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Immunological control of ticks and tick-borne diseases that impact cattle health and production

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1. ABSTRACT

The cattle industry is one of the most important agroeconomic activities in Mexico. The national herd is estimated to include approximately 33.5. million head of cattle. Ticks and tick-borne diseases are principal factors with a negative impact on cattle health and production. The most economically important tick species parasitizing cattle in Mexico are *Rhipicephalus microplus*, *R. annulatus*, and *Amblyomma mixtum*. Parasitism by ticks affects cattle health and production directly. Morbidity and mortality caused by tick-borne diseases augment the detrimental effect of tick infestation in cattle. Bovine babesiosis and anaplasmosis are the most important tick-borne diseases of cattle, which are caused by infectious agents transmitted by *R. microplus* and *R. annulatus*. However, there are no prophylactic therapies to control bovine babesiosis and anaplasmosis. Chemical control is the most common way to treat animals against ticks, and the use of acaricides can also help manage tick-borne diseases. However, the evolution of resistance to acaricides among cattle tick populations renders chemical control ineffective; which represents a challenge for sustainable ticks and tick-borne diseases control. The only anti-tick vaccine commercially available globally is based on the recombinant antigen Bm86. Because of its mode of immunity against *R. microplus* and *R. annulatus*, the Bm86-based vaccine also decreases the exposition of bovines to babesiosis and anaplasmosis. Research with Bm86-based vaccines documented high efficacy against *R. annulatus*, the efficacy levels against *R. microplus* varies according to the geographic origin of tick populations, and there is not effect against other ticks species such as *Amblyomma* spp. The impact of ticks and tick-borne diseases, the problem of chemical control due to acaricide resistance, and progress with anti-tick vaccine research efforts in Mexico are reviewed herein.

2. INTRODUCTION

The cattle industry is one of the most important and profitable agribusiness activities in the world (1). National cattle herds have grown in countries with the highest cattle inventory like Brazil, Australia, US, India, Argentina, and the European Union (2). Mexico ranks number 8 in the world for its cattle inventory and by 2015, the national herd was estimated to include approximately 33.5. million head of cattle; nearly 2.5. and 31 million were dairy and beef cattle, respectively (3). On average, the US imports 1 million head of cattle annually from Mexico (4). The value of beef exports to Mexico in 2011 totaled US $790 million (5). However, this activity is affected by ticks and tick-borne diseases.

Ticks are hematophagous ectoparasites that affect people, wild and domestic animals directly through their bites and blood consumption, and indirectly through the transmission of diverse pathogens, which include protozoa, bacteria, and viruses (5). The southern cattle fever tick, *Rhipicephalus microplus*, is considered the most economically important ectoparasite of livestock in tropical and subtropical world regions, and it is distributed in more than 50% of the Mexican territory (3). In Brazil, where the largest commercial cattle herd is found, yearly economic losses due to *R. microplus* were estimated to be US $3.2.4 billion (7). Recently, the estimated annual economic loss in animal agriculture associated with *R. microplus* parasitism in Mexico was US $573.6. million (8). Furthermore, *R. microplus* impedes advances with livestock genetic improvement programs because it is a vector of the infectious agents causing bovine babesiosis and anaplasmosis (9, 10). These tick-borne diseases result in significant morbidity and mortality wherever *R. microplus* is present in Mexico (11, 12).

Other economically important tick species affecting cattle in Mexico include *R. annulatus*, commonly known as the cattle fever tick, and *Amblyomma mixtum*. Even though it's not as widespread as *R. microplus*, *R. annulatus* also transmits babesiosis and anaplasmosis (13, 14). *A. mixtum* is a relatively big tick that consumes a large blood meal, and is a mechanical vector of anaplasmosis (11).

Chemicals able to kill ticks, also known as acaricides, are used widely to try to manage tick infestations in cattle. Chemical tick control is also an aid in efforts to mitigate the burden of tick-borne diseases. However, intensive use of acaricides selects for the evolution of resistance in tick populations (15). Acaricide resistance renders chemical control ineffective as the sole strategy for a sustainable tick and tick-borne disease management program. Vaccination against ticks is an alternative technology that could be integrated to prevent infestation and tick-borne disease in cattle. A vaccine containing the recombinant antigen Bm86 with efficacy against *R. microplus* and *R. annulatus*, which also had the ability to decrease the burden of Babesia parasites because of its mode of protection, was commercially available in Mexico until recently (16). Novel vaccines are needed to control ticks and tick-borne diseases. The impact of *R. microplus*, *R. annulatus*, *A. mixtum*, babesiosis, and anaplasmosis on cattle health and production, strategies to overcome the problem of chemical control due to acaricide resistance, and progress with anti-cattle tick vaccine research efforts in Mexico are reviewed herein.

3. ECONOMICALLY IMPORTANT TICKS THAT COMMONLY INFEST CATTLE IN MEXICO

3.1. *Rhipicephalus* spp.

