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Review

Background and perspectives of certain priority diseases affecting cattle farming in Mexico

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Abstract:

The review focused on concisely presenting the contributions that INIFAP researchers have developed, directly or in collaboration with researchers from other institutions, on different

aspects of the diseases that affect cattle farming in Mexico. It describes the research on viral diseases such as rabies and bovine viral diarrhea; bacterial diseases such as anaplasmosis, brucellosis, tuberculosis, paratuberculosis, leptospirosis and bovine respiratory disease, and among parasitic diseases, tick infestation and babesiosis. It identifies potential lines of research that can help mitigate the impact of diseases on production. It considers contributions on the development or adaptation of serological and molecular diagnostic techniques and the diagnosis of resistance to ixodicides. In addition, it indicates epidemiological parameters of the diseases and makes reference to the biologics generated, which include vaccines against rabies, anaplasmosis and babesiosis; bacterin against leptospirosis, and a bacterin-toxoid against pneumonia. It also discusses the evaluations of the use of BCG against tuberculosis and a new generation vaccine against brucellosis. The review concludes that the research of INIFAP in animal health must necessarily have the omic sciences as a perspective. This is the only way to complement the understanding of disease mechanisms, the development of new diagnostic techniques and the design of effective and safe vaccines. Therefore, the great challenge will be the involvement of the animal health area in the concept of "One Health".

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Introduction

The purpose of livestock production is to produce quality food that is affordable for society and obtained in a sustainable environment, which is difficult in the face of a growing need for meat and milk. INIFAP researchers maintain a constant attention to the demands of producers, through the generation of scientific knowledge and technological innovation in animal health problems.

In Mexico, the inventory is slightly more than 34 million cattle[(1)], which are exposed to viral, bacterial or parasitic pathogens, which often behave as co-infections or complexes. The distribution and frequency of diseases vary according to the interactions between the pathogen, the bovine host and the ecological conditions. Its occurrence causes different rates of morbidity, mortality and low productivity, with a detrimental effect on the use of the production potential, and inherently generates trade restrictions at both the national and

international levels. It has been estimated that a disease outbreak can affect 20% of the commercial activities related to the herd[2]. Diseases involve a wide variability in the cost-benefit ratio of prevention and control programs, which results in underestimates of the impact on production, and, consequently, in inconsistency in the information on losses. It is also important to note that certain bovine diseases affect the human population[3]. Each disease has a different economic burden that is determined through direct costs, indirect costs of consumption or loss of resources; in general terms, it includes human, structural and economic resources. The objective of this review is to present in a concise manner the contributions that INIFAP researchers have made, directly or in collaboration with researchers from other institutions, on different aspects of the main diseases affecting cattle in Mexico. At the same time, the aim is to identify lines of research to mitigate the impact of diseases on production.

Bovine paralytic rabies

Bovine paralytic rabies (BPR), also known in Spanish as *derriengue*, is an encephalitis caused by a negative-strand RNA virus of the *Rhabdoviridae* family and of the genus *Lyssavirus*. In Mexico, the main transmitter is the chiropteran *Desmodus rotundus*, a hematophagous bat, distributed in Latin America from the coasts of Mexico to the north of Argentina[4]. In Mexico, BPR is a frequent disease: 284 positive cases were diagnosed in 2019[5].

Contributions by INIFAP

Diagnosis. In endemic countries such as Mexico, diagnosis is critical for the prevention and control of rabies. The reference test is direct immunofluorescence (DIF); however, in tropical climates, the brain tissue frequently exhibits decomposition when handled, which makes it impossible to perform the diagnosis or leads to false negative results. Therefore, a real-time polymerase chain reaction test (RT-PCR) was developed based on the sequences of 40 virus isolates from different reservoirs and geographical areas of the country. For this purpose, primers were designed for the N gene, which is the most conserved gene of the virus. With the application of the test, sensitivity, specificity and predictive value rates of 86, 91 and 96 %, respectively, were obtained[6]. The virus was also detected in samples stored at 27 °C for 23 days. Thus, RT-PCR is currently accepted as an excellent alternative for virus diagnosis[7].

Molecular Epidemiology. INIFAP researchers have pioneered the antigenic and molecular characterization of the rabies virus. They performed the detection of antigenic variants using a panel of monoclonal antibodies obtained from the Pasteur Institute in Paris, France[8]. Subsequently, using monoclonal antibodies from the U.S. Center for Disease Control, they achieved molecular characterization of samples from humans and domestic and wild animals collected from 1990 to 1995. Thus, they recognized a new cycle, called "hypervariables", circulating in skunks in Baja California Sur. At the same time, antigenic and molecular variants circulating in vampires and other wildlife were identified[9,10]. In a collaborative study with researchers from the Pasteur Institute, the main epidemiological cycles of rabies in Mexico were determined using the Restriction Fragment Length Polymorphism (RFLP) technique[8]. In other research, using a portion of the P gene, it was discovered that a variant of the virus that circulates in cats also circulates in the bat *Tadarida brasiliensis*[11,12].

Vaccination. The use of gamma radiation with a Cobalt-60 source made it possible to maintain the potency, safety, stability, and shelf life of traditional vaccines[13-16]. In the application of a gene vaccine in dogs, it was possible to successfully replace the gene gun with an insulin syringe[17].

Edible rabies vaccines were generated using the N gene expressed in the tomato; however, a low level of immunogenicity was obtained[18]. In contrast to the G protein expressed in carrot embryogenic callus, it provided a 60% protection in mice[19]. Subsequently, an anti-rabies vaccine was produced in corn, whereby the protection was increased to 80% in sheep in the face of a challenge with a lethal virus[4].

Recent collaborative research has uncovered differences in Toll-like receptors (TLR) between chiropterans and terrestrial mammals[20]. The Nature series has published the hologenome of the vampire, and it has been inferred that the hematophagous bat has adapted to blood through a close relationship between its genome and the gut microbiome[21].

Perspectives. In tropical conditions, the maintenance of the cold chain is a serious inconvenience; therefore, a thermostable vaccine must be generated for massive use. Also, mass testing for neutralizing antibodies associated with protection must be carried out in order to evaluate the effectiveness of vaccines. In addition, it is essential to produce a good quality conjugate that will allow high sensitivity, specificity and lower cost of the test.

Bovine viral diarrhea

Bovine viral diarrhea (BVD) is a globally distributed disease that causes significant losses to livestock. The causal agent is a *Pestivirus* of the *Flaviviridae* family, which has an immunosuppressive effect that facilitates secondary or concomitant infections. It affects the digestive, respiratory and reproductive systems, and is a component of the bovine respiratory complex[22]. The virus has high genetic variability and is classified into two genotypes and several subgenotypes.

Contributions by INIFAP

Epidemiology. In Mexico, the first description of BVD was made in 1975, specifically in cattle with reproductive problems in which circulating antibodies were detected[23]. INIFAP studies have been limited to understanding the epidemiology and measurement of risk factors. However, the presence of subgenotypes 1a, 1b, 1c and 2a has been demonstrated in Mexico[24].

A report describes a sampling of dairy cattle in different states of the country, in which a seroprevalence of 78.8% was determined. In the same study, the significant risk factors were herd size, pens, intensive production, and long inter-calving periods[25].

Perspectives. The high prevalence of BVD suggests the opportunity to create lines of basic and applied research for the prevention and control. The ideal challenge would be the elimination of BVD, for which a vaccine should be developed with Mexican isolates representing the subgenotypes present. It would be desirable to develop new generation vaccines, as well as diagnostic techniques with high sensitivity and specificity for recognizing concomitant infections.

Bovine Anaplasmosis

Bovine anaplasmosis is a disease caused by the Gram-negative bacterium *Anaplasma marginale*, which affects mostly grazing cattle in tropical areas where the largest livestock populations are concentrated in extensive farms in Mexico. The disease can cause up to 25% of the total death losses of animals moved to the tropics for breeding programs[26]. The clinical form is manifested by anemia, jaundice, lack of appetite, loss of weight and milk

production, miscarriage in the third third, and death. At INIFAP, the Anaplasmosis Unit was founded by Dr. Ramón Aboytes Torres in 1994, where research on diagnosis, epidemiology, bovine immune response, *in vitro* culture of the bacteria, and the generation of vaccines is carried out.

Contributions by INIFAP

Diagnosis and epidemiology. Serological and molecular studies have been performed, and prevalence rates of 50% have been estimated in northern Veracruz[27]. Serological diagnosis was improved with the development of an indirect enzyme-linked immunosorbent assay (ELISAi), which has been adopted by SENASICA[28]. In molecular epidemiology studies using the *msp*1 α and *msp*4 genes as markers, Mexican strains have been observed to have a distribution that allows to assume their migration. Several strains of *A. marginale* present in Mexico were also found to be more similar to those characterized in Brazil than to U.S. strains[29]. More than 20 strains are stored in the laboratory, having been collected in different states of the Republic and used for testing conserved antigens[30,31].

Immunity and vaccines against anaplasmosis. *A. marginale* infects mature erythrocytes; this makes it behave as an extracellular bacterium, since it does not infect nucleated cells and, therefore, does not induce a typical Tc response with $CD8^+$ cells, but prompts a Th1 response. This Th1-type immune response model had been previously postulated[32]; at INIFAP, it was tested in calves that normally establish infection but resist the clinical occurrence of the disease. The model was also corroborated in adult cattle, in which a Th1-type response was observed, associated with the presence of IgG2, Interferon-γ and $CD4^+$ T-helper (Th) lymphocytes, which is essential for resistance to the clinical occurrence of anaplasmosis[33].

An inactivated immunogen was developed to induce protective immunity to a homologous challenge[28,34,35]. In the search for broad-spectrum alternatives, a strain of *A. marginale* from the state of Yucatan was identified, which was named "Tizimín" and characterized as a strain of low natural virulence[36]. This strain was used as a vaccine and was shown to protect against a heterologous challenge in cattle vaccinated with doses of $1x10^4$-$1x10^{10}$[37]. The inactivated immunogen has been used to vaccinate both local and imported animals in Veracruz and Tamaulipas, thus contributing to reduce the morbidity and mortality due to anaplasmosis. On the other hand, the use of live immunogen has been limited due to the difficulty to maintain it in liquid nitrogen. In Mexico, the most important biological vector of *A. marginale* is known to be the *Rhipicephalus microplus* tick[38,39]. Trans-ovarian transmission was demonstrated in the laboratory this was done with *R. microplus* larvae

that were fed on infected cattle and which subsequently transmitted the infection to susceptible cattle[40].

Genome studies. The first complete genome of *A. marginale* was published in 2005[41], revolutionizing the study of potential vaccine candidates against this bacterium. Today, there are 23 complete sequences, including seven Mexican strains[42,43]. Membrane proteins with vaccine potential have been analyzed for the development of immunogens[44], and trials have been conducted with recombinant proteins or synthetic peptides[31]. However, there is still no immunogen capable of fully protecting experimentally or naturally challenged cattle against this bacterium. Currently, in the Anaplasmosis Unit, studies focus on these sequences in order to include proteins associated with transport, signaling or metabolic pathways in the design of vaccines[30].

Perspectives. After the publication of the 23 genomes of *A. marginale*, the sequences are to be analyzed by bioinformatics procedures in order to establish criteria for the identification of vaccine candidates linked to vital or virulence functions. Currently, there are examples of multi-epitope vaccines and reverse vaccinology strategies; thus, INIFAP research group according is making use of these tools to design new vaccines against *A. marginale*[30]. It is very likely that proteins other than those already studied will be identified for inclusion in a vaccine. This may take place within a period of five years, at which time an immunogen will be widely and safely used.

