Theileriosis and Tick control management in different Mediterranean Livestock Production Systems

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Theileria annulata is a tick-borne protozoan parasite that causes Tropical or Mediterranean theileriosis, an economically important cattle disease that occurs around the Mediterranean region, the Middle East and Southern Asia. In several countries, *T. annulata* infection causes a major constraint to the development of cattle industry because the disease and the carrier state induced by the parasite can cause significant production losses such as reduction in milk yield and daily weight gain, abortions, mortality and treatment costs. The parasite is transmitted by ticks of the genus *Hyalomma*. During tick feeding, *T. annulata* sporozoites enter the bovine host and rapidly invade cells of myeloid lineage. After several rounds of multiplication, merozoites are produced and released after host cell destruction and subsequently invade erythrocytes. Infected erythrocytes are ingested by ticks during feeding and thousands of infective sporozoites are produced within the salivary glands of infected ticks. The onset of clinical signs is generally around nine to 25 days after feeding of the infected tick. The initial clinical signs are pyrexia and lymphadenopathy and this is accompanied by an increase in pulse and respiratory rates and anorexia. In the peracute form of the disease, susceptible animals die within three to five days following infection. Acute form of the disease lasts for one to two weeks and is often fatal in adult cattle. Immunity in cattle can be induced through either passive immunity or following vaccination. In such animals clinical signs are mild or absent and recovery is spontaneous, however a long-lasting carrier state develops in these animals which is characterized by the presence of low parasitaemia and this carrier cattle can maintain the *T. annulata* infection in the herd. This carrier state is also associated with significant production and economic losses of millions of euros per year, particularly in areas of tropical or temperate climate. In 1997, it was estimated that annual global costs associated with ticks and tick-borne disease in cattle were calculated between 13.9 billion and 18.7 billion US$. Since disease prevalence, tick distribution and control approaches are different between Mediterranean countries this paper aims to describe the possible control options, its limitations and new strategies for the control of the disease.

Occurrence and economic impact

The occurrence of theileriosis is reported in many countries around the Mediterranean basin and reflects the availability of suitable habitats for the ixodid tick species. Some regions of southern European countries, northern African and Asia Minor countries are endemically infected. Certain areas have an endemic stability which is associated with a high level of *T. annulata* infection in herds but rare clinical cases. Other regions have endemic instability, where only a proportion of the population becomes infected and immune and clinical cases occur in different age groups, particularly in adult cattle. Although it has been described in the first part of the 20th century in Europe, this disease is becoming increasingly studied and recognised since molecular diagnostic methods became available. In Portugal, epidemiological studies determined a prevalence of around 30%. The majority of reported clinical cases occur in young calves or imported animals, and a state of endemic stability is presumed to occur. In Spain, several studies have also

been performed and it was detected mainly in southern regions and Balearic Islands but cases were also detected in Northern regions. Some references exist from Italy, especially in Sardinia and the occurrence in Greece seems to be low. Tropical theileriosis is one of the most important diseases in Turkey and has been reported from all seven geographical regions with endemic stability in some regions. In Israel, the disease is also recognised as important for the cattle industry. The disease has been described in parts of Africa facing Mediterranean region such as Morocco, Algeria, Libya, Egypt and Tunisia.

Limited studies have been done on the economic impact of Tropical theileriosis in the Mediterranean region. A study from Cappadocia, Turkey was performed to evaluate some epidemiological aspects of the disease, the effectiveness of vaccination and also the economic losses due to clinical theileriosis. During two years 554 animals were studied including 346 vaccinated and 208 unvaccinated. Acute tropical theileriosis cases were diagnosed in 153 animals of which 86 died from the disease. Mortality contributed with approximately 75% of all costs, both from beef and dairy production animals' losses and 6.8% from calves' death. From recovered animals it was calculated a decreased production of meat and milk with a cost of 7,000 US$ (costs in US$ from February 2006). From all sampled animals, 342 (62.4%) were known to be vaccinated while 208 were unvaccinated. The vaccination cost per animal in Turkey was 10 US$. From those groups, only 13.3% of vaccinated animals died from tropical theileriosis while 51% of the unvaccinated died during the study period. Besides the vaccination, the study also takes in account the costs of acaricides in tick-infected animals. Costs from treatment of the 153 animals was 23,715 US$ including the use of several drugs such as Buparvaquone but also veterinary fees. Finally, a value of 81,600 US$ from credit and insurance payment corresponding to the number of dead animals increased the total loss to more than 750,000 US$. After insurance refund of 20.6%, the final loss was calculated in 598,133 US$. Another example is a study carried out in an endemic region of Tunisia during two successive summer seasons (2002 and 2003). A total of 56 calves from three farms were monitored. An infection prevalence of 92.86%, an overall cumulated incidence of 42.86% of clinical cases and a mortality rate of 12.5% were determined. The total costs due to disease and infection were estimated in € 9388.20 and 50.81% of those costs were due to asymptomatic infection and 23.64% to clinical cases and calves' death. A cost–benefit analysis of vaccination using a Tunisian attenuated cell line vaccine was also undertaken. Considering that the vaccine would cost € 183.73 and the modification of incomes due to vaccination was calculated in € 4360.50 (additional weight gain, reduced costs of treatment and death) the cost–benefit ratio of vaccination was 23.7. Besides the economic losses, animal diseases have also a major a social impact in many societies by reducing family income in livestock dependent communities. In such areas, as in some parts or northern Africa but also in southern Europe, Tropical theileriosis have a major socio-economic impact.