The distribution of *R. microplus* and *R. annulatus* in Mexico differs with the former species being more abundant. *R. microplus* occurs throughout the country with the exception of central and northern high plateau areas, which include unsuitable habitat (17). Widespread acaricide resistance, in some cases to multiple classes of acaricides within the same population, makes difficult the control of this cattle pest (18, 19), which results in a spillover of beyond cattle infestations (20). Parasitism of wild animals like the white tailed deer, *Odocoileus virginianus* (21), and the red deer *Cervus elaphus* (22) in regions where these species cohabitate with cattle promotes the maintenance of *R. microplus* populations despite chemical treatment in cattle. This requires the treatment of wild animals especially in those places close to the border with the US where an eradication program operates to avoid the establishment of *R. microplus* in free areas (23).

At the beginning of the now extinct national eradication campaign against cattle fever ticks in Mexico, *R. microplus* was found in all of the states of the country, except in Mexico city and Tlaxcala, (24). Currently, 47.8.8 % of the country, which corresponds to the Northern states of Baja California, Sonora and some other states of Central Mexico are *R. microplus*-free (3). In states bordering parts of the Gulf of Mexico to the east like Tamaulipas, Veracruz, and Tabasco, 90% or more of the cattle population can be infested with *R. microplus* (25, 26).

Following the initiation in 1907 of an intensive eradication program in the USA, the country was declared free of *R. microplus* and *R. annulatus* in 1943, except for a Permanent Quarantine Zone in south Texas on the border with Mexico along the Rio Grande where surveillance is maintained to buffer cattle fever tick incursions (27). Cattle fever tick outbreaks that occur in the Permanent Quarantine Zone tend to be caused by *R. annulatus* in the north of Laredo Tx, and in the southern part by *R. microplus* (28). In Mexico, *R. annulatus* has been identified in several states, however, this tick has preferences for high temperatures and low rainfall (29); therefore, is

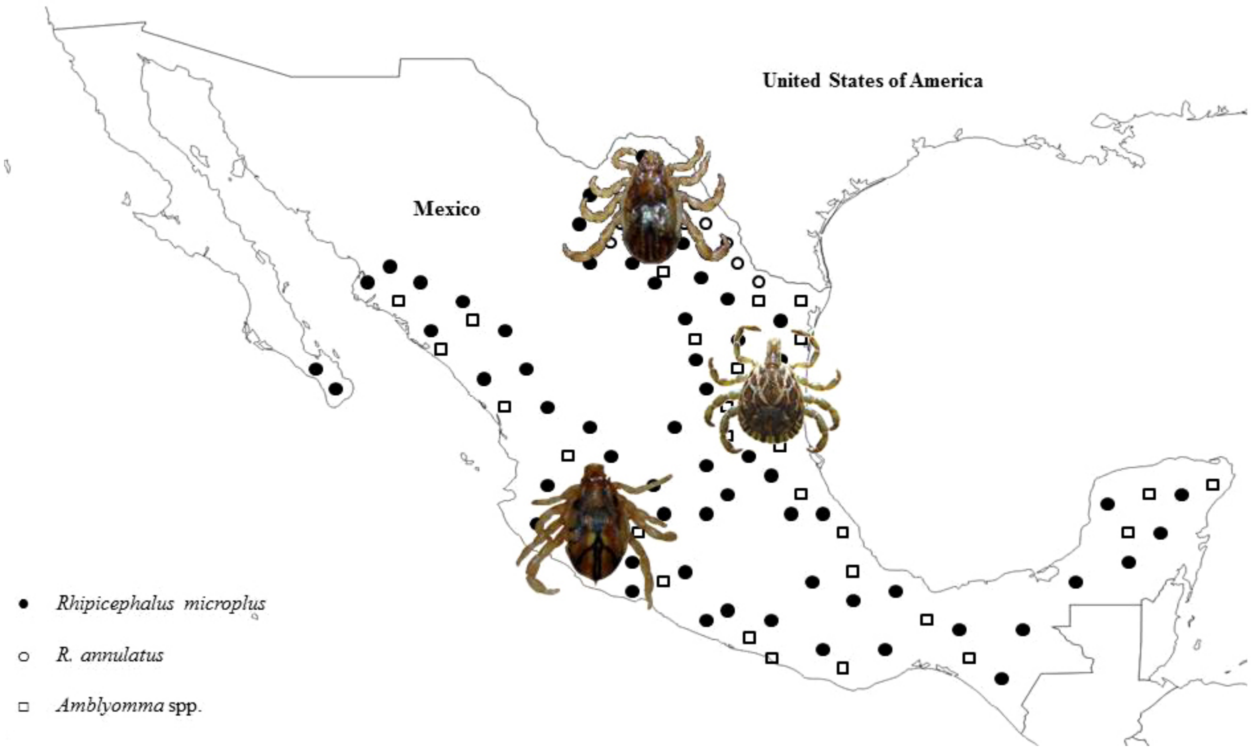

Figure 1. Geographical distribution of the most important ticks that infest cattle in Mexico.