Brucellosis

Brucellosis is an infectious disease caused by bacteria of the genus *Brucella* that affects different domestic species such as cattle, sheep and goats. The most important species that affects cattle is *Brucella abortus*[45]. In Mexico, brucellosis is the main zoonosis of bacterial origin. In cattle, the most notorious clinical signs are reproductive, including miscarriage and reduction of milk production, which have a high impact on cattle farming. For the purpose of controlling the disease in the country, there is a National Campaign against Brucellosis in animals, which applies the NOM-041-ZOO-1995 standard based on diagnosis and vaccination.

Nationally, *B. abortus* strains S19 and RB51 are used for immunization of cattle. S19 induces the presence of antibodies in serum and milk, but interferes with official diagnostic tests; therefore, the alternative is RB51[46-49]. For diagnosis, the most commonly used serological methods are the 8% card test and the rivanol test[50]; these tests detect antibodies against the components of the outer membrane of *Brucella*, directed against the O-chain of

the lipopolysaccharide (LPS), which is the most antigenic structure of the smooth strains[51].

Despite the efforts made in the campaign, brucellosis in Mexico continues to have an unfavorable effect on animal and human health. Prevalence in production units is higher than 20%; in humans, an average of 3,000 new cases are reported each year according to Ministry of Health of Mexico (Secretaría de Salud), CENAPRECE 2013-2018).

Contributions by INIFAP

At INIFAP, researchers have made relevant contributions to the campaign in multiple aspects. The diagnostic tests that are applied directly or indirectly have been the result of its research, and are endorsed by the Mexican Official Standard. The tests utilized are Rose Bengal, rivanol, complement fixation and ring in milk. However, with the use of these tests, it is difficult to differentiate between vaccinated and infected animals, especially in those that are revaccinated; this issue has been solved with the radial immunodiffusion (RID) test[52]. In turn, this test facilitated the development of other tests with greater sensitivity and specificity, such as ELISA and polarized fluorescence, in which the polysaccharide known as native hapten (NH) is used as antigen[49,53].

In relation to the pathogenesis of brucellosis, it was studied the survival and intracellular trafficking of the vaccine strain RB51 vs. field strains in phagocytic cells. Thus, a shorter survival time of the vaccine strain was observed, and a lower probability of causing disease was inferred[54].

The effect of revaccination and the management of infected herds were evaluated in order to demonstrate the effectiveness of the vaccines used in the campaign. Trials have also been carried out with new generation vaccines such as *rfbK* mutants[47,55,56]. The RB51 vaccine exposed the potential risk to public health, as it was shown to be eliminated in the milk and vaginal secretions of cows[57]. Although the RB51 vaccine strain displaced Strain 19, its real protective potential is still unknown, although its efficacy in eliminating reagent animals to conventional tests has been demonstrated. Vaccination *per se* has not been sufficient to reduce the high incidence of the bacterium in infected herds[46,47]. Vaccination with the RB51 strain has been described as not interfering with official diagnostic tests because it lacks the "O" chain. While some positive "outlier" responses have been observed, these have been attributed to contact with field strains that occurred during the studies[58,59]. The use of the rough mutants RB51 and *rfbK* as vaccines has been described as inducing adequate protection in a herd under medium prevalence conditions[56].

Perspectives. Despite the existence of the national campaign for the control of brucellosis, the prevalence and incidence of the disease remain at a level that has economic and social repercussions. Therefore, the prevention and control of brucellosis could be approached under the concept of "One Health"; this would involve producers and authorities in charge of animal and human health. Technically, projects should be continued to improve the efficacy and safety of existing vaccines and to develop new types of vaccines.

Tuberculosis

Bovine tuberculosis is a chronic disease caused by the mycobacterium *Mycobacterium bovis*, which belongs to the *Mycobacterium tuberculosis* complex. *M. bovis* affects a wide variety of species, including humans. In Mexico, tuberculosis is the second most important zoonosis of bacterial origin after brucellosis[60]. Control depends on the application of the Mexican Official Standard NOM-031-ZOO-1995 of the National Campaign Against Bovine Tuberculosis (*Mycobacterium bovis*)[61], whose strategy is based on the diagnosis and elimination of reagents. The diagnosis is performed with the tuberculin test, using as antigen the bovine purified protein derivative (PPD) made with *M. bovis* strain AN5. Bovine PPD is applied in the caudal fold or at par with avian PPD made with *M. avium* strain D4, in a comparative cervical test[62]. Animals positive to this test are sent to the slaughterhouse; the diagnosis is confirmed by specific bacteriological analysis and by histopathology of granulomatous lesions, which is established in NOM-031-ZOO-1995[61]. In Mexico, the prevalence is usually above 2.5 % in milk production units; it is lower in beef cattle, but in both systems it affects the commercialization of cattle. More than 15,000 new cases of tuberculosis are reported in humans each year (Secretaría de Salud, CENAPRECE 2017).

Contributions by INIFAP

INIFAP, through its researchers, has contributed to the development and application of the different diagnostic techniques applied in the campaign, which are endorsed by the Mexican Official Standard. An outstanding contribution is a study that proved that the tuberculin test does not identify animals in the terminal stages of tuberculosis. Therefore, complementary tests such as ELISA, Interferon-γ and spoligotyping were implemented to improve the reliability of the diagnosis and identify these anergic animals[62].

The use of sodium tetraborate in the isolation of mycobacteria was established as a routine procedure for the optimal preservation of samples for up to 90 days; this is also a contribution made by INIFAP researchers. Another contribution was the use of PCR and histopathological analysis with Ziehl-Neelsen staining, which improved the sensitivity and specificity of bacteriological culture[63]. A major achievement was the development of end-point PCR and Multiplex PCR tests, with which it is possible to differentiate between animals vaccinated with BCG and those infected with field strains[64,65].

In Mexico, there is no authorized vaccine to prevent tuberculosis in animals; however, INIFAP has conducted studies of the BCG vaccine used in humans to evaluate its protective capacity in animals. Laboratory animals have been used as models and preliminary tests have been carried out in cattle. In a study of calves vaccinated with BCG and challenged with a pathogenic strain of *M. bovis*, a marked reduction in granulomatous lesions was demonstrated. Therefore, its use has been suggested for the control of tuberculosis in high prevalence areas[66].

Perspectives. The scientific information that has been generated, in association with with the existence of a campaign for the control of tuberculosis with an Official Standard, suggests that an efficient control of bovine tuberculosis is feasible. However, the suitability of the use of BCG vaccine, which is currently the only vaccine in existence to prevent tuberculosis in cattle, must be solidly demonstrated. At the same time, an alternative line of research should be established for another vaccine that will not interfere with discrimination between vaccinated and infected animals, which would reduce the prevalence and allow efficient control of bovine tuberculosis.

Paratuberculosis

Paratuberculosis is a chronic infectious disease affecting cattle, sheep and goats. It is caused by *Mycobacterium avium* subspecies *paratuberculosis* (Map); it is characterized by granulomatous lesions in the small intestine. This disease causes nutrient malabsorption syndrome, loss of physical condition in infected animals, and a reduction of productive capacity. The etiological agent is eliminated in feces; therefore, the animals become infected by ingesting contaminated colostrum, milk, feed, or water. The slow spread of the disease and its chronic course cause periodic economic losses[67].

Contributions by INIFAP

Researchers at former CENID-Microbiología obtained a protoplasmic antigen from a strain called Map 3065, derived from a clinical case of a sheep. This antigen was used to standardize agar immunodiffusion techniques (IDGA) and enzyme-linked immunosorbent assay (ELISA)[68].

In Mexico, epidemiological indicators have been determined in production units (PU) in the states of Chihuahua, Coahuila, Sinaloa, Durango, San Luis Potosí, Jalisco, Aguascalientes, Guanajuato, Querétaro, Hidalgo, Puebla, Chiapas, and Veracruz. Prevalences ranged from 1.0 to 32.37 % in the different states; in each individual PU, prevalences ranged from 1.0 to 88.87 %. In another epidemiological study, the presence of paratuberculosis was associated with the sanitary conditions of each PU, which allowed the issuance of sanitary management recommendations for the control of the disease[67,69,70,71].

Another technique that was implemented was the fluorescence polarization assay (FPA), which improved the epidemiological sensitivity and specificity rates[72]. A PCR was also implemented, in which DNA is extracted from feces, milk, cheese, or tissues with lesions. Using this technique, cases of serology-negative animals are confirmed, which, if they remain in the herd, would be the main source of infection. Thus, PCR is useful as a confirmatory test for the disease.

In addition, a nested PCR (nPCR) has been standardized, for which primers were designed to amplify a region of the IS900 insertion sequence gene specific for Map. With nPCR, results are obtained in a shorter time, and high sensitivity and specificity are attained. It should be contrasted with bacteriological isolation, which regularly requires 16 weeks[73].

Perspectives. In order to understand the processes of humoral and cellular immunity of cattle to *M. avium* subspecies *paratuberculosis*, it is necessary to generate a line of research, and the challenge will be to develop an effective immunogen for the prevention of the disease.

Bovine respiratory disease

This is a multifactorial disease involving exposure to viral, bacterial, environmental and physiological stressors affecting the cattle. It has been described as the most common and costly disease afflicting cattle worldwide. Clinical manifestations include fever (>40°C),

nasal and ocular discharge, dyspnea, poor appetite, depression, prostration, and death. The economic impact due to morbidity, mortality, treatment costs, and lower production is substantial.

Bovine respiratory disease (BRD) involves infectious bovine rhinotracheitis (IBR), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus (BVDV), parainfluenza-3(PI3), and bovine herpes virus type 1 (BHV1). Viruses create conditions conducive to the colonization and replication of bacteria, facilitating their adhesion to infected cells. Thus, in cattle with viral infections and subjected to stressful conditions, severe respiratory infections associated with bacteria are present. The most frequent are *Mannheimia haemolytica, Pasteurella multocida*, and *Histophilus somni*; these are normally part of the microbiota of the upper respiratory tract. They possess various virulence factors, *M. haemolytica* produces a leukotoxin that affects ruminant leukocytes; *P. multocida* has an antiphagocytic capsule and lipopolysaccharides; *H. somni* can survive intracellularly and is capable of producing biofilm[74]. The complex is capable of altering the functions of alveolar macrophages, suppressing lymphocyte proliferation, inducing apoptosis, modifying cytokine expression, and triggering an inflammatory process[75].

Contributions by INIFAP

A collaborative project called "Pneumonic Complex in Ruminants", with the aim of determining the bacterial genera involved in BRD, their serotypes and resistance to chemotherapeutics, was developed between INIFAP former CENID-Microbiología and UNAM. This project allowed the isolation of *H. somnus* (*H. somni*)[76], *P. haemolytica* (*M. haemolytica*), and *P. multocida*. These bacteria were also serotyped and characterized for resistance to chemotherapeutics. Likewise, it was found that most of the *M. haemolytica* serotypes belonged to type A1, and those of *P. multocida*, to type A[77,78,79]; specifically, resistance to penicillin, ampicillin, and streptomycin was detected[80,81]. Subsequently, virulence factors such as leucotoxin were detected in *M. haemolytica*[82], and biofilm formation was evidenced in *P. multocida*, *M. haemolytica*, and *H. somni*[83]. In addition, *P. multocida* was observed to produce vesicles on the outer membrane[84].

