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Control of tropical theileriosis (*Theileria annulata* infection in cattle) in North Africa

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ABSTRACT

Tropical theileriosis (*Theileria annulata* infection) is a protozoan disease of cattle transmitted by *Hyalomma* ticks. This parasite is causing high losses in several countries in South Europe, North Africa and Asia. Indeed, both symptomatic and subclinical forms are present in infected animals causing live weight decrease, milk yield decrease, abortions and in some cases death. Due to its high medical and financial impact, the control of this disease is of paramount importance. It can be implemented through five control measures: (i) treatment of infected animals with theilericidal drugs and other symptomatic treatments (this option is used for the treatment of animals and is insufficient to eradicate the parasite), (ii) use of acaricides in animals which contain several side effects for humans, animals and the environment, (iii) roughcasting and smoothing of the outer and inner surfaces of the cattle buildings for endophilic tick species (this control option is expensive but leads to the eradication of the parasite from the farm), (iv) vaccination against ticks, a control option used with success against *Rhipicephalus* (*Boophilus*) species but not still available for *Hyalomma* ticks and (v) vaccination against the parasite with live attenuated vaccines. These control options were presented in the paper and their advantages and limits were discussed. The implementation of one (or more) of these control options should take into account other considerations (social, political, *etc.*); they sometimes cause the failure of the control action.

1. Introduction

Tropical theileriosis [*Theileria annulata* (*T. annulata*) infection] is a tick-borne protozoan disease transmitted by ticks which belong to the genus *Hyalomma*. It is one of the major constraints to cattle breeding development and intensification in several parts of the world[1]. *T. annulata* causes severe financial losses due to live weight decrease, a drop of milk yield, abortions and in some cases deaths[2]. Moreover, the treatment of this disease is very expensive. It not only needs the association of specific theilericidal drugs but also several non-specific treatments. These costs could be increased by the costs of carrier state. They are certainly lower but persistent and by far more frequent than clinical cases[2,3]. Indeed, the prevalence of carrier animals' status reaches 100% in enzootic stability state farms[4]. Due to all these losses, the implementation of a control program is a necessity that can never succeed if not adapted to the epidemiological context of the farm. In this review, we present the different control options of tropical theileriosis control in North Africa where the vector tick is mainly *Hyalomma scupense* (*H. scupense*) (Figure 1). These programs are also applicable to other regions of the world where *Hyalomma anatolicum* is the vector since the two ticks have roughly the same biology. The control programs consist of diseased animals' treatment and prevention.

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2. Treatment of tropical theileriosis

The treatment of diseased animals associates a theilericidal specific treatment and a full symptomatic treatment, that's why a deep clinical examination is requested in order to detect any abnormality.

