In addition, extracellular matrix genes such as: keratocan, osteoglycin, collagen, and lumican were up-regulated in infested-skin from *B. t. indicus* resistant Brahman cattle. In a study involving coagulation in skin from resistant and susceptible cattle infested with *R. microplus* (Carvalho et al., 2010b), susceptible hosts had an increased blood clotting time at tick hemorrhagic feeding pools in comparison to normal skin and the skin of resistant hosts.

Furthermore, the host resistant phenotype affects the transcript of genes associated with anti-hemostatic proteins in the salivary glands of *R. microplus*, with transcripts coding for anti-coagulant proteins expressed at a higher level in ticks fed on susceptible hosts compared to ticks fed on resistant hosts (Carvalho et al., 2010b).

In the same experiment where PBL gene expression was studied in infested indicine and taurine cattle, the authors examined the response to infestation and larval attachment in bovine skin (Piper et al., 2010).

The susceptible cattle displayed an intense cellular inflammatory response at the tick attachment site, i.e., genes involved in inflammatory processes and immune responses including those which encode for matrix proteins were up-regulated in tick-infested susceptible cattle, but not in resistant hosts.

Nascimento et al. (2010) constructed cDNA libraries from skin biopsies from resistant and susceptible cattle to evaluate the pattern of gene expression of three calcium-binding-proteins. The results showed that genes coding for translationally controlled tumor protein (*1-TPT1*), calcium channel protein transient receptor potential vanilloid 6 (*TRPV6*) and cysteine proteinase inhibitor (*CST6*) were highly expressed in susceptible cattle compared to resistant cattle (Nascimento et al., 2010).

Also, a microarray study using samples from tick infested cattle to evaluate the profile of gene expression during *R. microplus* larvae attachment showed differentially expressed genes involved in lipid metabolism, inflammation control and impairment of tick infestation in resistant cattle (*B. t. indicus* Nelore) (Carvalho et al., 2014).

Conversely, in susceptible cattle (*B. t. taurus* Holstein) the acute phase response appeared impaired but this study confirmed the up-regulation of calcium ion control genes which correlates with the calcium binding proteins reported by Nascimento et al. (2010).

Franzin et al. (2017) also report the up-regulation of protein S100G, another calcium binding protein, in susceptible cattle. An earlier qPCR study showed higher up-regulation of calcium signaling genes in a tick resistant composite breed in response to ticks most predominantly at 24 h post-infestation with larvae (Belmont Red) (Bagnall et al., 2009).

Calcium signaling, calcium binding and/or calcium ion control genes and their functions in tick resistant and tick susceptible cattle warrant further specific examination.

Another host gene expression study was reported recently demonstrating that resistant cattle (*B. t. indicus* Nelore breed) up-regulated the expression of fewer genes encoding enzymes producing volatile compounds that render them less "attractive" to ticks compared with susceptible cattle (*B. t. taurus* Holstein breed) (Franzin et al., 2017).

This finding is consistent with the theory associated with odor (Osterkamp et al., 1999) described above. The study also reported that resistant hosts when exposed to ticks mount an earlier inflammatory response than susceptible cattle (gene expression studies undertaken at 2 days post larval infestation using tick naïve cattle) (Franzin et al., 2017) which appears to disappear later (feeding nymphs at 9 days) but lingers in susceptible cattle.

Franzin et al. (2017) identified numerous novel immune response genes that were up-regulated in susceptible Holstein cattle including *FCER1A* the high affinity immunoglobulin epsilon receptor subunit alpha precursor which is known to be responsible for initiating an allergic response.

The up-regulation of complement *C1QTNF7* (C1q tumor necrosis factor-related protein 7 precursor), an inducer of pro-inflammatory activators (Kishore et al., 2004) also concurs with the conclusions of tick susceptible cattle responding in a pro-inflammatory manner (Piper et al., 2010).

Piper et al. (2008) had identified the up-regulation of Toll-like receptors (i.e., *NFkB*, nuclear factor kappa-light-chain-enhancer of activated B cells) that correlates with the activity of the complement C1Q identified by Franzin et al. (2017).

In addition, *BCL10* (B-cell CLL/lymphoma 10) was up-regulated in susceptible cattle (Franzin et al., 2017) and is also known to activate *NFkB* (Wang M. et al., 2007). In addition fibroleukin is known to have a dual prothrombinase and immunoregulatory activity and was up-regulated in tick naïve (tick susceptible) cattle by larvae (Franzin et al., 2017).

Immunoregulatory activity includes the suppression of T cell proliferation and cytokine production, mainly of Th1 and Th17 cells but not Th2 cells (Bézie et al., 2015), which is in contrast to other observations above suggesting that susceptible cattle mount a Th1 response. Functionally, Bézie et al. (2015) found that fibroleukin induced long-term allograft survival in a rat model through regulatory B cells which in turn suppress the proliferation of CD4+T cells. These cells are up-regulated in tick resistant cattle in the majority of studies undertaken. To summarize the results presented by Franzin et al. (2017), early responses to larvae (48 h post-infestation by naïve tick susceptible cattle) appear to show a mixed Th1 and Th2 response with tick susceptibility being associated with a Th1 response.

Several differences are noted among the comparisons of gene expression studies and as noted by Regitano et al. (2008) we generally concur that "*differences in gene expression of resistant cows compared to susceptible cows were breed-specific*." However, there are some consistencies identified in the above studies.

The presence of high densities of CD4+, CD25+, and γδ T cells are seen relatively consistently in resistant indicine cattle.

The up-regulation of keratins and collagens is also common in resistant indicine cattle, with some divergent upregulation in susceptible breeds in fewer studies. The up-regulation of IgGs in susceptible cattle was reported by most researchers. MHC Class II and calcium binding proteins seem to be mostly up-regulated in susceptible breeds, with the latter commonly associated with susceptible breeds studied in Brazil. The expression of chemokine ligands varied greatly between studies and breeds with no identifiable consistency.

Other genes that were up-regulated consistently in susceptible cattle include: apolipoproteins (lipid transport), lysozymes (anti-microbial, also found in macrophages and polymorphonuclear neutrophils), Toll like receptors, *NFkB* and NFkB activators *C1QTNF7* (complement C1q tumor necrosis factor –related protein 7) and *BCL10* (B-cell CLL/lymphoma 10) and several complement factors. IL10 is considered an anti-inflammatory cytokine perhaps induced early in susceptible cattle but IL10 appears to be associated with long term resistance in *B. t. indicus* Gyr cattle in Brazil (Domingues et al., 2014; Franzin et al., 2017).

Cytochrome P450 enzymes are a superfamily of hemoproteins known to be involved in the synthesis or metabolism of various molecules and chemicals within cells. A P450 gene called CYP4F3 (cytochrome 450 group 4, subfamily F, gene 3) is known to degrade leukotrienes which are the chemical mediators of allergic responses (Karasuyama et al., 2011).

CYP4F3 was downregulated in the skin of tick exposed resistant cattle (Brahmans, Piper et al., 2010) yet up-regulated in the skin of susceptible cattle (Holsteins, Carvalho et al., 2014) thus showing a correlation for the activity of this enzyme in tick susceptible cattle.

Differential expression of genes coding for other host modifying enzymes were associated with resistant and susceptible phenotypes.

Although not identified in more than one study, the up-regulation of these factors appears to correlate to the relevant phenotype and are thus worthy of further description. For example the following were identified in different studies as up-regulated in tick resistant breeds: Cathepsin B (Wang Y. H. et al., 2007), Cathepsin L2 precursor (cysteine proteases, mast cell mediators) (Nascimento et al., 2011), Cathepsin D (aspartyl protease, mast cell mediator) (Franzin et al., 2017), serine peptidase inhibitor clade A (inhibits neutrophil elastase), phospholipase A2, group

VII (platelet activating factor) (Piper et al., 2010), coagulation factors, and procollagen C-endopeptidase enhancer (metalloprotease inhibitor) (Piper et al., 2010); and conversely in tick susceptible breeds: serine peptidase inhibitor clade F (negative regulation of inflammatory response), spleen trypsin inhibitor, plasminogen activator (serine protease which produces plasmin which catalyzes the degradation of fibrin polymers in blood clots), prostaglandin D2 synthase (platelet aggregation inhibitor) (Piper et al., 2010), and phosphoprotein 24 (endopeptidase associated with platelet degranulation) (Franzin et al., 2017) were up-regulated.

Comparative transcriptomic studies of different life stages (larvae and adult females) have shown that ticks respond differentially according to whether they are sensing or feeding on a tick-susceptible or tick-resistant breed of cattle.

A microarray study based on a *R. microplus* EST database (Wang M. et al., 2007), using sensing larvae (not attached to the host but contained within a fabric bag and able to sense the host) and feeding, adult females, which were collected from naïve, tick-susceptible Holstein Friesian *B. t. taurus*, and tick-resistant Brahman *B. t. indicus* cattle has been reported (Rodriguez-Valle et al., 2010).

Ticks that were feeding on resistant cattle demonstrated the up-regulation of serpin 2, lipocalins and histamine binding proteins. A recent study utilized next generation sequencing to compare tick expression differences from larvae, nymph salivary glands and larval offspring of females - all fed on tick-resistant Nelore *B. t. indicus* and tick-susceptible Holstein *B. t. taurus* (Franzin et al., 2017).

That study showed an increased number of transcripts that included evasins, immunosuppressant proteins, lipocalins (including histamine, serotonin, and odorant binding proteins), and reprolysin metalloproteases from ticks associated with susceptible cattle, and an increased number of chitinases and cysteine proteases from ticks associated with resistant cattle. The analysis also included larvae exposed to volatile compounds prepared from the same breeds of cattle and showed that resistant breeds produce less attractive volatiles (Franzin et al., 2017).

The latter was thought to be correlated to the fact that ticks on susceptible cattle up-regulated odorant binding proteins.

Not enough studies have been undertaken to draw any similarities between the molecular profiles of ticks from susceptible vs. resistant hosts particularly when different stages and breeds are compared. It is clear however that the tick gene expression profiles associated with tick-resistant vs. tick-susceptible cattle appear to be divergent.

Acquired immunity to tick infestation is established after a period of susceptibility to a primary infestation (Wagland, 1978). As confirmed above, gene expression profiles from tick resistant breeds appear to be congruent with a T-cell mediated response, while susceptible cattle exhibit innate and inflammatory responses with higher levels of tick specific IgG1.

One quite consistent fact is that resistant cattle appear to be primed to respond to ticks with a higher presence of γδ T cells in the skin of tick naïve resistant cattle in comparison to susceptible. A formal meta-analysis of all gene expression studies where the data are in the public domain is theoretically possible but would likely be compromised by variation in the conditions of each of the studies.

Each gene expression comparison study was undertaken using quite different conditions. The variable factors include: environment, season, naïve vs. tick exposed cattle, the use of acaricides post exposure prior to artificial infestations, infestation protocols including frequency and numbers of larvae, and comparative breeds including within breed studies.

## Cellular Physiology Associated with Host Resistance

Granulocytes (or polymorphonuclear leukocytes) are white blood cells characterized by the presence of granules in their cytoplasm and which perform different immune functions. They include neutrophils (most abundant), eosinophils, mast cells, and basophils. The inflammatory profile of the host skin contributes to resistance or susceptibility to tick infestation. Marufu et al. (2013) showed that tick susceptibility (*B. t. taurus* Bonsmara cattle) is associated with an immediate type hypersensitivity reaction.

On the other hand, the resistance phenotype was linked to a delayed hypersensitivity reaction in *B. t. indicus* Nguni breed (Marufu et al., 2013) confirming the observations of Constantinoiu et al. (2010) with *B.t.taurus* Holstein Friesian (susceptible) and *B.t.indicus* Brahman (resistant) cattle.

Neutrophils are usually found in the blood stream and are the most abundant phagocyte. During host infection, neutrophils are quickly recruited to the site of infection i.e., skin in response to tick infestation. Neutrophils may favor infestation by destroying the extracellular matrix around the attached tick and thereby allowing access to blood for feeding (Tatchell and Bennett, 1969; Tatchell and Moorhouse, 1970).

Similar numbers of neutrophils were found to be recruited in Holstein (susceptible, *B. t. taurus*) and Nelore cattle (resistant, *B. t. indicus*) (Carvalho et al., 2010a), with slightly higher neutrophils in the skin of susceptible cattle at early stages of infestation using naïve cattle of the same breeds (Franzin et al., 2017).

Marufu et al. (2014) showed higher counts of neutrophils at the attachment sites of *R. microplus* in both resistant and susceptible breeds compared to noninfested skin, with higher counts also found in tick susceptible Bonsmara cattle compared to tick resistant Nguni cattle (Marufu et al., 2014).

Higher levels of neutrophils do not seem to show a protective role against *R. microplus* infestation and feeding larvae demonstrated a high ingestion of neutrophils in susceptible *B. t. taurus* Holstein Friesian cattle (Constantinoiu et al., 2010).

Moreover, activated neutrophils lead to a calcium ion influx which could correlate with the common up-regulation of calcium binding proteins in susceptible cattle gene expression studies.

Overall, higher neutrophil densities in the skin at the site of a tick attachment appear to be associated with the tick susceptible phenotype.

Eosinophils have long been known to be associated with parasite infections and allergy, with several immune functions having been only recently elucidated.

For example, recent evidence suggests that eosinophils suppress Th17 and Th1 responses via dendritic cell regulation and also activate basophil degranulation (Wen and Rothenberg, 2016).

Eosinophils may influence the tick resistant phenotype due to their role in the translocation of mast cell histamine and lysosomal enzymes to the feeding site lesion, and by impairing tick attachment (Schleger et al., 1981). *B. t. taurus* show higher eosinophil counts during secondary infestations compared with *B. t. indicus* breeds in early studies (Tatchell and Moorhouse, 1968).

Marufu et al. (2013) confirmed this observation with higher eosinophil counts in tick susceptible Bonsmara cattle, in contrast to other studies which showed higher eosinophil counts in tick resistant cattle (Nelore *B. t. indicus*) in Brazil and Australian Shorthorn breed (*B. t. taurus* tropically adapted cattle) (Schleger et al., 1976; Carvalho et al., 2010b).

Using the same breeds as Carvalho et al. (2010b), Franzin et al. (2017) showed higher eosinophil counts in susceptible cattle during the first infestation of tick naïve cattle, yet higher eosinophil counts at nymph feeding sites in the tick resistant breed. Suppression of Th1 responses by eosinophils may be logically associated with the response of resistant cattle and may also correlate with mast cell activity.

Mast cells (including tissue basophils) are a multifunctional cell population involved in maintaining local homeostasis of connective tissue, control of blood coagulation and defensive functions of innate and adaptive immunity. In addition mast cell dysfunction is associated with several chronic allergic/inflammatory disorders (da Silva et al., 2014).

Mast cells contain granules rich in histamine and heparin, and are the main effectors of allergic reactions. Host resistance to ticks appears to concur with mast cell functions such as allergic responses, wound healing and immune tolerance, and a potential mast cell dysfunction in tick-susceptible cattle.

However, one study comparing several breeds found that the Nelore *B. t. indicus* resistant breed had the highest number of mast cells in response to ticks while the Gyr *B. t. indicus* tick resistant breed had similar levels as two tick susceptible *B. t. taurus* breeds, Holstein and Brown Swiss (Veríssimo et al., 2008).

In contrast, previously Gyr *B. t. indicus* cattle showed a higher number of mast cells in the dermis compared to susceptible European breeds (Moraes et al., 1992; Veríssimo et al., 2008). A few mast cell activators (da Silva et al., 2014) have been noted in resistant host gene expression studies above including Cathepsins B, D, and L2, platelet activating factors, complement factor C3, IL10, IL2, and TNFα.

Basophils are known for their allergic effector function and were first described in response to ticks by Trager in 1939. Basophils notably accumulated at tick bite sites causing cutaneous hypersensitivity (Trager, 1939).

In the 1950s, it was confirmed that histamines are stored in basophil granules (Graham et al., 1955). Basophils have been associated with immunity against parasites including ticks and helminths, reviewed by Karasuyama et al. (2011). It is thus logical as described above for mast cells, that high levels of circulating basophils would be associated with the tick resistant phenotype, which has been confirmed by two groups (Carvalho et al., 2010a; Marufu et al., 2014; Franzin et al., 2017).

The release of histamine has been postulated as a mechanism which dislodges feeding ticks. This was confirmed when mice were injected with cultured mast cells which resulted in tick rejection following infestation of *Haemophysalis longicornis* ticks, with no tick rejection in mast cell deficient mice (Matsuda et al., 1987).

A recent review of basophil functions confirms their effector role in allergic reactions, however basophils also share features of innate and adaptive immunity (Steiner et al., 2016) which again associates well with the tick resistant bovine phenotype. Steiner et al. (2016) examined the ever expanding function of basophils including the modulation of several cytokines, Toll-like receptors and chemokines (including CXCL10, CCR1, CCR7 described as up-regulated.

The correlation of granulocyte activity (and their immune effector mechanisms) in the skin of tick resistant cattle could further be examined to attempt to correlate immunity with gene expression studies described above. The existence of tick histamine-binding salivary lipocalins have been associated with inhibiting histamines from their receptors (Paesen et al., 1999; Mans et al., 2008) with specific lipocalins up-regulated in resistant vs. susceptible breeds in response to ticks (Rodriguez-Valle et al., 2013).

In addition, the central role of histamine in tick resistance was supported by antihistamine administration to cattle which led to increased tick loads on both *B. t. taurus* (Hereford) and *B. t. indicus* (Brahman) breeds (Tatchell and Bennett, 1969).

In summary, these studies confirm that cattle breeds behave differently during *R. microplus* infestation, presenting various intrinsic mechanisms to provide protection against ticks. Overall, resistant cattle appear to be associated with increased mast cells, eosinophils, and basophils in the skin, while the recruitment of neutrophils is potentially associated with tick susceptibility. The release of histamines from these cells appears to be associated with the resistant phenotype.

Histamine is thought to inhibit tick attachment and leads to itching, which subsequently leads to more grooming and tick removal.

# Microbiota Role in Tick Resistance

The microbiome contributes to the architecture and function of tissues, host energy metabolism, and also plays an important role in the balance between health and disease as demonstrated recently for intracellular protozoa (Yilmaz et al., 2014; Bär et al., 2015).

In vertebrates, semiochemicals can be generated by the activity of the microbiota upon amino acids, short chain fatty acids or hormones secreted in body emissions, such as sweat, tears, sebum, saliva, breath, urine, and feces (Amann et al., 2014; Fischer et al., 2015).

This volatile repertoire is of paramount importance with evidence that they can direct host-vector specificity (Smallegange et al., 2011; Davis et al., 2013).

The variation in the host chemical production thus causes differential attractiveness to vectors between species and in turn, the bacterial profiles differ according to human genetic background (Benson et al., 2010; Prokop-Prigge et al., 2015).

Microbial composition can be affected by diet and other management strategies, such as those used for beef and dairy cattle (Durso et al., 2012; Thoetkiattikul et al., 2013).

The differences in the composition of their microbiota (Dowd et al., 2008; Durso et al., 2010, 2012; de Oliveira et al., 2013; Mao et al., 2015), as well as the distinct volatile organic compounds (VOC) produced by *B. t. taurus* and *B. t. indicus* cattle may corroborate the contrasting tick infestation phenotypes observed among these animals (Steullet and Gnerin, 1994; Osterkamp et al., 1999; Borges et al., 2015; Ferreira et al., 2015).

Although a few studies have demonstrated that host VOCs play a role in attracting *Rhipicephalus* spp. ticks (Louly et al., 2010; Borges et al., 2015; de Oliveira Filho et al., 2016; Franzin et al., 2017)

# DAMAGES CAUSED BY TICKS AND THEIR CONTROL

Ticks comprise veterinary problem because they transmit diseases, produce paralysis or toxicosis, and cause physical damage to livestock. Ticks' species are grouped into three families, Argasidae or soft ticks, Ixodidae or hard ticks and Nuttalliellidae (Klompen et al., 1996).

Ticks are very important to man and his domestic animals, and must be controlled if livestock production is to meet world needs for animal protein. Knowledge of the nature and habits of the tick and the disease agents it transmit helps in control (Stewart et al., 1981).

## Losses and control

A complex of problems related to ticks and tick-borne diseases of cattle created a demand for methods to control ticks and reduce losses of cattle (George et al., 2004).

Control of tick infestations and the transmission of tick-borne diseases remain a challenge for the cattle industry in tropical and subtropical areas of the world. Tick control is a priority for many countries in tropical and subtropical regions (Lodos et al., 2000).

Losses due to tick infestations can be considerable. In Australia alone in 1974, losses due to cattle tick (*Boophilus microplus*) were estimated to be USD 62 million (Springell, 1983).

Brazil loses around USD 2 billion per year (Grisi et al., 2002). Such losses can be cut considerably by adopting effective tick control measures.

There are three major reasons for controlling ticks in domestic animals: disease transmission, tick paralysis or toxicosis, and tick-caused physical damage. The main weapon for controlling ticks at present is the use of chemical acaricides (Drummond, 1983).

Ticks are responsible for severe economic losses both through direct effect of blood sucking and indirectly as vector of pathogens and toxins.

## 1. Direct effect

Feeding by large numbers of ticks causes reduction in live weight and anemia among domestic animals, while tick bites also reduce the quality of hides. Apart from irritation or anemia in case of heavy infestations, tick can cause severe dermatitis (FAO, 1998).

These parasites generate direct effects in cattle in terms of milk production and reduce weight gain (L′Hostis and Seegers, 2002; Peter et al., 2005).

### (1) Tick-bite paralysis

It is characterized by an acute ascending flaccid motor paralysis caused by the injection of a toxin by certain ticks while feeding. Examples are paralysis caused by the feeding of *Dermacentor andersoni*, sweating sickness caused by *Hyalomma truncatum*, Australian tick paralysis caused by *Ixodes holocylus*, and tick toxicosis caused by *Rhipicephalus* species (Drummond, 1983).

Tick paralysis is most common in late winter and spring when the adult ticks are active, but it can occur at any time if the weather is warm and humid (Stewart and de Vos, 1984).

Paralysis in cattle caused by *Ixodes holocyclus* and *Dermacentor andersoni* had also been reported by Doube and Kemp (1975) and Lysyk et al.(2005) respectively.

## (2) Physical damage

Ticks are attached to the body for a blood meal and may cause irritation and serious physical damages to livestock. Included are “tick worry”, irritation, unrest, and weight loss due to massive infestation of ticks; the direct injury to hides due to tick bites, loss of blood due to the feeding of ticks (Drummond, 1983).

## 2. Vector of pathogens

Ticks can be carrier, of pathogens, which they transmit from host to host during blood sucking and cause a large variety of diseases (FAO, 1998).

The major diseases include Babesiosis, Anaplasmosis, Theileriosis, and heart-water, East Coast fever; in addition, other diseases of lesser importance cause severe economic losses to the livestock industry (Drummond, 1983; Bram, 1983).

The presence, dynamics and amount of parasite stock in ticks exert a major influence on the kinetics of transmission of tick-borne parasitic diseases (Morel, 1980).

Generally the ticks become infested with the causative organisms of diseases while they are feeding on infected animals. Then the organism may be transmitted from stage to stage in the tick (an example is *Theileria parva* transmitted by *Rhipicephalus appendiculatus*), or from the female tick through the egg to the larvae—an increase of several thousand times in vector potential (an example is *Babesia equi* transmitted by Anocentor nitens). When the next stage or generation subsequently feeds on another animal, the organism is transmitted to that animal if it is susceptible to the disease (Drummond, 1983).

Tick born diseases generally affect the blood and/or lymphatic system (FAO, 1998). Tick fever organisms, like *Anaplasma marginale*, are significant causes of cattle morbidity in Australia, USA, China and other countries (CRC-VT, 2001).

Cattle tick *B. microplus*, economically impact cattle production by transmitting pathogens that cause Babesiosis (*B. bovis* and *B. bigemina*) and Anaplasmosis (*A. marginale*) (Peter et al., 2005).

## Chemical control of ticks

There are several methods being applied for controlling ticks and tick-borne diseases. The main weapon for the control of ticks at present is the use of chemical acaricides. Acaricides used to control ticks on livestock or in the environment are applied in such a manner that the ticks are killed, but will not harm livestock or applicators, the tissues of treated animals will not contain chemical residues, and the environment will not be adversely affected (Drummond, 1983).

The conventional control methods include the use of chemical acaricides with partially successful results but this treatment has certain implicit drawbacks, such as the presence of residues in the milk and meat and the development of chemical resistant tick strains (Willadsen and Kemp, 1988; Nolan, 1990).

The use of acaricides has disadvantages, such as the selection of resistant tick populations and harmful effects on the animals, human beings and the environment (García-García et al., 2000).

The development of new acaricides is a long and expensive process, which reinforces the need for alternative approaches to control tick infestations (Graf et al., 2004).

Certain herbal mixtures with 70% efficacy for tick control have also been reported by Regassa (2000).

## 1. Acaricides

Control of tick infestation through the use of acaricides is one of the methods that can be used to reduce the tick-borne diseases (Spickett and Fivaz, 1992).

A wide range of acaricides, including arsenical, chlorinated hydrocarbons, organophosphates, carbamates and synthetic pyrethroids are being used for controlling ticks on livestock. The performance of an acaricide in the control of ticks depends not only on the activity of a product, but on the quality and quantity of active ingredient deposited on cattle or delivered internally (George, 2000).

## 2. Arsenic

Use of arsenic was the first effective method for controlling ticks and tick-borne diseases, and was used in many parts of the world before resistance to the chemical became a problem (George, 2000).

It was first used for tick control in 1893 in South Africa (Bekker, 1960) that is inexpensive, stable, and water soluble, and there is an accurate vat-side test (Drummond, 1983).

Arsenic was the first acaricides to be widely used which is cheapest and most effective agent. Mostly it is used in the form of water soluble compounds like sodium arsenite. Usually $As_2O_3$ have been used for many years in dipping vats to control ticks, especially ticks of the genus *Boophilus*. Arsenic dips were used successfully to eradicate *Boophilus* ticks from the southern United States. Unfortunately, arsenic has a very short residual effectiveness (less than one to two days), and in most areas of the world *Boophilus* ticks have become resistant to arsenic (Drummond, 1983).

## 3. Chlorinated hydrocarbons

These are synthetic acaricides. Resistance to arsenicals was developed in many species of ticks (Matthewson and Baker, 1975; Angus, 1996) and it was replaced by chlorinated hydrocarbons (Graham and Hourrigan, 1977).

Chlorinated hydrocarbon acaricides are very persistent and have been used extensively throughout the world for controlling ticks. Of particular interest are benzene hexachloride toxaophene (Drummond, 1983).

Their mode of action is by interfering with nerve conduction of ticks (Solomon, 1983). Because of their high toxicity and long lifespan, these compounds have mostly been withdrawn from the market (Spickett, 1998).

## 4. Organophosphorous compounds

Organophosphates were introduced around 1950, as a replacement for the chlorinated hydrocarbons to which significant resistance had occurred (Shanahan and Hart, 1966).

These are esters of phosphoric acid and have a wide range of activities against ticks at very low concentration in companion and livestock animals. However, their residual effectiveness is usually shorter than that of chlorinated hydrocarbons, and the risk of causing acute toxicity in livestock is greater (Drummond, 1983).

Resistance in ticks was first recognized in 1963 and several tick species are now known to be resistant to organophosphorous acaricides (Wharton, 1967).

## 5. Carbamates

These are esters of carbamic acids and closely resemble the organophosphates (Spickett, 1998). They are a little more toxic than the organophosphates for mammals and are much more expensive.