From the isolates, strains were generated with which it was possible to formulate a bacterin-toxoid vaccine for the prevention of BRD, which was evaluated in ovine models[85,86,87]. The biologic generated is currently produced at INIFAP and is used in some ovine health programs.

Perspectives. The priority will be to develop biologics with domestic strains of IBR, BRSV and PI3 viruses, and combine them with live attenuated strains or subunits of *M.*

haemolytica, *P. multocida*, and *H. somni* to confer effective protection against BRD. In order to improve diagnosis, it will be necessary to initiate metabolomics studies to monitor metabolites during the course of the disease. It would also be desirable to create a line of research on the genetic resistance of cattle to BRD. Collaterally, transcriptomics would be a very useful tool to try to make a genetic selection and form BRD-resistant herds.

Leptospirosis

Leptospirosis is a zoonosis of worldwide distribution; it is caused by bacteria of the genus *Leptospira*. According to the DNA analysis, this genus includes 10 pathogenic, 5 intermediate, and 7 saprophytic species[88]. Serology recognizes more than 300 serovars[89]. Worldwide, *hardjo* is the most commonly detected serovar in cattle[90]. Small mammals are the main reservoirs of the bacterium, large herbivores are a source of infection, and humans can be accidental hosts[91].

Cattle are renal carriers of *Leptospira* spp. and therefore eliminate the bacteria through urine, contaminating the environment[92]. Leptospirosis causes reproductive disorders such as miscarriages, stillbirths, weak premature calves, and reduced milk production, resulting in considerable economic losses[93]. In Mexico, the first descriptions of leptospirosis in humans and cattle were made in 1928 and 1930, respectively[94].

Contributions by INIFAP

A collaborative work between INIFAP, UAM and UAEM reported the situation of bovine leptospirosis in Mexico. Prevalence rates were determined in different ecological zones of the country; in the arid and semi-arid zones, the prevalence was 37.8%; in the dry tropics, 45.9%; in the humid tropics, 63.8%, and in the temperate zone, 39.4%. The presence of the *hardjo*, *wolffi*, and *tarassovi* serovars was demonstrated in all regions. In the temperate region, the *icterohaemorrhagiae*, *portland-Vere*, *bratislava*, *pyrogenes*, *canicola*, and *pomona* serovars were detected[95]. The *grippotyphosa*, *mini*, and *tarassovi* serovars were isolated; this had not been done in Mexico[96,97]. In other epidemiological studies, the same serovars were identified, but prevalences varied widely from 31 to 91 %[98-103].

For diagnosis, INIFAP implemented the PCR technique, which allowed the detection of bacteria in urine collected from cattle with a history of reproductive problems[100].

Bacterins were generated to prevent the disease: one was added with adjuvant using liposoluble vitamins, which yielded satisfactory results[104]. Another bacterin was made with serovars isolated in the state of Chiapas that were not contained in commercial bacterins; an excellent level of protection was observed in susceptible cattle with this homologous biologic. INIFAP currently has a bacterin that has been validated in dairy herds[105].

Perspectives. The endemicity and high prevalence of leptospirosis in Mexico is evident; therefore, it is a real challenge to massify the use of microagglutination, which is the reference test for determining the *Leptospira* serovars present in the different regions, and then produce homologous bacterins that will effectively prevent leptospirosis. A line of research should be generated to develop molecular vaccines that can be used in any ecological region. It would also be advisable to implement an accurate methodology with high sensitivity and specificity, fast execution, and low cost. This can result in a better diagnosis that will increase the reproductive and productive parameters of cattle.

Ticks

Ticks are hematophagous ectoparasites capable of injecting toxins and transmitting to livestock different pathogens such as *A. marginale* and *Babesia* spp. with high morbidity and mortality rates. Of the various ticks identified in Mexico, the most important is *Rhipicephalus microplus*. Today they constitute a global problem due to their great adaptability to different ecological niches, it is considered that 65% of the national territory is infested with this tick.

Contributions by INIFAP

Epidemiology. An epidemiological study involving different states of the country corroborated that the distribution of *R. microplus* was essentially associated with environmental temperature, rainfall and water vapor[106]. In another research, greater efficiency and reproductive fitness was observed in ticks of a native strain collected in the field in Sinaloa, compared to a reference strain from CENID-SAI[107].

Biological control. For tick control, this strategy has been well documented in studies conducted at INIFAP. Among the evaluation of techniques for the collection of *R. microplus* tick larvae, the double-traveled flag technique was selected for various studies.

The effect of the recovery of *R. microplus* larvae using tropical legumes in the state of Morelos was evaluated[108]. Another study evaluated the anti-larvae effect using *Stylosanthes humilis, S. hamata, Cenchurus ciliaris*, and *Andropogon gayanus* grasses in artificially infested plots. A favorable effect was observed in *S. humilis* plots where only 3% of live larvae were recovered[109]. Other research using mature plants of *S. humilis* and *S. hamata* observed no anti-tick effect[110]. On the other hand, when evaluating crops of the legumes *Leucaena leucocephala* and *Macroptilium artropurpureum, S. humilis*, and *S. hamata*, a significant reduction in the number of larvae of *R. microplus* was observed[111]. Based on these findings, certain chemical compounds in *S. humilis* and *S. hamata* were identified as possible causes of the repellent effect[112]. Similarly, another study using *M. minutiflora* grass also showed a reduction in larval recovery [113].

Other strategies have involved the use of fungi or bacteria for tick control; thus, the use of the entomopathogenic fungus *Metarhizium anisopliae* demonstrated its ability to infect ticks and induce up to 100 % mortality, which allowed inferring that it could be a potential acaricide for the biological control of *R. microplus*[114]. On the other hand, in engorged adult ticks that were experimentally infected with *Staphylococcus saprophyticus* bacteria, it was able to induce tick mortality[115]. It was reported for the first time that the fungus *Aspergillus flavus* is capable of infecting 80 % of engorged adult ticks, the ovigerous masses and the larvae that emerge after hatching, under controlled conditions[116].

Resistance. This is one of the most studied topics at INIFAP; in one of the first studies it was demonstrated that in *R. microplus* tick populations resistant to organophosphates, there is an elevated expression of carboxylesterase enzymes[117]. Subsequently, some genes coding for esterases were characterized to provide molecular markers for discriminating ixodicide-susceptible and ixodicide-resistant tick strains[118]. Genes coding for carboxylesterases B were analyzed by PCR assays in individual *R. microplus* larvae, detecting polymorphisms upon protein translation[119,120]; an esterase was also identified in the "Coatzacoalcos" strain (Cz EST9)[121].

Another study sought to identify the association of gene mutations with pyrethroid resistance. Noting that the presence of the mutation is not associated with resistance in the dose-response form[122]. Studies on pyrethroid resistance attempted to correlate different diagnostic tests, and it was concluded that resistance is mediated by a mutation in the target gene *Kdr*[123]. The participation of cytochrome P450 has also been studied, and it has been observed to be expressed at high levels in pyrethroid-resistant strains[124]. However, a multifactorial process has been evidenced in the resistance of *R. microplus* to organophosphates and pyrethroids[125]. The first case of amitraz resistance was reported[126], and selection pressure with amitraz was described as increasing the level of resistance in field populations[127]. In addition, RT-PCR methodology was used to measure the expression of cholinesterase and carboxylesterase in acaricide-resistant ticks[128].

Immunological control. For tick control, immunogenic proteins derived from extracts of *R. microplus* ovaries obtained from cattle after immunization have been identified [(129)]. Other studies have characterized and evaluated homology to vitellogenin proteins[(130)] and ATAQ, both as potential vaccine candidates against *R. microplus*[(131,132)]. Certain immunization experiments against *R. microplus* and *R. annulatus* ticks have shown inconclusive results. However, similar studies have continued, such as the use of the protein subolesin, which was described as a potential target for developing a tick vaccine[(133,134)].

Perspectives. There is an undeniable need to place greater emphasis on research into the epidemiology of ticks; especially climate change is a factor that is favoring their greater spatial distribution and, therefore, the infestation of livestock not previously exposed to ticks. It is also imperative to develop molecular techniques for the rapid diagnosis of resistance to the different chemical principles of ixodicides. Collaterally, a line of research on biological control should be maintained, involving the identification and characterization of plants. A line of research on the development of immunogens from conserved proteins associated with vital tick functions should be a priority. The major challenge will be to implement an integrated program for the control of *R. microplus*.

Babesiosis

Bovine babesiosis or piroplasmosis is a parasitic disease caused by protozoa of the genus *Babesia* that invade the erythrocytes of the bovine host. In Mexico, the recognized species are *Babesia bovis* and *B. bigemina*, both transmitted by the *R. microplus* and *R. annulatus* ticks[(135)]. Approximately 70% of the country's 35,224 960 head of cattle[(136)] are permanently exposed to tick infestation. Thus, the prevalence of *Babesia* spp. varies between 50 and 96 %, which in turn explains the high risk of outbreaks occurring[(137)].

Babesiosis has been identified as the most important arthropod-borne disease of cattle[(138)]. In Mexico territory, losses are estimated at 573.61 million dollars per year due to ticks and the diseases they transmit[(139)]. However, there is no commercial vaccine, and no national production of diagnostic reagents. In addition to the above, the wide distribution of resistance to ixodicides and climate change are major factors contributing to the abundance of vectors and the facilitation of pathogen transmission[(140)].

Contributions by INIFAP

Diagnosis and epidemiology. INIFAP has implemented direct methods for the confirmatory diagnosis of babesiosis. Techniques for the identification of intraerythrocytic stages are routinely available. The most common is the peripheral blood smear with which *B. bovis* and *B. bigemina* are identified by means of microscopic observation; brain tissue imprints are also made, particularly for the detection of *B. bovis*[141,142]. Histopathological analysis of tissues collected at necropsy can also be performed[143,144,145]. Immunologically based indirect methods have been developed to detect circulating anti-*B. bovis* or anti-*B. bigemina* antibodies[143,146,147]. Defined and characterized parasitic antigens have been obtained for these procedures[148,149]. Advantages have been observed when compared to crude antigens with which a low specificity is regularly obtained in diagnostic tests; this occurs due to the similarity of epitopes present between different species of *Babesia*[146,150,151], and it can also generate cross-reactions with other species[148,151,152].

INIFAP research group has also improved the specificity of serological tests. For this purpose, genes coding for immunodominant, species-specific peptides have been cloned, and monoclonal antibodies have been used[148,149,153].

Other studies have identified the most conserved antigens for *B. bovis*[154-158], utilized for developing indirect ELISA tests for both species[159,160], which in turn were tools for serological monitoring of experimentally immunized animals [161,162,163]. These tests were also incorporated in seroepidemiological studies of cattle herds located in different cattle-raising areas of the country[160,164].