very likely that it is established in a delimited region in the Northeast part of the country, in the states of Tamaulipas and Coahuila. Tick collections done by personnel of the Campaign for Tick Control in the state of Tamaulipas and by ourselves, found *R. annulatus* in farms from the municipalities of Miguel Aleman, Mier and Guerrero, which are geographically located along the Mexican side of the Rio Grande. It is probable that *R. annulatus* ticks found in other states of Mexico may be due to the continuous mobilization of cattle into the country, but are not geographically established. Although engorged adult female *R. annulatus* are bigger than *R. microplus,* both ticks are quite similar and misidentification in the field is possible, which makes it difficult to determine the real distribution of *R. annulatus*.

3.2. *Amblyomma* spp.

The genus *Amblyomma,* comprises several tick species that have been identified in Mexico*,* with *A. maculatum, A. immitator, and A. mixtum* found commonly infesting bovines (24, 25). *A. mixtum* is the *Amblyomma* species found infesting cattle most frequently, and it is the second most important ectoparasite of bovines in the Gulf of Mexico after *R. microplus* (30). As reviewed by Nava *et al*. (31), after being described by Koch in 1844, Neumann (1899) considered *A. mixtum* as synonymous with *A. cajennense*. Therefore, all the information on *A. mixtum* in the last century referred to it as *A. cajennense*, which suggested distribution in the American continent from southern Texas and Florida to Northern Argentina and the Caribbean islands (29). Recently, the phylogeography of *A. mixtum* was reassessed and its current distribution comprises southern Texas through Mexico until Ecuador (31). *A. mixtum* is a three-host tick that completes its life cycle parasitizing birds and mammals (32). However, we have observed that larvae, nymphs, and adults can feed on cattle (30). Areas close to the Gulf of Mexico, where high concentration of cattle exists and high humidity and temperature persists during the year, providing suitable habitat for *A. mixtum* populations to thrive, and therefore infestations also occur in other animals such as equines, dogs, and wild animals like the white-tailed deer and collared peccary. People working with livestock, and also personnel involved in forestry and wildlife management in the Mexican tropics are frequently parasitized by *A. mixtum* (20). Although *Amblyomma* have been involved in the mechanical transmission of anaplasmosis, no scientific evidence indicates that *A. mixtum* is involved in diseases of importance in humns and animals. Additional research is required to determine if *A. mixtum* is a biological vector of pathogens of public health and veterinary importance. Figure 1 shows the distribution of ticks that affect cattle in Mexico.

4. TICK-BORNE DISEASES THAT IMPACT CATTLE HEALTH AND PRODUCTION

4.1. Anaplasmosis

Bovine anaplasmosis is an infectious, noncontagious disease, transmitted mainly by *Rhipicephalus* spp ticks, although biting insects may also transmit the causal agent, in particular in the absence of ticks (11, 33, 34). The etiological agent is the rickettsia *A. marginale*, Gram-negative bacteria that infects mature erythrocytes of cattle and other ungulates (9). While young calves acquire the infection, they are usually refractory to the clinical syndrome for up to one year of age (35). The clinical syndrome includes recumbency, jaundice, abortion in the last trimester of pregnancy, severe loss of weight, and death may ensue if the appropriate antibiotic and palliative drugs are not applied timely (9). As there are no pathognomonic signs, clinical disease can be confirmed by the direct identification of the organism (36). Direct identification can be performed by microscopic observation on blood smears stained with Giemsa or any other Romanowsky stain (37). While other methods have been used for direct staining of blood smears, Giemsa stain remains the preferred method (38). Amplification of major surface protein 5 gene (*msp5*) by nested PCR is an alternative for direct diagnostic, yet it is usually applied for experimental purposes (39). A duplex qPCR was documented to be more sensitive than the nested PCR and reverse line blot hybridization assay (40).

Anaplasmosis is a major cause of economic losses in cattle located in the tropical and subtropical regions of Mexico (41). Estimation of annual costs indicates that anaplasmosis may cause up to 25% of the total losses among beef cattle in official genetic improvement programs (11). Losses in imported breeding stock can reach 20% when cattle are acquired from anaplasma-free areas of the U.S. or elsewhere (11, 33). Economic losses are difficult to calculate, as there are several factors to be considered including weight loss, milk production losses, abortion, and mortality, thus anaplasmosis continues to be a scourge for the cattle industry in Mexico.

When clinical signs are timely detected, anaplasmosis can be treated specifically with tetracyclines (oxytetracycline) and imidocarb dipropionate. Oxytetracycline at dose of 22 mg/kg daily over a five-day period, or imidocarb dipropionate at 5mg/kg twice, seven days apart, have been reported to control but not to consistently eliminate *A. marginale*, which leaves animals as asymptomatic carriers (42). The inability to totally clear the infection with available drugs and the wide diversity and variability of *A. marginale* strains make difficult to eliminate the disease (43). Vaccination against infectious pathogens can be an effective way to control or eliminate diseases. The success of prophylactic approaches targeting *A. marginale* however, has been limited because of its antigenic and genetic diversity that involves mechanisms generating variants of outer membrane proteins resulting in pathogen persistence in cattle, which promotes transmission to other members of the herd.

