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Bovine Anaplasmosis: Diagnosis, Treatment and Control Strategies

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INTRODUCTION

Anaplasmosis is an infectious disease that affects bovines when the pathogen, *Anaplasma (A.) marginale*, is transmitted from a carrier animal to a healthy (free) one (Sarli et al. 2021). Clinical presentation is characterized by fever, anemia, a reduction in food intake and, therefore, decrease in meat and milk production (Khan et al. 2022). However, clinical signs are not always evident but, in acute phase, adult cattle may be severely affected causing a high mortality rate while recovered cattle as well as infected calves become carriers for life and a source of distribution, presenting rickettsemia cycles every 6 to 8 weeks (French et al. 1998). Transmission is made by ticks, flies, horseflies and fomites. This is a crucial point as the disease is one of the most common and important vector-borne diseases in bovine production (Amaro-Estrada et al. 2020; Bautista-Garfias et al. 2021). Early diagnosis allows a better control and reduction of economic losses (Railey and Marsh, 2021). Even though there is not a universal vaccine commercially available, but there are several approaches in curse around the world to develop effective immunogens (Salinas-Estrella et al. 2022b) and many work still needs to do in order to generate better control strategies that contribute to improve bovine, human and environmental health.

Etiological Agent

Bovine anaplasmosis is caused by *A. marginale*, which is a gram negative intracellular obligate pathogen belonging to the order *Rickettsiales* which infects bovine erythrocytes (Kocan et al. 2010). It was first described by Sir Arthur Theiler (1910a,b) as "marginal points" in erythrocytes, therefore given the name "*marginale*" based on its location (Theiler 1908, 1910a, 1910b). Previously, it was believed that *A. marginale* was some developing state of *Babesia* spp. but later Theiler proved, by isolation and replication of the pathogen, that it was an entirely different microorganism (Theiler 1908, 1911).

For a while, any intracellular bacteria were considered among the term rickettsiae, despite any other characteristic; later, classification based on phenotypical, morphological, ecological, epidemiological and clinical characteristics followed (Drancourt and Raoult 1994) and then some molecular studies brought more clarity over this matter. A few years ago, taxonomic analysis based on the information of several molecular markers, antigenic characteristics, as well as pathogeny, helped to confirm and define its classification as member of one of the two families that belong to Rickettsiales order: Ricketssiae and Anaplasmataceae (Dumler et al. 2001). Members of the second family include the genus *Anaplasma*, *Ehrlichia*, *Wolbachia* and *Neorickettsia*, several species of *Anaplasma* genus that infect animals (*A. marginale*, *A. marginale* subspecie *centrale*, *A. ovis*, *A. bovis*, *A. platys*) and a specie that may infect humans as well as several mammals, (*A. phagocytophilum* which was formerly known as *Ehrlichia phagocytophila*, *E. equi* and the human granulocytic ehrlichiosis (HGE) agent) (Drancourt and Raoult 1994; Dumler et al. 2001).

Bovine anaplasmosis is produced when the microorganism enters the circulatory system and replicates within the erythrocytes. Genetic diversity (de la Fuente et al. 2005, 2007; Rodríguez et al. 2009; Castañeda-Ortiz et al. 2015) may influence the severity of the signs producing an infection that can be mild to severe according to the immune status of the animals and the virulence of the strain (García-Ortíz et al. 2000; Rodríguez-Camarillo et al. 2008; Rodríguez et al. 2009; Kocan et al. 2010).