On the other hand, there was a notorious advance in direct diagnosis; molecular procedures that detect genetic material of the parasites were reported. These have included the use of nucleic acid probes or nucleic acid amplification techniques[165,166], which have been used in epidemiological studies in different cattle-raising regions of the country[167]. Using *B. bigemina* genomic DNA, a PCR with high analytical sensitivity was developed, for which the amplified product was hybridized with a non-radioactive DNA probe[168,169]. A multiple format was also implemented for the simultaneous detection of *B. bovis* and *B. bigemina*, to which the diagnosis of *A. marginale* was added[166,170,171]. DNA probes were used in epidemiological studies in Yucatán, Tabasco and Campeche[172,173]. They were also used for the monitoring of cattle inoculated with vaccine strains of *B. bovis* and *B. bigemina*[149,174]; as well as in the monitoring of susceptible animals introduced to endemic areas[175,176]. This same methodology proved useful for the detection of pathogen DNA in ticks[177], as well as for the specific identification of *B. bovis* and *B. bigemina* in the tick *R. microplus*[178,179].

Prevention. So far the best babesiosis prevention strategy in endemic regions is immunization with live attenuated vaccines, which can be derived from subinoculation into splenectomized calves, or from *in vitro* culture of *B. bovis* and *B. bigemina*[180]. The application of attenuated vaccines in susceptible cattle has been shown to induce a robust immune response in the face of attacks by highly virulent parasites[181,182].

INIFAP researchers have participated in the development and adaptation of *in vitro* culture of *B. bovis* and *B. bigemina*, and today attenuated strains of these protozoans are available in Mexico[183,184]. A review of the development in Mexico of the attenuated vaccine from *in vitro* culture can be carried out based on various studies. These include the demonstration of low virulence of *in vitro*-culture derived parasite clones that were inoculated into susceptible cattle[185]. When using the material as fresh immunogen, the appropriate dose was determined to be 1 x 10^7 erythrocytes infected with *B. bovis* or *B. bigemina*[186,187]. Another study showed the need to include both *Babesia* species to induce successful protection against the disease[188]. Similar results were obtained with the vaccination of cattle against a natural challenge in the tropics[189]. Subsequently, it was determined that material derived from *in vitro* culture that was removed from cryopreservation in liquid nitrogen (-196 °C) required increasing the dose to 1 x 10^8 infected erythrocytes of each species in order to protect cattle from challenge with virulent parasites[190]. The use of the vaccine was also evaluated in native cattle kept in farms with high endemicity and enzootic instability, where an excellent level of protection against babesiosis was also demonstrated[191]. In another study, the vaccine was spiked with *Lactobacillus casei* and evaluated against a natural challenge; increased levels of specific IgG1 against *B. bovis* and *B. bigemina*; however, the level of protection was analogous to that of the vaccine without the bacteria[192].

In vitro culture of *B. bovis* and *B. bigemina* is apparently a simple methodology; however, few laboratories in the world do it successfully. After more than 30 years of being established in Mexico, there was a low efficiency in the production of biomass. In recent years, INIFAP has positioned itself as a leading institution at the international level for innovations that have been integrated into the *in vitro* cultivation of *B. bovis* and *B. bigemina*. Bovine serum has been successfully removed from the culture medium and replaced by vital components such as insulin, transferrin, selenite, and putrescine. For the first time, the process was transferred to a perfusion bioreagent, thereby increasing the number of infected erythrocytes by 300%. This implied obtaining a high number of vaccine doses, compared to the traditional procedure[193,194,195]. The bioreagent-derived material evaluated as an immunogen conferred to cattle a level of protection above 80% in a field challenge[196]. That immunogen without the presence of serum proteins has been proposed to induce a response with greater immunological specificity[197]. At the same time, the incorporation of the bioreagent has generated a line of research on the use of soluble antigens derived from the culture supernatant. Recently, in INIFAP laboratory have

achieved for the first time the proliferation of *B. bigemina* in a culture medium free of animal components, and also successfully transferred it to the bioreagent –a procedure that represents a scale-up of the process for vaccine production–[198]. These changes will facilitate the continued development of subunit vaccines[199]. Due to their degree of invention, the innovations described above have caused two patents to be granted in favor of INIFAP, and a third one is pending. One of the granted patents is entitled "Serum-free *in vitro* culture composition for obtaining erythrocytes parasitized with *Babesia* spp." (Patent No. 347729), and the other is called "Process for the elaboration of vaccinal reagent of erythrocytes parasitized with *Babesia* spp. *Babesia bovis* or *Babesia bigemina*" (Patent No. 337161).

Perspectives. There is a need to generate highly sensitive diagnostic tests with the ability to identify *Babesia* strains resistant or susceptible to antibabesial compounds. It would also be relevant to implement a procedure to discriminate attenuated (vaccine, conventional, genetically modified) or virulent field strains. Dynamic mapping of distribution and frequency is essential for the timely application of babesiosis prevention or control procedures. Live vaccines are now the only way to prevent the disease, but it is imperative to maintain the omics sciences in order to generate more knowledge of the interactions between parasites and cattle. This knowledge will facilitate the development of subunit vaccines that may be safer and more easily scalable.

Conclusions

INIFAP has developed and adapted serological and molecular diagnostic tools that have contributed to programs for the prevention and control of cattle diseases. Techniques for the detection of resistance to ixodicides have also been implemented. The distribution and frequency of some of the most important diseases affecting cattle farming in Mexico have been determined. The biologics developed include vaccines against rabies, anaplasmosis and babesiosis, as well as a bacterin against leptospirosis and a bacterin-toxoid against pneumonia. In addition, a BCG vaccine against tuberculosis and a new generation vaccine against brucellosis have been studied. The animal health perspective on zoonotic diseases such as tuberculosis and brucellosis suggests directing scientific and technical efforts toward those diseases elimination. Research on the effect of climate change, especially on vector-borne diseases, should be developed through the protocols and methods of omics sciences, such as genomics, epigenomics, transcriptomics, proteomics, metabolomics and other omics derivatives. It is currently the most appropriate way to understand the mechanisms of disease, and, therefore, it generates more effective vaccines and allows designing more precise diagnostic tools, which will be essential to integral control

programs. Probably the biggest challenge will be to incorporate animal health research at INIFAP into the "One Health" concept. This has been defined as a multi-sectoral and transdisciplinary collaborative process at local, regional, national and global levels, based on the interconnections between humans, animals, plants and the environment[(200)].

Literature cited:

1. INEGI. Instituto Nacional de Estadística, Geografía e Informática. Encuesta Nacional Agropecuaria. 2019.

2. OIE. World Organisation for Animal Health. The economics of animal health: direct and indirect costs of animal disease outbreaks. Paris, France. 2016.

3. FAO. Food and Agriculture Organization. Animal production and health. Economical analysis of animal diseases. 2016.

4. Loza RE, Nadin DSA, Morales SE. Molecular and biological properties of rabies viruses circulating in Mexican skunks: focus on P protein. Rev Mex Cienc Pecu 2012;3(2):155-170.

5. SENASICA. Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria. Indicadores de la Campaña Nacional para la prevención y control de la rabia en bovinos y especies ganaderas. 2020.

6. Loza RE, Rojas AE, Banda RVM, Nadin DS, Cortez GB. Detection of multiple strains of rabies virus RNA using primers designed to target Mexican vampire bat variants. Epidemiol Infect 2005;133(5):927-934.

7. Rojas AE, Loza RE, Banda RVM, Hernández BE. Use of reverse transcription-polymerase chain reaction to determine the stability of rabies virus genome in brains kept at room temperature. J Vet Diagn Invest 2006;18(1):98-101.

8. Loza RE, Aguilar SA, Bahloul Ch, Pastoret PP, Tordo N. Discrimination between epidemiological cycles of rabies in Mexico. Archives of Med Res 1999;30(2):144-149.

9. De Mattos CC, de Mattos CA, Loza RE, Aguilar SA, Orciari LA, Smith JS. Molecular characterization of rabies virus isolates from Mexico: Implications for transmission dynamics and human risk. Am J Trop Med Hyg 1999;(61):587-597.

10. Loza RE, De Mattos CC, Aguilar S, De Mattos CA. Aislamiento y caracterización molecular de un virus rábico obtenido de un murciélago no hematófago en la Ciudad de México. Vet Méx 2000;31(2):147-152.

11. Nadin DSA, Loza RE. The molecular epidemiology of rabies associated with chiropteran hosts in Mexico. Virus Res 2006;117(2):215-226.

12. Loza RE, Rojas AE, Lopez J, Olivera FMT, Gomez LM, Tapia PG. Induction of protective immune response to rabies virus in sheep after oral immunization with transgenic maize. Vaccine 2012;3 (37):5551-5556.

13. Weimersheimer RJE, Loza RE. Desarrollo de un nuevo método para inactivación mediante radiación gamma, para la vacuna antirrábica V-319 Acatlán. Av Cienc Vet 1991;6(1):70.

14. Weimersheimer RJE, Loza RE. Estabilidad de la vacuna antirrábica V-319 Acatlán inactivada con radiación gamma (Cobalto-60). Téc Pecu Méx 1994;32(1):43-46.

15. Weimersheimer RJE, Loza RE. Caducidad de una vacuna antirrábica inactivada con radiación gamma (Cobalto-60a). Téc Pecu Méx 1996;34(3):172-174.

16. Weimersheimer RJE, Loza RE. Alternativa para inactivar vacunas antirrábicas, usando radiación gamma (Co-60). Vet Méx 1999;30(4):313-316.

17. Perrin P, Jacob Y, Aguilar SA, Loza RE, Jallet C, Desmézières E, *et al*. Immunization with DNA vaccine induces protection against rabies virus. Vaccine 2000;18(5-6):479-486.

18. Perea AI, Loza RE, Rojas AE, Olivera FT, De la Vara GL, Gómez LM. Expression of rabies virus nucleoprotein in plants at high-levels and evaluation of immune response in mice. Plant Cell Rep 2008;27(4):677-685.

19. Rojas AE, Loza RE, Olivera FMT, Gomez LMA. Expression of rabies virus G protein in carrots (*Daucus carota*). Transgenic Res 2009;18(6):911-919.

20. Escalera ZM, Zepeda MML, Loza RE, Rojas AE, Méndez OML, Arias CF, *et al*. The evolution of bat nucleic acid sensing Toll-like receptors. Mol Ecol 2015;24(23):5899-909.

21. Zepeda MML, Xiong Z, Escalera ZM, Runge AK, Thézé J, Streicker D, *et al*. Hologenomic adaptations underlying the evolution of sanguivory in the common vampire bat. Nat Ecol Evol 2018;2(4):659-668.

22. Grisset GP, White BJ, Larson RL. Structured literature review of responses of cattle to viral and bacterial pathogens causing bovine respiratory disease complex. J Vet Intern Med 2015;29(3):770-780.

23. Correa P, Brown LN, Bryner JH. Presencia de anticuerpos contra rinotraqueitis infecciosa, diarrea viral bovina, parainfluenza 3, brucelosis, leptospirosis, vibriosis y *Haemophilus somnus* en sueros de bovinos con problemas patológicos, reproductores y respiratorios. Téc Pecu Mex 1975;(29):26-33.

24. Gómez RN, Basurto AFJ, Verdugo RA, Bauermann FV, Ridpath JF. Genetic diversity of bovine viral diarrhea virus in cattle from Mexico. J Vet Diagn Invest 2017;29(3):362-365.

25. Milián SF, Hernández OR, Hernández AL, Alvarado IA, Díaz AE, Mejía EF, *et al.* Seroprevalence and risk factors for reproductive diseases in dairy cattle in Mexico. J Vet Med Anim Health 2016;8(8):89-98.

26. Rodríguez SD, García OMA, Jiménez ORJ, Vega MCA. Molecular epidemiology of bovine anaplasmosis with a particular focus in Mexico. Infect Genet Evol 2009;9:1092-1101.