## Application of chemicals

Various methods including dipping, spraying, ear tagging or pour on, have been used to apply chemicals to protect livestock against ticks. Direct application of acaricides to animals is the most popular method of controlling ticks on livestock (Drummond, 1983).

Applications of acaricide to tick-infested cattle via dipping or sprayer can be equally effective under ideal conditions with proper handling of equipments without injuring animals and subsequent dilution of a product (George, 2000).

## 1. Dipping

In this method, animals are immersed in a dipping tub containing solution of chemicals. By 1893 in Australia, Africa, and the United States the use of "dipping-vats" to immerse tick-infested cattle in a variety of chemical agents was a component of the effort to control the ticks and tick-borne diseases affecting cattle (Mohler, 1906; Matthewson and Baker, 1975).

A variety of tickicides including cottonseed oil, fish oil, crude petroleum, kerosene, creosote, tobacco extract, soap, and a combination of sulphur and kerosene were among the hundreds of possible acaricides tested for dipping (Mohler, 1906; Angus, 1996).

Infested cattle should be dipped in the organophosphate acaricide coumaphos (0.3% active ingredient) (Bram et al., 2002). In general, dipping vats provide a highly effective method of treating animals with acaricides for tick control. However, their immobility, high initial cost of construction, and the cost of the acaricides may make vats impractical for many small ranching operations.

Also, dipping vats must be managed carefully so that the dips are maintained at the proper concentration and the cattle are dipped properly (Drummond, 1983).

## 2. Spray

The application of fluid acaricides to an animal by means of a spray has many advantages and has been successfully practiced for controlling ticks on most of the animals (Barnett, 1961).

Spraying equipment is highly portable, and only small amounts of acaricides need to be mixed for a single application. However, spraying is generally less efficient in controlling ticks than immersion in a dipping vat because of problems associated with applying the acaricides thoroughly on all parts of the animal body (Drummond, 1983).

## 3. Spot treatment or hand dressing

There are predilections sites for certain tick species on part of the body which are not effectively treated by spray or dips. The inner parts of the ear, under part of the tail, the tail brush and the areas between the teats and the legs in cattle with large udder, are especially liable to escape treatment. Acaricides may be applied to these sites by hand is termed as hand dressing (Barnett, 1961) or spot treatment.

The application of insecticides with aerosols and in oils, smears, and dusts by hand to limited body areas is time-consuming and laborious, but in certain instances it may be more effective and economical (in terms of cost of acaricide) than treating the entire animal (Drummond, 1983).

## 4. Some other applications

Some other methods of applying acaricides are ear tags, neck bands, tail bands and pour-one, particularly for the pyrethroids with long residual activity. A mechanical applicator was also developed (Duncan, 1991).

In Kenya, an intraruminal ivermectin slow-release device provided 90 d protection against tick damage (Tatchell, 1992). Tick repellents used on livestock are limited (Mwase et al., 1990).

Ivermectin has been delivered orally in the case of *Boophilus annulatus* on cattle by Millar et al.(2001) as a single or double ruminal bolus as daily capsules to *B. miroplus* infested cattle. The control levels against standard engorging female ticks reached 99%. Despite this, the level of control of ticks on pastures grazed by treated cattle was insufficient to prevent cattle from becoming infested when grazed there later on.

Abdel-Shafy and Zayed (2002) examined the acaricidal effect of plant extract of neem seed oil (*Azadirachta indica*) on egg, immature and adult stages of *Hyalomma anatolicum excavatum*. This short communication on the potential use of azadirachtin for tick control is an extension from the large volume of literature on this material for control of crop pests and vectors of medical pathogens.

Azadirachtin was applied at concentrations of 1.6% through to 12.8% in water and applied to ticks in vitro for 1 min. The ticks were examined up to 15 d post treatment for mortality and reduced viability.

Abdel-Shafy and Zayed (2002) concluded that Neem can be used for tick control at economic concentrations of 1.6% to 3.2%. The work will need to be enlarged to test control of feeding ticks on cattle and possibly control of ticks by spraying the moulting and resting site of ticks in cattle pens.

## Safety measures

Appropriate directions and precautions given on the label, should be taken while applying the acaricides. According to U.S. Department of Agriculture (1967), these acaricides can be toxic to livestock and humans, can create residues in tissues of animals, and may be destructive to the environment if they are not used and handled in a safe and correct manner. The safe use of acaricides is essential to an efficient, well-run program for control of ticks.

To avoid accidents and misuse, it is necessary to continually review and employ safe use, precautions and procedures (Drummond, 1983). It is important to prepare dipping and spray fluids correctly to ensure that a dose of the active ingredient, lethal for the tick, is administered to cattle safely and without exceeding the limits for milk and meat residues.

Licking behavior and environmental contamination arising from pour-on ivermectin for cattle was studied by Laffont et al.(2001) and was found to be associated with unexpected residues in meat and dairy products and as an environmental contaminant via cattle dung. They recommended that the route of potential contamination of parasiticides be taken into account during product registration.

# RESISTANCE

Tick resistance to acaricides is an increasing problem and real economic threat to the livestock and allied industries. Most stock holders depend completely on acaricides to control ticks, but do not have access to guidelines on how to make a profit from their tick control program or how to detect and resolve problems with resistance to acaricides (George, 2000).

Resistance has led to instability and increased costs in areas where the one-host cattle ticks *Boophilus microplus* and *B. decoloratus* have acquired resistance to a variety of toxic chemicals. The point has now been reached where such resistance must be expected in these ticks within five to 10 years of the introduction of any new type of acaricide, unless control practices are changed (Wharton, 1983).

The evolution of tick resistance to acaricides has been a major determinant of the need for new products (George, 2000).

Fernandes (2001) worked on toxicological effects and resistance to pyrethroids in *Boophilus microplus* from Goias, Brazil. According to his research ticks were resistant to deltamethrin and cypermethrin, and showed the required level of mortality only to high concentrations of permethrin.

Considering the frequency with which synthetic pyrethroid resistance has been reported in Brazil, and elsewhere, the claim of resistance is very likely to be correct. Sseruga et al.(2003) studied the serological evidence of exposure to tick fever organisms in young cattle on Queensland dairy farms. They concluded that, in tick-infested areas, vaccination is the most effective means of protecting cattle against tick fever. Resistance detection, identification and characterization of resistance were briefly reviewed by Wharton (1983).

Resistance is usually recognized because of failure to obtain a satisfactory kill of the parasitic stages on treated animals. Failure is frequently due to inadequate treatment and many reports of resistance are unfounded. There is no doubt about resistance when cattle continue to be infested with large numbers of engorged ticks after frequent treatments. But the response of parasitic ticks to under strength acaricides is very similar to their response to low-level resistance or to high-level resistance in the early stages of its development. Thus field-spraying tests must be conducted under standardized conditions where resistance is suspected (Baker and Shaw, 1965).

A survey of cattle tick control practices in South Africa found that 35.7% of farmers using hand sprays have confirmed acaricide resistance compared with 25.8% and 23.9% of users of spray races and plunge-dips respectively (Spickett and Fivaz, 1992).

Confirmation of resistance must be made by laboratory tests. The traditional test for diagnosing resistance to acaricides in single host ticks is the larval packet test, in which larvae are placed in envelopes impregnated with acaricide (Wharton and Roulston, 1970). Mainly chemical companies in screening programmes have developed many methods of testing the effects of acaricides.

The stages most commonly used are the engorged females and the unfed larvae. The former usually provide the most useful information on potential acaricides, but unfed larvae are generally accepted as the logical stage to document resistance. Several engorged females provide sufficient larvae to test against a range of concentrations of several acaricides.

The response of susceptible ticks provides the baseline when resistance is suspected (Wharton, 1983). Resistance in *B. microplus* populations in Australia and South Africa to arsenic was observed about 50 years after use of the chemical began (Matthewson and Baker, 1975; Angus, 1996).

## Host resistance

Resistance to tick infestation varies among individuals and breed of cattle. It is known that in many subtropical and semi-arid environments in Africa indigenous dual purpose breeds are highly resistant to ticks, resulting in low infestation rates that cause significant direct losses (Norval et al., 1991).

The phenomena of host resistance to ticks and enzootic stability to tick born diseases are well documented (Perry et al., 1985; Latif and Pegram, 1992).

## ALTERNATIVE METHODS OF TICK CONTROL

Due to the present disadvantages of chemical acaricide products, the adoption of alternative methods could minimize such problems. Wharton (1983) briefly reviewed the alternative methods of tick control and concluded that the utilization of host resistance, while offering an attractive approach to tick control, raises many questions even with the relatively simple *B. microplus-Babesia* association.

Resistance is an acquired characteristic and each animal develops its own level of resistance in response to tick challenge; the level may be high (as in most zebu cattle) or low (as in most European cattle), but a wide range of resistance occurs in all breeds of cattle.

It is heritable, and selection and breeding for tick resistance are possible not only in zebu×European breeds, but also within European breeds. However, selection for resistance or culling for susceptibility must at present be based on tick numbers surviving on cattle exposed either naturally or artificially to tick challenge. This raises obvious problems for the cattle producer who is concerned about the effects of these ticks on production. Breeding of tick resistance cattle, pasture spelling, pasture burning and some special grasses have also been considered for tick control.

Bock et al.(1997) compared two breeds of cattle and reported that *Bos indicus* had innate resistance to infection with *babesia bovis*, *B. bigemina* and *Anaplasma marginale* as compared to *Bos taurus* breed.

Wharton (1983) reviewed that the most logical method of alleviating tick depredations would be to capitalize on host-parasite relationships that evolved in nature.

Cattle survived in Asia and Africa despite *Babesia*, *Theileria* and their *Boophilus* and *Rhipicephalus* vectors. Host resistance, expressed by an animal's ability to prevent the maturing of large numbers of ticks, and disease immunity, are survival mechanisms for the host and for external and internal parasites.

The problem is not only to utilize these attributes, but also to increase productivity. Resistance to *B. microplus* is associated primarily with zebu (*Bos indicus*) cattle. Considerable progress has been made in evolving resistant *Bos indicus*×*Bos taurus* beef and dairy cattle that limit the effects of ticks while retaining high productivity (Turner, 1975; Hayman, 1974; Mason, 1974).

Pasture spelling, pasture burning and use of certain grasses and legumes are also practiced for inhibition or killing of ticks (Branagan, 1973; Sutherst et al., 1982; Chiera et al., 1984).

Improvement of the nutrient value of pasture would allow cattle to develop a better resistance to tick infestation (Sutherst, 1983). Gladney et al.(1974) concluded that insect control based on sterile males or genetic manipulations offer little promise while pheromone attractants could be useful for domestic pets, or for ticks attached on specialized sites. Mbati et al.(2002) reported that farmers also used alternative methods such as used engine oil (12%), Jeyes fluid (24%), chickens (4%) and de-ticking (2%).

Kaaya and Hassan (2000) reported that the use of entomopathogenic fungi to control ticks may reduce the frequency of chemical acaricide use and the need for treatment for tick-borne diseases. They also conclude that mycopesticides are safer for the environment than conventional acaricides.

## Immunological control through vaccines

Several approaches have been used to actively immunize bovines against the cattle tick. The first attempts included the use of complex tick extracts (Willadsen et al., 1988).

For both, ticks and tick-borne diseases, vaccines have been developed or are in the course of being developed. Although tick-borne diseases are important in all domestic animals, vaccine development and production has so far focused on the economically important tick-borne diseases of cattle, such as Babesiosis (*B. bovis*, *B. bigemina*), Theileriosis (*T. parva*, *T. annulata*), Anaplasmosis (*A. marginale*) and Cowdriosis (*C. ruminantium*).

FAO has been implementing a coordinated multi-donor programme for integrated tick and tick-borne disease control in Eastern, Central and Southern Africa. Vaccine development, production and delivery were the main focus of that programme.

A three strain *Theileria parva* stabilize vaccine, known as the Muguga cocktail was developed. This vaccine has been used in combination with an antibiotic treatment, known as the infection-and-treatment method.

Later the mild Boleni strain was isolated for vaccine production in Zimbabwe and is currently being used without the treatment component (FAO, 1998). Scientists are working towards new and improved vaccine of tick fever to replace existing one. Existing vaccines have a high level of side effects, low coverage and can be expensive to make (CRC-VT, 2001).

Willadsen (2001) worked on the molecular revolution in the development of vaccines against ecto-parasites. He warns that there is a long way to go before the full potential of anti-tick vaccines will be reached and they will very likely need to be multiple antigen formulation. However, he and his coworkers pioneering work on the Bm86 based vaccines remain the best example of a commercial recombinant vaccine in the tick and tick-borne pathogens field.

The recombinant Bm86-containing vaccine against the cattle tick *Boophilus microplus* has proved its efficacy in a number of experiments, especially when combined with acaricides in an integrated manner (García-García et al., 2000).

Tellam et al.(2002) studied the reduced oviposition of *Boophilus microplus* feeding on sheep vaccinated vitelline. He used both vitelline and the protein GP80 with which it has immunological cross reactivity. Two vaccines against the tick *Boophilus microplus* have been developed. They are being field tested in Brazil in collaboration with FAO. Although there is a vaccine available, their efficiency is not 100%, which is why there is need to have an improved and absolute vaccine that can help cure animal stock against ticks (FAO, 1998).

Jenkins (2001) studied "Advances and prospects for subunit vaccines against protozoa of veterinary importance". Tick-borne diseases in his review were Babesiosis and Theileriosis. Because of the risk associated with using live parasites (such as the need for a cold chain, limited shelf-life, clinical reactions, reversion of attenuation), research towards subunit vaccines is rapidly advancing.

But their development is complicated by several factors, such as antigenic variation and strain diversity. The generation of a protective immune response furthermore depends on other factors, for instance delivery, adjutant, age of the animal, etc. The identification of protective tick antigens remains a major limitation in the development of further anti-tick vaccines as well as a significant scientific challenge.

García-García et al.(2000) reported the isolation of the Bm95 gene from the *B. microplus* strain A, and found that Bm95 antigen from strain A was able to protect against infestations with Bm86-sensitive and Bm86-resistant tick strains.

He suggested that Bm95 could be a more universal antigen to protect cattle against infestations by *B. microplus* strains from different geographical areas. Almazán et al.(2003) conducted research on identification of protective antigens for the control of *Ixodes scapularis* infestation using cDNA expression library immunization.

## Chemical Control

Control of ticks with acaricides may be directed against the free-living stages in the environment or against the parasitic stages on hosts. Control of ixodid ticks by acaricide treatment of vegetation has been done in specific sites (eg, along trails) in recreational areas in the USA and elsewhere, to reduce the risk of tick attachment to people.

This method has not been recommended for wider use because of environmental pollution and the cost of treatment of large areas. Dog kennels, barns, and human dwellings may also require periodic treatment with acaricides to control the free-living stages of ixodid ticks such as the kennel tick, *Rhipicephalus sanguineus*.

The free-living stages of argasid ticks, which infest specific foci such as fowl runs, pigeon lofts, pig sties, and human dwellings, are more frequently and more effectively treated with acaricides.

Treatment of hosts with acaricides to kill attached larvae, nymphs, and adults of ixodid ticks and larvae of argasid ticks has been the most widely used control method. A variety of ectoparasiticide products are available on the market to treat animals against ticks.

Some ectoparasiticides are applied as sprays, dips/washes, spot-on treatments, or impregnated tugs and collars and distributed cutaneously—on the skin surface. Others are administered orally and distributed systemically by the blood circulation. In general, ticks must attach to an animal and acquire a blood meal for the blood-distributed ectoparasiticides to be effective. Alternatively, cutaneously distributed ectoparasiticides have a potential to both kill the attached ticks and prevent the attachment of new ones.

Pyrethroids, including fipronil, permethrin, and permethrin combination products, are effective ectoparasiticides because of rapid penetration of arthropod cuticle and high accumulation in arthropod tissues. However, topically applied cutaneous ectoparasiticides may not achieve a uniform distribution, with some body parts not being covered to the same extent and concentration (eg, hind legs vs. the back).

This may be due to the distance from the original application site as well as by greater comparative loss of active ingredient from the legs because of routine life activities.

Pyrethroids are safe and effective in dogs; however, they are toxic to cats and fish. Systemically distributed ectoparasiticides, including afoxolaner, fluralaner, and sarolaner, tend to achieve more uniform distribution throughout the animal body and extremities but may take longer to reach the full efficacy and have somewhat lower speed of kill.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## Vaccines

An advance of potentially great importance has been the production, using biotechnology, of a promising vaccine against *R microplus*. The immunizing agent is a concealed tick antigen, not normally encountered by the host.

The immune mechanism it stimulates is different from that stimulated by exposure to ticks (ie, tick feeding). The antigen was derived from a crude extract of partially engorged adult female ticks. It stimulates the production of an antibody that damages tick gut cells and kills the ticks or drastically reduces their reproductive potential.

Prospects of developing similar vaccines against other ixodid tick vectors of cattle diseases of major veterinary importance are not clear. *Rhipicephalus* ticks are good candidates for such vaccines in that they are one-host ticks and show a marked preference for bovine hosts, which act as the principal reservoir of perhaps the most important group of disease agents (*Babesia* spp) these ticks transmit.

By contrast, most other tick vector species of agents that cause important cattle diseases (eg, anaplasmosis, heartwater, theileriosis) are three-host ticks, which infest not only cattle but also wild ungulate species, for which vaccination is not feasible. Moreover, many wild ungulate hosts of the vector ticks serve as reservoirs of these disease agents.

For these reasons, vaccines against nonboophilid vector ticks may be unable either to eradicate the ticks or to eliminate important sources of the disease agents they transmit.

## Control Strategies

Initially, the main uses of acaricides were for tick eradication, prevention of spread of ticks and tickborne diseases (quarantine), and eradication and control of tickborne diseases.

The eradication programs were successful in some ecologically marginal subtropical areas, such as southern USA and central Argentina where *Rhipicephalus* spp and babesiosis were eradicated, and southern Africa where East Coast fever (caused by *Theileria parva parva*) was eradicated.

The programs were less successful in the ecologically more favorable tropical areas of northeastern Australia, Central America, the Caribbean Islands, and East Africa.

In the areas where eradication was not achieved, costs of maintaining intensive tick control programs often have become prohibitive. For this reason, integrated biologic and chemical control strategies are being adopted. The effectiveness of these cost-containment strategies requires better knowledge of the dynamic associations among the disease agents, their vertebrate hosts, the tick vectors, and the environment.

Strict quarantine measures to prevent reintroductions are enforced in countries from which ticks and tickborne diseases have been eradicated. Climate-matching models, geographic information systems, and expert systems (models based on expert knowledge and artificial intelligence) are being used to identify unaffected areas in which tick pests could become established if introduced.

Control of these diseases will require use of the principles of endemic stability and development of improved recombinant vaccines. A current, promising strategy is the identification of receptor sites on the midgut of vector ticks and the development of antibodies that bind with these sites, thereby blocking tick-ingested tickborne pathogens from infecting the tick.

Cattle injected with receptor-site antigens may produce antibodies that feeding ticks ingest.

## Management of Host Animals

Food and shelter are essential requisites for wildlife. The residential landscape can be particularly attractive to white-tailed deer and conducive to mice and chipmunks, important hosts in the prevalence of ticks and Lyme disease.

One component of a tick management strategy is managing deer and small rodent activity in your yard. Some landscaping practices discussed in the previous section can also help manage key animals in the landscape. Stonewalls, woodpiles, and dense vegetation can harbor rodents.

## Host-Targeted Chemical Tick Control for White-tailed deer

The U.S. Department of Agriculture, Agricultural Research Service (ARS), developed passive self-treatment methods for white-tailed deer through both systemic (i.e. ivermectin-treated corn) and topical application technologies to kill ticks feeding on deer.

A device termed a ‘4-Poster’ was designed for the application of topical acaricides to white-tailed deer to prevent the successful feeding of adult ticks. It consists of a feeding station with four paint rollers that hold the pesticide.

Deer self treat themselves when, because of the design, they are forced to brush against the rollers as they feed on whole kernel corn. Computer simulations of various intervention scenariossuggested that acaricide applied to white-tailed deer (assuming 90% of deer are treated and 90% tick mortality on these deer) would prevent more cases of human Lyme disease except perhaps for the best use scenario of a Lyme disease vaccine.

Because white-tailed deer are the keystone species for adult blacklegged ticks and lone star ticks, the ‘4-Poster’ was evaluated on free-ranging deer in a multi-year (5 years treatment plus 2 additional years tick sampling) project in the northeastern United States for the control of both tick species at seven 2-mi2 sites in 5 states (MD, NJ, NY, CT, RI). Approximately one device was placed per 51 acres, although some minimally used 4-posters were redeployed near heavily used devices to increase host access. Treatments utilized a 2% oily formulation of amitraz and reduced blacklegged tick abundance by up to 81% and lone star ticks up to 99.5% in the treated communities in comparison with untreated areas after 3 or more years of use.

Similarly, the application of 10% permethrin to a 600-acre fenced population of deer resulted in a 91-100% reduction of larval, nymphal, and adult questing blacklegged ticks at the Goddard Space Flight Center, MD. While usage of the devices by deer was generally high (> 90 to 100%), utilization of the devices by deer can be low or sporadic when alternative food sources were available such as heavy acorn mast.

Maintenance of the feed and topical insecticide through the tick season is labor intensive. Nevertheless, according to computer simulations, this approach, in principal, could provide the greatest reduction in Lyme disease with the least direct community involvement (i.e. number of direct participating households) and may be an alternative to the application of area-wide acaricides and the maintenance of drastically reduced deer populations.

The '4-Poster' Deer Treatment Bait Station is licensed to the American Lyme Disease Foundation of Lyme, CT (www.aldf.com) and manufactured by C. R. Daniels, Inc. of Ellicott City, MD (www.crdaniels.com). The U.S. Environmental Protection Agency (EPA) has registered an oily 10% permethrin formulation of ready to use tickicide (Y-TEX '4-Poster' Tickicide®, Y-TEX Corporation, Cody, WY) especially for application to deer via the '4-Poster Deer Treatment Stations to control I. scapularis and A. americanum. Permethrin is the chemical used as a tick repellent on clothing and as an acaricide in some louse and scabies mite treatment products for human use. According to the 'Tickicide' label, the acaricide is not to be used less than 100 yards from any home, apartment, playground, or other place children might be present without adult supervision.

States may impose more restrictive requirements than the federal label. State pesticide registrations have been obtained in 47 of the 48 contiguous states except for New York, which has strict regulations against feeding deer. Approval requirements or regulations for use by state wildlife officials vary from state to state and use of the device raises some concerns among some state wildlife agencies. Although no cases have been observed in New England and only a single isolated occurrence in New York, Chronic Wasting Disease (CDW) has been shown to be transmitted via blood and saliva of infected deer, primarily in Michigan and other north central states. The use of the 4-poster will probably be most practical as part of a neighborhood or community coordinated program to reduce ticks and the risk of Lyme disease, managed under state use regulations, and combined with some form of a deer management program.

# Controlling Ticks and Tick-borne Zoonoses with Biological and Chemical Agents

**Ticks are obligate, blood-feeding ectoparasites of** vertebrate hosts. They feed by inserting specialized piercing mouthparts (figure 1) into the skin of the host, avoiding detection by using a salivary pharmacopeia of anti-inflammatories, analgesics, antihistamines, and anticoagulants (Ribeiro et al. 1985, Sonenshine 1993). Tick life histories can be categorized by the degree of intimacy between tick and host.

The nidicolous (from the Latin *nidis*, or nest) tick species, including almost all members of the family Argasidae and some members of the Ixodidae, spend their entire lives within dwellings—nests, burrows, caves—used by their hosts. The habitat specificity exhibited by nidicolous ticks typically is associated with a high degree of host specificity. The remaining, nonnidicolous tick species occupy habitats such as forests, savannas, grasslands, and shrublands, where they undergo long periods of diapause interspersed with short periods of host seeking or questing.

After attaching to a host and feeding for a few days to a few weeks, these ticks drop off, spend weeks to many months digesting the blood meal, molt into the next stage, and then repeat the process with a different host. Adult ticks reproduce and die after their blood meal. A typical nonnidicolous tick is the blacklegged tick (*Ixodes scapularis*), a species widespread in eastern and central North America. The life cycle of this tick lasts more than 2 years, during which individuals take three blood meals, one each as a larva, nymph, and adult, typically from three different host species (figure 2).

Many viral, bacterial, and protozoal species have evolved to take advantage of the nonnidicolous tick life history, using the tick as a vehicle for dispersing from one vertebrate host to another. In the tick, these microbes generally reproduce little until the tick begins feeding on a host, and they typically do not cause obvious disease. In the vertebrate host, however, the microbes reproduce, disseminate, and often cause disease.

Tick-borne microbial pathogens, which cause human and livestock diseases such as Lyme disease, anaplasmosis, ehrlichiosis, babesiosis, tick-borne encephalitis, Crimean–Congo hemorrhagic fever, Rocky Mountain spotted fever, Colorado tick fever, tick typhus, tularemia, heartwater, East Coast fever, and Nairobi sheep disease, have enormous negative impacts on human health and economic development worldwide. Because of the near absence of vaccines, inefficient diagnostic capabilities, and imperfect treatments for tick-borne illnesses, the major means of reducing the burden of tick-borne disease is reducing the abundance of ticks.

Attempts to control tick population abundance require at least two strategic decisions: what sort of control agent to use and how to deliver it. Control agents can be either chemical or biological, and delivery can be either to the environment (e.g., forest floor, pasture, lawn) where ticks seek hosts and undergo diapause or directly to the hosts on which ticks feed. In this overview, we will first briefly review the more traditional use of chemical control of ticks through environmental applications, but our treatment will be somewhat cursory, given recent reviews of this topic (Stafford and Kitron 2002).

Next, we will explore more recent advances in the delivery of chemical control agents to wildlife and livestock hosts for ticks. Last, after a brief general discussion of the strengths and limitations of biological control (biocontrol), we will describe recent developments in the use of biocontrol agents deployed against ticks both off-host and on-host, ending with our view of the future prospects for tick control.

## Traditional tick control with chemical insecticides

The primary means of reducing tick abundance is application of chemical insecticides into the environment where ticks seek hosts. Insecticides typically are highly lethal to ticks (although ticks are arachnids, not insects), and field applications generally are quite effective in reducing tick numbers (Sonenshine 1993, Stafford and Kitron 2002).

Conventional organophosphate, carbamate, and pyrethroid insecticides generally are inexpensive, and broadcast delivery of chemical pesticides can be effective in reducing tick numbers within localized areas (Schulze et al. 1991, 2001a).

Carbaryl and chlorpyrifos appear to be the most widely used insecticides for controlling ticks (Stafford and Kitron 2002), but their high toxicity to vertebrates has triggered the banning of some products by federal agencies and the discontinuation of others by manufacturers (Schulze et al. 2001a).

Somewhat less toxic alternatives, including the synthetic pyrethroids, can be effective in lower doses than organophosphate and carbamate compounds, at least over the short term. For example, a single application of deltamethrin, a synthetic pyrethroid, along a band extending from a lawn–forest edge to 7 meters (m) into the forest resulted in a reduction in tick density of 90% or more over the ensuing 9 days (Schulze et al. 2001a). However, synthetic pyrethroids are not devoid of toxic effects on wild vertebrates, particularly when used in combination with other insecticides (Thompson 1996).