Epidemiology

A. marginale is commonly found in tropical and subtropical regions around the world, which have the perfect conditions for developmental cycle of biological and mechanical vectors of the pathogen but, due to climate change, it can also be found on template regions because different species of vectors are prevalent in that climatic region (Kocan et al. 1992; Kocan et al. 2004). Prevalence of this disease has been

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reported in several countries of all continents, either by serologic or molecular analysis (Bautista-Garfias et al. 2021). The presence of the pathogen is associated with the presence of ticks –the biological vector– or flies –one of the mechanical vectors– (Scoles et al. 2005). Approximately 30 tick species have been reported as vector of bovine anaplasmosis, including *Rhipicephalus* (*R.*) *microplus*, *R. decoloratus*, *R. simus*, *R. evertsi evertsi*, *Dermacentor* (*D.*) *andersoni*, *D. variabilis*, *D. reticulatus*, *D. albipictus* and *Hyalomma* (*H.*) *marginatum rufipes* (Stiller et al. 1989; Samish et al. 1993; Jongejan and Uilenberg 2004; Kocan et al. 2004; Scoles et al. 2005, 2008; Zivkovic et al., 2007; Hove et al. 2018; Khan et al. 2022).

Other factors that may contribute to prolong the presence of *Anaplasma sp*. circulation include the possibility of infection to wild ruminants (Kocan et al. 2010), mobilization and introduction of infected cattle to a free herd, and some malpractice while doing procedures with contaminated instruments (Reinbold et al. 2010a; Zabel and Agusto 2018; Abdisa 2019; Bautista-Garfias et al. 2021).

The clinical effects of disease are more frequent in adult animals mobilized from a non-endemic herd to an endemic herd. Calves are often resistant to the pathogen presenting clinical sings of disease in rare occasions while adults are the group that present higher mortality rates at first contact with the pathogen (Rodríguez et al. 2009).

Life Cycle

Once *A. marginale* infect the erythrocytes, it replicates within them from one initial body to eight forming a type of morulae called inclusion body. The initial bodies contained in the inclusion body are released and multiply sequentially in other erythrocytes until high percentages of infection are reached. The initial bodies do not cause lysis of the erythrocytes when exit the bovine cells (Rodríguez-Camarillo et al. 2008). Prepatent period ranges from 7 to 60 days producing as many as 70% parasitezed red blood cells during acute presentation (Kocan et al. 2010). Instead of being destroyed by lysis in the blood vessels, macrophages in the spleen detect infected erythrocytes, and produces a diminution on the circulating cells (Kocan et al. 2004; Rodríguez et al. 2009). After acute phase has passed, bovines that recovers become carriers and may be a source of infection for other animals (Kocan et al. 2010).

When ticks feed from infected cattle they acquire the initial bodies present in erythrocytes (Fig. 1) and, once in the midgut of the tick, the initial bodies are released and infect the cells of the intestinal epithelium where they begin a cycle of development (Kocan et al. 2004). One (or more) cycles of *A. marginale* development in tick intestinal cells can pass and other tick tissues become infected (Cobaxin-Cárdenas et al. 2019), including salivary glands from where the pathogen is transmitted to vertebrates during feeding (Kocan et al. 2004). The first form of *A. marginale* seen within the colony is the reticulate (vegetative) form that divides by binary fission forming large colonies that may contain hundreds of organisms. The reticulate forms change to dense forms, which is the infective form and can survive outside the cells. Cattle become infected with *A. marginale* when the dense form is transmitted during tick feeding through salivary glands (Kocan et al. 2004).

Transmission

Anaplasma transmission in cattle occurs both biologically and mechanically. Biological transmission requires feeding of ticks on carrier animals while in mechanical transmission there is no multiplication of the bacteria in the vector and only infected blood is transferred from one animal to another (Foil, 1989; Kocan et al. 1981, 2010).

Biological Transmission

Several tick species have been associated with *Anaplasma* transmission, however, *D. andersoni* and *Rhipicephalus spp.* are the most studied (Kocan et al. 1992; Futse et al. 2003; Scoles et al. 2008). Transmission of *A. marginale* by *Dermacentor spp., R. microplus* and *R. annulatus* have already been reported (Kocan et al. 2004). Biological transmission can be transstadial, from one stage to another, or intrasstadial, within the same stage (Stich et al. 1989; Samish et al. 1993; Ribeiro and Lima, 1996; Cobaxin-Cárdenas et al. 2019; Vimonish et al. 2020).