Highly effective commercial anaplasmosis vaccines are unavailable in Mexico or elsewhere. Research efforts involved tests with live and inactivated vaccine preparations. The use of an inactivated infected blood-based vaccine resulted in lack of solid protection against challenge with heterologous strains, and the presence of neonatal isoerythrolysis due to presence of isoantibodies in the cow's colostrum (44). Other studies showed that protection is possible when inactive purified organisms from more than one strain are used in an immunogen preparation, providing solid protection against a field heterologous challenge (45). This immunogen preparation was produced by Mexico's National Institute for Forestry, Agricultural and Livestock Research (INIFAP), and used as an alternative for the protection of imported livestock by ranchers in the northern region of Veracruz state.

The use of attenuated or naturally avirulent organisms has been tested too. Some of these preparations included *A. marginale* sbsp. *centrale* (46), an organism declared exotic and not allowed to be used in Mexico. Naturally avirulent organisms have been tested in Australia and Mexico. Testing of the *A. marginale* Dawn strain in an immunization trial resulted in protection against heterologous challenge (47). In Mexico, the Yucatán strain was tested inducing solid protection and absence of secondary effects (33).

Immunoprotection efforts involving live agents pose the risk for transmission of other blood-borne pathogens. Thus, recent research efforts have focused on tests with recombinant or subunit vaccines. The major surface proteins (MSP's) were discovered more than 30 years ago (48). MSP1a, an adhesion protein to which its ligand on the erythrocyte is yet to be discovered, presents an amino terminal extracellular variable region composed of short peptides very similar among themselves that can repeat in variable numbers (49). This finding led to the discovery that more than two hundred repeats can be present in the amino terminal extracellular variable region (50, 41). Other proteins within the same complex (MSP2 and MSP3) can recombine with a number of pseudogenes that code for the central extracellular domain of the protein through a gene conversion mechanism in such a manner that generates as many variants needed for the pathogen to persist during the host's entire life (51)

Type four secretion system proteins (T4SS) have also been studied as candidate antigens. This secretion system, associated with the transfer of proteins and DNA among bacteria and from bacteria to other host cells, is highly conserved in Gram-negative bacteria. Some T4SS components such as VirB7, VirB9, VirB10, VirB11, and VirD4, along with outer membrane proteins of *A. marginale*, have been used as part of vaccine preparations and evaluated for their immunoprotective potential (52, 53). Recombinant proteins have shown poor immunoprotection, which at best is equal to that provided by inactivated vaccines against homologous challenge. The use of cross-linked bacterial outer membrane proteins enhanced immunogenicity (54). However, the production of such vaccines would be costly and cumbersome as the bacterial membranes have to be obtained from purified initial bodies. Genomics approaches are enabling research that could realize the potential to develop safer and efficacious vaccination technology against bovine anaplasmosis (41).

4.2. Babesiosis

Bovine babesiosis, also known as piroplasmosis or tick fever, is an infectious disease transmitted by ticks and is caused by protozoans of the genus *Babesia* (55), which are intraerythrocytic parasites that cause fever, hemolytic anemia, sometimes hemoglobinuria, and nervous signs. Infected animals may present several forms of the disease that go from subclinical to hyperacute (56). The signs vary according to the pathogenicity and virulence of the species and strains involved. The susceptibility of animals is affected by several factors such as age, breed, and immunological status. Animals show clinical manifestations such as fever of 41 to 42^{o}C, hemoglobinuria, jaundice, constipation, dehydration, muscular trembling, weakness, prostration, and death 8 to 14 days after infestation with infected ticks. Nervous signs as ataxia, incoordination and coma are evident in animals infected with *B. bovis*. Few hours before death, the temperature falls to subnormal levels. Recovery of sick animals is followed by the apparent elimination of the parasite from blood, with a subclinical infection that can last for several years (57).

Bovine babesiosis has a world distribution, and is common in regions with tropical and subtropical weather. In Mexico, it is associated with the presence of the vector ticks *R. microplus* and *R. annulatus* (58). Another way of transmission is by inoculation of blood from one infected animal to a healthy one by fomites (hypodermic needles or surgery tools) (58).

In the mammal host, each sporozoite transmitted by the tick invades the red blood cells and develops first, into an intracellular trophozoite. From each trophozoite, two pear-shaped merozoites are formed (59). Each merozoite abandons the red cell and immediately invade another one, continuing this cycle until the host dies or the parasite is eliminated. The destruction of the red cells produces anemia and hemoglobinuria. The tick acquires the infection when it feeds on an infected animal (58). Between 16 and 24 hours after dropping from the host, transovarian transmission occurs in the tick and its progeny becomes infected (60). Once the emerging larvae infest a host and start feeding, multiple fission cycles start in several organs, including salivary glands with formation of thousands of infective sporozoites (61).