In addition, all of the commercial insecticides are lethal to many invertebrates, including pollinators and predators on arthropod pests (Schauber et al. 1997). The nature and extent of undesirable, nontarget effects on invertebrates are rarely determined in field trials of the efficacy of chemical insecticides for tick control (for an exception, see Schulze et al. 2001b), but they could be considerable. Finally, repeated insecticide applications can cause the evolution of insecticide resistance (Roush 1993). These shortcomings of broadcast chemical insecticides have led to a search for alternative methods of controlling ticks.

## Host-targeted tick control with chemical insecticides

Chemical pesticides delivered directly to livestock hosts (e.g., via cattle and sheep dips) have been employed successfully for many years (reviewed by George et al. 2004). Although clearly effective at reducing transmission of tick-borne pathogens to livestock, repeated heavy applications of pesticides to hosts can cause considerable mortality in nontarget arthropods through environmental contamination (Gassner et al. 1997).

Moreover, evolved resistance to insecticides, which is a well-known problem with mosquitoes, is a persistent issue for tick species such as *Boophilus microplus* that are chronically exposed by virtue of their close association with cattle to which the insecticides are applied (Foil et al. 2004, George et al. 2004).

Only recently have researchers explored the delivery of pesticides directly to wildlife hosts for ticks as a means of reducing the risk of tick-borne human illness. Targeting wildlife hosts of ticks with insecticides poses many challenges, owing to the variety of vertebrate hosts for many species, their ability to disperse, the potential impact of insecticides or bait attractants on host populations, and the potential proximity of hosts to residential areas. For blacklegged ticks in North America, new devices that deliver insecticide to hosts for adult ticks (largely deer), or to hosts for immature ticks (largely rodents), are currently being tested (table 1).

The four-poster deer feeder is a device that attracts deer to a bin of corn; to feed on the corn, the deer must rub their heads and necks against paint rollers strategically placed around the bin and impregnated with insecticide. Two recent field studies designed to assess the impacts of four-posters on the abundance of blacklegged ticks in Lyme disease–endemic areas of Maryland concluded that tick populations were reduced substantially following several years of continuous deployment (Carroll et al. 2002, Solberg et al. 2003). Carroll and colleagues (2002) found that three sites at which 25 four-posters per site had been operated for 5 years had densities of nymphal blacklegged ticks 69%, 76%, and 80% lower than on unmanipulated control sites.

The study by Solberg and colleagues (2003), which consisted of one treatment and one control (untreated) site, also claimed a reduction in tick numbers, but that work was unreplicated. Moreover, tick numbers were markedly low on the treatment site before the deployment of four-posters, weakening the conclusions drawn. To date, no studies have been performed in the suburban landscapes where most cases of Lyme disease occur (Barbour and Fish 1993).

The difficulty of keeping nontarget animals such as raccoons and children from visiting the four-posters, and the high cost of installing and maintaining these devices, are important challenges for this technique. Another obstacle is that the detection of chronic wasting disease (a degenerative brain disease caused by aberrant proteins called prions) in deer within Lyme disease–endemic zones has led some states to ban the feeding of deer. Despite the potentially high efficacy of four-posters, the feeding ban, intended to reduce deer-to-deer transmission of prions, makes widespread use of these devices unlikely.

Two devices for delivering insecticide to small rodents are commercially available. One consists of a cardboard tube with permethrin-impregnated cotton that can be retrieved by rodents to their nests. Field tests of this product have produced mixed results, but generally do not support its efficacy in reducing numbers of host-seeking ticks (Stafford and Kitron 2002).

An early study conducted in coastal Massachusetts (Mather et al. 1987) documented a marked reduction in the numbers of immature blacklegged ticks on white-footed mice in areas provided with abundant bait tubes, compared with untreated areas, but the impact of the bait tubes on host-seeking nymphal ticks was not assessed. This is unfortunate, because host-seeking nymphal ticks are primarily responsible for transmission of Lyme disease spirochetes and other pathogens to humans. Another test of this product in coastal Massachusetts (Deblinger and Rimmer 1991) consisted of the deployment of 2000 tubes in a 7.3-hectare site over 3 years.

Although the apparent lack of host-seeking nymphs and of complaints about human tick bites was interpreted as confirmation of the efficacy of the product, the lack of replication and of control sites weakens this claim. The most comprehensive field deployments of these tubes over multi-year periods in Connecticut and New York showed no significant reduction in numbers of host-seeking ticks (Daniels et al. 1991, Stafford 1992).

The other device is a small plastic box ("bait box") with holes that allow access by small rodents. Rodents are attracted by a food source within the box, but to reach the food they must contact a wick that applies fipronil, a pesticide marketed as "Frontline." A field test of this product, conducted by the device's inventors on more than 300 different properties in Connecticut, supported its efficacy. Numbers of host-seeking nymphal ticks, a key risk factor for tick-borne diseases, were reduced by more than 50% on bait box–treated properties compared with untreated properties (Dolan et al. 2004).

Unfortunately, as in one of the four-poster studies described above, considerably lower numbers of ticks on the treatment sites preexisted the deployment of bait boxes (Dolan et al. 2004), suggesting that conditions unrelated to the bait box treatment may have played a role in the apparent reduction in tick numbers caused by the device. A clearer result of deploying bait boxes, however, was the reduction in the proportion of nymphal blacklegged ticks infected with *Borrelia burgdorferi* (the spirochete that causes Lyme disease) and *Anaplasma phagocytophilum* (the bacterium that causes human granulocytic ehrlichiosis, or anaplasmosis) by 67% and 64%, respectively.

Reduced infection prevalence in nymphal ticks would serve to reduce risk of human exposure to tick-borne infections, and would be expected from a device that preferentially targets ticks on rodents, which are the principal reservoirs for these pathogens.

Although host-targeted chemical insecticides show promise as a means of reducing the abundance of ticks, more thorough testing of safety and efficacy will be required. The efficacy of deer- or rodent-targeted interventions will undoubtedly be enhanced by high, even saturation, densities of devices. However, the health consequences of massively supplementing food for either rodents or deer are unknown.

Certainly, supplemental foods can induce strong population growth in rodents (Boutin 1990), with unpredictable net effects on disease transmission. The potential impact of seasonally or chronically available supplemental corn on deer population dynamics is unknown. In addition, very little is known about the degree to which host-targeted pesticides and their toxic breakdown products accumulate in avian and mammalian predators and scavengers on the target organisms.

## Classical and augmentative biocontrol

The most promising alternatives to chemical pesticides are biological control (biocontrol) agents, which are species that consume target pest organisms via predation, herbivory, or parasitism. Biocontrol agents typically are nontoxic to humans and to nontarget wildlife (for a few exceptions, see below).

Moreover, biocontrol agents are expected to coevolve with their target organisms, reducing the likelihood that resistance will evolve. Although biocontrol programs have a mixed record of success and include some spectacular failures (e.g., the decimation of island endemic birds by mongooses [*Herpestes javanicus*] released to control introduced rats), biocontrol appears promising, but understudied, for the control of ticks.

The predominant form of biocontrol is “classical biocontrol,”whereby nonnative predators, herbivores, or parasites (including parasitoids and pathogens) are introduced to control nonnative pest species. The vast majority of classical biocontrol efforts have been directed at exotic plants and insect pests of agricultural products. The most widely recognized danger of classical biocontrol is that the biocontrol agent will attack nontarget organisms, particularly native taxonomic relatives of the exotic target species (Stiling 2004, Louda et al. 2005).

Attacks on nontarget species are expected to be minimal when the biocontrol agent is a specialist on the target species, and much effort is devoted to confirming that a potential biocontrol agent is indeed a specialist on the target pest. Unfortunately, many biocontrol agents identified during prerelease screening as specialists have attacked nontarget organisms once they were released into new ecological communities with alternative hosts, particularly when the target pest became scarce (Lynch et al. 2002).

In addition, owing to their dependence on the target organism, specialist biocontrol agents are likely to become ineffective or disappear entirely when the abundance of target organisms declines, and this interdependence can cause damaging fluctuations in the abundance of the pest. Other dangers include displacement of native predators or parasites on the pest species, for example, when native ladybird beetles are displaced following introduction of exotic ladybirds to control aphids (Evans 2004).

Some biocontrol agents unintentionally provide large food subsidies to predators, with potentially serious consequences. For instance, native deer mice (*Peromyscus maniculatus*) feast on gall flies (*Urophora*), which were released to control noxious knapweeds (*Centauria*). Deer mice with access to this unintended food supplement can undergo explosive population growth (Ortega et al. 2004), with possible negative consequences for human health, given that high deer mouse density is correlated with high hantavirus disease incidence in humans (Yates et al. 2002).

Despite these shortcomings, classical biocontrol has seen some well-known successes, such as the use of myxoma virus to control rabbits introduced to

Australia (Hayes and Richardson 2001) and the resurgence of populations of *Entomophaga maimaiga*, a fungal pathogen of gypsy moths (*Lymantria dispar*), nearly 100 years after its release (Hajek et al. 1990). Efforts to reduce the probability of unpleasant surprises are ongoing (Louda et al. 2003, Kimberling 2004).

Far less attention has been paid to "augmentative biocontrol,"whereby native predators, herbivores, or parasites are used to control a target pest species. Augmentative biocontrol is typically attempted with insect predators or parasitoids, reared in insectaries, that are released in massive numbers ("inundative" biocontrol) to suppress insect pests (Collier and Van Steenwyk 2004).

A major benefit of augmentative biocontrol is that it does not involve introducing species outside their native geographic ranges, and thereby avoids problems that accompany species introductions. Its major limitation arises from the inability of the native natural enemy to control the pest without being augmented repeatedly. In other words, if the pest and control agent coexist naturally, but the pest (by definition) is normally not controlled by the agent, the effectiveness of the control agent seems in doubt.

Poor control under normal (unaugmented) conditions, however, can be caused by low natural contact rates between the control agent and the target. A key challenge for augmentative biocontrol is to alter the abundance or distribution of the control agent to maximize contact rates with the target species.

## Use of biological agents to control ticks

Natural enemies of ticks include insectivorous birds, parasitoid wasps, nematodes, *Bacillus thuringiensis* bacteria, and deuteromycete fungi (largely *Metarhizium anisopliae* and *Beauvaria bassiana*) (Samish and Rehacek

1999). The potential of each of these taxa as biocontrol agents will be discussed in turn.

Mammals and birds typically consume ticks during self-grooming. For example, laboratory studies demonstrate that significant numbers of larval blacklegged ticks are consumed by white-footed mice (*Peromyscus leucopus*) during self-grooming (Shaw et al. 2003). Nevertheless, a high proportion of ticks encountering mice survive and feed to repletion, and abundance of blacklegged ticks is positively correlated with that of mice (Ostfeld et al. 2001).

Some vertebrates attack ticks in the environment. Wild turkeys (*Meleagris gallopavo*) consume a very high proportion of the immature blacklegged ticks they encounter while grooming (Ostfeld and Lewis 1999), and might reduce tick numbers. In the case of host species, such as turkeys, that groom a high proportion of the ticks that attempt to feed from them, abundance of ticks could be suppressed by high host abundance.

To our knowledge, such negative correlations between the abundances of specific hosts and ticks have not been explored. Unfortunately, enhancing the numbers or distribution of turkeys as biocontrol agents seems infeasible. Moreover, turkeys are an important host for lone star ticks (*Amblyomma americanum*) (Kollars et al. 2000), and high abundance of turkeys could facilitate populations of this tick species, which is a vector of human monocytic ehrlichiosis. Other birds also consume host-seeking ticks in the environment.

On the basis of a modest study using small enclosures and exclosures to manipulate helmeted guineafowl (*Numida meleagris*) on lawns (Duffy et al. 1992), this bird has reached cult status as a biocontrol agent for blacklegged ticks and is credited with reducing the transmission of Lyme disease bacteria to people (figure 3). However, tick reduction by guineafowl was restricted to the adult stage (Duffy et al. 1992), which transmits a small minority of Lyme disease cases to people (Barbour and Fish 1993), and to lawns, which maintain far smaller populations of ticks than do brushy and wooded habitats (Ostfeld et al. 1996).

Recently, we extended Duffy and colleagues' (1992) study by comparing numbers of adult and nymphal blacklegged ticks on properties with and without free-ranging guineafowl in a highly Lyme disease–endemic zone in southeastern New York State. We found that although reduced abundance of adult ticks in the presence of guineafowl suggested that the birds do attack this life stage, the presence of guineafowl did not significantly reduce the density of nymphs (box 1), which transmit the vast majority of Lyme disease cases (Barbour and Fish 1993).

We hypothesize that the provision of food (grain) to guineafowl attracts small rodents, which might import immature ticks onto properties containing the birds, and that this might counteract the suppressive effects of predation by the fowl on adult ticks.

The most obvious vertebrate consumers of ticks are oxpeckers (*Buphagus* spp.), pan-African birds that specialize on ticks feeding on both wild and domestic large mammals. The daily intake of ticks by oxpeckers is reported to be in the hundreds (adult ticks) to thousands (nymphs) (Samish 2000). However, neither a reduction in tick populations by natural populations of oxpeckers nor the feasibility of augmenting their numbers has been demonstrated.

Parasitoid wasps and flies are among the most effective agents in the biocontrol of insects (Kimberling 2004). Parasitoids are often, although not always, specialists on one host taxon (or a small number of host taxa), and typically have sufficiently high fecundity to allow rapid population response to host abundance. Both of these features enhance their potential as control agents. The chalcid wasp, *Ixodiphagus hookeri*, a parasitoid that specializes on ixodid ticks, was introduced to Massachusetts islands and the northwestern United States several times in the early 20th century to control populations of the American dog tick, *Dermacentor variabilis*, and the Rocky Mountain wood tick, *Dermacentor andersoni*. Although systematic monitoring following these mass releases was not conducted, spotty sampling revealed that the parasitoid became established in the blacklegged tick population on Naushon Island, Massachusetts (Mather et al. 1987).

Neither immediate nor long-term control of ticks was reported, however, and recent studies suggest that *I. hookeri* will persist only when blacklegged ticks and their deer hosts are hyperabundant, which tends to occur on islands and adjacent mainland sites in southern New England (Stafford et al. 2003).

The reported threshold effect of host abundance suggests that, in fact, tick populations control those of parasitoids from the bottom up, rather than parasitoids exerting top-down control of their hosts. Nevertheless, inundative releases of *I. hookeri* have shown promise in controlling tick populations infesting cattle in Kenya (Mwangi et al. 1997), and a modeling study by Knipling and Steelman (2000) suggests that massive environmental releases of laboratory-reared parasitoids could reduce tick populations locally.

Unfortunately, the production of inundative quantities of *I. hookeri* might be prohibitively expensive, given the high costs of maintaining tick colonies, which would be necessary to raise these obligate tick parasitoids.

Several species of steinernematid and heterorhabditid nematodes, including those used commercially to control insect pests, are also pathogenic to ticks. Juvenile nematodes invade ticks either through natural orifices or using digestive enzymes and mechanical force to penetrate the host cuticle. Once in the host haemocoel, they release mutualistic bacteria that attack and kill the tick (Zhioua et al. 1995).

The pathogenicity of nematodes is generally restricted to engorged adult female ticks (Samish and Glazer 2001), probably due to ease of access through the genital pore or through stretched and thin cuticle. However, because these nematodes do not complete their life cycles within the tick host (Samish and Glazer 2001), ticks are incapable of sustaining nematode populations in either laboratory or natural environments.

Moreover, commercially available entomopathogenic nematodes appear not to survive winter temperatures within temperate regions where many tick-borne diseases of humans occur (Samish and Glazer 2001). As is the case for parasitoids, release of nematodes to control ticks is likely to be successful only temporarily and in highly localized areas.

However, systematic searches may identify native entomopathogenic nematodes with greater potential as biocontrol agents. Potentially, native entomopathogenic nematodes could use nontick arthropods as reservoir hosts (Donald Strong, Bodega Marine Laboratory, University of California, Davis, personal communication, 6 August 2005) and opportunistically attack ticks during the postfeeding diapause.

Although several bacterial species are pathogenic to ticks, the usefulness of bacteria as biocontrol agents is poorly studied. *Bacillus thuringiensis*, which is used as a biocontrol agent for many insects, is pathogenic to ticks, but apparently must be ingested to be effective (Samish and Rehacek 1999, Zhioua et al. 1999).

Because ticks tend to ingest only host blood, inducing ticks to ingest these bacteria seems impractical, and the prospects for *B. thuringiensis* as a biocontrol agent seem poor. Recent surveys of microbes naturally infecting blacklegged ticks and American dog ticks (Martin and Schmidtmann 1998, Grindle et al. 2003) reveal a rich flora including spore-forming and crystal-forming bacteria that, if found to be entomopathogenic, could be developed as potential biocontrol agents.

## Biocontrol of ticks using entomopathogenic fungi

Dozens of species of eumycete and deuteromycete fungi are known to attack and kill ixodid ticks, but only a handful of species have been extensively studied. *Metarhizium anisopliae*(figure 4) and *Be. bassiana*, in particular, appear to cause significant tick mortality in laboratory assays. Both of these taxa have virtually worldwide distributions (Humber 1992), although much genetic variation exists among geographic areas. Fungus-caused mortality varies substantially among different tick species and life stages, fungal taxa, and modes of application.

Mortality tends to be higher in engorged or engorging ticks than in unfed ticks; higher in adults than in nymphs or larvae; higher in *Ixodes* than in *Boophilus*, with *Amblyomma* and *Rhipicephalus* highly variable; higher in oil-based than in water-based delivery mode; and higher in laboratory than in field assays. (A table reviewing responses by 12 species of ticks to 9 species of entomopathogenic fungi is available from the authors.) The mechanisms that underlie these sources of variation are poorly understood.

Numerous laboratory assays of both *M. anisopliae* and *Be. bassiana* support their lethal effects on several tick species of epidemiological and veterinary importance. In addition, strong and demographically significant sublethal effects of these fungi on ixodid ticks have been noted by several researchers (reviewed in Hornbostel et al. 2004).

A combined field and laboratory study using *M. anisopliae* on *Ixodes scapularis* revealed that fungal exposure reduced the body mass of engorging female ticks and the mass of their egg clutches by up to 50% (Hornbostel et al. 2004). Engorged larvae and nymphs treated with fungus molted into significantly lighter nymphs and adults, respectively, compared to untreated controls. These results suggest that these fungal pathogens reduce tick fitness (fecundity and body mass), with likely negative impacts on population growth beyond that imposed by direct mortality alone.

Field deployments of both fungal species in mesh bags in pastures and directly on cattle in Kenya and Brazil suggest that these biocontrol agents have great promise in reducing tick burdens on livestock (Kaaya et al. 1996, Bittencourt 2000, Kaaya and Hassan 2000).

Mortality rates tend to be moderate to high for adult and immature livestock ticks from the genera *Boophilus*, *Rhipicephalus*, and *Amblyomma* exposed to entomopathogenic fungi in pastures or stables. The potential for replacing livestock dips using chemical insecticides with those employing fungal spores in solution seems quite high. We are not aware of any demonstrations of toxic effects of these fungal solutions on livestock or other terrestrial vertebrates, although nontarget effects of *M. anisopliae* and *Be. bassiana* on fish eggs have been reported (Genthner and Middaugh 1995).

Isolated cases of human disease caused by entomopathogenic fungi have been reported, especially in immunocompromised individuals (DeGarcia et al. 1997, Tucker et al. 2004).

Broadcast delivery of *Be. bassiana* and *M. anisopliae* to pastures can dramatically reduce the population density of ticks known to infest cattle in Brazil (Bittencourt 2000) and Kenya (Kaaya 2000, Maranga et al. 2005). For example, Kaaya (2000) found a five- to tenfold reduction in the numbers of ticks on cattle following the spraying of fungi on pasture vegetation, and Maranga and colleagues (2005) demonstrated a synergistic effect of adding a “cocktail” of both species, compared with either species alone. To our knowledge, only two studies have employed broadcast delivery of fungal spore solution into forested habitat to assess its potential in controlling ticks.

Benjamin and colleagues (2002) sprayed aqueous solution of *M. anisopliae* spores onto forest understory vegetation at a site in southeastern New York State where Lyme disease is hyperendemic. They sprayed in autumn, to coincide with the peak activity period for adult blacklegged ticks, and targeted the understory rather than the forest floor because adult blacklegged ticks tend to climb up vegetation to a height of a meter or more to seek a host. Autumn spraying and avoidance of the forest floor were also thought to minimize impacts on nontarget arthropods. After field spraying, exposed ticks retrieved to the laboratory experienced a 53% mortality rate, as compared with control ticks (sprayed with water only), which experienced only 3% mortality (Benjamin et al. 2002).

A follow-up study (Hornbostel et al. 2004) demonstrated that similar field spraying of *M. anisopliae* solution resulted in 36% control of adult ticks, where the percentage of control is defined as [1 – (*n* in treatment group after treatment / *n* in control group after treatment)] • 100 (Abbott 1925).

This degree of control via direct mortality, combined with potent sublethal effects on ticks, suggests that field spraying of fungus solution has promise as a means of substantially reducing tick abundance. The use of oil-based fungal solutions for field applications, and additional spraying during spring and summer to target immature ticks, seems likely to improve efficacy, although this regimen could increase nontarget effects.

In addition, the use of entomopathogenic fungi supplemented with low doses of pyrethroids or other chemical insecticides might enhance tick control without the need to apply large quantities of chemical agents. Hornbostel and colleagues (2005) demonstrated that, although an expected synergistic effect of *M. anisopliae* and permethrin on blacklegged tick mortality did not occur, these two agents did not interfere with each other's impact on ticks.

The potential drawbacks of broadcast applications of fungal biocontrol agents need to be assessed more fully. Laboratory studies indicate that entomopathogenic fungi are likely to have nontarget effects (Ginsberg et al. 2002), but the scope and magnitude of these effects in the field are not well understood. In addition, the strains of fungi released at a site are rarely, if ever, demonstrated to occur naturally at that site. The impacts of releasing exotic genotypes of a native species are unknown.

For certain fungal strains, spore viability may degrade rapidly in direct sunlight, potentially reducing the effectiveness of broadcast applications in summer, when immature ticks are most abundant. Optimal temperature and moisture conditions also may be required for infection. Finally, although the evolution of resistance by ticks to entomopathogenic fungi might appear unlikely, we are not aware of any direct tests.

With the exceptions of the Kenya (Kaaya et al. 1996, Kaaya and Hassan 2000) and Brazil (Correia et al. 1998) studies on livestock mentioned above, application of entomopathogenic fungi directly to hosts has not been undertaken systematically.

As for the host-targeted chemical agents described above, a major challenge for host-targeted biocontrol agents is efficient delivery to hosts while avoiding food subsidies that might boost host numbers. To assess the ability of entomopathogenic fungi applied to wildlife hosts to control tick populations, Hornbostel and colleagues (2005) used wood nest boxes attached to tree trunks at a height of 1.5 m as a platform for delivering fungal solution to white-footed mice. These nest boxes (figure 5; described in Burns et al. 2005) are known to be used by mice as supplements to natural nesting sites in underground burrows or tree holes.

Hornbostel and colleagues (2005) periodically sprayed aqueous fungal solution onto cotton nesting material within the nest boxes and monitored the effects, both on the tick burdens on rodent hosts and on the abundance of host-seeking ticks, after a 1-year lag. The authors expected that the dark nest boxes would promote spore longevity and that prolonged contact of moist nesting material with tick hosts would encourage fungal infection.

Although laboratory treatment of cotton nesting materials with identical fungal solution was highly lethal to larval blacklegged ticks engorging on white-footed mice, field applications were only modestly effective. Abundance of nymphal ticks in 2003, following nest-box spraying targeting larval ticks in 2002, was significantly reduced only in localized areas around the nest boxes most heavily used by mice (Hornbostel et al. 2005).

We expect that the effectiveness of nest-box targeted biocontrol could be strongly enhanced by creating a saturated abundance of nest boxes (only about 35% of mice in field plots were known to use the nest boxes), and by deploying this method in suburban forests where mice tend to be abundant and relatively few alternative hosts for larval ticks occur.

## Box 1. A field study of the effects of guineafowl on blacklegged ticks.

In the eastern and central United States, the blacklegged tick, *Ixodes scapularis*, is the primary vector of *Borrelia burgdorferi*, *Babesia microti*, and *Anaplasma phagocytophilum*, the etiological agents of Lyme disease, babesiosis, and human granulocytic ehrlichiosis, respectively. The burden of all three diseases could potentially be alleviated by reducing the abundance of the tick vector, particularly in peridomestic settings where exposure can be high (Barbour and Fish 1993). The use of insectivorous helmeted guineafowl (*Numida meleagris*) for tick control is an increasingly popular tactic, widely promoted in newsletters and Web sites dedicated to Lyme disease. The promotion of guineafowl appears to be based largely on the study by Duffy and colleagues (1992), who compared the abundances of adult blacklegged ticks inside and outside small enclosures (containing guineafowl) and exclosures placed on lawns, and concluded that the presence of guineafowl was associated with lower tick density. As recognized by the authors, the study by Duffy and colleagues was limited in its ability to assess the impacts of guineafowl on disease transmission, because the research (a) was restricted to lawns, whereas tick abundance is dramatically higher in forested, ornamental, and ecotonal habitats (Ostfeld et al. 1996), and (b) assessed only the abundance of adult ticks, which transmit a small minority of Lyme disease cases (Barbour and Fish 1993).

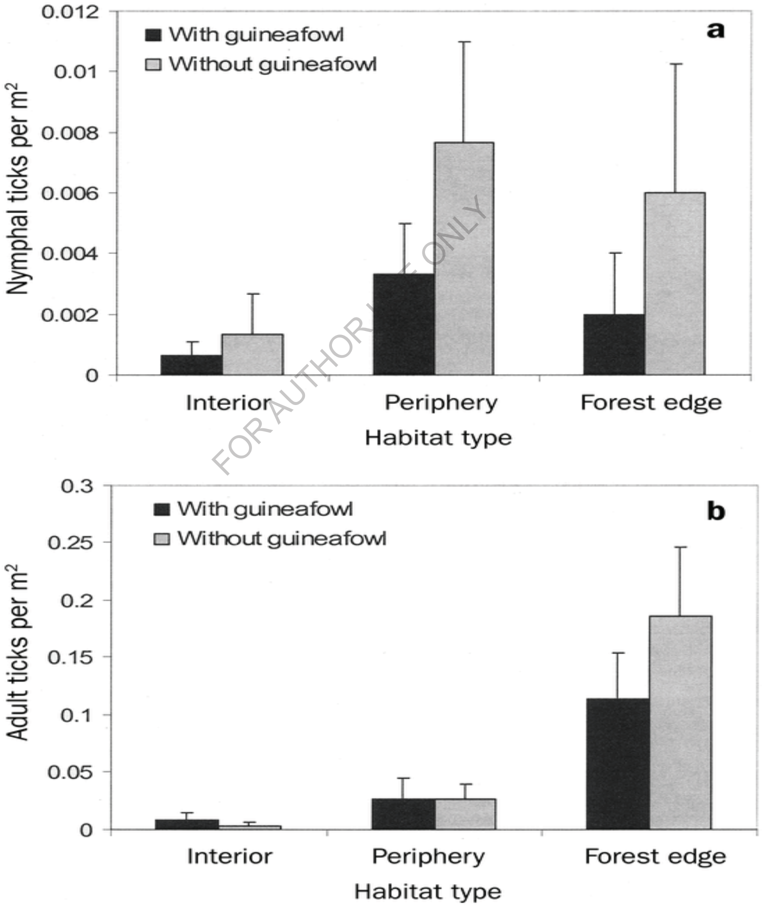
To extend the scientific assessment of the effectiveness of helmeted guineafowl in reducing the risk of human exposure to tick-borne infections, we selected matched domestic properties in Dutchess County, New York, with and without free-ranging guineafowl, and sampled the abundance of both nymphal and adult ticks in major habitat types on these properties. Ten "treatment" properties were chosen on the basis of the following criteria: free-ranging guineafowl had been present for over a year, no other tick control methods were used, and no other domestic fowl were present. Each treatment property was paired with a control property located within 1 kilometer but not directly adjacent to the treatment property, to ensure that there was no spillover guinea fowl activity. Control properties fulfilled the same criteria as treatment properties, except that no guineafowl were present. Treatment and control sites were in similar landscapes and had similar com positions, with lawns, ornamental vegetation, and forested habitat. We sampled tick abundance by dragging a 1-square-meter ($m^2$) white corduroy cloth along premeasured transects in each three habitat types: the lawn interior, the lawn periphery 1 to 2 from the forest edge, and the forest periphery 3 to 5 m from the lawn edge. Sampling at each site occurred once a week for 3 weeks during the seasonal peak in nymphal host-seeking activity (20 June–10 July 2004) and again during the peak in adult activity (16–31 October 2004).