The relationship of *A. marginale* with ticks is complex and presents variations among different strains, vectors and environmental conditions. For example, it has been shown experimentally that only 10 infected ticks (*Dermacentor*) are enough to transmit *A. marginale* (Scoles et al. 2005). However, transmission has been experimentally described through *R. simus* ticks but was not achieved with *H. excavatum, R. sanguineus or R. annulatus.* Additionally, not all strains of *A. marginale* are transmitted efficiently by ticks (de la Fuente et al. 2001).

Intrasstadial and transstadial transmission are mainly important in three-host ticks, because they leave their host at the end of each stage. Until a while ago, it was considered that transovarial transmission of *A. marginale* did not occur in ticks; otherwise it has been reported that *R. microplus* larvae hatched from infected females were able to transmit the bacteria to cattle despite not having been fed previously. Transmission was confirmed molecularly by monitoring the variable region of *msp1a* (Amaro-Estrada et al. 2020) (Fig. 2). There are still several aspects to investigate, for example, temperature and strains involved seem to be relevant issues; but it is important to consider transovarial transmission in control strategies of bovine anaplasmosis.

Another form of transmission in cattle is transplacental transmission, which may occur during gestation, when the carrier cows suffer immunosuppression and an increase in rickettsemia occurs, allowing the bacteria to cross the placental barrier (Gale et al. 1996; Zabel and Agusto 2018). It has also been observed that some calves show clinical signs and do not survive, but others do not show signs but remain as reservoirs (Rey-Valeirón et al. 2003).

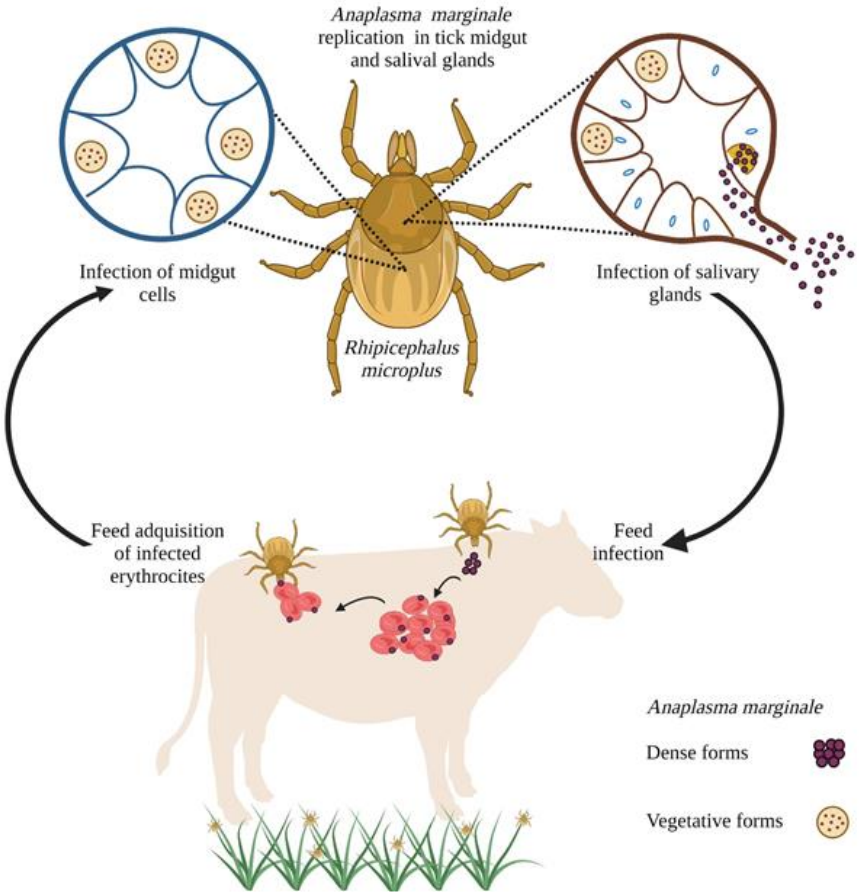

Fig. 1: Life cycle of *A. marginale* (made in Biorender.com under de license ZF250QYQMI).