Control Options

Current strategies for the control of tropical theileriosis in herds are based on several approaches including (i) the treatment of clinical cases with buparvaquone, (ii) vector control and improvement of farm conditions, (iii) vaccination with live attenuated vaccines and more recently, (iv) selection of more resistant or tolerant cattle breeds against disease. New strategies are getting increasingly attention from researcher, namely the development of anti-tick vaccines and the use of sub-unit vaccines against *Theileria annulata*.

Treatment of clinical cases

Tropical theileriose was a fatal disease until the discovery of menoctone, the first substance with anti-*Theileria* activity followed by the development of parvaquone and buparvaquone, very effective against *Theileria* infections in cattle. Buparvaquone is currently the most effective substance for treatment of both *T. parva* and *T. annulata* infected animals. A single dose of 2.5 mg/kg or 5 mg/kg intramuscularly rapidly eliminates schizonts and piroplasms in experimentally infected calves. This substance has been used with great success in Tunisia and Turkey since 1980s, with mortality reduction down to 10-12% after treatment. Buparvaquone is not registered with a maximum residue limit (MRL) in European Medicines Agency so the product cannot be commercialized in the European

Union for the use in food producing animals. An increase in the rate of treatment failures of tropical theileriosis has been observed in recent years, raising the possibility of resistance to buparvaquone. Recent scientific reports demonstrated the existence of buparvaquone-resistant *Theileria annulata* populations in Turkey, in Tunisia and in Iran. There is a clear need to evaluate the effectiveness of buparvaquone against field parasite populations in endemic regions and determine the underlying reasons for possible drug resistance cases when present. Since this is the only substance available to avoid high mortality, new research investment should be put in the study of new drugs.

Tick control

Elimination of endophilic ticks such as *Hyalomma scupense* from farms can be accomplished by the destruction of shelters of several off-host stages such as egg laying females, freshly hatched larvae, hibernating nymphs and freshly moulted adults. Roughcasting and smoothing all the outer and inner wall surfaces of cattle buildings and cleaning the surrounding areas could aid in eradicating this tick species. However, this option needs a financial investment for most farmers.

The use of acaricides has been a major component of integrated tick control and consists of spraying the cattle with a substance suitable for elimination of all stages of several tick species responsible for the transmission of different tick-borne pathogens. Different substances can be used such as pyrethroids (deltamethrine, cypermethrine, flumethrine), organophosphates (trichlorfon, phoxim, coumaphos) and formamidines (amitraz) although this is dependent from national legislation. However, tick control using acaricides is unsustainable due to possibility of creating resistant ticks, environmental, milk and meat products contamination.

Anti-tick vaccines can represent the best tool for control, not only against *T. annulata* infection but also all other tick-borne pathogens. These are a type of transmission-blocking vaccines aimed to target vector molecules to block pathogen transmission from vectors to mammalian hosts. The effect of such vaccines could be obtained by reducing vector populations and consequently the exposure of susceptible hosts to vector-borne pathogens, reducing the capacity for pathogen transmission or a combination of these factors. The use of recombinant *Rhipicephalus microplus* BM86/BM95 antigens has demonstrated the advantages of using anti-tick vaccine as a cost-effective and eco-smart alternative. Vaccination of cattle with the recombinant HAA86 antigen, a BM86 ortholog of *Hyalomma anatolicum anatolicum*, did not only protect against homologous tick challenge but also reduced tick transmission of *Theileria annulata*, thus protecting the animals against lethal exposure. However studies with *H. scupense* vaccine candidates (Bm86 and its ortholog Hd86) showed no activity against adult *H. scupense*. Therefore, new studies are needed for new vaccines since these have shown some promising efficacy and are an environmentally secure option.

Vaccination against Theileria annulata

Immunization of animals started in the 1930s through the inoculation of blood with *T. annulata* merozoites, but it was only during the 1960s that cell cultures were established *in vitro* and the immunization of cattle with schizont infected cells was attempted. Those cell cultures can be established from tissues of infected animals or by *in vitro* infection of leukocytes with sporozoites extracted from ticks and kept indefinitely in culture. *T. annulata* isolates can vary in virulence, so their attenuation is required, which is achieved after 60 to 300 passages in cell culture over a period of several months to two years.