Nymphal tick densities were extremely low in lawn interiors, and somewhat higher at the lawn periphery and within the forest edge (see the figure). Despite an apparent trend toward lower nymphal densities in all three habitat types in properties with guineafowl, these differences were not statistically significant. We observed no significant difference between total tick densities on properties with and without guineafowl (Wilcoxon $z$ value = 1.26, $p = 0.21$).

Densities of adult ticks were extremely low in lawn interiors and lawn peripheries, and considerably higher within the adjacent forest (see the figure). The density of adult ticks in properties with guineafowl was significantly lower than adult tick density in control properties (Wilcoxon $z$ value = 2.03, $p = 0.043$). This difference was due entirely to lower abundance in the forest edge habitat.

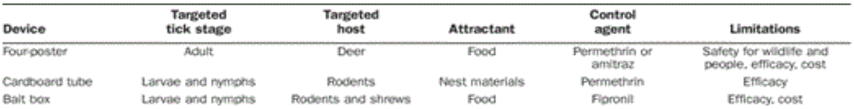
These results, combined with the experimental study by Duffy and colleagues (1992), suggest that guineafowl actively consume adult, but not nymphal, blacklegged ticks. Nymphs are probably too tiny to be sought or detected by these birds. Chronically reduced density of adult ticks could cause reduced abundance of nymphs even without direct attacks on nymphs by guineafowl. This could occur via a reduction in the number of adult females ovipositing on properties with guineafowl. However, reduced abundance of adult ticks was not associated with significant reduction in abundance of nymphs, the stage responsible for transmitting the vast majority of Lyme disease cases (Barbour and Fish 1993). Guineafowl foraging behavior in peridomestic settings appears to be highly localized and largely confined to lawns and adjacent forests. Adult female ticks that survive predation pressure by guineafowl, find a host, and feed to repletion are likely to be transported to sites for oviposition away from the location at which they quested. Consequently, we expect local density of adult ticks to have little impact on the future density of immature stages, including nymphs.

It is possible that, in fact, guineafowl do consume nymphal ticks, but our failure to detect a significant effect on nymphal abundance was due to a concurrent increase in immigration of immature ticks on mobile hosts. According to this scenario, the provision of food (grain) for guineafowl might attract granivorous rodents, such as white-footed mice and eastern chipmunks, from adjacent forests into forest–lawn edges or the lawns themselves. If this attraction occurred during the season of larval feeding, replete larvae could drop off hosts and molt into nymphs, which would later quest on these edges or lawns. Such an importation conceivably could counteract local reduction in numbers due to predation by guineafowl. Alternatively, the lack of statistical significance might be a consequence of a relatively small sample of properties. A power analysis indicated that, if the magnitude of the difference in nymphal density between properties with and without guineafowl were maintained, a sample of 27 pairs of properties would result in significance at the 0.05 level. In sum, our results weaken the argument that guineafowl are an effective means of biologically controlling human risk of exposure to tick-borne pathogens. Modest and statistically insignificant reductions in nymphal tick abundance, combined with the potential for food provision to attract small mammalian hosts, indicate that popular recommendations to use these birds to protect human health are not supported.



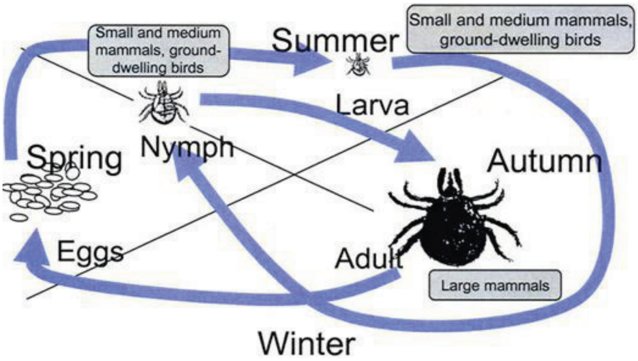
Mean (+ 1 SE [standard error]) density of (a) nymphal and (b) adult blacklegged ticks within three different habitat types on properties that either did or did not maintain free-ranging guinea fowl. The three habitat types correspond to the interior of lawns, the lawn periphery within 1 to 3 meters (m) of the forest edge, and the forest edge within 3 to 5 m of the lawn. The reduction in density of host-seeking adult ticks was statistically significant, but that of host-seeking nymphs was not.

**Table 1.**

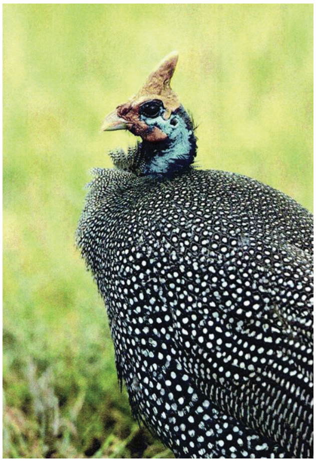


| Device | Targeted tick stage | Targeted host | Attractant | Control agent | Limitations |
|---|---|---|---|---|---|
| Four-poster | Adult | Deer | Food | Permethrin or amitraz | Safety for wildlife and people, efficacy, cost |
| Cardboard tube | Larvae and nymphs | Rodents | Nest materials | Permethrin | Efficacy |
| Bait box | Larvae and nymphs | Rodents and shrews | Food | Fipronil | Efficacy, cost |

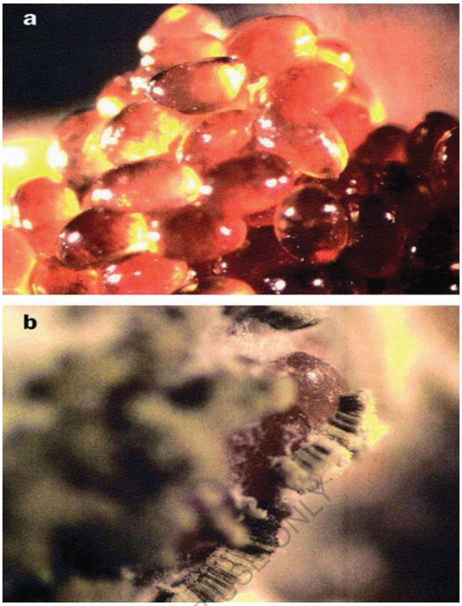
Photomicrograph of a nymphal blacklegged tick (*Ixodes scapularis*), dorsal view. Inset shows a ventral view of the mouthparts, with the jagged-edged central hypostome and the two palps on each side. These mouth-parts are embedded in the host while the tick takes a blood meal. Photomicrograph: R. Ostfeld laboratory.



Generalized life cycle of the blacklegged tick in North America. The four stadia are egg, larva, nymph, and adult. The predominant hosts for each feeding stage are indicated in boxes, although larvae and nymphs in particular are known to parasitize dozens of different mammalian, avian, and reptilian hosts, and adults feed on several medium to large mammals in addition to deer. Modified from Van Buskirk and Ostfeld 1995.



The helmeted guineafowl *(*Numida meleagris*)*. The cult status of this bird as a tick predator, and hence a protector of people from exposure to tick-borne infections, appears to be unwarranted. Although evidence suggests that these birds eat adult ticks, they appear not to reduce the numbers of nymphs (the stage responsible for most cases of Lyme disease) sufficiently to have a strong protective effect. Photograph: *WildeyesImages.com*.



Egg masses produced by blacklegged ticks in the laboratory. (a) Eggs produced by a female that had not been treated with the fungus *Metarhizium anisopliae*; these eggs hatched normally. (b) Eggs produced by a female that had been treated with *M. anisopliae*; these eggs died. Photographs: R. Ostfeld laboratory.

A nest box (top) attached at chest height to a tree on an experimental plot at the Institute of Ecosystem Studies in Millbrook, New York. These nest boxes are frequently colonized by white-footed mice (*Peromyscus leucopus*, below), which are a primary host for larval blacklegged ticks and the principal natural reservoir for Lyme disease spirochetes (*Borrelia burgdorferi*). Treating the cotton nesting material in these nest boxes with Metarhizium anisopliae resulted in modest, local reductions in the abundance of nymphal ticks. Photographs: R. Ostfeld laboratory.

## Biological control of ticks

Ticks have numerous natural enemies, but only a few species have been evaluated as tick biocontrol agents (BCAs). Some laboratory results suggest that several bacteria are pathogenic to ticks, but their mode of action and their potential value as biocontrol agents remain to be determined. The most promising entomopathogenic fungi appear to be *Metarhizium anisopliae* and *Beauveria bassiana*, strains of which are already commercially available for the control of some pests.

Development of effective formulations is critical for tick management. Entomopathogenic nematodes that are pathogenic to ticks can potentially control ticks, but improved formulations and selection of novel nematode strains are needed. Parasitoid wasps of the genus Ixodiphagus do not typically control ticks under natural conditions, but inundative releases show potential value.

Most predators of ticks are generalists, with a limited potential for tick management (one possible exception is oxpeckers in Africa). Biological control is likely to play a substantial role in future IPM programmes for ticks because of the diversity of taxa that show high potential as tick BCAs.

Considerable research is required to select appropriate strains, develop them as BCAs, establish their effectiveness, and devise production strategies to bring them to practical use.

Virulence of 11 native strains of entomopathogenic fungi; *Metarhizium anisopliae* (three strains), *Beauveria bassiana* (six strains) and *Lecanicillium psalliotae* (two strains) collected from different parts of Iran, were studied against different developmental stages of Rhipicephalus (Boophilus) annulatus.

After the exposure of ticks to the fungal strains in different concentrations (i.e. 10(3), 10(5), 10(7) conidia/ml), various parameters such as mortality rate and reproductive efficiency of engorged females, mortality of unfed tick larvae and eclosion percentage of infected eggs were evaluated to determine the fungal virulence.

Based on the obtained results, five strains including M. anisopliae (IRAN 437 C and DEMI 001), B. bassiana (IRAN 403 C) and L. psalliotae (IRAN 468 C and IRAN 518 C) were found to be virulent to various stages of tick developmental cycle. Mortality rate of engorged females was found to be dose-dependent with regard to the conidial concentration used. Total mortality rates of 90-100%, 70% and 56.6% were observed for M. anisopliae (IRAN 437 C and DEMI 001), *B. bassiana* (IRAN 403 C) and L. psalliotae (IRAN 468 C), 6-11 days post inoculation (PI) with 10(7) conidia/ml, respectively. Most strains were able to inhibit egg laying by females in the range of 0-26% in different conidial concentrations.

The results indicated that the mean egg laying of treated engorged tick females exposed to M. anisopliae (IRAN 437 C) was less than the mean values of those treated with other fungal strains. Results revealed 89.1%, 35.5% and 56.3% decrease in egg hatchability and 88.69%, 78.15% and 59.74% reduction in reproductive efficiency of the ticks using 10(7) conidia/ml of *M. anisopliae* (IRAN 437 C), *B. bassiana* (IRAN 403 C) and *L. psalliotae* (IRAN 468 C), respectively. In general, the entomopathogenic effects of native M. anisopliae and *B. bassiana* against various developmental stages of R. (B.) annulatus were confirmed in the present work. Likewise, although *L. psalliotae*, which was introduced for the first time as an entomopathogenic fungus against tick, had not more than 13.3% mortality effect against adult females, but its effect on egg hatchability and reproductive efficiency was remarkable.

## Entomopathogenic Fungi for Tick Control

Food security is one of the main concerns worldwide, where cattle play a fundamental role in the supply of milk and meat (Falvey, 2015). Cattle production in Mexico is an activity of social and economic importance that is carried out throughout the national territory, occupying more than 110 million hectares, with 1.1 million registered livestock farms (SIAP (Servicio de Información Agroalimentaria y Pesquera), 2020).

This large area of the national territory dedicated to livestock has an impact on the use of natural resources and can affect the quality and preservation of ecosystems (González-Padilla et al., 2019). Bovine livestock in the country has an inventory of 35.2 million cattle heads (SIAP (Servicio de Información Agroalimentaria y Pesquera), 2018), and is based mainly on direct grazing in extensive production systems (Castillo-Gallegos et al., 2005); where one of the main threats are ticks and the pathogens they transmit, affecting productivity, health and well-being. It has been estimated that more than 80% of cattle population worldwide is exposed to tick infestations (Snelson, 1975; Giles et al., 2014), where the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini), *R*. (*B*.) *annulatus* (Say), and *Amblyomma mixtum* (Koch) are considered the most important livestock ticks in Mexico.

Previously, tick control has been based on therapeutic interventions using chemical treatments (acaricides and endectocides). These methodologies have definitely contributed to improving productivity and welfare; however, the intensive and frequent use, and inappropriate use as well, of these chemicals has resulted in the development of acaricidal resistance in ticks (Fernández-Salas et al., 2012a,b,c; Alonso-Díaz et al., 2013a).

Tick resistance has been reported for almost all the main chemical acaricides (Alonso-Díaz et al., 2013a; Rodríguez-Vivas et al., 2014a) and this phenomenon, added to an exacerbated chemical control problem, has had other consequences such as environmental and food contamination by secondary chemical metabolites, spread of ticks into free zones, restrictions on cattle export and increase in diseases transmitted by these parasites (De Castro, 1997; Domínguez-García et al., 2016; Rodríguez-Vivas et al., 2017).

Results have shown that dependence on these chemical products, as the only form of control, is neither economically nor ecologically sustainable. Sustainable cattle production needs strong changes, such as considering both agroecology-oriented and novel tick-control approaches (Alonso-Díaz et al., 2014). This latter has motivated the exploration of alternative methods for tick control (Samish et al., 2004), such as the use of entomopathogenic fungi (EPF). Biological control by EPF is one of the most promising options for tick control (Polar et al., 2005).

The most widely used EPF species against cattle ticks are *Metharizium anisopliae* s.l., *Beauveria bassiana* and *Akanthomyces lecanii* (formerly, *Lecanicillium lecanii*) (Fernandes et al., 2012; Romo-Martínez et al., 2013). EPF show clear advantages, such as being environmentally safe, can be mass-produced, and have the ability to infect their hosts through the cuticle rather than wait for ingestion in order to cause infection (Rajula et al., 2020). It has also been reported that EPF may affect the entire tick cycle (free-living and parasitic stages) (Fernández-Salas et al., 2017, 2018, 2019), a characteristic that allows broadening the spectrum of use in a tick control strategy. The research requires a transdisciplinary approach in order to be able to integrate the necessary knowledge on the use of EPF in the control of ticks.

Through this integration, it will be possible to identify links between the studies carried out, generate research hypotheses to improve biological control and design viable EPF application schemes based on experiences that help guide future studies in the use of these fungi against livestock ticks. The objective of this paper is to review the use of EPF as an alternative control method against cattle ticks in Mexico.

## Cattle Ticks

Ticks are obligate blood-feeding ectoparasites that infest 80% of the cattle worldwide (Giles et al., 2014; Grisi et al., 2014). These ectoparasites are one of the most important health problems for the livestock industry and are responsible for high economic losses around the world, putting food safety at risk (Rodríguez-Vivas et al., 2017).

In addition to having direct effects on their hosts, ticks are also the most important group of parasitic arthropods as vectors of pathogens that affect domestic animals and wildlife (Pérez de León et al., 2020). Tick-borne pathogens are the main cause of transboundary livestock diseases (e.g., bovine babesiosis, anaplasmosis, theileriosis, and heartwater disease), which are among the diseases listed as notifiable by the World Organization for Animal Health (Esteve-Gasent et al., 2020).

Estimated annual global costs associated with ticks and the pathogens transmitted by them range between US$ 13.9 billion and US$ 18.7 billion (De Castro, 1997). Ticks that affect cattle around the world belong to two families: Ixodidae and Argasidae. The first, also known as hard ticks, includes all species from *Amblyomma, Dermacentor, Haemophysalis, Hyalomma, Ixodes*, and *Rhipicephalus*; while the second family or soft ticks, includes the *Ornithodoros* and *Otobius* ticks (Figure 3).

Figure 3

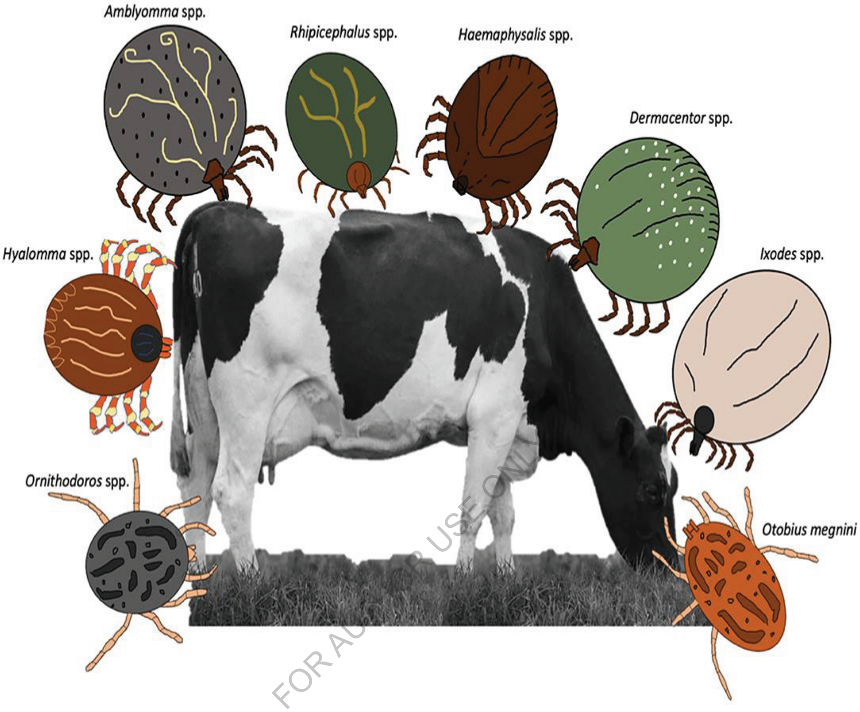


FIGURE 3. Ticks infesting cattle around the world.

## Cattle Ticks in Mexico

In Mexico there are 82 species of ticks that parasitize domestic and wild animals (Higa et al., 2020). The main ticks of domesticated cattle belong to the Ixodidae family. Among these, *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini), *R.* (*B.*) *annulatus* (Say), and *A. mixtum* (Koch 1844) have been reported with a high prevalence in cattle farms across the country. However, there are other ticks such as *Dermacentor albipictus, R. sanguineus, Anocentor nitens* and *Otobius megnini* that also have a considerable livestock impact (Martínez et al., 2019).

In Mexico, the economic losses caused only by *R. microplus* were US$ 573.61 million per year (Rodríguez-Vivas et al., 2017). Although, some other ticks such as *Otobius megnini* which is present throughout the country, are also very important in livestock inspections for the export market (Martínez et al., 2019).

Cattle Fever Ticks *Rhipicephalus microplus* and *Rhipicephalus annulatus*

Cattle fever ticks (CFT) *R. microplus* and *R. annulatus* remain endemic in Mexico (Esteve-Gasent et al., 2020). Both ticks have similar biological processes and morphology; however, their geographic distribution is different (Estrada-Peña and Venzal, 2006; SENASICA, 2013). While *R. microplus* is present in tropical and subtropical regions, *R. annulatus* is endemic to arid and semiarid regions (Northern Mexico) (SENASICA, 2013) (Figure 4).

CFT are present in 65% of the national territory and have the capacity to infest mainly cattle, but they have also been reported to infest equines, deer and other wild animals (CFSPH (The Center For Food Security and Public Health), 2007; Rodríguez-Vivas et al., 2013a). These ticks have the ability to transmit notifiable animal diseases in cattle, such as anaplasmosis and babesiosis (Klafke et al., 2020). For this reason, CFT have special attention and constant vigilance in the border area between Mexico and the United States, in order to prevent their spread in free-tick areas (Lohmeyer et al., 2011), where there has already been an increase in infestations or outbreaks (Pound et al., 2010; Araya-Anchetta et al., 2015). The biological cycle of *R. microplus* is shown in Figure 5.

Figure 4

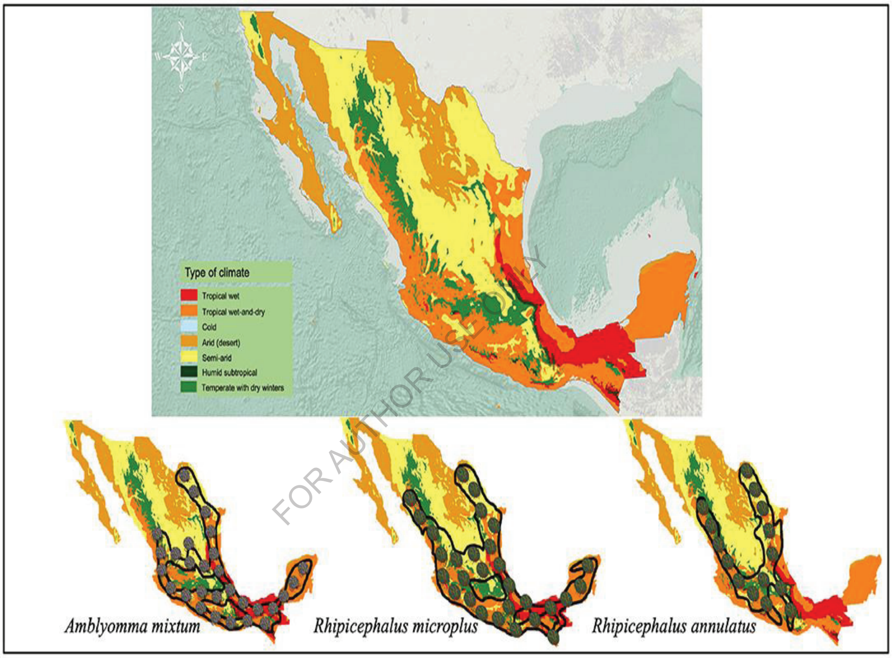


FIGURE 4. Climate zones of Mexico and their relationship with the distribution of the main ticks that affect cattle in Mexico. Imaged edited according to information from SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales) (2003) and SENASICA-SAGARPA (2015).

Figure 5

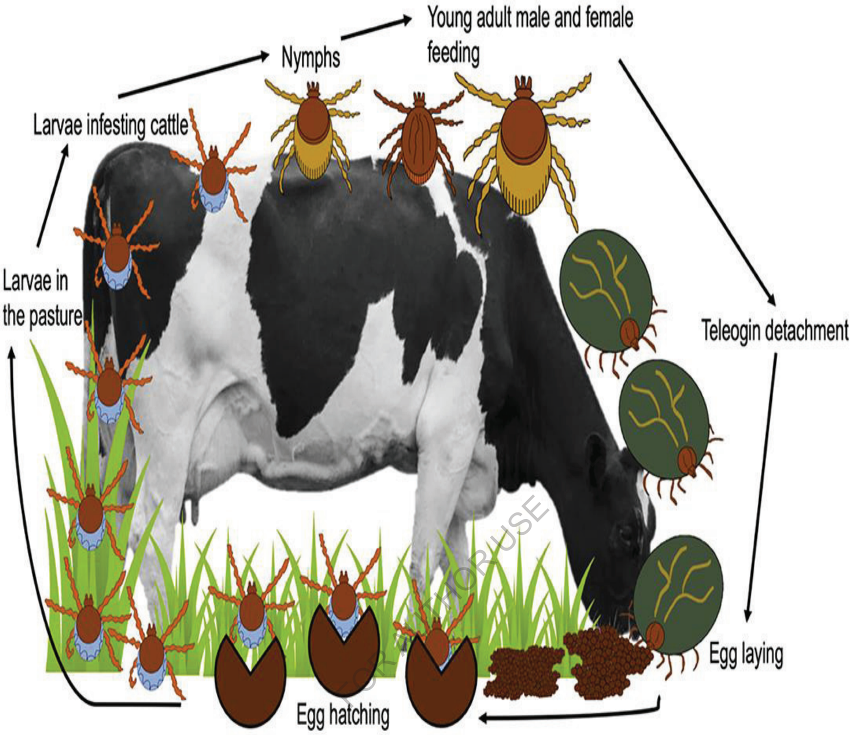


FIGURE 5. Life cycle of *R. microplus*.

### ***Amblyomma mixtum***

*A*. *mixtum* has a similar distribution to that of *R*. *microplus* in Mexico (Figure 4) (SENASICA, 2013); where, concomitant infestations are common in ~86% of farms (Alonso-Díaz et al., 2013a). Currently, it seems that *A*. *mixtum* has a greater distribution, since this species has been able to adapt to various ecological niches, including semi-arid grasslands and subtropical secondary forests (Estrada-Peña et al., 2004); in addition to its great capacity to occupy the ecological niches of other ticks (i.e., *R*. *microplus* under high pressure from acaricides) (Alonso-Díaz et al., 2013b).

This ectoparasite has a heteroxenous life cycle and is a generalist species that infests livestock, humans and, wildlife in Mexico (Aguilar-Domínguez et al., 2019; Higa et al., 2020). It causes economic losses due to the large amount of blood taken from its hosts and the transmission of infectious diseases to domestic/wild animals (*Anaplasma marginale*) and humans (*Rickettsia ricketsii*) (Alonso-Díaz et al., 2013b; Aguilar-Domínguez et al., 2019).

Additionally, other potentially zoonotic species such as *Rickettsia amblyommatis* have been detected in *A*. *mixtum* from Mexico (Sánchez-Montes et al., 2016; Merino et al., 2020), making this parasite one of the most important tick species in veterinary medicine and public health in the country (Pérez de León et al., 2020). The biological cycle of *A*. *mixtum* is shown in Figure 6.