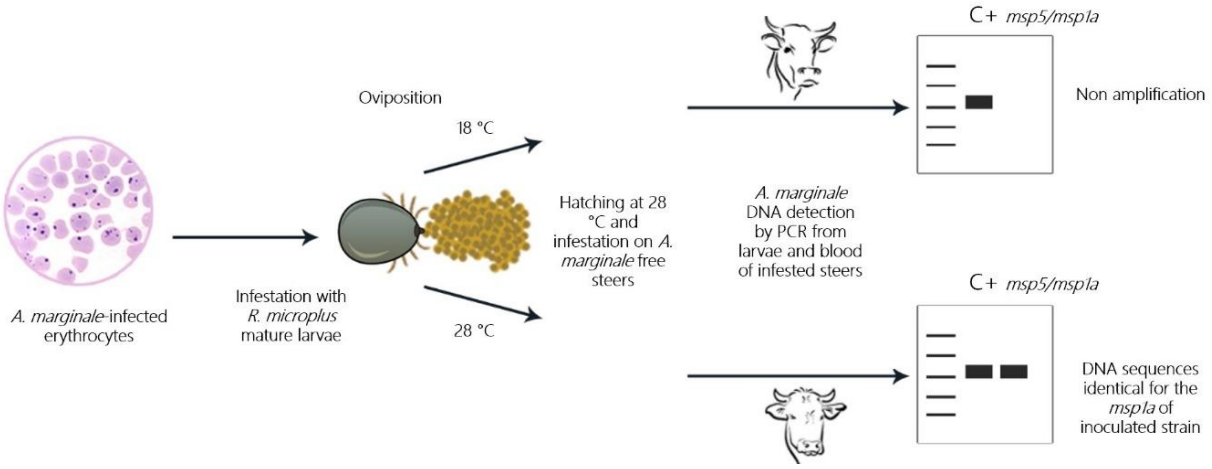

Fig. 2: Representation of transovarian transmission of *A. marginale* under controlled conditions.

Mechanical Transmission

Mechanical transmission of anaplasmosis occurs through contaminated fomites or when blood is transferred from one host to another. While blood sucking flies have shown a role of apparent little importance in the transmission of *A. marginale*, they can also participate in the horizontal transmission of bovine anaplasmosis in the absence of ticks (Kocan et al. 2010). Blood sucking flies are important as mechanical vectors of *A. marginale*, and by themselves as they feed on cattle's blood and lower the conversion efficiency due to disturbance in behavior of animal (Scoles et al. 2005).

Hematophagous arthropods such as *Tabanus bovinus and T. fuscicostatus* (horseflies), *Haematobia irritans* (horn fly) and *Stomoxys calcitrans* (stable fly) have been associated with infection, but appear to be at least 100 times less efficient than ticks fed on infected animals (Scoles et al. 2005; 2008). However, that low efficiency can be compensated by the large number of flies that can infest cattle (Bautista-Garfias et al. 2021).

Within the mechanical transmission, human malpractices does happen and probably accounts for many of the cases observed in the absence of ticks or where flies are under control. Vaccination with a single syringe and same needle, insertion of hormone implants and ear-tagging are customary practices in places where there are many animals (Kocan et al. 2010). It has been proven that needles used in procedures such as vaccinations are capable of transmitting *Anaplasma* from one infected animal to two subsequent ones (Reinbold et al. 2010a).

Bovine Immune Response

Infected erythrocytes are recognized by macrophages in reticular tissues and vascular sinusoids present in the red pulp of the spleen. The optimal capture activity by macrophages occurs until high concentrations of infected erythrocytes are reached (Rodríguez et al. 2008; Salinas-Estrella et al. 2022b). Non-specific phagocytosis has been demonstrated in the spleen and may explain splenomegaly present in the infections (Palmer et al. 1999).