The disease is found in tropical and subtropical regions where the tick vector is present. Two situations are required for the occurrence of a babesiosis outbreak: 1) by exposition of highly susceptible animals (introduction of susceptible animals to enzootic areas or introduction of ticks to tick-free areas by infested animals) or by climate change that favors the establishment of ticks to new areas, and 2) by the occurrence of enzootic instability (62), a situation in which young calves are not infected even if they are exposed to ticks, therefore immunity against babesiosis is not developed.

Enzootic stability areas are the ones in which tick population can vary during the year, but the amount of infective ticks is enough to guaranty that all the calves are exposed to *Babesia* infection before nine months of age, therefore the antibodies acquired with the ingested colostrum and the resistance due to the age protects calves against clinical infection, developing a state of immunity in the presence of parasites. A large population of infected ticks is not required to maintain the enzootic stability even though in the field a very low percentage of the ticks transmit babesiosis.

The areas in which there is enzootic instability are those where the tick population is reduced significantly from one season to another one, propitiating that some animals do not get exposed to the infection until they are older than 9-months old, when they are very susceptible to the disease, and sometimes after they are two-years old, generating a very strong reaction that ends up with the death of the animals. The severity of the reaction is directly related with the proportion of susceptible animals (62).

The diagnosis is based on clinical signs, clinical history and the presence of the vector. However, laboratory techniques to confirm the presence of the parasite are required (63, 12). The observation of blood smears stained with Giemsa under the microscope is the most useful procedure. Thick smears of organs such as brain or kidney are useful to observe *B. bovis*. Serological techniques are used in research and epidemiological studies. The most common are the Complement Fixation (CF), Indirect Immunofluorescence (IFI) and ELISA (12). Other techniques consist in DNA detection by PCR.

A large number of compounds have been used for treatment of babesiosis, some of them are very effective and a unique application is required to eliminate the parasite. Some compounds are derived from quinolones, which can be very effective, however, its use is limited due to toxicity. Currently, diamidine derivatives are the most effective and used for treatment of babesiosis (47).

To obtain an effective control, actions directed to the tick vector, the intraerythrocytic parasite and the cattle host are required: a) Control of the vector consists in transmission cycle disruption by treating cattle against ticks. This is a method used commonly as part of an integral program of vector control; b) Control of cattle mobilization consists cattle mobilization control in order to prevent introduction of infected animals into babesiosis free regions; c) Chemoprophylaxis, It may be useful, but is expensive and unpractical to use as definitive or unique control strategy; d) Use of resistant animals, it is known that zebu cattle (*Bos indicus*) are more resistant to tick infestation and the infection by *Babesia* spp. than European cattle. Although, this has been used in some countries to maintain an enzootic stability, the low productivity of zebu breed, makes the strategy unpopular among farmers; e) Immunization offers the best results in the control of bovine babesiosis. Premunition has been used widely, with the aim to confer immunity trough a controlled infection; however, due to the risk of transmission of other diseases, is not recommended. In Australia and several other countries, a prophylactic method is been used for several years and consists in the attenuation of live organisms through passages in esplenectomized calves (47). This practice is limited due to maintenance of cold chain and the risk of transmission of other diseases (64). Despite the fact that vaccination with attenuated, live organisms confers strong immunity, since they are made from infected red blood cells, there is always the risk of contamination with other pathogens, which makes them impractical and unsafe. Recombinant vaccines against bovine babesiosis have not been developed yet in part due to limited research on vaccine candidate antigens involved in red blood cell invasion and tick transmission and in part due to high costs of vaccination trials. Sequencing and annotation of the *B. bovis* genome allows genome mining strategies aimed at identifying ideal vaccine candidates (65).

5. CHEMICAL CONTROL AND TICK RESISTANCE

Chemical control focuses on treatments with formulations of synthetic molecules known as acaricides to eliminate tick stages in the parasitic phase of their life cycle infesting cattle (66). Treatment methods include dipping vats, spraying, pour-on, and parenteral (67). However, the decision-making process triggering acaricide use can vary significantly, tending to be reactive to heavy infestations of cattle, and the ticks generally remain taxonomically unidentified and their susceptibility to the selected treatment unknown (68, 69). Understanding the epidemiology of acaricide susceptibility/resistance is fundamental for the success of area-wide tick management programs (14). Although in Mexico co-infestation with *R. microplus* and *A. mixtum* is common in cattle ranches along the Gulf of Mexico coast (26, 30), chemical treatment is usually directed against *R. microplus* (26).