27. Cossío BR, Rodríguez SD, García OMA, García TD, Aboytes TR. Bovine anaplasmosis prevalence in northern Veracruz State, Mexico. Prev Vet Med 1997;(32):165-170.

28. Rodríguez SD, García OMA, Hernández SG, Santos CN, Aboytes TR, Cantó AJ. *Anaplasma marginale* inactivated vaccine: dose titration against a homologous challenge. Comp Immunol Microbiol Infect Dis 2000;(23):239-252.

29. Jiménez OR., Vega MCA, Oviedo ON, Rojas REE, García OMA, Preciado TJF, *et al*. Diversidad genética de la región variable de los genes *msp1a* y *msp4* en cepas de *Anaplasma marginale* de México. Rev Mex Cienc Pecu 2012;3(3):373-387.

30. Rodríguez CSD, Quiroz CR, Aguilar DH, Vara PJE, Pescador PD, Amaro EI, *et al.* Immunoinformatic analysis to identify proteins to be used as potential targets to control bovine anaplasmosis. Int J Microbiol. 2020;2020:8882031.

31. Barrera MAI, Cossío BR, Gutiérrez PJA, Tello LAT, Preciado de la Torre JF, *et al*. Immunolocalization of Vir B11 protein in the *Anaplasma marginale* outer membrane and its reaction with bovine immune sera. Rev Mex Cienc Pecu 2018;9(4):769-791.

32. Brown WC, Zhu D, Shkap V, McGuire TC, Blouin EF, Kocan KM, *et al*. The repertoire of *Anaplasma marginale* antigens recognized by CD4(+) T-lymphocyte clones from protectively immunized cattle is diverse and includes major surface protein 2 (MSP-2) and MSP-3. Infect Immun 1998;66(11):5414-22.

33. Barigye R, Garcia OM, Rojas RE, Rodriguez SD. Identification of IgG2 specific antigens in three Mexican strains of *Anaplasma marginale*. Ann NY Acad Sci 2004;1026:84-94.

34. Rodríguez CSD, García OMA, Cantó AGJ, Hernández SG, Santos CN, Aboytes TR. Ensayo de un inmunógeno experimental inactivado contra *Anaplasma marginale*. Tec Pecu Mex1999;37(1):1-12.

35. Orozco VLE, Rodríguez SD, Cantó AG, López FR, Jiménez OR, García OM. *Anaplasma marginale* field challenge: protection by an inactivated immunogen that shares partial sequence of msp1α variable region with the challenge strain. Vaccine 2007;(25):519-525.

36. García OMA, Aboytes TR, Hernández SG, Cantó AJG, Rodríguez SD. *Anaplasma marginale*: Diferentes grados de virulencia en dos aislados mexicanos. Vet Méx 2000;31(2);157-160.

37. Rodríguez CSD, García OMA, Rojas REE, Cantó AGJ, Preciado TJF, Rosario C, *et al*. *Anaplasma marginale* Yucatan (Mexico) strain. Assessment of low virulence and potential use as a live vaccine. Annals NY Acad Sci. 2008;(1149):98-102.

38. Piercy PL. Transmission of anaplasmosis. Ann NY Acad Sci 1956;64:40-48.

39. Shimada MK, Yamamura MH, Kawasaki PM, Tamekuni K, Igarashi M, Vidotto O, *et al*. Detection of *Anaplasma marginale* DNA in larvae of *Boophilus microplus* ticks by polymerase chain reaction. Ann NY Acad Sci. 2004;(1026):95-102.

40. Amaro EI, García OMA, Preciado TJF, Rojas REE, Hernández OR, Alpírez MF, *et al*. Transmission of *Anaplasma marginale* by unfed *Rhipicephalus microplus* tick larvae under experimental conditions. Rev Mex Cienc Pecu 2020;11(1):116-131.

41. Brayton KA, Kappmeyer LS, Herndon DR, Dark MJ, Tibbals DL, Palmer GH, *et al*. Complete genome sequencing of *Anaplasma marginale* reveals that the surface is skewed to two superfamilies of outer membrane proteins. Proc Natl Acad Sci USA 2005;102(3):844-9.

42. Quiroz CRE, Amaro EI, Martínez OF, Rodríguez CSD, Dantán GE, Cobaxin CM, *et al*. Draft genome sequence of *Anaplasma marginale* strain Mex- 01-001-01, a mexican strain that causes bovine anaplasmosis. Microbiol Resour Announc. 2018;7(16):e01101-18.

43. Martínez OF, Quiroz CRE, Amaro EI, Dantán GE, Preciado Torre JF, Rodríguez CS. Whole-genome sequencing of Mexican strains of *Anaplasma marginale* an approach to the causal agent of bovine anaplasmosis. Int J Genomics 2020;2020:5902029.

44. Dark MJ, Lundgren AM, Barbet AF. Determining the repertoire of immunodominant proteins via whole-genome amplification of intracellular pathogens. PLoS One. 2012;7(4):e36456.

45. Díaz AE. Epidemiología de la brucelosis causada por *Brucella melitensis*, *B. suis* y *B. abortus* en animales domésticos. Revue Scientifique et Technique 2013;32(1):43-51.

46. Herrera LE, Hernández AL, Díaz AE. Study of brucellosis incidence in a bovine dairy farm infected with *Brucella abortus*, where cattle was revaccinated with RB51. International J Dairy Sci 2007;2(1):50-57.

47. Herrera LE, Palomares RG, Díaz AE. Milk production increase in a dairy farm under a six-year brucellosis control program. Ann New York Acad of Sci 2008;(1149):296-299.

48. Leal HM, Jaramillo ML, Hernández AL. Producción de interferón gamma en cultivos de sangre completa en respuesta a antígenos de *Brucella abortus* en bovinos vacunados con RB51. Téc Pecu Méx 2007;45(2):147-159.

49. Aparicio BA, Díaz AE, Hernández AL, Pérez GR, Alfonseca SE, Suárez GF. Evaluación serológica y bacteriológica de un hato bovino con brucelosis y revacunado con dosis reducida de *Brucella abortus* cepa 19. Téc Pecu Méx 2003;41(2):129-140.

50. Alton GG, Forsyth JRL. Brucellosis. Medical microbiology. INRA 2003;(28):512-525.

51. Muñoz PM, Marín CM, Monreal D, González D, Garin BB, Díaz R, Mainar JRC, Moriyón I, Blasco JM. Efficacy of several serological tests and antigens for diagnosis of bovine brucellosis in the presence of false-positive serological results due to *Yersinia enterocolitica* O:9. Clin Diagn Lab Immunol 2005;12(1):141-51.

52. Díaz AE, Marín C, Alonso UB, Aragón V, Pérez OS, *et al*. Evaluation of serological tests for diagnosis of *Brucella melitensis* infection of goats. J Clin Microbiol 1994;(32):1159-1165.

53. Ramirez PC, Díaz AE, Rodriguez PC, Morales LA, Alvarez OG, Gómez FR. Improved performance of *Brucella melitensis* native hapteno ver *Brucella abortus* OPS trace ron goat antibody detection by the fluorescence polarization assay. Vet Immun and Immunophatol 2008;123(3-4):223-229.

54. Arellano RB, Díaz AE, Leal HM, Hernandez L, Gorvel JP. Intracellular trafficking study of a RB51 *B. abortus* vaccinal strain isolated from cow milk. Vet Microbiol 2004;98(3-4):307-312.

55. Diaz AE, Hernández L, Suarez GF. Protection against brucelosis in goats, five years after vaccination with reduced-dose *Brucella melitensis* Rev-1 vaccine. Tropical Anim health and Prod 2004;3 (2) 117-121.

56. Cantú A, Díaz AE, Hernández AL, Adams GL, y Suárez GF. Estudio epidemiológico de un hato bovino con prevalencia media de brucelosis, vacunado con las mutantes rugosas de *Brucella abortus* RB51 y rfbk. Vet Mex 2007;38(2):197–206.

57. Fuentes DMD, Vitela MI, Arellano RB, Hernández CR, Morales AJF, Cruz VC. Presence of *Brucella abortus* vaccinal strain RB51 in vaginal exudates of aborted cows. Res J Dairy Sci 2007;1(1-4):13-17.

58. Leal HM, Díaz AE, Pérez R, Hernández L, Arellano RB, Alfonseca E, *et al*. Protection of *Brucella abortus* RB51 vaccine in cows introduced in a herd with active brucellosis, with presence of atypical humoral response. Comp Immunol Microbiol Infect Dis 2005;28(1):63-70.

59. Díaz AE, Arellano RB, Herrera LE, Leal HM, Suárez GF. Characterization of the transitory immune response in cows immunized with RB51 and its implication on diagnosis within brucellosis endemic zones. Intl. J. Dairy Sci 2007;2(4):364-371.

60. Gutiérrez JA, Casanova LG, Romero TC, Sosa GS, Cantó AG, Mercado PM, *et al.* Population structure of *Mycobacterium bovis* isolates from cattle in México. Prev Vet Med 2012;106(1):1-8.

61. Norma Oficial Mexicana NOM-031-ZOO-1995. Campaña Nacional Contra la Tuberculosis Bovina (*Mycobacterium bovis*).1995.

62. Díaz OF, Banda RV, Jaramillo ML, Arriaga DC, González SD Estrada, CC. Identificación de bovinos portadores de *Mycobacterium bovis* aplicando técnicas inmunológicas y moleculares. Vet Méx 2003;34(1):14-25.

63. Estrada CC, Díaz OF, Arriaga DC, Villegas SN, Pérez GR, González SD. Concordancia de la PCR y métodos rutinarios para el diagnóstico de la tuberculosis bovina. Vet Méx 2004;35(3):225-235.

64. Ramírez CIC, Santillán FMA, Arriaga DC, Arellano RB, Morales AJF. Empleo de la PCR-Multiplex para diferenciar caprinos vacunados con *M. bovis* BCG de infectados con *M. bovis* de campo. Tec Pecu Méx 2004;42(3):419-428.

65. Ramírez CIC, Santillán FMA, Arellano RB, Morales AJF, Tenorio GVR. Detección de secuencias nucleotídicas de *Mycobacterium bovis* a partir de ADN de moco nasal de caprinos inoculados experimentalmente. Vet Mex 2006;37(2):191-195.

66. González SDV, Díaz OF, Jaramillo ML, Pérez GR, Padilla UJ, Santillán FMA, *et al.* Evaluación de diferentes inmunógenos contra la tuberculosis bovina mediante presencia de lesiones a la necropsia. Vet Méx 2007;38(3):271-284.

67. Guzmán RCC, Santillan FMA, Córdova LD. Prevalence and possible risk factors for caprine paratuberculosis in intensive dairy production units in Guanajuato, Mexico. J Vet Med Anim Health 2016;8(11):156-162.

68. Martínez CAG, Santillán FMA, Guzmán RCC, Favila HLC, Córdova LD, Díaz AE, Hernández AL, Blanco OM. Desarrollo de un inmuno ensayo-enzimático (ELISA), para el diagnóstico de paratuberculosis en bovinos. Rev Mex Cienc Pecu 2012;3(1):1-18.

69. Milián SF, Santillán FMA, Zendejas MH, García CL, Hernández AL, Cantó AG. Prevalence and associated risk factors for *Mycobacterium avium* subsp. paratuberculosis in dairy cattle in Mexico. J Vet Med Anim Healt 2015;7(10):302-307.