When the erythrocyte is phagocytized, it initiates antigen processing by denaturing and partially digesting pathogen proteins into short peptides that are presented on MHC class II molecules on the macrophage surface to CD4+ T-helper (Th) cells (Brown et al. 1998a). Th cells involved in the immune response include Th1 cells, specialized in the production of IL2 and interferon gamma, which stimulate the synthesis of IgM and IgG2 synthesis by B cells and activate macrophages (Zaraza and Kuttler, 1971; Ristic and Carson, 1977; Arulkanthan et al. 1999; Rodríguez-Camarillo et al. 2008; Silvestre et al. 2018). However, there is also a response of Th2 cells that secrete IL4, IL6 and IL10. The cytokines also stimulate B cells towards the production of IgG1 (Rodríguez et al. 2008; Kocan et al. 2010). B cells can also be activated by recognizing an immunogen in its free, untransformed form through their antigen receptors or surface antibodies (Brown et al. 1998b; Zhao et al. 2016).

Studies on the different capacities of bovine immunoglobulin G isotypes indicate that IgG2 is related to the resolution of infectious processes (McGuire et al. 1991). This capacity has been confirmed in the case of bovine anaplasmosis, since these antibodies from immune animals are able to opsonize infected erythrocytes and be more rapidly phagocytized (Cantor et al. 1993), unlike IgG1 which is not able to mediate phagocytosis by neutrophils or monocytes (McGuire et al. 1991; Brown et al. 1998a).

Cattle that recover from acute infection remain infected with low rickettsemia, but do not show severe clinical signs of the disease, so they are considered protected against a homologous challenge (Palmer et al, 1999; Rodríguez-Camarillo et al, 2008), except when there is a concomitant disease inducing a state of immunosuppression. The persistence of the parasite occurs despite the development of a protective immune response (French et al. 1998).

Diagnosis

Clinical diagnosis is based on the anamnesis, clinical signs, presence of vectors, cattle management practices, time of the year, and other aspects considered in the clinical history. Early diagnosis may improve the possibility of a good outcome and reduce the economic losses in an affected herd (Railey and Marsh, 2021). The disease is characterized by fever, lethargy, isolation, anemia, and a diminution of food intake, which, altogether, translates into a drop in the production (either meat or milk), affecting reproductive activity due to the effects of a low energy and oxygen transport (Rodríguez-Camarillo et al. 2008; Quiroz-Castañeda et al. 2016; Railey and Marsh, 2021).

When a presumptive diagnosis is made then it is necessary to confirm it with laboratory tests. The first option is microscopic observation of the pathogen in blood smears stained with Giemsa. Disadvantages of this technique include; 1) the person analyzing the smear should have expertise in it 2) moment of the cycle during which sample is analyzed as a sample taken at the beginning or the end of the infection may not be quantifiable (Barigye et al. 2004; Kocan et al. 2010; Railey and Marsh, 2021).

Serological tests are recommended in different scenarios: a) for monitoring the development of antibodies at first infection or immunization; b) for epidemiological purposes: either when the herd is stable and located at an enzootic region, or when an outbreak occurred; c) in occasions when trading exchange requires it in order to mobilize only free of contact bovines (Rodríguez Camarillo et al. 2008; Rodríguez et al. 2009; Salinas-Estrella et al. 2022a).

The molecular diagnosis facilitates the identification of pathogen, when the infection is not quantifiable at microscope. It also allows the procurement of DNA for genomic identification. Several genes are used as target for molecular amplification by PCR including *msp5* gene as the elected gene for standard detection, followed by *msp1a*, *msp4* (Torioni de Echaide et al. 1998; Rodríguez et al. 2009; Singh et al. 2012; Bacanelli et al. 2014; Amaro-Estrada et al. 2020). Other molecular test have been developed for *A. marginale* detection, such as multiplex PCR (Figueroa et al. 1993), qPCR (Carelli et al. 2007), duplex qPCR (Decaro et al. 2008), PCR followed by restriction fragment length polymorphisms (PCR-RFLP) (Noaman et al. 2009), reverse line-blot hybridization (RLBH) (Guillemi et al. 2015) and loop-mediated isothermal amplification (LAMP) (Giglioti et al. 2019).