Figure 6

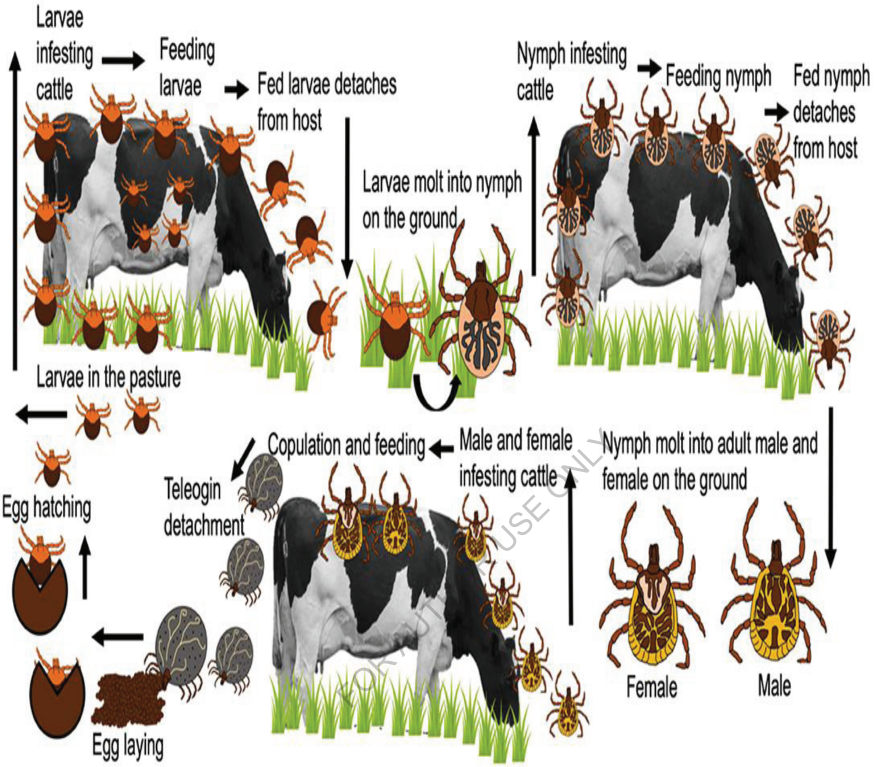
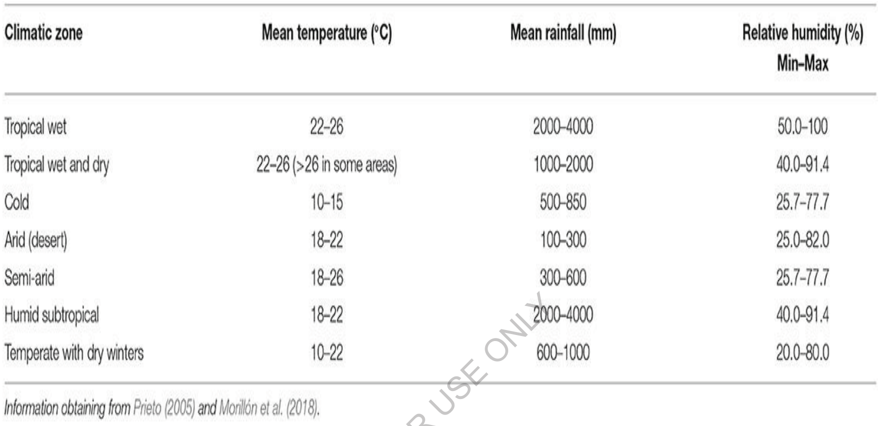


FIGURE 6. Life cycle of *A. mixtum*.

Table 2 shows the temperature, rainfall and relative humidity per climate zone in Mexico to observe the characteristics of each ecological niche where the main ticks are distributed.

Table 2



| Climatic zone | Mean temperature (°C) | Mean rainfall (mm) | Relative humidity (%) Min–Max |
|---|---|---|---|
| Tropical wet | 22–26 | 2000–4000 | 50.0–100 |
| Tropical wet and dry | 22–26 (>26 in some areas) | 1000–2000 | 40.0–91.4 |
| Cold | 10–15 | 500–850 | 25.7–77.7 |
| Arid (desert) | 18–22 | 100–300 | 25.0–82.0 |
| Semi-arid | 18–26 | 300–600 | 25.7–77.7 |
| Humid subtropical | 18–22 | 2000–4000 | 40.0–91.4 |
| Temperate with dry winters | 10–22 | 600–1000 | 20.0–80.0 |

*Information obtaining from Prieto (2005) and Morillón et al. (2018).*

TABLE 2. Annual mean temperature, rainfall and relative humidity per climate zone in Mexico.

## Impact of Climate Change on the Epidemiology of Ticks

Climate change is viewed as a long-term change in average weather patterns that have come to define Earth's local, regional and global climates (NASA-GCC, 2019). Perhaps, some of the most important alterations caused by climate change are warmer temperatures in temperate zones, altered precipitation patterns, increased frequency and severity of extreme weather events (hurricanes or droughts), and sea level rise (Kutz et al., 2009; Polley and Thompson, 2009).

These last changes have affected, directly or indirectly, the biology and ecology of a great number of organisms on the planet; therefore, these climate variations have impacted on the habits and biological cycles of ectoparasites

(Cumming and Van Vuuren, 2006; Kutz et al., 2009), including ticks (Pérez de León et al., 2012). In this regard, some authors in Mexico have mentioned that *R. microplus* can present between four to five successful generations per year in tropical and subtropical areas (Rodríguez-Vivas et al., 2005).

Ticks have had the ability to evolve, adapt and spread within the changing climatic conditions, which, for the most part, have favored the dynamics and population movement of these arachnids in different geographical areas (Barré and Uilenberg, 2010). This situation has led to the presentation of relatively new infestations in some livestock areas, or the diagnosis of diseases transmitted by these vectors, which were not common for certain latitudes in the past (Estrada-Peña, 2008; Montero et al., 2016).

Climate change can also affect domestic or wild hosts (Barré and Uilenberg, 2010; Rodríguez-Vivas et al., 2013a), which influences the geographical distribution of ticks, their infestations and the diseases they transmit in non-endemic areas (Giles et al., 2014). The presence of CFT has been frequently reported in tick-free zones or quarantine zones in the US. The risk of introducing ticks into or outside the quarantine zone is mainly high due to the movement of tick host species, such as the white-tailed deer (Pound et al., 2010; Webb et al., 2010), the nilgai antelope (Cárdenas-Canales et al., 2011), stray cattle and interactions between *R. microplus* and exotic weeds along the transboundary region with Mexico (Racelis et al., 2012; Esteve-Gassent et al., 2014).

Likewise, the red deer (*Cervus elaphus*) has been reported as a wild host for the *R. microplus* tick (Rodríguez-Vivas et al., 2013a), helping it to spread within the Mexican territory. Obviously, the movement of these hosts is also closely related to human activities, the temperature increases in some areas, and the scarcity of water.

All these characteristics can participate in a possible complex change in the ecology of ticks, since their biological cycles can be affected by these conditions.

## Tick Control

Tick control is mainly based on the use of chemical acaricides, which in recent decades have played a crucial role in the sustainability of the livestock production. However, since the development of the first broad-spectrum parasiticides, they have been used extensively by farmers in order to control or eliminate parasites.

When ectoparasiticides are administered correctly (dosed and targeted), they are effective and have wide safety margins for both the animals and the people who apply them. However, there are factors such as resistant or multiresistant parasites and/or incorrect ways of applying the medications, which decrease their effectiveness (Alonso-Díaz et al., 2014).

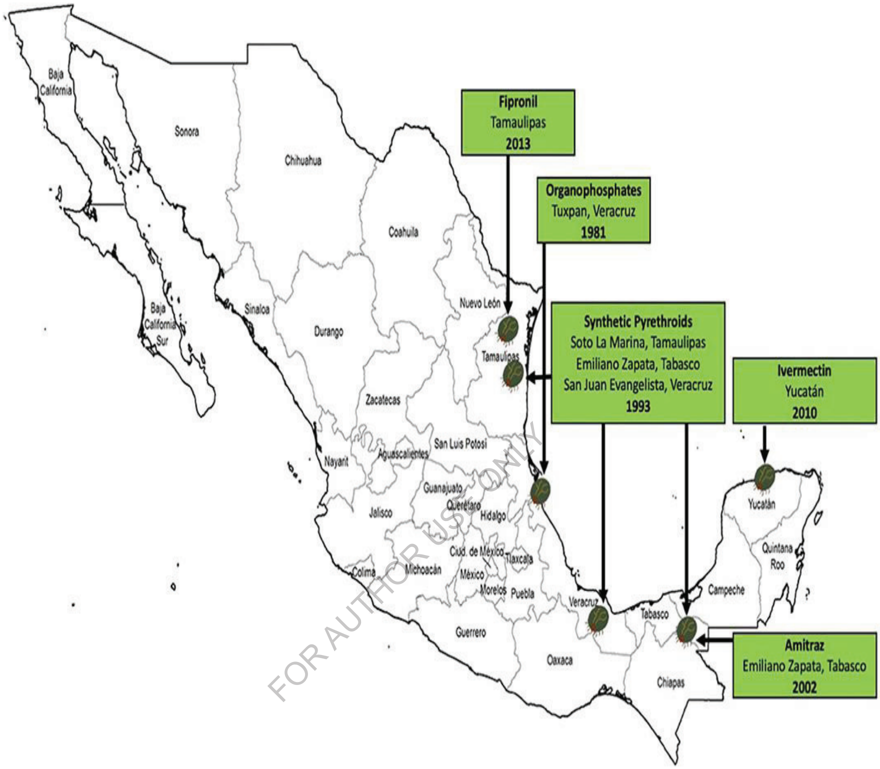
Currently, global results reveal that parasite control schemes based on a rigorous and exclusive use of chemical applications are not sustainable. The continuing propagation of these serious problems on a large scale involves many people in the pharmaceutical industries, professionals, farmers and in public health. It should be noted that chemical acaricides are and will be the fundamental basis of tick control, that is why they should be considered as a precious resource for cattle farming, since the cost of having an acaricide on the market implies expenses of more than 250 million dollars and between 8 and 12 years of research (De Alva, 1995; Omkar, 2016).

## Acaricide Resistance of Livestock Ticks in Mexico

One of the biggest concerns that has arisen on cattle farms across the country is the ability of ticks to resist the deadly effects of the chemicals used for their control. Tick resistance to acaricides is defined as "the specific heritable trait or traits in a tick population, selected as a result of the population's contact with an acaricide. This translates into a significant increase in the percentage of the population that survives after exposure to a certain concentration of this acaricide" (Rodriguez-Vivas et al., 2018).

In Mexico, several investigations have been conducted to identify and monitor populations of resistant and multiresistant ticks to acaricides, and to know the risk factors associated with the presence of this growing problem (Fernández-Salas et al., 2012a,b,c; Alonso-Díaz et al., 2013a; Higa et al., 2020). Figure 7 shows the first cases of ticks resistant to acaricides in Mexico. Table 3 shows a summary of the epidemiological studies of resistant or multiresistant ticks by state over time in the country, highlighting *R. microplus*, which has developed resistance to all the main types of acaricides. Multiple acaricide resistance is an alarming phenomenon in Mexico, considering that there are no new synthetic compounds on the market with a novel mode of action to control multidrug resistant ticks (Esteve-Gasent et al., 2020).

Figure 5



**Fipronil**
Tamaulipas
**2013**

**Organophosphates**
Tuxpan, Veracruz
**1981**

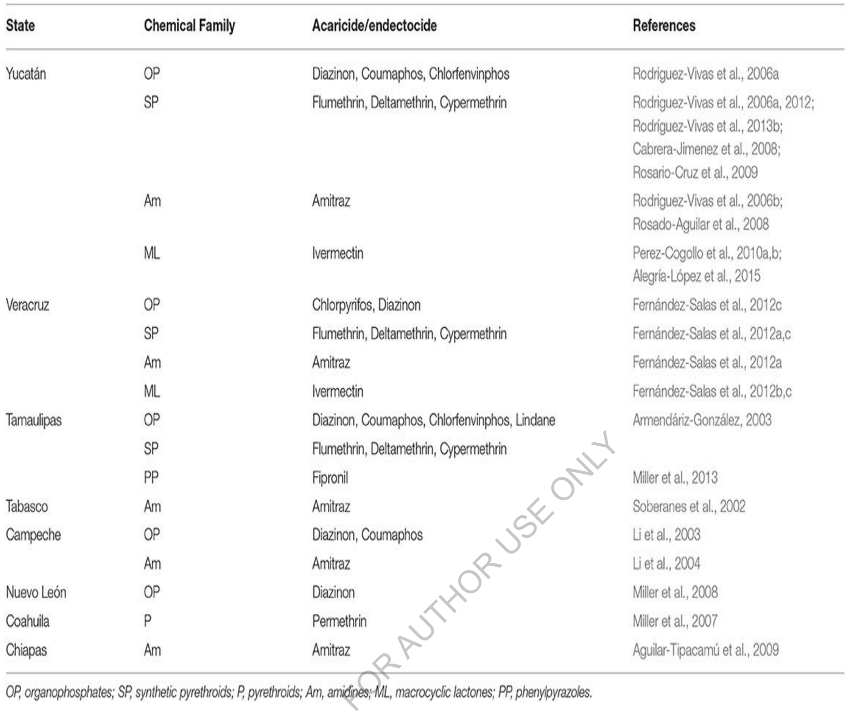
**Synthetic Pyrethroids**
Soto La Marina, Tamaulipas
Emiliano Zapata, Tabasco
San Juan Evangelista, Veracruz
**1993**

**Ivermectin**
Yucatán
**2010**

**Amitraz**
Emiliano Zapata, Tabasco
**2002**

FIGURE 7. First reports of *R. microplus* tick populations resistant to acaricides in Mexico (Ortiz et al., 1995; Soberanes et al., 2002; Perez-Cogollo et al., 2010a; Miller et al., 2013).

Table 3



| State | Chemical Family | Acaricide/endectocide | References |
|---|---|---|---|
| Yucatán | OP | Diazinon, Coumaphos, Chlorfenvinphos | Rodriguez-Vivas et al., 2006a |
| | SP | Flumethrin, Deltamethrin, Cypermethrin | Rodriguez-Vivas et al., 2006a, 2012;<br>Rodríguez-Vivas et al., 2013b;<br>Cabrera-Jimenez et al., 2008;<br>Rosario-Cruz et al., 2009 |
| | Am | Amitraz | Rodriguez-Vivas et al., 2006b;<br>Rosado-Aguilar et al., 2008 |
| | ML | Ivermectin | Perez-Cogollo et al., 2010a,b;<br>Alegría-López et al., 2015 |
| Veracruz | OP | Chlorpyrifos, Diazinon | Fernández-Salas et al., 2012c |
| | SP | Flumethrin, Deltamethrin, Cypermethrin | Fernández-Salas et al., 2012a,c |
| | Am | Amitraz | Fernández-Salas et al., 2012a |
| | ML | Ivermectin | Fernández-Salas et al., 2012b,c |
| Tamaulipas | OP | Diazinon, Coumaphos, Chlorfenvinphos, Lindane | Armendáriz-González, 2003 |
| | SP | Flumethrin, Deltamethrin, Cypermethrin | |
| | PP | Fipronil | Miller et al., 2013 |
| Tabasco | Am | Amitraz | Soberanes et al., 2002 |
| Campeche | OP | Diazinon, Coumaphos | Li et al., 2003 |
| | Am | Amitraz | Li et al., 2004 |
| Nuevo León | OP | Diazinon | Miller et al., 2008 |
| Coahuila | P | Permethrin | Miller et al., 2007 |
| Chiapas | Am | Amitraz | Aguilar-Tipacamú et al., 2009 |

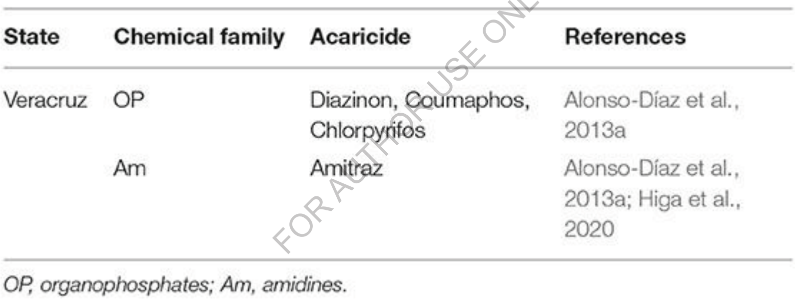
*OP, organophosphates; SP, synthetic pyrethroids; P, pyrethroids; Am, amidines; ML, macrocyclic lactones; PP, phenylpyrazoles.*

TABLE 3. Main reports of resistance of *R. microplus* to acaricides by state in Mexico.

This type of resistance has been reported in different regions of Mexico and the most common in *R. microplus* are: coumaphos, flumethrin, and amitraz; chlorfenvinphos, flumethrin, and amitraz; diazinon, deltamethrin, and amitraz (Rodriguez-Vivas et al., 2007); permethrin, coumaphos, and fipronil; permethrin, coumaphos, fipronil, and amitraz (Miller et al., 2013); amitraz, cypermethrin, and ivermectin (Fernández-Salas et al., 2012a,b); and coumaphos, cypermethrin, amitraz, ivermectin and fipronil (Rodríguez-Vivas et al., 2014a). Although less studied, multiresistant strains of *A. mixtum* to acaricides have also been detected (Table 4).

As for *R. annulatus*, there is insufficient evidence to know the resistance degree of this tick to chemical acaricides in Mexico; however, some studies suggest that it may be underdiagnosed as in some other countries (Klafke et al., 2020). Recently, the first evidence of permethrin resistance in *R. annulatus* strains was reported near the US-Mexico border, in Maverick County, Texas (Klafke et al., 2020). It is important to consider that populations of *R. annulatus* resistant to pyrethroids (Ziapour et al., 2017; Aboelhadid et al., 2018) and ivermectin have already been reported in other countries.

Table 4



| State | Chemical family | Acaricide | References |
|---|---|---|---|
| Veracruz | OP | Diazinon, Coumaphos, Chlorpyrifos | Alonso-Díaz et al., 2013a |
| | Am | Amitraz | Alonso-Díaz et al., 2013a; Higa et al., 2020 |

*OP, organophosphates; Am, amidines.*

TABLE 4. Reports of *A. mixtum* resistant to acaricides in Mexico.

Since acaricides will continue to be the basis of tick control, their lifespan and effectiveness need to be extended. To achieve this, it is suggested to know, evaluate and adopt other alternative control strategies in order to design an adequate integrated control scheme for ticks. It has been mentioned that the best way to control ticks in cattle farms is to combat them simultaneously in different ways (Alonso-Díaz et al., 2014; Pérez de León et al., 2020). By doing this, the parasites have less ability to defend themselves and develop resistance.

## Entomopathogenic Fungi

EPF are a species of fungal pathogens for arthropods (Rajula et al., 2020). They are considered cosmopolitan saprophytic organisms that live in diverse ecosystems and climates (e.g., tropical, temperate, arid and artic), where they interact with arthropods in many terrestrial and aquatic habitats (Skinner et al., 2014).

It is estimated that there are between 750 and 1,000 EPF placed in more than 100 genera (Mantzoukas and Eliopoulos, 2020; Rajula et al., 2020), which play an important role in the dynamics of arthropod populations in natural ecosystems (Maina et al., 2018). EPF comprise a wide range of genera and species with high morphological, phylogenetic and ecological diversity (Araújo and Hughes, 2016), and their interactions with arthropods are of great interest for environmental microbiology, determination of the balance of ecosystems, biodiversity, evolution of eukaryotic organisms and insect pest control (Semenova et al., 2020).

These fungi have the ability to evolve to be more successful in their biological cycle, and they are also capable of colonizing and using arthropods as a substrate for their reproduction (Humber, 2008). Among the arthropods to which they have adapted throughout this evolutionary transition are ticks, which is why they have been studied as a biological control for these pest arachnids. Biological control using EPF represents one of the most promising approaches for sustainable tick control schemes and can therefore be included as a component in an integrated pest management strategy for tick control. In addition, EPF have some advantages over conventional acaricides, such as: cost-benefit ratio, absence of harmful or secondary effects to non-target organisms, reduction of chemical residues in the environment and foods of animal origin, and short time between fungal generations (high production) (Porfirio and Schwentesius, 2016).

On the other hand, EPF can protect biodiversity in the natural ecosystem and can be used in combination with synthetic chemical products, since their residues have no known adverse effects on the environment, and are self-perpetuating under ideal environmental conditions (Maina et al., 2018). For example, Webster et al. (2105) reported that the combination of *M. anisopliae* with commercial acaricides (cypermethrin and chlorpyriphos) enhance the tick control against *R. microplus* (97.9% of efficacy). EPF have also been shown to play additional roles in nature, including endophytism, antagonism of plant diseases, promoting plant growth, and rhizosphere colonization (Jaber and Ownley, 2018).

The most studied EPF worldwide as biological control for ticks are *Metarhizium anisopliae* s.l., *B. bassiana* and *A. lecanii* (formerly, *Lecanicillium lecanii*) (Fernandes et al., 2012; Romo-Martínez et al., 2013). This is consistent with the reports that exist in Mexico (Ojeda-Chi et al., 2011; Fernández-Salas et al., 2018); however, some other EPF have been reported in the country, such as *Isaria* (*Paecilomyces*) *fumosorosea* (*fumosoroseus*), which has also stood out for its effectiveness (Ángel-Sahagún et al., 2010).

Currently, the taxonomic identification and reporting of *M. anisopliae* and *B. bassiana* strains are based on the studies proposed by Bischoff et al. (2009) and Rehner et al. (2011), respectively. These proposals are supported by various studies of molecular phylogeny of multiple loci and taxonomic classification, where various monophyletic lineages have been identified concluding that both species of fungi actually comprise a complex of species, which, in many cases, they are difficult to delimit without molecular tools and analysis.

After these studies, where the taxonomy of the species is clarified, the strains of these EPF that have not been reidentified according to this current taxonomy should be reported as sensu lato (s.l.), and those that have been reidentified and delimited with the taxonomic techniques and proposed molecular phylogenetic studies will be reported as sensu stricto (s. str).

The EPF strains used in Mexico for the control of cattle ticks have been identified through morphological analysis of their reproductive structures, and some through molecular analysis. However, in the case of *M. anisopliae* and *B. bassiana*, some of the molecular identification techniques used were not sufficient for the delimitation of the monophyletic lineages and, for other strains, the information from the molecular analyzes is not available. Therefore, in the present review, the strains of these fungi used in Mexico will be considered as *M. anisopliae* sensu lato (s.l.) and *B. bassiana* sensu lato (s.l.) as well.

## Infection Mechanism of EPF

The basic advantages related to the infection mode of EPF, compared to commercial acaricides, correspond to their ability to use different mechanisms to colonize and kill ticks. Fungi use enzymatic, toxicological and mechanical invasion systems, which suggest a difficulty for ticks to develop resistance to EPF. Furthermore, it is known that they can target almost all stages of the arthropod life cycle, which means another great advantage as a member of pest control schemes (Srinivasan et al., 2019).

According to Beys-da-Silva et al. (2020), the infection mode of EPF in ticks occurs as follows:

(1) recognition of the susceptible host;

(2) adhesion of conidia and germination on host cuticle;

(3) development of specific structures (germ tube and appressorium);

(4) penetration through the host's cuticle;

(5) intense fungal growth and death of the host; and

(6) production of conidia after hyphae emergence through the host cuticle. Figure 8 schematizes the infection mode of EPF in ticks.

Figure 8

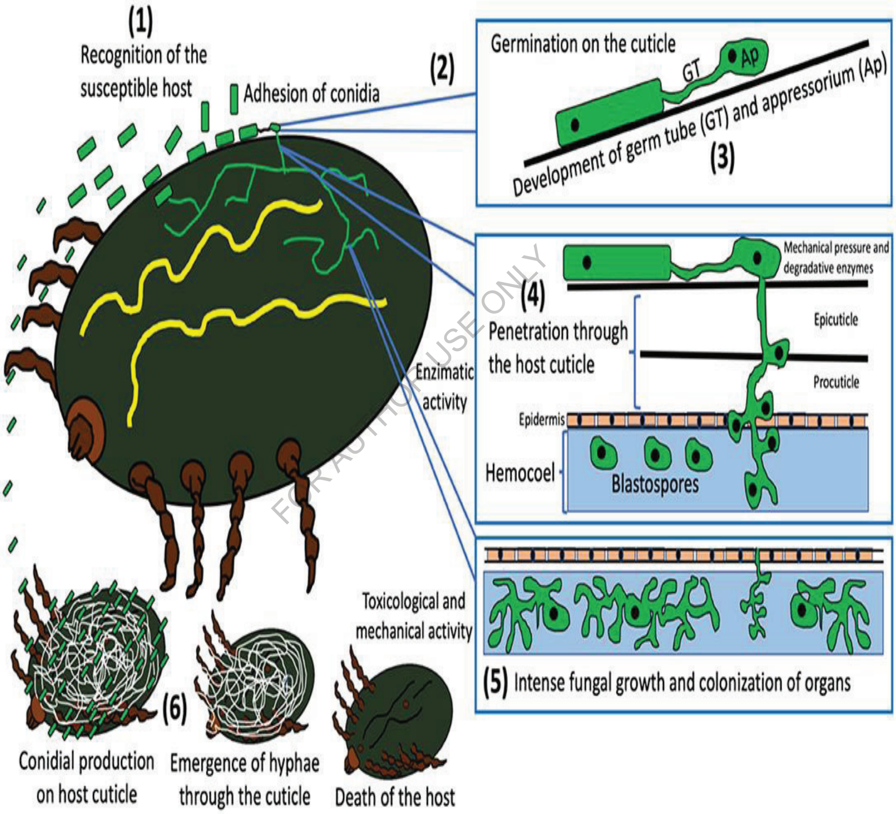


FIGURE 8. General infection process of entomopathogenic fungi in ticks and their biological mechanisms.

## Recognition of Susceptible Host, Adhesion of Conidia and Germination on the Host Cuticle

Aerial fungal conidia adhere to the host's cuticle through hydrophobic mechanisms (Ortiz-Urquiza and Keyhani, 2013), which are mainly mediated by proteins on the surface of the conidia named hydrophobins (St. Leger et al., 1992; Skinner et al., 2014) and adhesins (i.e., Mad1 and Mad2 identified in *M. anisopliae*) (Wang and St Leger, 2007; Valero-Jiménez et al., 2016).

It is important to mention that homologous proteins of these adhesins have been reported in *B. bassiana* (Gao et al., 2020), but it has been suggested that some genes that encode these proteins between the two main EPF (*M. anisopliae* and *B. bassiana*) could be different (Chen et al., 2018). Beys da Silva et al. (2010a) reported that lipolytic activity due to the action of enzymes such as lipase and esterase in ticks, could also contribute to the recognition and adhesion of conidia during the infection of *R. microplus* by *M. anisopliae*.

## Penetration Through the Host's Cuticle

Once the conidia are attached, they will germinate under optimal humidity and temperature conditions, producing a germination tube followed by a peg or appressorium for penetration into the host's cuticle (Skinner et al., 2014; Brunner-Mendoza et al., 2019). The penetration process begins and is aided by the production of several hydrolytic cuticular enzymes such as lipases, proteases and chitinases, and the mechanical pressure exerted by the appressorium (Brunner-Mendoza et al., 2019).

Lipolytic enzymes including lipases, act primarily on the epicuticle, followed by proteases and chitinases, according to the presence of polymeric substrates in the different portions of the cuticle (Beys da Silva et al., 2010a,b). Among the proteases that can act at this level are subtilisins, trypsins, chymotrypsins, metallopeptidases, aspartyl peptidases, and exopeptidases (Semenova et al., 2020); where their expression from fungi such as *M. anisopliae* will depend specifically on the composition of the cuticle and hemolymph (Freimoser et al., 2005).

EPF such as *M. anisopliae* and *B. bassiana* can express up to 11 different subtilisins, one of the most important being the Pr1 subtilisin-like peptidases, which intervene in the arthropod pathogenesis, causing hydrolysis of the cuticle and providing nutrients to the fungus (Gao et al., 2020; Semenova et al., 2020). To our knowledge, there are few reports elucidating the participation of these proteases during the infection process of cattle ticks (*R. microplus, R. annulatus* and *A. mixtum*) by EPF.