70. Morón CFJ, Cortéz RC, Santillán FMA. Figueroa SB, Gallegos SJ. Prácticas de manejo asociadas con la seroepidemiología de paratuberculosis ovina en San Luis Potosí. Agroproductividad 2015;8(6):30-36.

71. Gallaga MEP, Arellano RB, Santillán FMA, Favila HLC, Córdova LD, Morales RJ, Díaz AE. Situación epidemiológica de la paratuberculosis en las principales regiones caprinas del Estado de Puebla, México. Quehacer Científico en Chiapas 2017;12(1):36-45.

72. Torres VR, Santillán FMA, Córdova LD, Martínez MOL, Guzmán RCC. Comparison of fluorescence polarization assay and enzyme-linked immunosorbent assay for the diagnosis of bovine paratuberculosis. J Vet Med Anim Health 2019;11(5):94-89.

73. Jaimes NG, Santillán FMA, Hernández COA, Córdova LD, Guzmán RCC, Arellano RB, *et al*. Detección de *Mycobacterium avium* subespecie paratuberculosis, por medio de PCR-anidada a partir de muestras de heces de ovino. Vet Méx 2008;39(4):377-386.

74. Panciera RJ, Confer AW. Pathogenesis and pathology of bovine pneumonia. Vet Clin Food Anim 2010;(26):191–214.

75. Rivera RJJ, Kisiela D, Czuprynski CJ. Bovine herpesvirus type 1 infection of bovine bronchial epithelial cells increases neutrophil adhesion and activation. Vet Immunol Immunopathol 2009;131(3-4):167-176.

76. Aguilar RF, Trigo TE, Jaramillo ML, Sánchez MH. Aislamiento de *Haemophilus somnus* a partir de pulmones neumónicos de bovinos. Téc Pecu Méx 1986;(52):67-73.

77. Trigo TFJ: El Complejo respiratorio infeccioso de los bovinos y ovinos. Ciencia Veterinaria 1987;(4):1-37.

78. Jaramillo ML, Aguilar RF., Trigo TF. Serotipificación de *Pasteurella haemolytica* y determinación de los tipos cápsulares de *Pasteurella multocida*, aisladas de pulmones neumónicos de becerros en México. Vet Méx 1987;(18):185-188.

79. Jaramillo ACJ, Hernández CR, Suárez GF, Martínez MJJ, Aguilar RF, Jaramillo ML, Trigo TFJ. Characterization of *Mannheimia* spp strains isolated from bovine nasal exudate and factors associated to isolates, in dairy farms in the Central Valley of México. Res Vet Sci 2008;84(1):7-13.

80. Salas TE, Aguilar RF, Trigo TF, Jaramillo ML. Sensibilidad de aislamientos de *Pasteurella haemolytica* y *Pasteurella multocida* aislados de bovinos y ovinos a varios agentes antimicrobianos. Téc Pecu Méx 1987;25(2):243-249.

81. Pijoán AP, Aguilar RF. Resistencia y sensibilidad a antimicrobianos en cepas de *Pasteurella haemolytica, P. multocida* y *Haemophilus somnus*, aisladas en becerras lecheras en establos de Tijuana. Vet Méx 2000; 31(2)154-156.

82. Méndez LM. Detección de leucotoxina en aislamientos de *Mannheimia haemolytica* obtenidos de exudados nasales y pulmones neumónicos de bovinos productores de leche [Tesis Licenciatura]. México, D.F: Universidad Nacional Autónoma de México; 2010.

83. Pérez RN. Estudio de la capacidad de producción de biopelícula y resistencia a antimicrobianos en cepas de *Pasteurella multocida*, *Mannheimia haemolytica* e *Histophilus somni* [Tesis licenciatura]. México, DF: Universidad Nacional Autónoma de México; 2010.

84. Fernández RMA, Vaca S, Reyes LM, de la Garza M, Aguilar RF, Zenteno E, *et al*. Outer membrane vesicles of *Pasteurella multocida* contain virulence factors. Microbiology Open 2014;3(5):711-717.

85. Morales AJF, Jaramillo ML, Oropeza VZ, Tórtora PJ, Espino RG. Evaluación experimental de un inmunógeno de *Pasteurella haemolytica* en corderos. Vet Mex 1993;24(2):97-105.

86. Aguilar RF, Jaramillo ML, Trigo TF, Suárez GF, Morales AF. Evaluación de la protección contra la pasteurelosis neumónica en corderos vacunados con diferentes antígenos de *Pasteurella haemolytica* A1. Vet Méx 1997;28(3):221-229.

87. Jaramillo ML, Aguilar RF, Suárez GF, Trigo TFJ. Challenge exposure of sheep immunized with live vaccine and culture supernatant of *Mannheimia haemolytica* A1: Effects of revaccination. Small Ruminant Res 2007;70(2-3):209-217.

88. Marquez A, Djelouadji Z, Lattard V, Kodjo A. Overview of laboratory methods to diagnose leptospirosis and to identify and to type leptospires. Int Microbiol 2017;20(4):184-193.

89. Victoriano AF, Smythe LD, Gloriani BN, Cavinta LL, Kasai T, Limpakarnjanarat K, *et al*. Leptospirosis in the Asia Pacific region. BMC Infect Dis 2009;(9):147. https://doi.org/10.1186/1471-2334-9-147

90. Chideroli RT, Gonçalves DD, Suphoronski SA, Alfieri AF, Alfieri AA, de Oliveira AG, *et al*. Culture strategies for isolation of fastidious *Leptospira* Serovar Hardjo and molecular differentiation of genotypes Hardjobovis and Hardjoprajitno. Front Microbiol 2017;(8):2155.

91. Haake DA, Levett PN. Leptospirosis in humans. Curr Top Microbiol Immunol 2015;387:65-97.

92. Barbosa C, Martins G, Lilenbaum W. Infectivity and virulence of leptospiral strains of serogroup Sejroe other than Hardjo on experimentally infected hamsters. Braz J Microbiol 2019;50(4):1129-1132.

93. Ellis WA. Leptospirosis as a cause of reproductive failure. Vet Clin North Am Food Anim Pract 1994;10(3):463-78.

94. Varela G, Roch E. Leptospirosis en la República Mexicana. Salud Públ Méx1965;7(2):189-193.

95. Luna AMA, Moles CLP, Gavaldón RD, Nava VC, Salazar GF. Estudio retrospectivo de seroprevalencia de leptospirosis bovina en México considerando las regiones ecológicas. Rev Cubana Med Trop 2005;57(1):28-31.

96. Cantú CA, Banda RVM. Seroprevalencia de leptospirosis bovina en tres municipios del sur de Tamaulipas. Téc Pecu Méx 1995;33(2):121-124.

97. Carmona GCA, León LL, Castillo SLO, Ramírez OJM, Ko A, Luna PC, *et al*. Detección de *Leptospira santarosai* y *L. kirschneri* en bovinos: nuevos aislados con potencial impacto en producción bovina y salud pública. Vet Méx 2011;42(4):277-288.

98. Moles CLP, Cisneros PMA, Gavaldón RD, Rojas SN, Torres BJI. Estudio serológico de leptospirosis bovina en México. Rev Cubana Med Trop 2002;54(1):24-27.

99. Segura CVM, Solis CJJ, Segura CJC. Seroprevalence of and risk factors for leptospiral antibodies among cattle in the state of Yucatan, Mexico. Trop Anim Health Prod 2003;35(4):293-299.

100. Banda RV, Orozco VL, Urrutia VR. Use of polymerase chain reaction for the identification of *Leptospira* sp. in urine of carriers. Rev Cubana Med Trop 2005;57(1):47-48.

101. Escamilla HP, Martínez MJJ, Medina CM, Morales SE. Frequency and causes of infectious abortion in a dairy herd in Queretaro, Mexico. Can J Vet Res 2007;(71):314-317.

102. Zárate MJP, Rosete FJV, Ríos UA, Barradas PFT, Olazarán JS. Prevalencia de leptospirosis y su relación con la tasa de gestación en bovinos de la zona centro de Veracruz. Nova Scientia 2015;7(14):202-217.

103. Ojeda CJJ, Espinosa AE, Hernández GPA, Rojas MC, Álvarez MJA. Seroprevalencia de enfermedades abortivas de bovinos. Ecosistemas y Recursos Agropecuarios 2016;3(8):243-249.

104. Banda RVM, Loza RE, Mejía SP. Eficiencia del hidróxido de aluminio, vitaminas liposolubles y levamisol, empleados en una bacterina de leptospira en vaquillas, para la generación de anticuerpos específicos. Téc Pecu Méx 1991;29(3):139-143.

105. Orozco VLE, López FR, Moles CLP, Quiroz VJ. Evaluación de una bacterina homóloga contra la leptospirosis bovina. Rev Cubana Med Trop 2005;57(1):38-42.

106. Estrada PA, García Z, Sánchez HF. The distribution and ecological preferences of *Boophilus microplus* (Acari: Ixodidae) in Mexico. Exp Appl Acarol 2006;38(4):307-316.

107. Gaxiola CS, García VZ, Cruz VC, Portillo LJ, Vázquez PC, Quintero MMT, *et al*. Comparison of efficiency and reproductive aptitude indexes between a reference and field strains of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* in Sinaloa, Mexico. Rev Bras Parasitol Vet 2009;18(4):9-13.

108. Fernández RM. Comparación de cuatro técnicas de colecta de larvas de *Boophilus microplus* bajo condiciones de campo en infestación controlada. Tec Pecu Mex 1996;34(3):175-182.

109. Fernandez RM, Cruz VC, Solano VJ, García VZ. Anti-tick effects of *Stylosanthes humilis* and *Stylosanthes hamata* on plots experimentally infested with *Boophilus microplus* larvae in Morelos, Mexico. Exp Appl Acarol 1999;23(2):171-175.

110. Cruz VC, Fernández RM, Solano VJ, García VZ. Anti-tick effect observed in mature plants of tropical legumes *Stylosanthes humilis* and *S. hamata*. Parasitol 1999;23(1-2):15-18.

111. Fernández RM, Preciado Torre JF, García VZ, Cruz VC, Saltijeral OJ. Evaluación estacional de la recuperación de larvas de *Boophilus microplus* en cuatro leguminosas forrajeras en parcelas experimentalmente infestadas. Tec Pecu Mex 2004;42(1):97-104.

112. Muro CF, Cruz-Vázquez C, Fernández-Ruvalcaba M, Molina-Torres J, Soria CJ, Ramos PM. Repellence of *Boophilus microplus* larvae in *Stylosanthes humilis* and *Stylosanthes hamata* plants. Parasitol Latinoam 2003;58(3-4):118-121.

113. Fernandez RM, Preciado TF, Cruz VC, Garcia VZ. Anti-tick effects of *Melinis minutiflora* and *Andropogon gayanus* grasses on plots experimentally infested with *Boophilus microplus* larvae. Exp Appl Acarol 2004;32(4):293-9.

114. Fernández RM, Zhioua E, García VZ. Infectividad de *Metarhizium anisopliae* en contra de cepas de garrapata *Boophilus microplus* sensible y resistente a los organofosforados. Tec Pecu Mex 2005;43(3):433-440.

115. Miranda ME, Cossio BR, Quezada DMR, Sachman RB, Reynaud E. *Staphylococcus saprophyticus* is a pathogen of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*. Biocontrol Sci Technol 2010;20(10):1055-1067.