Control Strategies

Control of bovine anaplasmosis includ the actions towards control of tick-vector and immunoprophylaxis. The eradication of ticks has not been very practical as intense dipping or spraying with acaricides has resulted in resistance and failure in effectivity (Rodriguez-Vivas et al. 2017). According to some studies, rotational grazing is a controversial practice as it works best if pastures are left fallow for at least 60 days (Nicaretta et al. 2020), while some other studies proved that its success depends on extensive knowledge of local climatic conditions, plus robust data on seasonal peaks in tick populations (Hüe et al. 2021). Therefore, to be successful, it is important to apply grazing management whenever there is sufficient robust data from the area (Hüe et al. 2021).

Horizontal transmission due to blood transfer from one animal to another by flies can be reduced by a number of management practices, such as the use of repellents, insecticides, biopesticides, manure management, traps, etc. (Scoles et al. 2005; Cook 2020).

Iatrogenic transmission is one of the most unattended forms of dispersion of diseases. Changing needles between animals, disinfection of ear-taggers and other tools that get in contact with blood are the most simple and economical practices to curtail this form of horizontal transmission (Reinbold et al. 2010a).

On the immunoprophylaxis spectrum, there have been many attempts to induce protection against disease. "Premunition" is described as the inoculation of whole or diluted blood into adult or young animals (Todorovic et al. 1975). Inoculation of blood from animals with ongoing clinical disease is a dangerous option and close monitoring is critically important as it usually induces a severe clinical syndrome which may be fatal. Diluted blood from an infected carrier or a patent animal can be used, but the number of infected erythrocytes is unknown and the monitoring period varies from one inoculation to the next (Todorovic et al. 1975). Best results from practice are observed when calves between the ages of 6 and 9 months are the recipients of the infected blood (Kuttler 1979). Animals immunized through this way usually develop a sub-clinical syndrome that protects the recipient as long as the animal remains infected. The procedure should preferably be carried with local strains. *A. centrale* has been used in many countries but in México, it is banned as it is considered as an exotic pathogen (Rodríguez et al. 2009). Close monitoring in clinical variables but specifically packed cell volume (PCV) and vaginal mucosa coloration (VMC) in dairy calves, correlate with presence of bovine anaplasmosis, thus these parameters can be used as guide to apply timely treatment and significantly reduce losses in valuable dairy stock (Heller et al. 2022).

Treatment of bovine anaplasmosis can be done with two basic antibiotics, oxytetracycline and imidocarb dipropionate (Coetzee et al. 2005; Reinbold et al. 2010b; Sarli et al. 2021).

Vaccine Developments

The first description of *A. centrale* as a live immunogen (Theiler 1911) demonstrated that it can ameliorate the clinical signs of *A. marginale* infection. This strain has been used in many countries for the prevention of bovine anaplasmosis (usually as trivalent including *Babesia* (*B.*) *bovis, B. bigemina and A. centrale*). There are 23 complete genome sequences of *A. marginale* available at NCBI which can be used to design a vaccine that can be applied in animals of all ages and protect against many strains that have been reported yet (Rodríguez-Camarillo et al. 2020). Attenuation of naturally pathogenic strains was one of the first efforts to generate immunity, but some of these strains were passed into "unnatural" hosts such as deer or sheep with variable results (Zaraza and Kuttler, 1971; Kuttler and Zaugg, 1988). Strains of *A. marginale* of naturally low virulence have also been tested (Bock et al. 2003; Rodríguez-Camarillo et al. 2008) with promising results, yet not a single one of these options has reached the commercial use, because of the ever-present risk of transmission of blood associated pathogens with implicit disastrous results (Rogers et al. 1988). Inactivated vaccines tested so far while inducing solid immunity to homologous challenge, are yet to induce the same level of protection against heterologous strains (Salinas-Estrella et al. 2022b). Proteins that in their native form have induce solid immunity have failed to induce immunity as recombinant counterparts (Sarli et al. 2020) and even in some cases, animals inoculated with them showed worse clinical syndromes (Ducken et al. 2015). An ear implant was designed to deliver *Msp1a* tandem repeat K;S as the antigen in a scheme that comprised three vaccination doses (prime-boost like) (Curtis et al. 2020).