In this regard, Golo et al. (2015) reported that spores of *M. anisopliae* s.l. expressed the Pr1 gene and that there was an increase in the specific activity of Pr1, when the fungus was cultured in *R. microplus* larvae; however, these authors concluded that increased Pr1 activity in conidia and its expression levels were not associated with significant changes (up or down) in larval mortality. It was recently reported that five of the 11 members of the Pr1 family are essential for the maintenance of the total extracellular activity of Pr1, which is necessary for the degradation of the host cuticle during hyphal invasion of EPF (such as *B. bassiana*), providing capacity for a broadest host spectrum (Gao et al., 2020).

We suggest that it is necessary to continue evaluating the participation of subtilisins produced by EPF during the infection process against cattle ticks. A full understanding of the pathogenicity and/or virulence mechanism is essential for the development of an effective biological control scheme.

## Fungal Growth and Death of the Host

After penetration and once inside the host, the EPF develop hyphal bodies and blastopores that multiply and disseminate through the hemolymph to invade different tissues (Maina et al., 2018; Beys-da-Silva et al., 2020) using circulation as a vehicle for colonization and for nutrient absorption (Valero-Jiménez et al., 2016; Brunner-Mendoza et al., 2019).

During this event, different virulence factors act on host colonization in order to spread inside the arthropod's body, causing its death. Among the most important factors are mycotoxins such as Beauvericin, Beauverolides, Bassiannolide (by *B. bassiana, V. lecanii*, and *Paecilomyces* spp.) and Dextruxins A, B, C, D, E, F (by *M. anisopliae*), which act as poisons for the host (Maina et al., 2018).

These fungal toxins might cause flaccid paralysis, cellular alterations and inhibit the normal functioning of muscle tissues, the middle intestine and the Malpighian tubes (Mora et al., 2017). After the dead of the host and when the nutrients within it are depleted, the fungus open the integument, forms aerial mycelia and carries out sporulation on the corpse, initiating the dispersal of its conidia (Valero-Jiménez et al., 2016).

As we can see, EPF can have a wide variety of toxins that could affect the biology of livestock ticks. Although the general mechanisms of infection have been described for most of the useful EPF against arthropods, including ticks, the scientific community needs to understand the complexity of the molecular mechanisms in each infection phase, which is not completely elucidated. A transdisciplinary approach is required to incorporate different tools, such as genomics, transcriptomics, proteomics and metabolomics in order to better comprehend the mechanism of pathogenicity in EPF against ticks.

It is important to note that not all EPF populations have the same capacity to produce all the enzymes or toxins reported in the scientific literature (Schrank and Vainstein, 2010), neither in variety nor in quantity. For example, in *R. microplus*, the immersion or inoculation of dextruxin A from *M. anisopliae* on engorged ticks neither affected their biological parameters nor caused paralysis (Golo et al., 2011); but other study reported an acaricidal effect of distinct destruxins from Beauveria feline against *R. microplus* (Morais-Urano et al., 2012).

In addition, different environmental and nutritional factors have been identified as the main triggers of the genetic expression of these components (Campos et al., 2005; Fang et al., 2005). The production capacity of these toxins and enzymes is reflected in the time of death of the tick, which varies according to the EPF strain, the type of fungus (genus and species), the treated tick genus, the method of application and the number of infectious spores (Fernández-Salas et al., 2017; Mantzoukas and Eliopoulos, 2020).

Pathogenicity or virulence factors give EPF the ability to be specific to one or other pest arthropod, a characteristic that helps control them, without affecting other organisms beneficial to nature (Kirkland et al., 2004). It should be noted that EPF are considered "non-specialized" mite pathogens, and it has not been conclusively demonstrated that they develop specificity against ticks to the extent of causing epizootics (Fernandes et al., 2012), as has happened with some insects such as *Lymantria dispar* (Lepidoptera: Erebidae), *Diprion pini* (Hymenoptera: Diprionidae), *Dendrolimus pini* (Lepidoptera: Lasiocampidae), *D. punctatus* (Lepidoptera: Lasiocampidae), *Malacosoma disstria* (Lepidoptera: Lasiocampidae), *Fiorinia externa* (Hemiptera: Diaspididae) (Augustyniuk-Kram and Kram, 2012).

However, several authors have mentioned the high capacity of these organisms to become specialists for certain arthropods, without losing their ability to be general pathogens (Beys-da-Silva et al., 2020). In this regard, a recent study has found an increase in mortality caused by Mexican strains of *M. anisopliae* s.l. on *R. microplus* and *A. mixtum*, after submitting them to four passages using these ticks as substrate (Romero-Pérez, 2020).

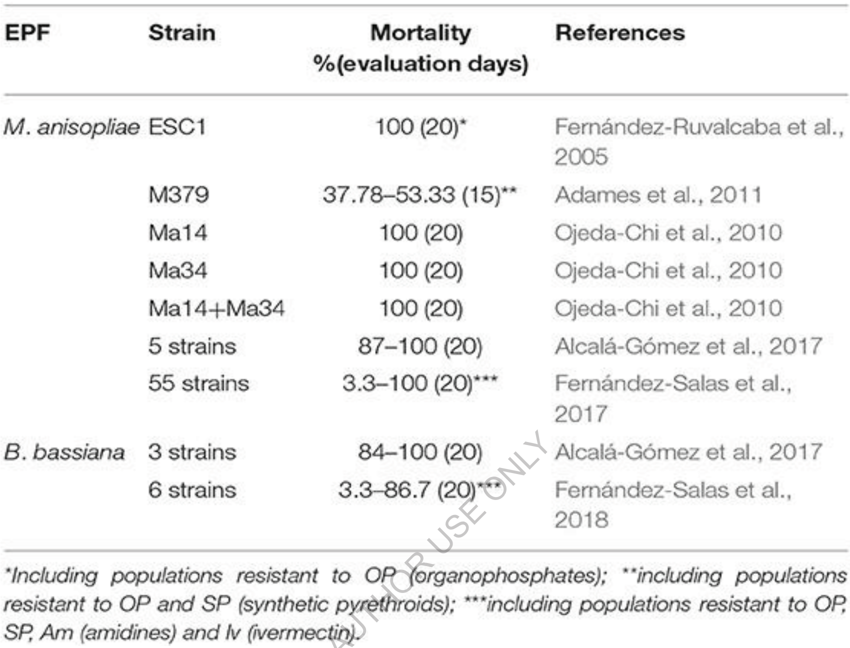
Furthermore, Adames et al. (2011) reported in Mexico that four to seven passages of *M. anisopliae* s.l. on *R. microplus* increase its virulence against this tick. Although more studies are needed in this regard (e.g., what are the molecular or metabolic mechanisms that are triggered to increase this virulence?), the mortality behavior that these EPF develop in their evaluation against ticks is interesting. This possibility shows a promising outlook to maintain or improve virulence in those Mexican EPF strains that show favorable characteristics such as high sporulation, resistance or tolerance to UV rays, thermotolerance and/or probable specificity against ticks.

## Entomopathogenic Fungi as Livestock Tick Pathogens

### Laboratory Tests

The evaluations of *in vitro* studies regarding the acaricidal effect of EPF against livestock ticks (including resistant populations) and their reproduction in Mexico are shown in Tables 5–7. Most laboratory studies have demonstrated the effectiveness of EPF and, in some cases, their potential to control susceptible and resistant/multiresistant ticks; however, the following details can also be observed and summarized.

Table 5

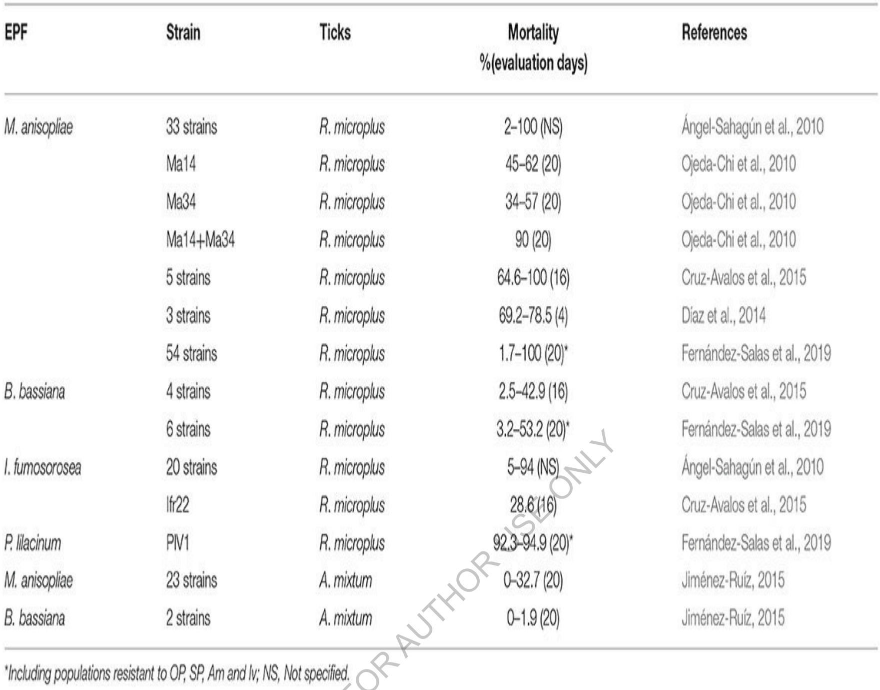


| EPF | Strain | Mortality %(evaluation days) | References |
|---|---|---|---|
| *M. anisopliae* | ESC1 | 100 (20)* | Fernández-Ruvalcaba et al., 2005 |
| | M379 | 37.78–53.33 (15)** | Adames et al., 2011 |
| | Ma14 | 100 (20) | Ojeda-Chi et al., 2010 |
| | Ma34 | 100 (20) | Ojeda-Chi et al., 2010 |
| | Ma14+Ma34 | 100 (20) | Ojeda-Chi et al., 2010 |
| | 5 strains | 87–100 (20) | Alcalá-Gómez et al., 2017 |
| | 55 strains | 3.3–100 (20)*** | Fernández-Salas et al., 2017 |
| *B. bassiana* | 3 strains | 84–100 (20) | Alcalá-Gómez et al., 2017 |
| | 6 strains | 3.3–86.7 (20)*** | Fernández-Salas et al., 2018 |

**Including populations resistant to OP (organophosphates); **including populations resistant to OP and SP (synthetic pyrethroids); ***including populations resistant to OP, SP, Am (amidines) and Iv (ivermectin).*

TABLE 5. Laboratory evaluations of the EPF effectiveness (dosage: 1 × $10^8$ conidia/ml) against engorged female of *R. microplus* (including resistant populations) in Mexico.

Table 6

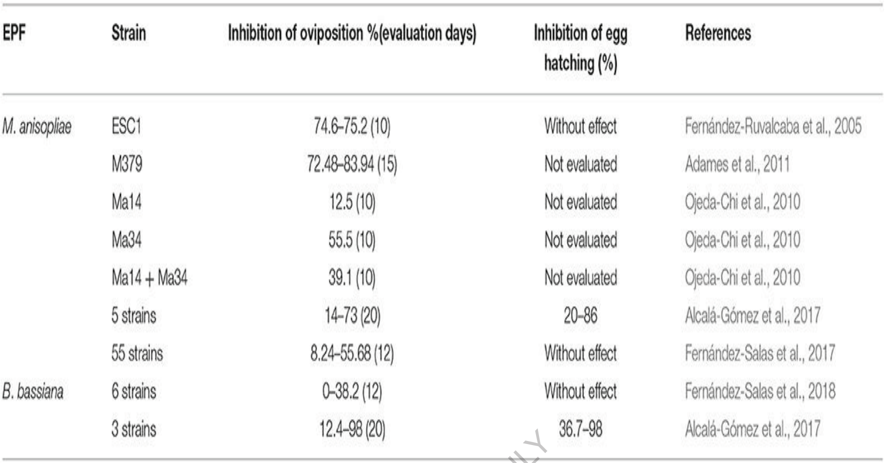


| EPF | Strain | Ticks | Mortality %(evaluation days) | References |
|---|---|---|---|---|
| *M. anisopliae* | 33 strains | *R. microplus* | 2–100 (NS) | Ángel-Sahagún et al., 2010 |
| | Ma14 | *R. microplus* | 45–62 (20) | Ojeda-Chi et al., 2010 |
| | Ma34 | *R. microplus* | 34–57 (20) | Ojeda-Chi et al., 2010 |
| | Ma14+Ma34 | *R. microplus* | 90 (20) | Ojeda-Chi et al., 2010 |
| | 5 strains | *R. microplus* | 64.6–100 (16) | Cruz-Avalos et al., 2015 |
| | 3 strains | *R. microplus* | 69.2–78.5 (4) | Díaz et al., 2014 |
| | 54 strains | *R. microplus* | 1.7–100 (20)* | Fernández-Salas et al., 2019 |
| *B. bassiana* | 4 strains | *R. microplus* | 2.5–42.9 (16) | Cruz-Avalos et al., 2015 |
| | 6 strains | *R. microplus* | 3.2–53.2 (20)* | Fernández-Salas et al., 2019 |
| *I. fumosorosea* | 20 strains | *R. microplus* | 5–94 (NS) | Ángel-Sahagún et al., 2010 |
| | Ifr22 | *R. microplus* | 28.6 (16) | Cruz-Avalos et al., 2015 |
| *P. lilacinum* | PlV1 | *R. microplus* | 92.3–94.9 (20)* | Fernández-Salas et al., 2019 |
| *M. anisopliae* | 23 strains | *A. mixtum* | 0–32.7 (20) | Jiménez-Ruíz, 2015 |
| *B. bassiana* | 2 strains | *A. mixtum* | 0–1.9 (20) | Jiménez-Ruíz, 2015 |

**Including populations resistant to OP, SP, Am and Iv; NS, Not specified.*

TABLE 6. Laboratory evaluations of the EPF effectiveness (dosage: 1 × $10^8$ conidia/ml) against larvae of *R. microplus* and *A. mixtum* (including resistant populations) in Mexico.

Table 7



| EPF | Strain | Inhibition of oviposition %(evaluation days) | Inhibition of egg hatching (%) | References |
|---|---|---|---|---|
| *M. anisopliae* | ESC1 | 74.6–75.2 (10) | Without effect | Fernández-Ruvalcaba et al., 2005 |
| | M379 | 72.48–83.94 (15) | Not evaluated | Adames et al., 2011 |
| | Ma14 | 12.5 (10) | Not evaluated | Ojeda-Chi et al., 2010 |
| | Ma34 | 55.5 (10) | Not evaluated | Ojeda-Chi et al., 2010 |
| | Ma14 + Ma34 | 39.1 (10) | Not evaluated | Ojeda-Chi et al., 2010 |
| | 5 strains | 14–73 (20) | 20–86 | Alcalá-Gómez et al., 2017 |
| | 55 strains | 8.24–55.68 (12) | Without effect | Fernández-Salas et al., 2017 |
| *B. bassiana* | 6 strains | 0–38.2 (12) | Without effect | Fernández-Salas et al., 2018 |
| | 3 strains | 12.4–98 (20) | 36.7–98 | Alcalá-Gómez et al., 2017 |

TABLE 7. Effect of EPF at laboratory level (dosage 1 × $10^8$ conidia/ml) on the reproductive parameters of *R. microplus* in Mexico.

## Most Studies Have Been Using *R. microplus* as a Model

There is only one report on the acaricidal effect of EPF against *A. mixtum* larvae and to our knowledge, there are not reports on the acaricidal effect on *R. annulatus* in Mexico. *R. microplus, R. annulatus* and *A. mixtum* are the most prevalent ticks on cattle farms across the country, causing great economic losses.

Therefore, it is important to evaluate the acaricidal effect of fungi on a higher diversity of ticks in order to identify strains that have a wide or reduced action spectrum, since this information could help design tick control schemes in the field (i.e., 86% of cattle farms have cohabiting *R. microplus* and *A. mixtum* in Mexico), explore the EPF infection mechanisms in each tick genus, and elucidate some biological aspects of the fungal-host immune system interaction.

In recent studies, several strains of *M. anisopliae* s.l. were highly effective against larvae of *R. microplus*, but not against *A. mixtum* larvae (Jiménez-Ruíz, 2015; Fernández-Salas et al., 2017). In other countries, a high variability in the virulence of EPF against different genera of ticks and different tick populations of the same genus has been reported (Fernandes et al., 2012; Perinotto et al., 2012). Webster et al. (2017) also reported that field populations of *R. microplus* show high variation in their susceptibility to *M. anisopliae*.

It is necessary to understand the factors that are involved in this differentiated response, and especially to be able to find a strain of EPF with a broad acaricidal effect against different genera of ticks and different field populations.

## Acaricidal Activity of EPF against Resistant and Multiresistant Populations of *R. microplus*

An essential goal of implementing EPF in tick control programs is the mitigation of the economic and sanitary impact of the resistance that these arthropods have developed. When a tick population is resistant to chemical acaricides, it is inappropriate to neglect the possible interference of tick resistance mechanisms in the EPF performance (Perinotto et al., 2012).

In Mexico, several strains of *M. anisopliae* s.l. induced a mortality of 90–100% in populations of *R. microplus* multiresistant to acaricides (OP, SP, Am) and ivermectin (Fernández-Salas et al., 2017, 2018, 2019). In fact, some studies reported a higher susceptibility to the EPF effect in *R. microplus* resistant to OP and SP than in susceptible strains (Adames et al., 2011). Fernández-Ruvalcaba et al. (2005) reported a similar mortality caused by *M. anisopliae* s.l. in *R. microplus* populations susceptible and resistant to OP.

In general, *M. anisopliae* strains showed high acaricidal effects against resistant or acaricide-susceptible *R. microplus* and no differences in effects were observed between tick populations (Table 5). This is important because the resistance mechanism used by ticks in order to avoid the toxic effects of chemical acaricides did not affect the action mechanism of the EPF strains. This supports the opinion of some authors, who state that resistance to biological agents as EPF is less likely to occur compared to resistance to chemical acaricides (Polar et al., 2005).

## Most Studies Have Been Targeting on a Single Stage of the Tick Life Cycle

A great advantage of EPF, compared to chemical acaricides, is that they can attack almost all stages of the arthropod life cycle, making them a unique component in integrated pest management approaches (Rajula et al., 2020). The few reports that exist on *A. mixtum* in Mexico were made in larvae, but the effect of EPF on adult ticks remains unknown. Regarding *R. microplus*, only a few EPF strains have been evaluated in engorged adult ticks and in larvae.

A greater susceptibility of tick larvae to the lethal effect of EPF has been reported compared to engorged females (Fernandes and Bittencourt, 2008; Fernandes et al., 2012); however, it seems that in the bioassays carried out in Mexico there is a possible tendency of susceptibility in engorged adult ticks than in the larval stage. It would be interesting to evaluate the factors that could intervene within this possible tendency with Mexican EPF strains, considering different factors such as the nutritional, genetic, origin and biological conditions of both fungi and ticks. Regarding tick reproduction, EPF have shown a significant inhibition of oviposition (most strains cause more than 50% effectiveness in a short time) (Table 7).

However, the fungal effect on egg hatching inhibition percentages is not reported or has not been evaluated. This could be different in the field, since in the laboratory, the egg mass is generally removed from the engorged female between 10 to 15 days from the beginning of oviposition in order to evaluate hatching, which avoids future contact of these eggs with the spores produced on the surface of teleogin corpses, a situation that would not occur in the field. Therefore, it is recommended to include in the evaluations the direct application of EPF in the egg mass to determine its effect on its viability. In addition, it is also advisable to carry out simultaneous studies that include all stages of the tick life cycle.

## Most Studies Have Been Based in the Use of *M. anisopliae* s.l. and *B. bassiana* s.l. Strains

Most of the studies carried out in Mexico have used *M. anisopliae* s.l. as the main EPF against ticks and, to a lesser extent, *B. bassiana* s.l. and *I. fumosorosea*. However, according to genetic diversity studies, other fungi have been identified that could cause high mortality effects in ticks, such as *Purpureocillium lilacinum* (Fernández-Salas et al., 2019).

Therefore, it is highly recommended to continue research that identifies and evaluates the effect of different genera and species of EPF against ticks in livestock. Also, it is important to highlight that the main states where these fungi have been isolated and evaluated are located in the tropical and subtropical areas of Mexico, which stand out for their extensive livestock activity and reports of tick resistance to conventional chemical acaricides.

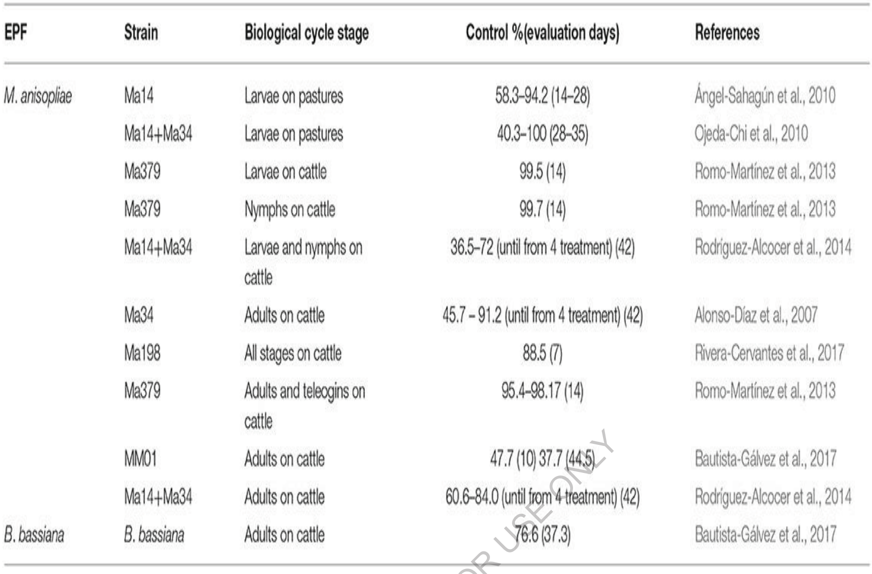
On the other hand, these studies have allowed not only to have different EPF strains effective against ticks, but also to isolate strains from different sources and the standardization of techniques through various bioassays in distinct laboratories in the country. Taking all these aspects into account for future research, it will help to improve the EPF's effect against ticks, and include them as an important tool in tick control programs.

It should be noted that the main EPF used in the studies have been isolated from different orders of insects, ticks and culture soils. In recent studies, EPF isolates have been made directly from grassland soils (Bautista-Gálvez et al., 2017; Fernandez-Salas et al., 2020), investigating whether this native characteristic may influence the tickcide effect. So far, the results have been promising (Fernández-Salas et al., 2017, 2019), attributing them to coexistence with ticks and the evolutionary adaptation of EPF to various nutritional substrates. In Mexico, studies in which EPF are isolated directly from tick corpses are scarce, although it would be interesting to carry out investigations in search of naturally colonized ticks to determine their virulence and effectiveness under controlled conditions.

## Field Tests

Field studies evaluating the acaricidal effect of EPF against livestock ticks in Mexico are presented in Table 8. These studies have shown satisfactory control efficiency of *M. anisopliae* s.l. against different stages of *R. microplus* when applied both in pastures as in cattle; however, the following details can also be observed and summarized.

Table 8



| EPF | Strain | Biological cycle stage | Control %(evaluation days) | References |
|---|---|---|---|---|
| *M. anisopliae* | Ma14 | Larvae on pastures | 58.3–94.2 (14–28) | Ángel-Sahagún et al., 2010 |
| | Ma14+Ma34 | Larvae on pastures | 40.3–100 (28–35) | Ojeda-Chi et al., 2010 |
| | Ma379 | Larvae on cattle | 99.5 (14) | Romo-Martínez et al., 2013 |
| | Ma379 | Nymphs on cattle | 99.7 (14) | Romo-Martínez et al., 2013 |
| | Ma14+Ma34 | Larvae and nymphs on cattle | 36.5–72 (until from 4 treatment) (42) | Rodríguez-Alcocer et al., 2014 |
| | Ma34 | Adults on cattle | 45.7 – 91.2 (until from 4 treatment) (42) | Alonso-Díaz et al., 2007 |
| | Ma198 | All stages on cattle | 88.5 (7) | Rivera-Cervantes et al., 2017 |
| | Ma379 | Adults and teleogins on cattle | 95.4–98.17 (14) | Romo-Martínez et al., 2013 |
| | MM01 | Adults on cattle | 47.7 (10) 37.7 (44.5) | Bautista-Gálvez et al., 2017 |
| | Ma14+Ma34 | Adults on cattle | 60.6–84.0 (until from 4 treatment) (42) | Rodríguez-Alcocer et al., 2014 |
| *B. bassiana* | *B. bassiana* | Adults on cattle | 76.6 (37.3) | Bautista-Gálvez et al., 2017 |

TABLE 8. Field evaluations (on cattle and pastures) of the EPF effectiveness against various biological stages of *R. microplus* ticks in Mexico.

## Some Field Studies Have Demonstrated the Effectiveness of *M. anisopliae* on *R. microplus* Free-Living Stage (Larvae)

Worldwide, tick control has been based on therapeutic treatments mainly directed at parasitic stages of *R. microplus*. However, this tick spends between 80 and 90% of its life cycle outside the host in pastures (Needham and Teel, 1991); therefore, a good strategy to reduce the negative effects of tick infestations on cattle is to reduce free-living populations.

*M. anisopliae* conidia can be applied to livestock farm pastures (as in crops) to control free-living stages. In Mexico, the aerial dispersal of *M. anisopliae* conidia in pastures reduced the number of *R. microplus* larvae from 40 to 100% (Ángel-Sahagún et al., 2010; Ojeda-Chi et al., 2010).

However, it is necessary to investigate the acaricidal effect using another genus of EPF (e.g., *B. bassiana, I. fumosorosea* or *P. lilacinum*). Recently, our research group evaluated the acaricidal activity of about 60 EPF strains isolated from paddocks against *R. microplus* in the Mexican tropics (Fernández-Salas et al., 2017, 2018).

EPF adapted both to the regional environmental conditions in which they were used, as well to the ticks that served as substrate for fungi development. Therefore, these EPF strains could also be useful in the implementation of biological control programs against ticks. In addition, several isolates showed thermotolerance and resistance to UV-R, which makes them possible candidates for field evaluation. Likewise, it is necessary to evaluate the fungal strains with high efficiency against other genera of ticks (*A. mixtum* and *R. annulatus*) present in pastures of cattle farms in Mexico.

## Most *in vivo* Studies Have Demonstrated the Efficacy of *M. anisopliae* s.l. Applied to Naturally Infested Cattle

Most of the field studies carried out in Mexico showed that when EPF conidia were sprayed on cattle, high control percentages against parasitic stages of *R. microplus* were observed (Table 8). However, it is known that EPF could decrease its acaricidal efficacy against ticks under field conditions due to biotic and abiotic factors that affect its virulence and pathogenicity. Fungal growth, conidia production, survival, germination, pathogenicity, virulence and the production of bioactive compounds can be strongly influenced by exposure to ultraviolet solar radiation (Wong et al., 2019).

Therefore, it is recommended that when using EPF for tick control in the field, strategies to counteract the negative effects of these factors need to be considered in order to improve the tickcide effect. Among the main strategies are the use of protectors and dispersers of conidia from highly virulent and pathogenic isolates, the selection of isolates adapted to the climatic conditions where they will be used (i.e., native isolates with a greater natural tolerance to UV-R) and proper application of EPF in order to avoid high temperatures and UV-R.