116. Miranda ME, Cossio BR, Martínez IF, Casasanero OR, Folch J. Natural occurrence of lethal aspergillosis in the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Acari:Ixodidae). Parasitology 2012;139(2):259-263.

117. Miranda ME, Cossio BR, Tellez AM, García VZ, Rosario CR, Ortiz EM. An enzymatic marker for ixodicide resistance detection in the cattle tick *Boophilus microplus*. Agric Res 1995;(3):000-008.

118. Rosario CR, Miranda ME, García VZ, Ortiz EM. Detection of esterase activity in susceptible and organophosphate resistant strains of the cattle tick *Boophilus microplus* (Acari: Ixodidae). Bull Entom Res 1997;87(2):197-202.

119. Hernandez R, He H, Chen AC, Waghela SD, Ivie GW, George JE, Wagner GG. Identification of a point mutation in an esterase gene in different population of the southern cattle tick, *Boophilus microplus*. Insect Biochem Mol Biol 2000;30(10):969-977.

120. Hernandez R, Guerrero F, George JE, Wagner GG. Allele frequency and gene expression of a putative carboxylesterase-encoding gene in a pyrethroid resistant strain of the tick *Boophilus microplus*. Insect Biochem Mol Biol 2002;32(9):1009-1016.

121. Pruett JH, Guerrero FD, Hernandez R. Isolation and identification of an esterase from a mexican strain of *Boophilus microplus* (Acari: Ixodidae). J Econ Entomol 2002;95(5):1001-1007.

122. Guerrero FD, Li AY, Hernandez R. Molecular diagnosis of pyrethroid resistance in mexican strains of *Boophilus microplus*. J Med Entomol 2002;39(5):770-776.

123. Rosario CR, Guerrero FD, Miller RJ, Rodriguez VRI, Tijerina M, Dominguez GDI, *et al*. Molecular survey of pyrethroid resistance mechanisms in mexican field population of *Rhipicephalus* (*Boophilus*) *microplus*. Parasitol Res 2009;105(4):1145-1153.

124. Cossio-Bayugar R, Miranda-Miranda E, Ortiz-Najera A, Neri-Orantes S. *Boophilus microplus* pyrethroid resistance associated to increased levels of monooxygenase enzymatic activity in field isolated Mexican ticks. J Biol Sci 2008;8(2):404-409.

125. Cossio-Bayugar R, Miranda-Miranda E, Ortiz-Najera A, Neri-Orantes S, Olvera-Valencia F. Cytochrome P-450 monooxygenase gene expression supports a multifactorial origin for acaricide resistance in *Ripicephalus microplus*. Res J Parasitol 2008;3(2):59-66.

126. Soberanes CN, Santamaria VM, Fragoso SH, Garcia VZ. Primer caso de resistencia al amitraz en la garrapata del ganado *Boophilus microplus* en México. Tec Pecu Mex 2002;40(1):81-92.

127. Rosado AJA, Rodriguez VRI, Garcia VZ, Fragoso SH, Ortiz NA, Rosario CR. Development of amitraz resistance in field populations of *Boophilus microplus* (Acari: Ixodidae) undergoing typical amitraz exposure in the Mexican tropics. Vet Parasitol 2008;152(3-4):349-353.

128. Cossio BR, Miranda ME, Portilla SD, Osorio MJ. Quantitative PCR detection of cholinesterase and carboxylesterase expression levels in acaricide resistant *Rhipicephalus* (*Boophilus*) *microplus*. J Entomol 2009;6(2):117-123.

129. Ramírez RPB, Rosario CR, Domínguez GDI, Hernández GR, Lagunes QRE, Ortuño SD, *et al*. Identification of immunogenic proteins from ovarian tissue and recognized in larval extracts of *Rhipicephalus* (*Boophilus*) *microplus*, through an immunoproteomic approach. Exp Parasitol 2016;170:227-235.

130. Granjeno CG, Hernandez OR, Mosqueda J, Estrada MS, Figueroa JV, Garcia Vazquez Z. Characterization of a vitellogenin gene fragment in *Boophilus microplus* ticks. Ann NY Acad Sc 2008;1149(1):58-61.

131. Almazán C, Lagunes R, Villar M, Canales M, Rosario CR, Jongejan F, *et al*. Identification and characterization of *Rhipicephalus* (*Boophilus*) *microplus* candidate protective antigens for the control of cattle tick infestations. Parasitol Res 2010;(106):471-479.

132. Lugo CCS, Hernandez OR, Gomez RN, Martinez VM, Castro SE, Lagunes QR. Genetic diversity of the ATAQ gene in *Rhipicephalus microplus* collected in Mexico and implications as anti-tick vaccine. Parasitol Res 2020;(119):3523-3529.

133. Lagunes R, Dominguez D, Quiroz H, Martinez M, Rosario R. Potential effects on *Rhipicephalus microplus* tick larvae fed on calves immunized with a subolesin peptide predicted by epitope analysis. Trop Biomed 2016;33(4):726-738.

134. Merino CJO, Gómez RN, Barrera MI, Lagunes QR. Análisis *in silico* del gen subolesina como posible vacuna contra garrapatas *Rhipicephalus microplus*. Ecosistemas y Recur Agropecuarios 2019;6(16):129-136.

135. Álvarez JA, Figueroa JV. Desarrollo de una vacuna viva atenuada para el control de la babesiosis bovina en México. Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria. Reunión CENAPA. Morelos, México. 2005:8-15.

136. SIAP. Servicio de información agroalimentaria. 2019.

137. Álvarez JA, Cantó GJ. Epidemiología de la babesiosis. En: H. Quiroz editor Parasitología. Vol. Conmemorativo de la Sociedad Mexicana de Parasitología. S.C. México, D.F.; 1985:55-72.

138. Bock R, Jackson L, De Vos A, Jorgensen W. Bovine babesiosis. Parasitol 2004;(129): 247-269.

139. Rodríguez VRI, Grisi L, Pérez de León AA, Silva VH, Torres AJFJ, Fragoso SH, *et al*. Evaluación del impacto económico potencial de los parásitos del ganado bovino en México. Rev Mex Cienc Pecu 2017;8(1):61-74.

140. Rodríguez VR, Rivas AL, Chowell G, Fragoso SH, Rosario CR, García Z, *et al*. Spatial distribution of acaricide profiles *Boophilus microplus* strains susceptible or resistant to acaricides in southeastern Mexico. Vet Parasitol 2007;146(1-2):158-169.

141. Álvarez MJA, Rojas MC. Hematología diagnóstica. En: Campos RR y Bautista GR editores. Diagnóstico de helmintos y hemoparásitos de rumiantes. AMPAVE; 1989:145-158.

142. Bolio GME, Figueroa MJV, Álvarez MJA, Rojas MC, Vega MCA, López RM. Examen de laboratorio para parásitos de la sangre. En: Rodríguez-Vivas RI editores. Técnicas para el diagnóstico de parásitos con importancia en salud pública y veterinaria. AMPAVE-CONASA. México, D.F.; 2015:129-157.

143. Alvarez MJA, Rojas MC, Figueroa MJV. Diagnostic tools for the identification of *Babesia* sp. in persistently infected cattle. Pathogens 2019;8(3):143.

144. Canto AGJ, Figueroa MJV, Ramos AJA, Rojas EE, Garcia TD, Alvarez MJA, *et al*. Evaluation of cattle inoculated with *Babesia bovis* clones adhesive *in vitro* to bovine brain endothelial cells. Ann New York Acad Sci 2006;1081(1):397-404.

145. Nevils MA, Figueroa MJV, Turk JR, Canto AGJ, Le V, Ellersieck MR, *et al.* Cloned lines of *Babesia bovis* differ in their ability to induce cerebral babesiosis in cattle. Parasitol Res 2000;86(6):437-443.

146. Figueroa MJV, Alvarez MJA, Buening GM, Cantó AG, Hernandez OR, Monroy B, *et al*. Antibody Response to *Babesia bigemina* infection in calves measured ELISA and immunoblotting techniques. Rev Lat Amer Microbiol 1992;34(4):47-55.

147. Rojas RE, Domínguez P, García M, Cruz-Vázquez C, Figueroa MJV, Ramos AJA. Prevalencia e incidencia de *Babesia bovis* y *Babesia bigemina* en un hato bovino en Axochiapan, Morelos. Avan Invest Agropec 2004;8(2):1-8.

148. Figueroa MJV, Buening GM, Kinden DA, Green TJ. Identification of common surface antigens among *Babesia bigemina* isolates using monoclonal antibodies. Parasitol 1990;100(2):161-175.

149. Figueroa MJV, Buening GM, Kinden DA. Use of monoclonal antibodies for the identification of a common surface antigen of *Babesia bovis*. Ann NY Acad Sci 1998;849(1):433-437.

150. Figueroa MJV, Precigout E, Carcy, B, Gorenflot A. Identification of common antigens in *Babesia bovis, B. bigemina*, and *B. divergens*. Ann NY Acad Sci 2006;1081:382-396.

151. Figueroa MJV, Precigout E, Carcy B, Gorenflot A. Identification of a coronin-like protein in *Babesia* species. Ann NY Acad Sci 2006;1026(1):125-38.

152. Goff WL, Johnson WC, Molloy JB, Jorgensen WK, Waldron SJ, Figueroa MJ, *et al.* Validation of a competitive enzyme-linked immunosorbent assay for detection of *Babesia bigemina* antibodies in cattle. Clin Vaccine Immunol 2008;15(9):1316-1321.

153. Ushe TC, Palmer GH, Sotomayor L, Figueroa MJV, Buening GM, Perryman LE, *et al.* Antibody response to a *Babesia bigemina* rhoptry-associated protein 1 surface-exposed and neutralization-sensitive epitope in cattle. Infect Immun 1994;62(12):5698-5701.

154. Borgonio V, Mosqueda J, Genis AD, Falcon A, Alvarez JA, Camacho M, *et al*. msa-1 and msa-2c gene analysis and common epitopes assessment in Mexican *Babesia bovis* isolates. Ann NY Acad Sci 2008;1149(1):145-148.

155. Perez J, Perez JJ, Vargas P, Alvarez JA, Rojas C, Figueroa JV. Sequence conservation of the 12D3 gene in Mexican isolates of *Babesia bovis*. Transbound Emerg Dis 2010;57(1-2):57-60.

156. Figueroa MJV, Buening GM, Mishra V, McElwain TF. Screening of a *B. bigemina* cDNA library with monoclonal antibodies directed to surface antigens. Ann NY Acad Sci 1992b;(653):122-130.

157. Figueroa MJV, Lira AJJ, Vargas UP, Rojas MC, Alvarez MJA. Cloning and sequencing of the *rap-1α1* gene from Mexican isolates of *Babesia bigemina*. J Vet Sci Technol 2017;(8):4.

158. Palacios MJM. Comparación de la prueba de iELISA mediante el uso de las proteínas r12d3 y rRAP–1 como antígeno contra *Babesia bigemina*. [Tesis licenciatura]. México, Universidad Autónoma del Estado México; 2019.

159. Castañeda ARO, Rojas MC, Figueroa MJV, Álvarez MJA. Ensayo inmunoenzimático con antígeno recombinante MSA-1 para el diagnóstico de *Babesia bovis*, Memorias VIII Congr Int Epidemiol, León, Gto. 2013:275-279.