Chemical classes used for tick control since commercial acaricides became available include arsenicals, organochlorides, organophosphates, pyrethroids, amitraz, macrocyclic lactones, insect grown regulators (IGRs), and phenilpirazolons (fipronil) (70, 71, 72). To avoid the inappropriate use of acaricides, the official norms were published by Mexican Agricultural authorities in order to be executed (NOM-019-ZOO-1994). These norms established strategic and systematic treatments based on the knowledge of population dynamics determined by tick collections to identify the seasons of higher or less abundance, and to conduct programs where a certain amount of ticks are allowed to keep enzootic stability of tick-borne diseases (73). Therefore, cattle can be infested with a reduced number of ticks to keep immunological memory, which otherwise would result in enzootic instability, and outbreaks of tick-borne diseases with significant mortality and economic loss to the producer (71, 12).

Sole reliance on the use of chemicals to manage tick populations puts a strong selection pressure for the emergence of resistance, which has a genetic basis and therefore is inherited to subsequent generations (18). How fast resistance is developed in a population depends on the intensity of selection, frequency and dominance of resistance genes (74).

Multiple resistance to acaricides represents a big problem worldwide (75), especially in tropical areas where cattle production is one of the most important agribusiness activities. In Mexico, studies on tick resistance started in the 90s, when populations of *R. microplus* resistant to pyrethroids (76), organophosphates and organochlorines (77) were detected. Due to these problems, amidine acaricides like amitraz were marketed to aid in tick control efforts, but very soon resistance to amitraz was detected in the Southern state of Tabasco (78). *R. microplus* populations in Tabasco were also found to be resistant to pyrethroids and organophosphates.

Commercial formulations of macrocyclic lactones can be applied to cattle parenterally and as pour-ons to treat gastrointestinal nematode infections and tick infestations in Mexico (72, 73). However, after 10 years of intensive use, resistance to ivermectin was detected in the southern state of Yucatan (79, 80). Resistance in *R. microplus* appears to be linked to the mode of use of ivermectin products to treat gastrointestinal parasitic infections in cattle (81). The frequency of multiple acaricide resistance cases in *R. microplus* infesting cattle continues to increase in Mexico, and in some regions is common to find tick populations resistant to organophosphates, pyrethroids, and amitraz, with a prevalence of resistance to one or several acaricides from 19-95 % in the south of the country (82). In Tamaulipas, a strain of *R. microplus* was found to be resistant to permethrin, coumaphos, amitraz, and fipronil. This was the first report of fipronil resistance in *R. microplus*, which occurred not too long after the introduction to the Mexican veterinary market of a commercial product containing that acaricide (19).

Research on acaricide resistance in Mexico has focused on *R. microplus* despite co-infestation of cattle with *A. mixtum* in important livestock production regions of the country. Only one work on *A. mixtum*, formerly referred to in Mexico as *A. cajennense*, was done where a high frequency of *A. mixtum* populations in the state of Veracruz were found to be resistant to organophosphates and amitraz (26). Where geographically applicable, the infestation of cattle with *A. mixtum* needs to be assessed and considered, and the susceptibility of this tick species to acaricides must be determined to ensure effective chemical control.

The rapid implementation of practical countermeasures is challenging once producers are fully aware of the presence of ticks infesting their cattle that are resistant to acaricides. A reactive approach to use more of the same acaricide, or intensify the use of products containing other acaricide classes can accelerate the development of multiple acaricide resistance. Area-wide integrated tick management strategies offer a sustainable alternative to the reliance on chemical control, and provide opportunities to solve the problems of acaricide resistance (83, 84).

6. IMMUNOLOGICAL TICK CONTROL

6.1. Immunization with the Bm86-based vaccines

Acaricide resistance was one of the drivers for research on immunological tick control through vaccination of susceptible cattle that in the middle 80's resulted in the discovery of Bm86, a gut membrane protein found in the intestine of *R. microplus* (85, 86). The recombinant version of the Bm86 molecule was developed as the antigen in the commercial vaccine TickGard that was launched in Australia (87). The Bm86 protein sequence obtained from a Cuban strain of *R. microplus* was used to express the recombinant protein in the yeast *Pichia pastoris* at the Center for Biotechnology and Genetic Engineering in Cuba (88), and subsequently the vaccine was marketed in several Latin-American countries under the commercial name Gavac (89). Although the function of the Bm86 protein remains to be fully determined, previous research showed that it is involved in blood coagulation, and cell growth (90). The effects on *R. microplus* feeding on cattle vaccinated with Bm86 include a reduction in the number of engorging females, their weight, and reproductive capacity, which at the population level reduces the tick numbers after several generations (87, 88).

The immunization of cattle against *R. microplus* under field conditions was conducted in the state of Tamaulipas using a Bm86-based vaccine in combination with a chemical acaricide in a farm of 800 cross-bred cattle where tick resistance to pyrethroids and orgonaphosphates had been detected. Cattle were treated with amitraz before immunization. The combined treatment with acaricide plus vaccination decreased tick numbers and control reached almost 100%; this was associated with an extension of the interval between chemical treatments of up to 132 days in comparison with the control group that required a treatment every 30 days (91).