The development of live attenuated vaccines from infected cell lines has resulted in some control of the disease in countries such as Israel, Iran, India, Turkey and Tunisia. Clinical and parasitological evidence indicates that attenuated vaccines confer immunity for at least six months and that thereafter a gradual decline of immunity follows if the animals are not exposed to the parasite during that period, and it may therefore be necessary to consider revaccination. It is also necessary to use vaccines produced with local isolates, either to direct the immunological response to these parasites or due to the concern of altering local parasite population. Cattle immunised with these vaccines develop a solid immunity against homologous challenge and a certain

degree of resistance was recorded against heterologous strains. However, this partial protection against heterologous genotypes can alter the population structure and may cause the formation of genetically diverse populations. The protectiveness of vaccine against repeated challenge in an endemic region and the influence of vaccination on the field parasite population are currently unknown. Besides, recent field reports from Turkey indicated an increasing number of the breakthrough cases in vaccinated animals, which resulted in severe theileriosis and mortality. Based on these data, it is obvious that attenuated live cell line vaccines are protective against certain parasite genotypes existing in each particular region and producing region specific live attenuated vaccines should possibly be used as an alternative for the control of tropical theileriosis in endemic regions. However, the need of a cold chain and the technical expertise in handling and administering the vaccine correctly and possibility to pose a risk to pregnant cows due to transient pyrexia post-vaccination that limits the use of attenuated live cell line vaccines worldwide.

It is therefore imperative to use subunit vaccines for the development of safer and more effective vaccines intending to stimulate a protective immune response against all isolates of *T. annulata*. Subunit vaccines have been primarily focused on the immunodominant sporozoite surface antigen (SPAG-1) but other antigens have also been studied such as immunodominant surface antigen of merozoite (Tams1) and *T. annulata* surface protein (TaSP). Some experiments showed only partial protection of calves compared with the level of protection using the attenuated cell line. Despite the great advances in genomics and proteomics, this knowledge has not yet been applied in the development of efficient recombinant vaccines in the control of tropical theileriosis. As a consequence, there is still a need for the use of attenuated vaccines in tick-transmitted disease control strategies in various parts of the world.

Selection and breeding for resistance

An alternative strategy for the control of tropical theileriosis is the use of tolerant or resistant cattle breeds in animal selection programs. Advances in technology have exponentially increased the genetic information on livestock and pathogens and allowed the identification of genes involved in resistance to certain diseases that could be used as markers for the identification of animals with resistance phenotypes. Various studies have provided evidence of genetic resistance to protozoan parasites such as *Theileria parva* and *Trypanosoma brucei* that cause respectively East Coast Fever and Trypanosomiasis. Resistance to *Theileria annulata* has also been studied in European cattle such as Holstein breed (*Bos taurus*) which is extremely susceptible to this parasite, creating a major barrier to the improvement of cattle farming in endemic areas using this breed.

Several indigenous *Bos indicus* breeds living in endemic areas show some degree of resistance to *T. annulata* compared to non-indigenous susceptible cattle such as Holsteins. Experimental infections gave us more information on resistance to *T. annulata* in two breeds, Kenana of Sudan and Sahiwal of India, both from endemic areas. Those breeds exhibit a certain level of resistance against tropical theileriosis compared to Holstein, but it was not known whether the resistance has a genetic basis or was a resistance to ticks. *In vitro* studies to compare cellular immune response between animals of tolerant and susceptible breeds have allowed confirming that part of breed differences depend on cells involved in the immune responses. Those studies have indicated that differences in resistance are based on different interaction of macrophages with the schizont form, namely in regulation of lymphoproliferation and production of proinflammatory cytokines, but also with phenotypic differences of the infected cells.

The strategy of animal selection and breeding has been adopted as a national policy in India, which has led to a decrease in exotic breeds in dairy herds. There is evidence to suggest that the reduction of imported breeds, such as Holstein, is also contributing to the reduction of the incidence of tropical theileriosis in this country. Autochthonous cattle breeds from southern Europe, northern Africa and Asia Minor living in *Theileria annulata* endemic areas have certainly developed mechanisms that allow them to coexist with this protozoan parasite. Since there is considerable

evidence of a genetic component in resistance to parasites, there is a strong assumption of a greater resistance of those breeds to *Theileria* infection. There is an opportunity to use new genomic tools to obtain more information on resistance markers to determine association with the occurrence of theileriosis in those breeds.

Final remarks

Prophylactic measures such as the use of acaricides or attenuated vaccines and the treatment of animals with buparvaquone have allowed the reduction of economic losses resulting from tropical theileriosis. However, these methods are not available in certain endemic areas or are very expensive. Currently there is also an increased risk of vector dissemination due to climate changes and new geographic areas can be affected. A requirement to sustainable disease reduction is the combination of all possible control measures within an integrated strategy and this requires an increased effort focused in new scientific advances and also political awareness.

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