## No Adverse Effects Were Reported in Animals and/or Operators

An important point that should be taken into account when using EPF is the safety in their use. None of the field studies conducted has reported adverse reactions in cattle or operators. This is consistent with Zimmermann (2007), who mentioned that EPF do not pose risks to animals, humans or the environment, concluding that this control method is a safe and sustainable alternative. On the contrary, the use of chemical acaricides can be highly harmful to beneficial species or non-target organisms, humans, animals and the environment (Fernández-Salas et al., 2012a).

Therefore, having an alternative control will help reduce the use of these chemicals and keep these situations at a low risk of presentation, based on the premise that EPF with affinity for a target organism are less capable of causing harm to non-target organisms (Goettel and Johnson, 1992). However, in Mexico, it is recommended to complement field studies with evaluations of the beneficial or negative impact of EPF on the ecosystems of livestock farms.

## Integrated Pest Management of Livestock Ticks

Through many years of experience in treating ticks, studies have concluded that applying a single treatment will not maintain efficient and sustainable control in the long term. Invariably, the product used will exhibit inefficiencies in killing ticks due to their ability to become resistant. In Mexico, it has been mentioned that none of the previous strategies (chemical and non-chemical) by themselves have been sufficient to sustainably control ticks, such as *R. microplus* (Romo-Martínez et al., 2013; Fernández-Salas et al., 2019).

Therefore, it is necessary to integrate two or more methodologies in order to be able to attack ticks on several fronts and take care, among themselves, of the effectiveness of the products or techniques used. Integrated pest management is defined as the systematic application of two or more technologies that are compatible with each other, with the environment and that are profitable to control populations of arthropod pests that negatively affect livestock (Bram, 1994). EPF are compatible with various tick control products, including chemical acaricides, without losing their acaricidal capacity (Sousa et al., 2011; Kiss et al., 2012; Romo-Martínez et al., 2013). Therefore, the integration of EPF in a tick control scheme is totally feasible.

## Proposals for Integrated Tick Control in Mexico

Research evaluating integrated pest management schemes, including the EPF for tick control in Mexico, is scarce. There is a very important gap that requires more research, since it has been mentioned that integrated tick control is the best way to establish sustainable and successful livestock in order to increase the productive capacity of the animals (Rodríguez-Vivas et al., 2014b).

Proposals for the use of EPF within an integrated tick pest management should be designed according to several factors, listed below.

### The Climatological Characteristics of the Region Where the Control Will Be Implemented

The population dynamics of ticks depends mainly on the conditions of temperature, relative humidity and rainfall. Therefore, the distribution of ticks throughout the year can be predicted since climatic factors are responsible for this characteristic.

### The Ticks Present in the Control Area

Different tick genera may show differences in the biological cycles, so their presence in the bovine body and in the pastures is distinct throughout the year (e.g., *R. microplus* and *A. mixtum*, which coexist in the Mexican tropics).

### The Toxicological Response/Behavior of Ticks

It is necessary to know the susceptibility status of the ticks to be treated, since, as mentioned above, chemical acaricides are and will be the basis of tick control programs, including those of integral management and the success of the establishment of these protocols will depend on their proper use.

### The Availability and Compatibility of Various Methods for Tick Control

It is important to consider all available and proven alternatives for tick treatment and use them in combination with each other and with chemical acaricides.

One of the main advantages that is present in Mexico for the design of tick control protocols through integrated management is that climatic characteristics of the country have well-defined patterns (Estrada-Peña et al., 2006).

However, there are few studies where the population dynamics of ticks of livestock importance have been determined in Mexico through the seasons of the year and in different states (Estrada-Peña et al., 2006; Alonso-Díaz et al., 2007; González-Cerón et al., 2009; Almazán et al., 2016). For this reason, adequate proposal designs for integrated tick management for all ecological regions of the country are limited.

## Plant extract and Ticks control

The cattle tick, *Rhipicephalus* (*Boophilus*) *microplus*, is an economically important ectoparasite of livestock and creates major problem for milk producers in tropical and subtropical countries including India. It causes severe economic losses by blood loss, reduction in weight gain, direct damage to skin and hides and also by serving as a vector of infectious diseases (Ghosh et al. 2006; Ghosh and Nagar 2014).

In order to manage this ectoparasite, different groups of chemicals viz., synthetic pyrethroids, organophosphates, formamidines and macrocyclic lactones are used widely. Such strategies are aimed to prevent damage and forestall an epidemic by keeping the populations below a critical threshold level. However, widespread use of these chemicals causes serious ecological problems. The use of chemicals to control ticks on cattle usually generates hundreds of gallons of residues (3–4 liters of solution per animal) which are often discarded indiscriminately, leading to soil and water contamination (Gromboni et al. 2007).

These chemicals kill non-target organisms and threaten human health due to the toxic residues in milk and meat (Graf et al. 2004). Another most serious problem of intensive use of these chemical acaricides is the development of resistant tick populations which causes failure of the chemical-based tick control program (Rosario-Cruz et al. 2005; Rodriguez-Vivas et al. 2006a, 2006b).

To tackle the problem of resistance and other environmental issues linked with chemical control, efforts have been made to develop sustainable immunological means for controlling ticks and tick-borne diseases. As a landmark development, two commercial vaccines against *R.* (*B.*) *microplus* were developed and marketed (de la Fuente et al. 1998, 1999; Willadsen 2004).

The vaccine efficacy was reassessed after 10 years of introduction and significant protection was reported with reduced frequency of acaricidal usage (de la Fuente et al. 2007). Encouraging data have also been developed to control other economically important tick species, *Hyalomma anatolicum* (Azhahianambi et al. 2009; Jeyabal et al. 2010; Binod Kumar et al. 2012).

However, the efficacy of the vaccine was found highly variable (de La Fuente et al. 2000; Canales et al. 2009) and farmers' expectation has not been met. Moreover, the vaccine is not giving significant protection against multi-species tick infestations, a common problem faced by the livestock owners of Asia and Africa. Other studies involving fungal biopesticides and entomopathogenic nematodes proved unsuccessful (Benjamin et al. 2002; Polar et al. 2005).

Thus, there is a keen interest in the development of alternative ecofriendly anti-tick natural products. Amongst the natural products, plant extracts and essential oils have been shown to have significant activity against economically important tick species (Borges et al. 2003; Pereira and Famadas 2006; Fernandes and Freitas 2007; Kamaraj et al. 2010; de Souza Chagas et al. 2012; Juliet et al. 2012; Sunil et al. 2013) including acaricide resistant species (Ghosh et al. 2011, 2013). Moreover, these botanicals are found to contain a mixture of active substances which can delay or prevent the development of resistance to herbal products (Wang et al. 2007).

To identify the best plants having anti-tick activity, seven plants with reported pharmacological, insecticidal and other medicinal properties were selected. For example, *Datura* spp. have been reported to possess insecticidal, antifeedant (Georges et al. 2008; Ramya and Jayakumararaj 2009), acaricidal (Mateeva et al. 2003; Fang Ping et al. 2006; Shyma et al. 2014), repellent, oviposition deterrent (Kumral et al. 2010; Swathi et al. 2012), fungicidal (Mdee et al. 2009), antiasthamatic, anticancer, anticholinergic and anti-inflamatory, pharmacological (Soni et al. 2012) properties.

The plant, *Argemone mexicana* possess anti-anthelmintic, anti-inflammatory, wound healing, anti-bacterial, anti-fungal (Bhattacharjee et al. 2006; Satish et al. 2007; Mohana and Raveesha 2007; Habbu et al. 2007), anti-termite (Elango et al. 2012), anti-mite (Thakur et al. 1995) and also anti-malarial activities (Willcox et al. 2007). A number of properties of *Ocimum sanctum* plant such as anti-microbial, anti-diabetic, hepato-protective, anti-inflammatory, anti-carcinogenic, radio-protective, immune-modulatory, neuro-protective, cardio-protective, mosquito repellent (Mondal et al. 2009) and acaricidal (Esther et al. 1995) have been reported.

The *Barleria prionitis* plant is found to have anti-inflammatory (Khadse and Kakde, 2011), anti-arrhythmic (Singh et al. 2003), and is also used in urinary infection, jaundice, hepatic obstruction, dropsy and in boils and glandular swellings (Jaiswal et al. 2010). Likewise, the acaricidal properties of *Citrus maxima* and *Ocimum sanctum* were also reported (Chungsamarnyart and Jansawan 1996). The *Tinospora cordifolia* and *Sphaeranthus indicus* plants have also been reported to possess insecticidal and pharmacological activities. However, the acaricidal potentiality of these plants against chemical acaricides resistant ticks has never been studied. Accordingly, the present experiment was conducted with an objective to identify plants having significant activity against *R.* (*B.*) *microplus* including chemical acaricide resistant species, a problem which needs to be tackled strategically.

Based on 72 h in vitro screening criterion, of the nine plant extracts tested against IVRI-I line of *R.* (*B.*) *microplus* using AIT, the 95 % ethanolic extracts of *A. mexicana* and *D. metel* were identified for further study as they caused significant mortality of 55.0 and 65.0 %, respectively, within 72 h. The other seven extracts did not show any acaricidal activity within 72 h. It was interesting to note that extracts of identified plants prepared in 50 % ethanol preparation did not show any toxic effect on tick survival. The data revealed that the antitick active compounds of these plants were richly present in 95 % ethanolic extract (Table 9).

Table (9): Names and parts of plants used for extraction and anti-tick activity

| **Sl. no.** | **Scientific name of plants** | **Common name** | **Parts used for extraction** | **% Mortality in 72 h[c] (Mean ± SE)** |
|---|---|---|---|---|
| 1 | *Argemone mexicana* | Mexican prickly poppy | Whole plant[a] | 55.0 ± 5.0 |
| | | | Whole plant[b] | 0.0 ± 0.0 |
| 2 | *Barleria prionitis* | Danti | Whole plant[a] | 0.0 ± 0.0 |
| 3 | *Citrus maxima* | Bara Nimbu | Fruit[a] | 0.0 ± 0.0 |
| 4 | *Datura metel* | Dhatura | Fruit[a] | 65.0 ± 5.8 |
| | | | Fruit[b] | 0.0 ± 0.0 |
| 5 | *Ocimum sanctum* | Tulsi | Whole plant[a] | 0.0 ± 0.0 |
| 6 | *Sphaeranthus indicus* | Akarkara | Whole plant[a] | 0.0 ± 0.0 |
| 7 | *Tinospora cordifolia* | Guduch | Stem[a] | 0.0 ± 0.0 |

[a] 95 % ethanolic extract
[b] 50 % hydro-ethanolic extract
[c] 10 % concentration of the extract

The dose–response data of *D. metel* fruits extract indicated that 4–9 % concentrations were highly efficacious and a significant percent mortality in the range of 70.0 ± 5.8 to 100.0 ± 0.0 was recorded ($p < 0.001$) within 14 days post treatment. Besides mortality, the rate of oviposition was inhibited up to 41.4–100 % in treated ticks. The dose response study using 2–10 % of *A. mexicana* extracts gave a different profile with 25–90 % mortality at the same concentration. The mortality was never at 100 % level even at the highest tested concentration of 10 % and the effect on reproductive function was in the range of 9.3–65.5 % which was about 30 % low in comparison to *D. metel* extract-treated ticks at similar concentration (Table 10).

Table (10): Dose-dependent efficacy of 95 % ethanolic extract of *Datura metel* fruits and *Argemone mexicana* whole plant extract against IVRI-I line of *Rhipicephalus* (*B.) microplus*

| **Conc. (%)** | ***D. metel*** | | ***A. mexicana*** | |
|---|---|---|---|---|
| | **% Mortality in 14 days (mean ± SE)** | **% Inhibition of oviposition** | **% Mortality in 14 days (mean ± SE)** | **% Inhibition of oviposition** |
| 2 | $45.0 \pm 5.0^{b}$ | 27.4 | 0.0 ± 0.0 | 3.4 |
| 3 | $50.0 \pm 5.8^{b}$ | 38.3 | 10.0 ± 5.8 | 9.9 |
| 4 | $70.0 \pm 5.8^{c}$ | 41.4 | 25.0 ± 9.6 | 9.3 |
| 5 | $75.0 \pm 9.6^{c}$ | 43.1 | 30.0 ± 5.8 | 6.2 |
| 6 | $85.0 \pm 5.0^{c}$ | 42.0 | $45.0 \pm 12.6^{a}$ | 12.5 |
| 7 | $85.0 \pm 5.0^{c}$ | 63.0 | $55.0 \pm 5.0^{b}$ | 20.7 |
| 8 | $90.0 \pm 5.8^{c}$ | 56.1 | $70.0 \pm 10.0^{c}$ | 29.9 |

| **Conc. (%)** | ***D. metel*** | | ***A. mexicana*** | |
|---|---|---|---|---|
| | **% Mortality in 14 days (mean ± SE)** | **% Inhibition of oviposition** | **% Mortality in 14 days (mean ± SE)** | **% Inhibition of oviposition** |
| 9 | $100.0 \pm 0.0^c$ | 100.0 | $80.0 \pm 8.2^c$ | 43.3 |
| 10 | $100.0 \pm 0.0^c$ | 100.0 | $90.0 \pm 5.8^c$ | 65.5 |
| Control | $10.0 \pm 5.8$ | – | $0.0 \pm 0.0$ | – |

$^a$Significant at $p < 0.05$
$^b$Significant at $p < 0.01$
$^c$Significant at $p < 0.001$

Analyzing the dose response data of the identified 95 % ethanolic extracts, the $LC_{90}$ and $LC_{50}$ values of *D. metel* fruit extract were determined as 7.13 (95 % confidence interval of 6.17–8.23) and 2.7 % (95 % CI 2.4–3.04), respectively. The $LC_{90}$ and $LC_{50}$ values of *A. mexicana* extract were calculated as 11.30 % (95 % confidence interval of 10.31–12.37) and 6.01 % (95 % CI 5.56–6.49), respectively, against the reference susceptible IVRI-I line of *R.* (*B.*) *microplus* (Table 11).

Dose response data of *D. metel* fruit extract indicated the gradual increase in the mortality pattern with slope of 2.99 ± 0.32 and $R^2$ value of 0.927 suggesting that 92.7 % data were correlated with log concentration. Based on the $LC_{50}$ value and % IO, *D. metel* fruit extract was found more effective than *A. mexicana* whole plant extract. Both the extracts caused reproductive toxicity in treated ticks in a dose-dependent manner with slope values of 70.08 ± 20.57 and 94.23 ± 24.28 where 62.4–68.3 % IO data were correlated with different concentrations (Table 11).

Table (11): LC$_{50}$ and LC$_{90}$ with 95 % confidence interval of identified *Datura metel* and *Argemone mexicana* extracts obtained by AIT against susceptible IVRI-I line of *Rhipicephalus* (*B.*) *microplus*

| **Extracts** | **Variables** | **Slope ± SE** | $R^2$ | **LC$_{50}$ (%) (95 % CI)** | **LC$_{90}$ (%) (95 % CI)** |
|---|---|---|---|---|---|
| *D. metel* | Mortality | 2.99 ± 0.32 | 0.927 | 2.7 (2.4–3.04) | 7.13 (6.17–8.23) |
| | Egg masses | −65.88 ± 15.44 | 0.722 | | |
| | RI | −0.45 ± 0.12 | 0.683 | | |
| | % IO | 94.23 ± 24.28 | 0.683 | | |
| *A. mexicana* | Mortality | 4.67 ± 0.25 | 0.980 | 6.01 (5.56–6.49) | 11.30 (10.31–12.37) |
| | Egg masses | −51.91 ± 15.64 | 0.611 | | |
| | RI | −0.32 ± 0.09 | 0.630 | | |
| | % IO | 70.08 ± 20.57 | 0.624 | | |

### Acaricidal activity of identified extracts against resistant tick lines

The 95 % ethanolic extracts of *D. metel* showed a lethal effect of 65.0 ± 9.6 and 70.0 ± 5.8 % against resistant IVRI- 4 and 5 lines, respectively. While 50.0 ± 5.8 and 55.0 ± 9.6 efficacies were noted in *A. mexicana* extracts treated ticks. Besides lethal effects, both the extracts were found to interfere with the reproductive function of treated ticks and adversely affected normal oviposition. The data revealed that the extract of *D. metel* showed slightly lesser effect on ovipsition of line-4 and line-5 ticks in comparison to the effect on susceptible line-1 but the effect was higher in comparison to the effect of *A. mexicana* extract (Table 12).

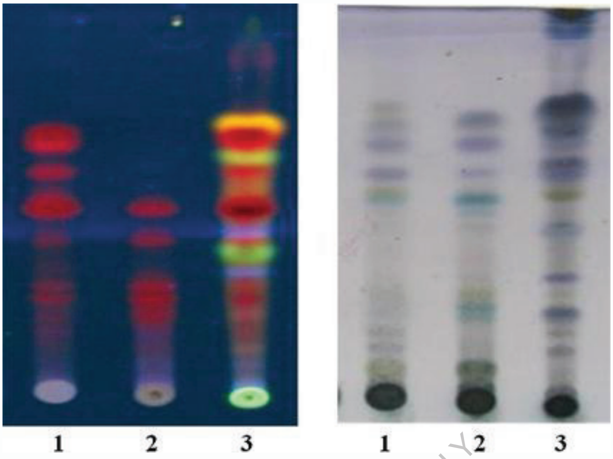
Table (12): Efficacy of 95 % ethanolic extracts of *Datura metel* and *Argemone mexicana* against IVRI-1, IVRI-4 and IVRI-5 lines of *Rhipicephalus*. (*B.*) *microplus*

| **Plant extract** | **Conc. %** | **Variables** | **% Mortality in tick lines** | | |
|---|---|---|---|---|---|
| | | | **Susceptible IVRI-I** | **Deltamethrin resistant IVRI-4** | **Multi-acaricides resistant IVRI-5** |
| *D. metel* | 7.13 | Mortality | 90.0 ± 5.8 | 65.0 ± 9.6 | 70.0 ± 5.8 |
| | | % IO | 53.5 | 42.2 | 37.0 |
| *A. mexicana* | 11.30 | Mortality | 90.0 ± 5.0 | 50.0 ± 5.8 | 55.0 ± 9.6 |
| | | % IO | 33.8 | 31.9 | 29.3 |

Phytochemical and physicochemical profile

Phytochemical tests showed presence of alkaloids and glycosides in 95 % ethanolic *D. metel* fruit extract and alkaloid, terpenoids, flavonoids and phenolics in *A. mexicana* whole plant extract. The fruit of *D. metel* contains total ash of 11.18 %; acid insoluble ash 1.31 %; alcohol soluble extract 5.55 % and water soluble extractive 28.53 %, whereas, *A. mexicana* extract contains total ash of 13.29 %; acid insoluble ash of 0.87 %; alcohol soluble extractive of 12.83 % and water soluble extractive of 28.87 %. Qualitative HPTLC studies of *D. metel* fruit extracts showed the presence of different bands having retention factor (Rf) ranges from 0.13 to 0.70 at UV- 366 nm and 0.04–0.84 at visible light. In case of *A. mexicana* extracts, presence of different bands having Rf. ranges from 0.10 to 0.77 at UV- 366 nm and 0.08–0.92 at visible light was noticed (Fig. 4).

**Fig. 4**



HPTLC profile of *Datura metel* (*1* and *2*) and *Argemone mexicana* (*3*) extracts under UV- 366 nm and visible light after derivatization with anisaldehyde-sulfuric acid reagent [Solvent System: Toluene : Ethyl acetate : Formic acid (80: 20: 5 V/V)]

As per the recent reports from different parts of India, the cattle tick, *R.* (*B.*) *microplus* has developed resistance to a number of acaricides. For example, Kumar et al. (2011) reported an average resistance factor (RF) of 6.1 to diazinon in the ticks collected from the northern sub-temperate trans-gangetic plains and high average RF values of 26.65 in the ticks collected from tropical middle-gangetic plains. Indiscriminate use of pyrethroid compounds on animals for tick control in middle, lower and trans-gangetic plains regions has led to the development of resistance against deltamethrin with average RF of 8.7, 5.8 and 4.13, respectively, while ticks inhabiting at middle and trans-gangetic plains, and in western dry regions were found to be resistant to cypermethrin with average RF values of 3.21, 2.9 and 5.24, respectively (Sharma et al. 2012).

High resistance to deltamethrin (average RF 14.36) was reported in ticks infesting cattle and buffaloes of western dry regions of the country (Sharma et al. 2012). Singh et al. (2010) reported more than 90 % resistant ticks collected from commercial dairy farms of Ludhiana, Punjab, a northern state and advocated the need for adopting the alternative tick control strategy.

To tackle the problems many methods have been recommended (Ghosh et al. 2007) and phyto-acaricide has been considered as a viable option. Plants provide a number of natural compounds which can intervene on all biological processes of insects interrupting their life cycle and are considered as an important part of ethno-veterinary practices (Habeeb 2010; Zaman et al. 2012). In comparison to synthetic acaricides, the botanicals are usually less toxic to mammals, have no residual effects and have less chance of development of resistant tick populations (Chungsamarnyart et al. 1991).

The possibility of using botanicals for the control of arthropods of veterinary importance has recently been reviewed by Ghosh and Ravindran (2014) and a few plants were identified as most promising against ticks. In the present study, the anti-tick potentiality of two plant extracts was identified to explore further the possibility of development of plant-based herbal acaricides for the control of resistant tick species.

Previously, ethanolic leaf and seed extracts of *Datura stramonium* were reported for acaricidal, repellent, and reproductive inhibitory properties against two-spotted spider mites, *Tetranychus urticae* and *Panonychus ulmi* (Kumral et al. 2010, 2013), flat mite, *Brevipalpus phoenicis* and the coconut eriophyid mite, *Aceria guerreronis* (Acari: Eriopyhidae) (Guirado et al. 2001; Thevan et al. 2005). Shyma et al. (2014) reported significant acaricidal activities of *D. stramonium* extracts against *R.* (*B.*) *microplus*. Kuganathan and Ganeshalingam

(2011) reported toxic effect of the leaf extracts of *D. metel* plants at various concentrations on grasshoppers and red ants. The anti-feedant activity of aqueous leaf extract of *D. metel* exhibited significant larval mortality against *Helicoverpa armigera* after 24 h of exposure (Ramya and Jayakumararaj 2009).

However, none of the studies have addressed the activity of the solvent guided extracts of *D. metel* or *D. stramonium* against the arthropods resistant to synthetic acaricides.

In the present experiment, alkaloids and glycosides were detected in *D. metel* extract which acted synergistically and gave strong anti-tick activity. While analyzing the acaricidal activities of *D. metel* the logistic regression analysis of the identified extracts showed that the independent variable, an increase in the dose of extracts, was associated with a significant increase in the mortality rate of treated ticks with significant inhibition in reproduction. Significantly, the 95 % ethanolic extracts at $LC_{90}$ concentration killed 65 % of treated ticks of IVRI-4 line possessing mutation at SII 4–5 region of sodium channel gene and over-expressed esterases (Kumar Rinesh et al. 2013) and 70 % ticks of line-5 having over-expressed Est1 enzymes (Ghosh unpublished data). As reported by Pavel (2004), Berkov et al. (2006) and Aronson (2009), the insecticidal properties of the extracts may be due to the involvement of certain alkaloids as anticholinergics (scopolamine, hyoscyamine, meteloidine, atropine), terpenoids and flavonoids. The alkaloids, terpenoids, flavonoids and phenolics in *A. mexicana* have been reported to have diuretic, antihelmintic, anti-inflammatory, wound healing, anti-bacterial, antifungal and anti-termite activities (Bhattacharjee et al. 2006; Satish et al. 2007; Mohana and Raveesha 2007; Habbu et al. 2007; Elango et al. 2012; Thakur et al. 1995).

The anti-tick activity of the extracts against chemical acaricide resistant ticks has given an ideal opportunity to work further on the plant metabolites involved in the process.

In a recent review Ghosh and Ravinbdran (2014) presented a list of plants reported to have anti-tick activity. It was observed than most of the cases after identification of anti-tick activity further work has not been conducted. While initiating work on screening of natural products for anti-tick activity, thorough literature survey was undertaken about the plants details, availability, nutritional and ecological requirements of potential plant parts and the most suitable plants were selected for the study.

The two plants based anti-tick formulations (Patent application nos. 2452/DEL/2010 & 2196/DEL/2011) with data on availability of raw materials, agronomical requirement, safety, stability and information on marker compound were generated recently. Both the patentable formulations were used consecutively for 27 generation on susceptible IVRI-1 line without any significant changes in the susceptibility pattern/LC50 value (Ghosh et al. 2014).

The preliminary studies indicated that both the plants have potential for developing acaricides to counter-act problems associated with the chemical acaricides. However, studies on the possibilities of large scale economical harvesting of the plants, stability pattern of active components at different environmental conditions and safety aspects of the extracts containing alkaloid, terpenoids, flavonoids and phenolics need to be undertaken in phased manner.

## Repellent properties of *Rotheca glabrum* plant extracts against adults of *Rhipicephalus appendiculatus*

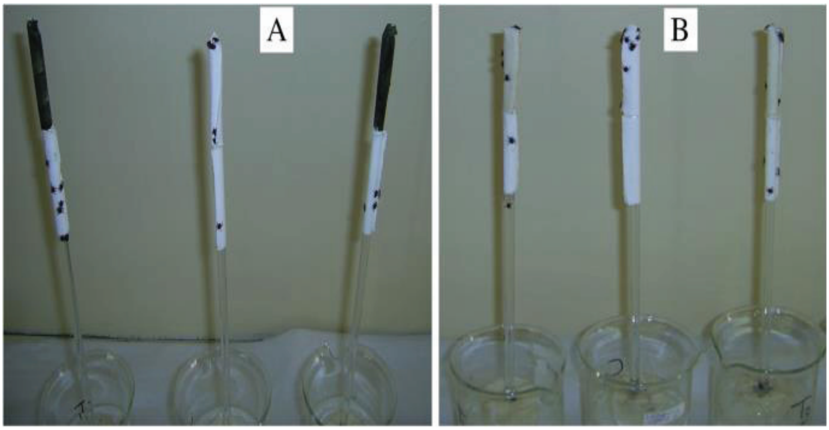
*Rotheca glabrum* (formerly known as *Clerodendrum glabrum* [Verbenaceae]) is used by local communities in the Limpopo Province of South Africa to control ticks on livestock and was selected from the database of the ARC-Onderstepoort Veterinary Institute.

Its leaves were extracted using organic solvents ranging from polar to non-polar solvents (methanol, acetone and dichloromethane (DCM)). In addition, the traditional soap-water (infusion) and water-based (decoction) methods were used. The tick repelling activity was determined against the adult stage of the livestock tick *Rhipicephalus appendiculatus*.

In the tick-climbing repellency bioassay a 30% acetone extract had a significant ($p \leq 0.05$) repellent effect against adults of *R. appendiculatus*. The extract was still active at a lower concentration of 10%. The hexane fraction from the *R. glabrum* acetone extract had a higher tick repellency activity than the positive controls Amitix and Bayticol at the same concentrations. Unfortunately, the activity decreased after 2.5 h, probably due to volatility of the biologically active compound(s) within the extract.

The acetone extract repelled the ticks, but the soap-water infusion of *R. glabrum* leaves did not repel the ticks (Fig. 5a and b).