An immunization trial conducted with cattle under controlled conditions against *R. annulatus*, resulted in an efficacy close to 100% (92), which demonstrated for the first time the high effect of the Bm86 antigen against other tick species. These results indicated that vaccination with Bm86 in combination with systematic chemical treatment could be useful to control and even eradicate *R. annulatus* in regions where this tick is established in relatively small geographical areas (93).

Vaccination with Bm86 was conducted in several parts of the world for about two decades with satisfactory results in areas where tick resistance was a problem. In a farm located in Tamaulipas Mexico, where tick vaccination was part of an integrated tick control program lasting more than 10 years, the efficacy of the vaccination was documented by an 80% decrease in the tick population, and a 67% reduction in acaricide treatments, which was associated with a significant reduction in the number of cases of babesiosis (89). However, during commercialization, the Bm86-based vaccine faced several challenges including poor acceptance of the technology in extensive beef cattle farms due in part to perception that it did not have a "knockdown" effect comparable to acaricides, and the need to still use acaricides to other ticks like *Amblyomma* spp. Gavac is not available in the veterinary medicine market of Mexico since 2012. Therefore, strategies to control ticks infesting cattle are focused again on the use of acaricides, and the research to develop a vaccine that can be integrated for sustainable cattle tick management is required.

6.2. Anti-tick vaccine research on antigens other than Bm86

Subolesin is an anti-tick vaccine candidate antigen discovered in *Ixodes scapularis* by cDNA expression library immunization (cDNA-ELI) following screening of protective clones in a mouse infestation model (94). Subolesin was conserved in all tick developmental stages and gut, salivary glands, and reproductive organs of not only *I. scapularis* but also in *Amblyomma* spp., *Dermacentor variabilis, D. marginatus*, and *R. microplus* (95). Immunization of mice, rabbits and sheep with subolesin and challenge infestation with larvae, nymphs and adults, respectively, decreased tick infestation levels, which indicates that this candidate antigen could be used in a polyvalent anti-tick vaccine formulation (96).

In vivo efficacy against cattle ticks was tested in trials where animals immunized with subolesin were infested with *R.microplus* (97, 98) *and R. annulatus* (97, 21). Furthermore, pathogen DNA levels in ticks decreased significantly in cattle immunized with subolesin that were infested with *R. microplus* and then challenged with *B. bigemina* and *A. marginale*, this observation indicates that the use of subolesin as an immunogen could decrease tick infestation levels in cattle and avoid pathogen transmission simultaneously (98).

RNA interference (RNAi) is a research tools that has been adapted for the analysis of vaccine candidates (95, 18, 99). This methodology was used to evaluate selected cDNAs in unfed adult *R. microplus* ticks. After RNAi, tick subolesin (*sub*) and ubiquitin (*ubn*) were selected. These genes were expressed and recombinant proteins used to immunize calves that were then challenged with *R. microplus* and *R. annulatus* larvae. Positive controls were immunized with adjuvated Bm86 and negative controls with adjuvant only. Immunoprotection with both antigens was >50% in comparison to 60% with Bm86 (97). However, specific antibody levels tested by indirect ELISA showed that the immunological response was not as strong as in previous studies where antibody titers were higher after the second immunization. Protection against *R. annulatus* afforded by subolesin was 60%, while the efficacy in the control group immunized with Bm86 was 100% (97). These results supported the concept of using a Bm86-based vaccine as part of an integrated tick management program in the US-Mexico border (100).

The aforementioned hypothesis was tested by performing a trial where 5 cattle were immunized with the Bm86-based vaccine Gavac according to label instructions, and another group of 5 cattle was injected with adjuvant only to assess efficacy against a strain of *R. annulatus* causing outbreaks in Texas. All the animals were challenged with 4,500 larvae of *R. annulatus* that were feed until repletion. Efficacy was 99 and 91% at 8 weeks and 5.5. months, respectively, after the initial immunization (100). The results of this study indicated the feasibility to include an anti-tick vaccine as part of an integrated cattle fever tick eradication program. Moreover, continued vaccination at the herd level could allow maintaining cattle in quarantined pastures where *R. annulatus* was detected.

Ferritin 2 (Ferr2), a secreted protein expressed in the tick gut functions as transporter of non-heme iron, a metabolic product of the high amounts of blood consumed during engorgement and detoxification of tick tissues, which makes it an essential molecule for tick survival (101). Its expression profile involves all tick developmental stages and Ferr2 does not have orthologs in vertebrates. An RNAi experiment to silence ferr2 in *I. ricinus* resulted in a significant impact on tick feeding, oviposition, and hatching (102). Recombinant Ferr2 used to immunize cattle following the methodology used in previous experiments showed efficacy of 64% against *R. microplus,* and was 72% efficacious against *R.annulatus* (102). Thus, Ferr2 is a suitable candidate for inclusion in formulations against cattle fever ticks.