160. Castillo PIM, Lira A JJ, Castañeda ARO, Cantú CA, Mejía EF, Polanco MDJ, *et al*. Comparación de pruebas serológicas para el diagnóstico epidemiológico de babesiosis bovina transmitida por garrapatas. Entomol Mex 2017;4:611-616.

161. Figueroa MJV, Santamaria RM, Lira AJJ, Vargas UP, Castañeda ARO, Alvarez MJA, *et al*. Determination of the immunogenicity conferred in cattle by inoculation of *Babesia bigemina* recombinant antigens. J Vet Sci Technol 2018;9. doi:10.4172/2157-7579-C2-039.

162. Alvarez MJA, Lopez U, Rojas MC, Borgonio VM, Sanchez V, Castaneda ARO, *et al.* Immunization of *Bos taurus* steers with *Babesia bovis* recombinant antigens MSA-1, MSA-2c and 12D3. Transbound Emerg Dis. 2010;57:87-90.

163. Reyes SRM, Bautista GCR, Castañeda ARO, Vargas U P, Álvarez MJA, Rojas MC, et al. Babesiosis: Field assessment of protection in cattle immunized with a mixture of *Babesia bovis* recombinant proteins. Quehacer Científico en Chiapas 2016;11(2):36-46.

164. Santamaria RM, Lira AJJ, Vargas UP, Álvarez MJA, Rojas MC, Figueroa MJV. Validation of an indirect ELISA using recombinant proteins as antigen to identify animals exposed to *Babesia bigemina*. Transbound Emerg Dis 2020;67(S2):201-207.

165. Aboytes TR, Buening GM, Figueroa MJV, Vega MCA. El uso de zonas de ADN para el diagnóstico de hemoparásitos. Rev Cubana Cienc Vet 1991;22(3):173-181.

166. Figueroa MJV, Buening GM. Nucleic acid probes as a diagnostic method for tick-borne hemoparasites of veterinary importance. Vet Parasitol 1995;57(1-3):75-92.

167. Ramos AJA, Alvarez MJA, Figueroa MJV, Solis J, Rodriguez VRI, Hernandez OR, *et al*. Evaluation of the use of a *Babesia bigemina* DNA probe in an epidemiological survey. Mem Inst Oswaldo Cruz 1992;87(3):213-217.

168. Figueroa MJV, Chieves LP, Johnson GS, Buening GM. Detection of *Babesia bigemina*-infected carriers by polymerase chain reaction amplification. J Clin Microbiol 1992;30(10):2576-2582.

169. Figueroa MJV, Chieves LP, Johnson GS, Goff WL, Buening GM. Polymerase chain reaction-based diagnostic assay to detect cattle chronically infected with *Babesia bovis*. Rev Lat Amer Microbiol 1994;36(1):47-55.

170. Buening GM, Aboytes TR, Figueroa MJV, Allen LW. A PCR amplification/DNA probe assay to detect *Anaplasma marginale* carriers. Proc. 96th Ann Meet US Anim Health Assoc. Louisville, Kentucky. 1992:287-294.

171. Figueroa MJV, Chieves LP, Johnson GS, Buening GM. Multiplex polymerase chain reaction assay for the detection of *Babesia bigemina*, *Babesia bovis* and *Anaplasma marginale* DNA. Vet Parasitol 1993;50(1-2):69-81.

172. Figueroa MJV, Alvarez MJA, Ramos AJA, Vega MCA, Buening GM. Use of a multiplex PCR assay to diagnose hemoparasite-infected bovine carriers in Mexico. Revue Élev Méd vét Pays trop 1993;46(1-2):71-75.

173. Alvarez MJA, Ramos AJA, Figueroa MJV, Mosqueda GJJ, Vega MCA, Buening GM. Descriptive epidemiology of anaplasmosis and babesiosis in cattle farms from Campeche Mexico. 75th Ann Meet CRWAD. Chicago, Ill. 1994:56.

174. Figueroa MJV, Alvarez MJA, Canto AGJ, Ramos AJA, Mosqueda GJJ, Buening GM. Comparative sensitivity of two tests for the diagnosis of multiple hemoparasite infection of cattle. Ann NY Acad Sci 1996;791(1):117-127.

175. López M, Figueroa MJV, Ramos AJA, Mosqueda GJJ, Rojas, REE, Vega MCA, *et al*. Infection and seroconversion of susceptible animals introduced into a babesiosis endemic area. Ann NY Acad Sci 2008;1149(1):131-135.

176. Figueroa MVJ, Cantó AGJ, Álvarez MJA, Lona R, Ramos AJA, Vega MCA. Capacidad protectora en bovinos de una cepa de *Babesia bigemina* derivada del cultivo *in vitro*. Téc Pecu Méx 1998;(36):95-107.

177. Sparagano OAE, Allsopp MTEP, Mank RA, Rijpkema SGT, Figueroa MJV, Jongejan F. Molecular detection of pathogen DNA in ticks: A review. Exp Applied Acarol 1999;23(12):929-960.

178. Rojas RE, Mosqueda GJJ, Álvarez MJA, Hernández OR, Ramos AJ, Rojas MC, *et al*. Transmissibility of *Babesia bigemina* and *Babesia bovis* attenuated strains by *Rhipicephalus microplus* ticks. Rev Mex Cienc Pecu 2011;2(3):267-281.

179. Figueroa MJV, Lira JJ, Polanco MDJ, Álvarez MJA, Rojas MC, Bautista GCR. Diferenciación de *Babesia bovis* y *Babesia bigemina* mediante el uso de una prueba molecular en ADN extraído de garrapatas repletas. Entomol Mex 2015;(2):706-713.

180. Bock RE, de Vos AJ, Lew A, Kingston TG, Fraser IR. Studies on failure of T strain live *Babesia bovis* vaccine. Aust Vet J 1995;72(8):296-300.

181. Shkap V, de Vos AJ, Zweygarth E, Jongejan F. Attenuated vaccines for tropical theileriosis, babesiosis and heartwater: the continuing necessity. Trends Parasitol 2007;(23):420-426.

182. Shkap V, Kocan K, Molad T, Mazuz M, Leibovich B, Krigel Y, *et al*. Experimental transmission of field *Anaplasma marginale* and the *A. centrale* vaccine strain by *Hyalomma excavatum*, *Rhipicephalus sanguineus* and *Rhipicephalus* (*Boophilus*) annulatus ticks. Vet Microbiol 2009;134(3-4):254-260.

183. Figueroa MJV, Cantó AGJ, Juárez FJ, Ruiz LF. Cultivo *in vitro* de *Babesia bovis*: establecimiento y condiciones óptimas de multiplicación. Téc Pecu Méx 1984;(46):46-52.

184. Monroy BM, Romero OG, Torres AR, Álvarez MJA, Canto AGJ, Vega MCA. Establecimiento en México del cultivo *in vitro* de *Babesia bigemina*. Téc Pecu Méx 1987;(25):141-50.

185. Hernández OR, Álvarez MJA, Buening GM, Cantó AGJ, Monroy BM, Ramos AJA, *et al*. Diferencias en la virulencia y en la inducción de protección de aislamientos de *Babesia bigemina* derivados de cultivo *in vitro*. Téc Pecu Méx 1990;28(2):51-61.

186. Cantó AGJ, Figueroa MJV, Álvarez MJA, Ramos AJA, Vega MCA. Capacidad inmunoprotectora de una clona irradiada de *Babesia bovis* derivada del cultivo *in vitro.* Téc Pecu Méx 1996;34(3):127-135.

187. Figueroa MVJ, Cantó AGJ, Álvarez MJA, Lona R, Ramos AJA, Vega MCA. Capacidad protectora en bovinos de una cepa de *Babesia bigemina* derivada del cultivo in vitro. Téc Pecu Méx 1998;36:95-107.

188. Vega MCA, Figueroa MJV, Rojas REE, Ramos AJA, Cantó AGJ. Insuficiente inmunidad cruzada en bovinos por *Babesia bigemina* y/o *Babesia bovis* derivadas del cultivo *in vitro*. Téc Pecu Méx 1999;37(1):13-22.

189. Cantó AG, Figueroa MJV, Ramos AJ, Álvarez MJA, Mosqueda GJJ, Vega MC. Evaluación de la patogenicidad y capacidad protectora de un inmunógeno fresco combinado de *Babesia bigemina* y *B. bovis*. Vet Méx 1999;30(3):215-20.

190. Alvarez MJA, Ramos AJA, Rojas RE, Mosqueda GJJ, Vega MCA, Olvera MA, *et al*. Field challenge of cattle vaccinated with a combined *Babesia bovis* and *Babesia bigemina* frozen immunogen. Ann NY Acad Sci 2004;1026(1):277-283.

191. Ojeda JJ, Orozco Flores R, Rojas C, Figueroa JV, Alvarez JA. Validation of an attenuated live vaccine against babesiosis in native cattle in an endemic area. Transboun Emer Dis 2010;57(1-2):84-86.

192. Bautista GCR, Lozano AR, Rojas MC, Alvarez MJA, Figueroa MJV, García GR, *et al*. Co-immunization of cattle with a vaccine against babesiosis and *Lactobacillus casei* increases specific IgG1 levels to *Babesia bovis* and *B. bigemina*. Parasitol Int 2015;64(5):319-323.

193. Rojas MC, Rodriguez VRI, Figueroa MJ, Acosta VKY, Gutiérrez RJ, Alvarez MJ. *In vitro* culture of *Babesia bovis* in a bovine serum-free culture medium supplemented with insulin, transferrin, and selenite. Exp Parasitol 2016;(170):214-219.

194. Rojas MC, Rodriguez VRI, Figueroa MJV, Acosta VKY, Gutiérrez REJ, Alvarez MJA. Putrescine: essential factor for *in vitro* proliferation of *Babesia bovis*. Exp Parasitol 2017;(175):79-84.

195. Rojas MC, Rodriguez VRI, Figueroa MJV, Acosta VKY, Gutiérrez REJ, Bautista GCR, *et al*. *Babesia bigemina*: advances in continuous *in vitro* culture using serum free medium, supplemented with insulin, transferrin, selenite and putrescine. Parasitol Int 2018;67(3):294-301.

196. Rojas MC, Rodriguez VRI, Figueroa MJV, Bautista GCR, Castaneda ARO, Lira AJJ, *et al*. Bovine babesiosis: Cattle protected in the field with a frozen vaccine containing *Babesia bovis* and *Babesia bigemina* cultured *in vitro* with a serum-free medium. Parasitol Int 2018;67(2):190-195.

197. Brown WC, Palmer GH. Designing blood-stage vaccines against *Babesia bovis* and *B. bigemina*. Parasitol Today 1999;15(7):275-281.

198. Álvarez, M.J.A, Figueroa, MJV, Ueti, MW, Rojas MC. Innovative alternatives for continuous *in vitro* culture of *Babesia bigemina* in medium free of components of animal origin. Pathogens 2020;9(5):343.

199. Alvarez MJA, Rojas MC, Figueroa MJV. An Overview of current knowledge on *in vitro Babesia* cultivation for production of live attenuated vaccines for bovine babesiosis in Mexico. Front Vet Sci 2020;(7):364. doi:10.3389/fvets.2020.00364.

200. Mackenzie SJ, Jeggo M. The one health approach. Why is it important? Trop Med Infect Dis 2019;4(2):88.