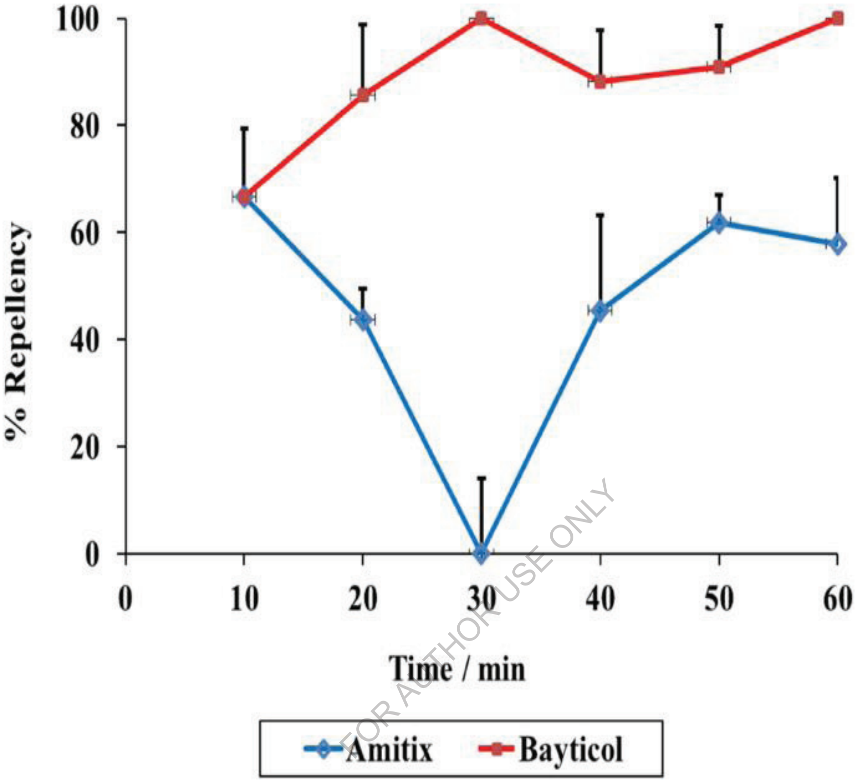
**Fig. 5**



Example of a repellent (**a**) and a non-repellent (**b**) extract. Treatment bioassays, the top stained filter papers were coated with *R. glabrum* extract. All the bottom filter papers contained no extract or solvent. Control bioassays, unstained filter papers at the top were treated with the negative control (solvent). Rods containing repellent acetone extract resulted in *R. appendiculatus* adults being repelled from the treated filter papers to the neutral filter papers, whereas no repellency was observed in the control bioassays (**a**). The soap-water infusion of *R. glabrum* and solvent control did not repel the ticks from climbing up the rods to the top filter paper (**b**)

Bayticol and Amitix positive controls on adults of *R. appendiculatus* led to an average of 89 and 45% repellency, respectively (Fig. 6).

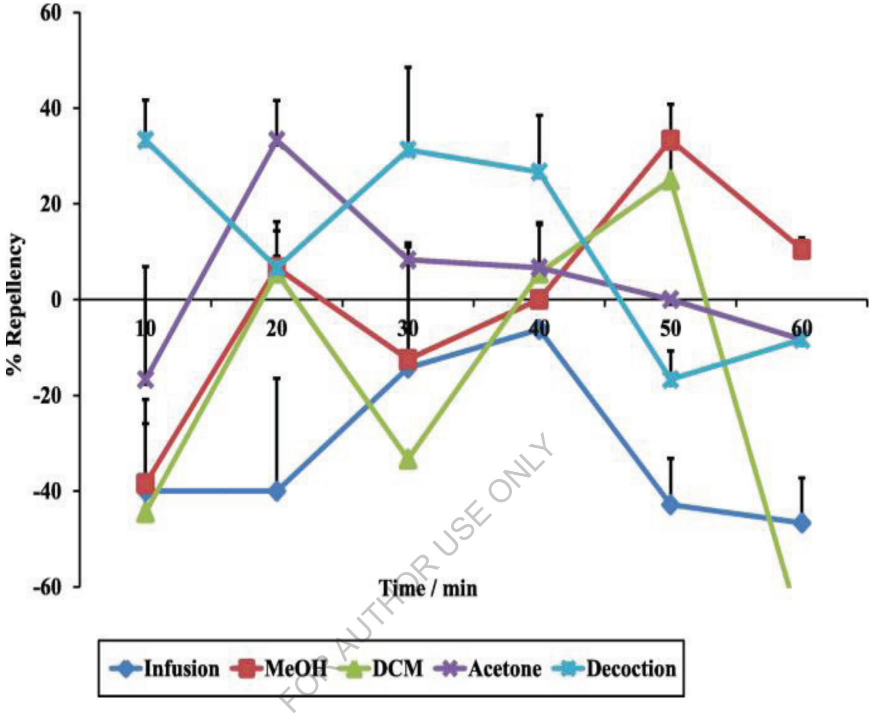
**Fig. 6**



Percentage repellencies of the *R. appendiculatus* adults against 30% concentration of the positive controls as calculated from the adopted formula (Ibrahim and Zaki, 1998)

The repellent activity of the different negative controls against adults of *R. appendiculatus* was examined over time (Fig. 7). With the exception of one time, the repellency was generally below 5% for the organic extractants. The repellency of the decoction was the highest and diminished over time. The soap-water mixture attracted the ticks rather than repelling them.

**Fig. 7**

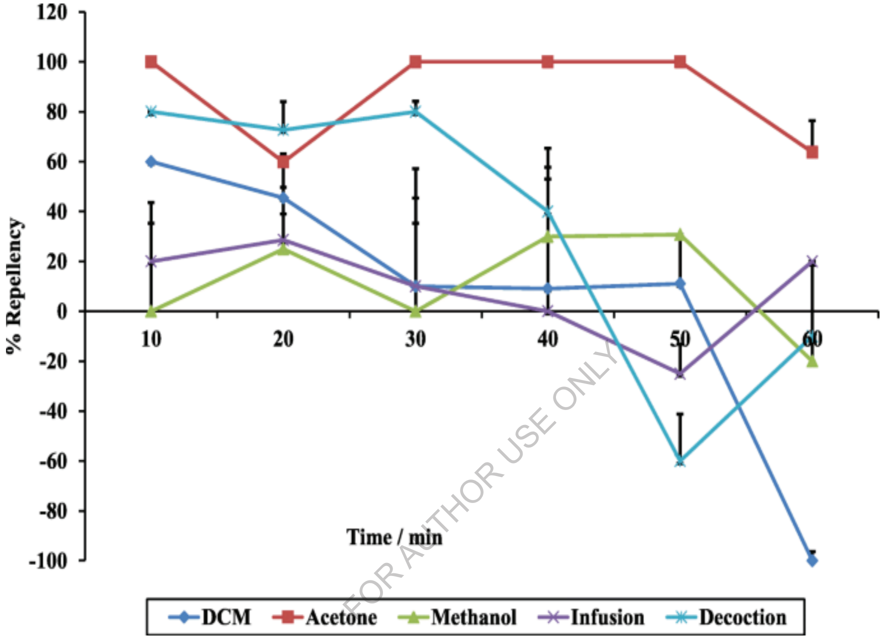


Percentage repellences against *R. appendiculatus* adults for the negative controls (dichloromethane, acetone, methanol, soap-water infusion and the decoction)

## Crude extracts

The repellency response of *R. appendiculatus* adults to different 30% crude extracts of *R. glabrum* was determined (Fig. 8). It was surprising that the DCM extract attracted 100% of ticks after 1 h. It was also noteworthy that the decoction initially had a good repellency activity against the ticks, but decreased after 50 min, although the difference was not statistically significant (Table 1). On the contrary, the repellency effect of the acetone extract differed significantly from the control treatment with a *p*-value of less than 0.05 (two-tailed test).

**Fig. 8**



Percentage repellency of 30% crude extracts against *R. appendiculatus* adults over time

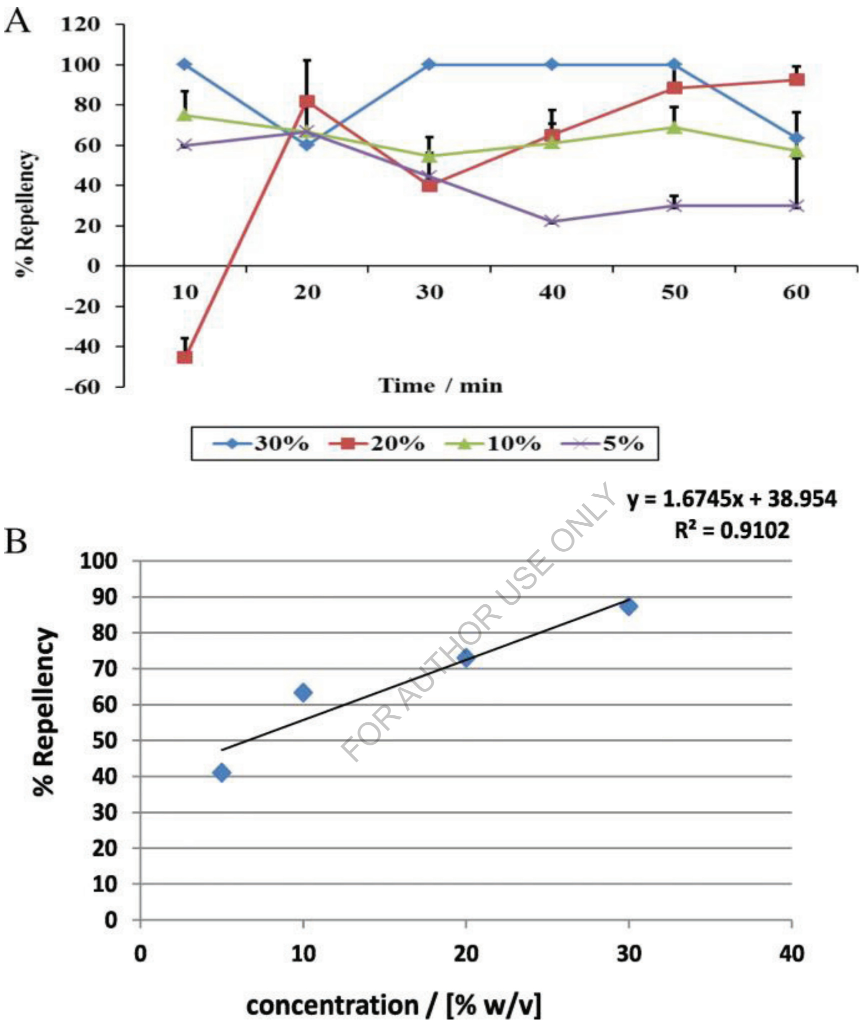
**Table (13): Summary of the repellency effects and statistical analysis of the 30% crude extracts when compared to the negative controls**

| **Decoction** | **Infusion** | **MeOH** | **Acetone** | **DCM** | | |
|---|---|---|---|---|---|---|
| Average % repellency | | 33.7 | 8.93 | 11 | 87.3 | 5.94 |
| *t*-test | Equal mean variance | 0.408 | 0.0031 | 0.415 | 1.5e-5 | 0.391 |

| **Decoction** | **Infusion** | **MeOH** | **Acetone** | **DCM** | | |
|---|---|---|---|---|---|---|
| | Unequal mean variance | 0.419 | 0.0016 | 0.416 | 1.6e-5 | 0.395 |
| p (2-tail) value | | 0.298 | 0.0131 | 0.69 | 0.00507 | 0.174 |
| Mann-Whitney U test | | $p > 0.05$ | $p < 0.05$ | $p > 0.05$ | $p < 0.05$ | $p > 0.05$ |

The significant repellency of the 30% crude acetone extract led us to evaluate activities at lower concentrations. The 30% acetone extract retained 100% repellency up to the 50-min time point except at the 20 min mark where it dropped briefly to 60% (Fig. 9a). As the concentration decreased to 5%, the extract lost its average repellency activity. The $EC_{50}$ of 6.6% was calculated from the trend line graph of average activities (Fig. 9b).

**Fig. 9**

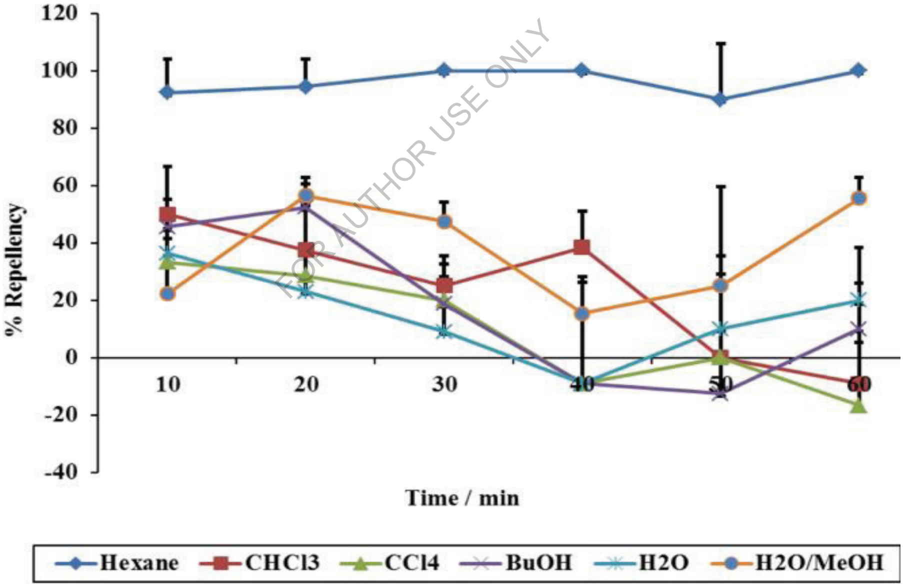


Repellent effects of acetone crude extracts of *R. glabrum* against *R. appendiculatus* adults. **a** Percentage repellency of four concentrations. **b** Relationship between average percentage repellency at different concentrations to determine $EC_{50}$ of 6.6%

## Activity of the acetone fractions

Percentage repellencies of the six different fractions were determined (Fig. 10). The hexane fraction had an average repellency of 96.2%, followed by 35% water-in-methanol (36.8%), chloroform (24.0%), butanol (18%), water (14.3%) and, lastly, carbon tetrachloride (9.27%). From these results, it is clear that the bioactive principles are non-polar compounds. The repellency activity value for the hexane fraction was significantly greater than those of the two positive controls, Bayticol (89%) and Amitix (45%) (Fig. 6). although one should remember that different concentrations of hexane fraction were used.

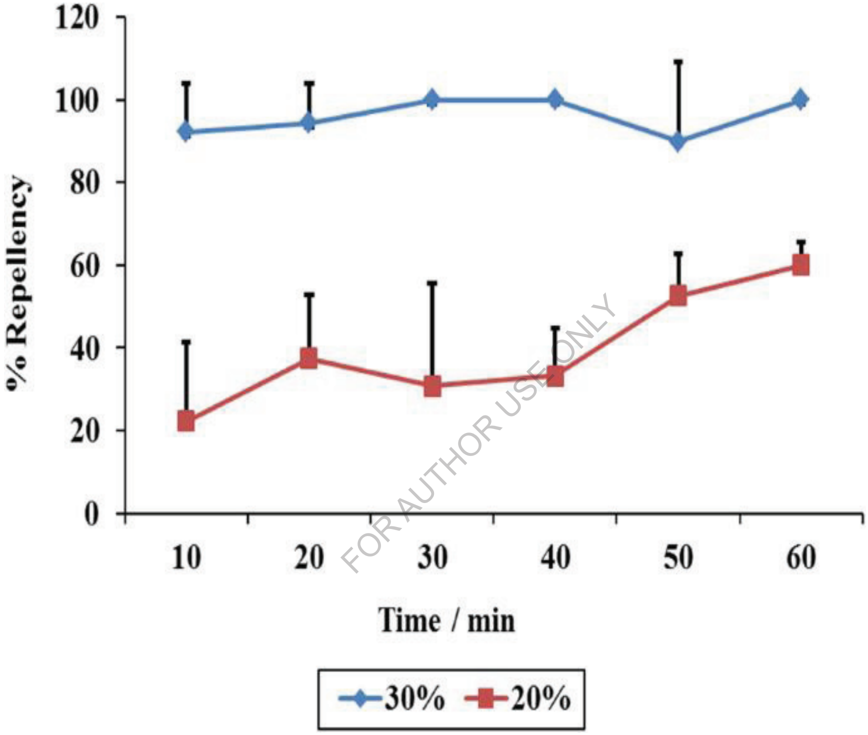
**Fig. 10**



Percentage repellencies of different 30% solvent-solvent fractions against *R. appendiculatus* adults measured over a 60-min time period

To determine the efficiency of lower hexane concentrations, the experiment was repeated using 20% hexane. The average repellency drastically dropped to 40% and no lower concentrations were tested (Fig. 11).

**Fig. 11**

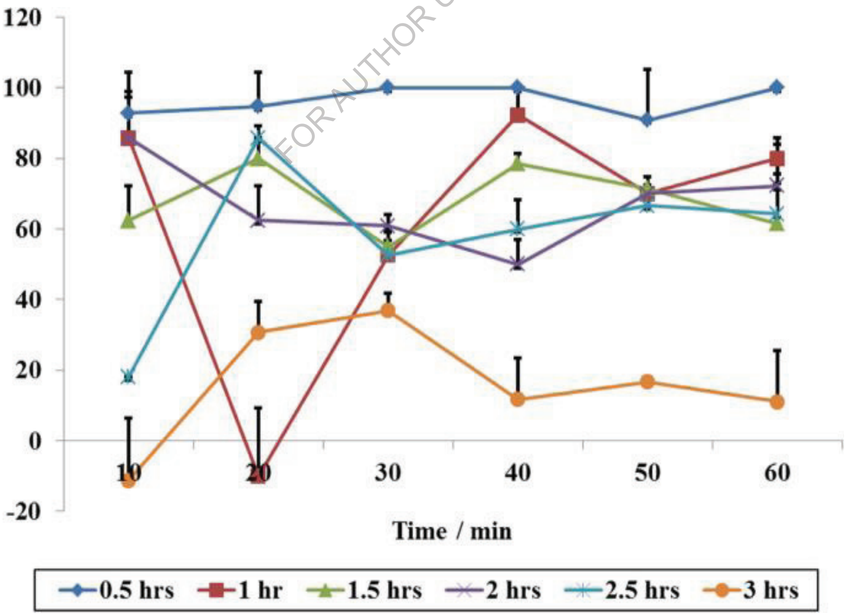


Percentage repellency levels of two hexane fraction concentrations (30 and 20%) against *R. appendiculatus* adults

Evaluation of volatility of the bioactive components

Another aspect considered was longevity of the repellency. Thus, five different drying times (up to 3 h) of the filter paper coated with 30% hexane fractions were explored and the percentage repellencies were determined (Fig. 12). As expected, the average percentage repellencies for 0.5 h drying time gave the highest average activity over the course of the test period of 96.17%. As the drying times increased, the repellency effects dropped to as low as 18% at 3 h, thus indicating that the repellent compounds are volatile. Due to volatility and low concentrations of bioactive components encountered in the present study, *R. glabrum* essential oils may be the best option for effective and practical control of ticks in the field.

**Fig. 12**



Percentage repellency of 30% hexane fractions against *R. appendiculatus* adults when different drying times of the experimental filter papers were explored

### Attempts to isolate the active compounds

Open column vacuum liquid chromatography fractionation on silica gel was conducted in an attempt to isolate the repellent compounds from the bioactive fraction. Solvents of increasing polarity with the non-polar hexane, ethyl acetate, acetone and methanol, were used for further fractionation, resulting in four sub-fractions. The sub-fractions were tested for repellency activity and the ethyl acetate gave the highest repellency activity of 73.6%, followed by acetone (65.9%), hexane (15.0%) and methanol (12.5%). However, when further fractionation of the ethyl acetate sub-fraction was conducted, the resulting fractions lost repellency activities, against *R. appendiculatus* adults.

As bioassay guided fractionation of the acetone extract continued, the hexane subfraction had higher activities (96.2%) than the commercial products. The hexane sub-fraction lost activity while the ethyl acetate sub-fraction retained activity of 73.6%. These activities indicate that the non-polar and intermediate polarity compounds found in the hexane fraction and ethyl acetate fraction had an influence on repellence. By combining the hexane (65.5%), DCM/ethyl acetate (58.7%) and ethyl acetate (57.2%) sub-fractions obtained after conducting further fractionation of the ethyl acetate sub-fraction, the original activity (73.4%) was re-established to prove the existence of synergism between different compounds.

## Prospects for the future

Each of the tick-control methods described herein has strengths and limitations. Numerous studies have demonstrated the efficacy of broadcast and host-targeted chemical pesticides for tick control. The potential drawbacks of this approach include damage to nontarget organisms, the development of insecticide resistance, and human health consequences.

The nontarget mortality and toxicity of chemical insecticides can be reduced by avoiding organophosphate and carbamate compounds and by applying insecticides directly to hosts. Also, for multihost ticks, rapid development of insecticide resistance seems less likely than in shorter-lived arthropods or in single-host ticks facing constant chemical exposure.

We suspect that, in the case of *Ixodes* vectors of human pathogens, a combined strategy of applying insecticides to deer (e.g., via four-posters) and rodents (e.g., via bait boxes) might be highly effective with limited collateral damage.

More generally, the use of integrated pest management against ticks should be pursued more vigorously. For tick vectors of livestock pathogens that plague tropical and subtropical areas, an important issue is whether the application of entomopathogenic fungi instead of chemical agents will reduce or eliminate the evolution of resistance by ticks. If so, the means of making fungal agents affordable and widely available should be pursued.

Prospecting for additional biocontrol agents would seem a promising direction for further research. The primary reasons for testing *B. thuringiensis*, *M. anisopliae*, and *Be. bassiana* against ticks appear to be their known efficacy against other arthropods and their commercial availability, rather than their primacy as tick control agents or evidence of their ability to control ticks in nature.

Rarely are acarologists or medical entomologists aware of the major causes of mortality in tick populations, and pursuit of these causes might reveal heretofore unrecognized natural enemies. In particular, fungal or bacterial taxa that naturally infect ticks and that might be mildly pathogenic at natural abundances might have promise as biocontrol agents, particularly if they can be deployed inundatively.

Although considerable genetic variation is known to occur within species used as biocontrol agents (Freimoser et al. 2003), little use has been made of this information for the control of ticks. For example, genetic strains of *M. anisopliae* that differ in host specificity are characterized by strong variation in the complex set of proteins they secrete to invade and kill arthropod hosts (Freimoser et al. 2003).

Strains of *M. anisopliae* show particularly dramatic genetic and phenotypic variation in the expression of subtilisin proteinases, which are used to penetrate arthropod cuticles and release nutrients (Freimoser et al. 2005). The potential for either discovering natural strains or artificially selecting strains with extremely high pathogenicity to ticks (but low pathogenicity to insects) seems high. The effectiveness of strain combinations, as opposed to single strains, has not to our knowledge been tested in the lab or field.

Application of biocontrol agents to white-tailed deer as a means of reducing Lyme disease risk has not been attempted but seems to hold promise, particularly in light of successes with biocontrol agents delivered to livestock. In general, adult ticks in the process of engorging on host blood are most susceptible to attack by nematodes, bacteria, and fungi, and deer-targeted delivery would take advantage of this vulnerability.

Delivery systems to hosts for adult ticks that avoid nutritional subsidies (for example, those deployed along deer trails or in areas used for resting) are likely to be more palatable to wildlife management agencies than are those that accompany feeding stations. Similarly, biocontrol delivery systems to hosts for immature ticks that avoid nutritional subsidies (for example, those using collectible nesting materials or nest boxes) should be pursued.

Both *M. anisopliae* and *B. bassiana* appear largely nontoxic to people and to other terrestrial vertebrates, but their impacts on nontarget arthropods and aquatic vertebrates have not been addressed adequately in the field. Further studies of the efficacy of broadcast spraying of fungal spore solution in reducing tick numbers, combined with monitoring of impacts on other arachnids, insects, and other nontarget taxa, should be undertaken. Continued research is needed into methods of maximizing contact rates between these fungi and ticks while minimizing contact rates between fungi and nontarget organisms.

In our opinion, the efficacy of *M. anisopliae* and *Be. bassiana* as tick control agents has been sufficiently well established to warrant aggressive pursuit of efficient and safe delivery systems. Prospecting for or selecting fungal strains with high specificity toward particular target tick species will maximize control while minimizing nontarget effects. Targeted research and development of deployment strategies, whether areawide, host targeted, or a combination of both, should be undertaken.

Timing the deployment seasonally to coincide with vulnerable life stages of ticks, with periods when risk to nontarget organisms is minimized, or with conditions favoring maximum longevity of fungal pathogens should be a part of any strategy. The promise of tick biocontrol using entomopathogenic fungi, which has also been supported by recent studies on *Anopheles* mosquitoes (Blanford et al. 2005, Scholte et al. 2005), can only be realized with renewed commitment and strong financial support.

# CONCLUSION

It is concluded that ticks cause great economic losses to livestock in the world and adversely effect livestock host in several ways and parasitized a wide range of vertebrate hosts, and transmit a wider variety of pathogenic agents than any other group of arthropods.

In the area of tick control, much has been achieved, but much more remains to be done. The availability of vaccine is very small. The ability to induce an effective, sustained immunological response is crucial but needs improvement.

Problems of acaricide resistance, chemical residues in food and the environment and the unsuitability of tick resistant cattle for all production systems make the current situation unsatisfactory and require the development of absolute control through effective vaccine.

Taking advantage of recent advances from new approaches and technologies as applied to the field of vector biology, such as transcriptomics, proteomics, immune-molecular characterization, elucidation of naturally acquired resistance, and the development of innovative arthropod and animal models, may lead to improved investigations of naturally acquired resistant breeds against tick and tick-borne pathogens.

Immune-proteomic, sialotranscriptome and reverse genetics/gene editing (RNAi, CRISPR/Cas9) may help to identify new vaccine candidates that resist ticks and tick-borne pathogens. By understanding the tick:host interface and the most common denominators of immunity to ticks, this acquired immune response could be manipulated to improve the efficacy of novel anti-tick vaccines.

Conversely, the knowledge obtained may assist in the selection of tick resistant cattle or the manipulation of susceptible cattle to develop a protective tick immune response. Furthermore, the in depth analysis of host microbiota and volatile organic compounds could lead to probiotic or diet changes or inhibitory chemicals which could render susceptible cattle less attractive to ticks.

Future research may lead to a combination of several of these technologies as novel tick and tick-borne disease control options by first identifying viable biological targets and dissecting pathways leading to vaccination or pharmaceutical therapies or cattle management opportunities for tick control.

Reducing tick abundance is likely to remain the most effective method for preventing tick-borne diseases. Most recent research on reducing tick numbers has focused on the targeted delivery of chemical insecticides to particular hosts. These methods seem promising, but more rigorous tests are needed. Several methods of biocontrol of ticks, including parasitoids and some bird predators, have been shown to reduce tick numbers in some situations. Perhaps the most promising method of biocontrol is the targeted use of fungal pathogens, which has been shown to reduce tick numbers both directly (through mortality) and indirectly (through reductions in fitness). These preliminary successes demonstrate the importance and potential of rigorous research into novel and existing methods of biological control of ticks.

Attempts were made to isolate the repellent compound from the acetone extract of *R. glabrum.* The process produced very good results up to a late stage in the bioassay-guided fractionation process. At that point, the repellent activity was lost.

When two fractions were combined, the repellent activity was regained. These results provide strong evidence for the existence of a synergisticactivity of different compounds. It may be better to concentrate on extracts that would kill ticks rather than on extracts that would repel ticks.

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