As mentioned before, live organisms would seem to induce complete immunity against homologous and heterologous challenge. Based on this premise, targeted mutagenesis was used for deletion of the phage head-to-tail connector protein (phtcp) gene in *A. marginale* (that is cultivable on tick cells) (Hove et al. 2022). The resulting mutant did not cause disease and exhibited attenuated growth in its natural host (cattle). When its ability to confer protection against wild-type *A. marginale* was challenged, all animals receiving the live mutant did not develop clinical signs or anemia, or erythrocyte infection. In contrast, animals immunized with whole cell inactivated vaccine developed similar disease as the non-vaccines following *A. marginale* infection (Hove et al. 2022). This is very innovative approach as the organism is cultivable on tick cell lines, thus it is not exposed to cattle pathogens and cannot transmit them and it can be reproduced on demand. The mutation seems to be stable, thus it will not reverse the virulence in the host and finally it may protect a wide array of heterologous strains upon testing (Hove et al. 2022).

Perspectives

New diagnostic techniques will need to be developed or implemented at ranch-site if we want to treat an animal on the

spot, when no microscope or serology result is available and the animals is suffering with the clinical symptoms of anaplasmosis (Giglioti et al. 2019; Salinas-Estrella et al. 2022a,b).

In reference to the treatment, two different oxytetracycline-resistance genes (otrA, and otrB) were detected recently by PCR in samples from infected cattle and sheep with *A. marginale* in the Northwest and Southwest of Iran (Shahbazi et al. 2021). While this discovery opens the possibility of oxytetracycline resistance, there have been no reports of such resistance when treating bovine anaplasmosis. What is known is that long-term treatment of *Anaplasma* carriers with oxytetracycline does not necessarily result in elimination of the carrier state (Curtis et al. 2020). This is an area of clinical interest as resistance to oxytetracyclines could be the next great challenge when treating cattle against anaplasmosis in the absence of an effective vaccine (Shahbazi et al. 2021).

Regarding immunoprophylaxis, there are numerous studies aimed at inducing long-term immunity against bovine anaplasmosis. Molecular diversity and antigenic variability have proven to be an obstacle to the induction of protective immunity against all strains studied so far. Although studies have been conducted with immunorelevant proteins, these proteins may not be conserved in all strains, so that robust protection may be induced against some strains but not others (Quiroz-Castañeda et al. 2016; Salinas-Estrella et al. 2022b). Native, recombinant proteins and peptides have been tested as immunogens, with results that range from full protection against homologous challenge to no protection or even a worse clinical syndrome against heterologous strains (Ducken et al. 2015; Curtis et al. 2020; Sarli et al. 2020).

Live *A. centrale* vaccines are still used in many countries, sometimes with disastrous results in terms of transmission of other blood-borne pathogens (Rogers et al. 1988). Still, live agents that can establish a subclinical infection, may seem to be one of the best alternatives for induction of solid protection. Therefore, genetically modified *A. marginale* tested in susceptible cattle may be one of the best options, as it can be grown on tick cell lines and is not in contact with bovine pathogens, therefore unable to transmit them. It can be produced on demand, and is likely to protect against heterologous strains (Hove et al. 2022).

An effective vaccine is a demand from cattle producers in many countries that perhaps can now be fulfilled with mastered techniques; even if is not a universal vaccine it can be applied to local strains that can more fully cover the antigenic spectrum of a region and be produced by government laboratories for the benefit of each country's cattle industry (Salinas-Estrella et al. 2022b).

Conclusion

Bovine anaplasmosis is a widely distributed and economically important infectious disease. To know the clinical signs, the economic effects, the diagnostic and treatment options and the several attempts to develop a protective universal vaccine gives a broad description of the problem this disease represents for bovine health and production around the world. Its control has been tried multiple times since its discovery in 1910. Several approaches are still in progress to develop effective control strategies. With the climate change and its effects on the habitats in the last years, studies on the distribution of biological vectors and the participation of new species pf ticks as vectors has to be made in order to determine the best course of action to control and prevent bovine anaplasmosis.

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