Bm95, and homologous protein of Bm86, which was isolated from an Argentinean strain of *R. microplus,* has 39 and 21 differences at the nucleotide and amino acid level than the Bm86 obtained from the Australian strain (103). This protein demonstrated to be efficacious against a population of *R. microplus* refractory to immunization with Bm86 (103). MSP1 is one of five major surface proteins (MSPs) that have been described on *A. marginale* from bovine erythrocytes and was found to be conserved in tick salivary glands. MSP1 is a heterodimer composed of two structurally unrelated polypeptides: MSP1b and MSP1a. MSP1a is an adhesin for bovine erythrocytes in both native and cultured tick cells (104). A recombinant protein comprising the Bm95 immunogenic peptides fused to the *A. marginale* MSP1a N-terminal region (Bm95-MSP1a) was surface exposed on the *Escherichia coli* membrane, which resulted in a simple and cost-effective process for the production of vaccine preparations involving the propagation and fermentation of the recombinant *E. coli* strain followed by cell harvest, disruption, and debris separation (105). Using this system, production of the subolesin-MSP1a fusion protein was scaled up in the *E. coli* expression system. Vaccine efficacy in cattle immunized with bacterial fractions containing the chimeric proteins BM95-MSP1a and SUB-MSP1a against *R. microplus* was 64% and 81%, respectively. These results demonstrated the feasibility of immunization with bacterial membranes containing chimeric proteins to control cattle fever tick infestations (106).

Some candidate antigens like subolesin, TROSPA, and Silk are proteins that decrease tick infestations, but are also involved in tick-pathogen interactions. TROSPA, identified in *Ixodes scapularis* as a receptor for *Borrelia burgdorferi* (107) increased the levels of its coding mRNA in response to infection with *B. bigemina* in *Rhipicephalus* ticks (108). Silk, a protein present in tick and spider salivary glands reduced the *A. marginale* infection in tick salivary glands after the *silk* gene silencing by RNAi (109), Subolesin reduced the *A. marginale* and *B. bigemina* infection levels in *R. microplus* (98). The efficacy of these proteins was tested in cattle immunized, and infected with *A. marginale* and *B. bigemina* (110). Immunization with subolesin and silk resulted in a general anti-tick efficacy of 60 and 62 %, respectively; and TROSPA did not affect tick infestation. Subolesin and Silk decreased significantly the DNA levels of *A. marginale* in tissues of ticks fed on immunized cattle that were also infected, while subolesin decreased the levels of *B. bigemina.* Although clinical signs of infection were not detected in cattle, the results demonstrated the possibility of using proteins that interact with ticks and pathogens to reduce tick infestation, and to diminish infection rates with tick-borne pathogens such as *A. marginale* and *B. bigemina* (110). More studies are required to test polyvalent vaccines for cattle that are efficacious against ticks and tick-borne pathogens (111, 112).

Efforts on cattle tick vaccine development have focused on *R. microplus*, where the efficacy with the Bm86 antigen ranges from 50 to 75%, and very high levels of protection against *R. annulatus* are achieved consistently. But, no available vaccines to reduce the infestations of *Amblyomma* spp. ticks exist. With *A. americanum,* protective clones from a cDNA library were selected by RNAi, and the expressed proteins were used to immunize cattle that were challenged with *A. americanum* adult ticks. One of the proteins resulted in tick protection higher than 55%, similar to the efficacy obtained with subolesin, which was used as positive control (113). Other proteins with potential as candidate antigens for vaccine development have been identified (114). However, they have not been tested in cattle. Research to identify candidate antigens that could be developed as a vaccine against *A. mixtum* is needed. Success with these efforts would benefit the livestock industry in Mexico, and in other parts of the American continent where *A. mixtum* infests cattle and other animals.

7. SUMMARY AND PERSPECTIVES

Ticks and tick-borne pathogens represent a major economic problem for the cattle industry in Mexico. Currently, anti-tick vaccines are not commercially available, hence the tick control relays on the use of acaricides, increasing the tick resistance, with high exposition of cattle to the pathogens *A. marginale* and *Babesia* spp. Continued research is required to advance on development of anti-tick and tick-borne pathogen vaccines. Although new antigens protective against ticks and tick-borne pathogens have been discovered, most of them have been evaluated only in controlled conditions, therefore it is required to evaluate these antigens in natural infestations. An ideal vaccine to be used in conditions similar than the Mexican tropics should be able to target several tick species and the pathogens causing anaplasmosis and babesiosis. However, problems with ticks and tick-borne diseases, including those that affect cattle, seem to be getting more complex due to the high diversity of involved organisms. The genomes of *A. marginale, B. bovis, B. bigemina* and *R. microplus* offer tools to search for new tick and tick-borne pathogen vaccine candidates. The next generation anti-tick vaccines with a better efficacy than the existent Bm86-based are expected in the near future and may help decreasing the problematic of cattle industry which has an urgent need of anti-tick vaccines as a sustainable technology with significant positive impact on integrated cattle tick and tick-borne diseases management in Mexico and other countries with similar environments for ticks and tick-borne pathogens in cattle.

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