Due to the absence of specific symptoms, the high death risk and

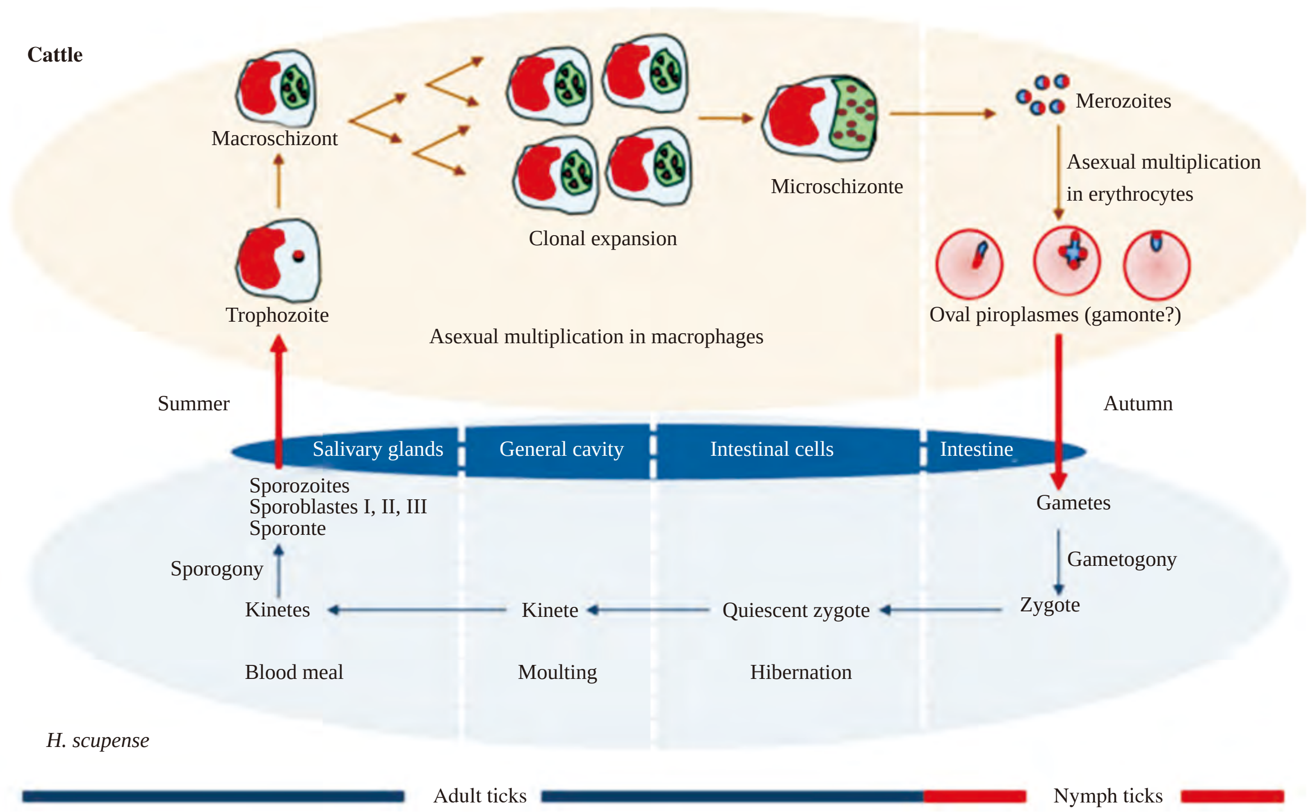

Figure 1. Biological life cycle of *T. annulata*.

the high cost of theilericidal molecules, the treatment should be carried out after laboratory confirmation of the infection. Moreover, hemopathogen co-infections are frequent in enzootic regions for example co-infections between *Babesia* spp. and *T. annulata* and *Anaplasma marginale* are possible[5].

2.1. Theilericidal treatment

To date, the best theilericidal drugs belong to the hydroxynaphtoquinones family: parvaquone and buparvaquone. Parvaquone (Parvexon ND, Bimeda) is mainly active drug against schizontes; it should be injected intramuscularly at the dose of 20 mg/kg[6]. Buparvaquone (Butalex ND, Schering-Plough; Teldex ND, Médivet) is active against both schizontes and piroplasmes; it is injected intramuscularly at the dose of 2.5 mg/kg. Its efficacy after a single injection was estimated to 92%, which is higher than parvaquone[6]. Nevertheless, it is more expensive (approximately $33 per adult cattle in Tunisia). A second injection after 72 h is recommended if no clinical improvement is observed. Injected precociously, this drug leads to a reestablishment of acute cases but is poorly effective against hyperacute and late treated forms; this is due to the severity of anaemia and different tissues lesions. In Tunisia, treatment failure in diseased animals was estimated to be 12%[7]. In cattle with low packed cell volume (PCV) and high parasitaemia, the injection of theilericidal drug may lead to a massive erythrocytes destruction and in several cases the treated animal die due to very low PCV. It is important to estimate both parasitaemia and PCV and then to decide whether to implement a blood transfusion. In non-infected cattle, transfusion is needed when their PCV is lower than 15%[8], but a higher threshold value is to be considered in *T. annulata* infected cattle since the theilericidal injection will induce a destruction of infected erythrocytes. For this reason, the treatment is not a suitable tool for parasite eradication from a farm.

In Tunisia, cases of resistance against buparvaquone were reported in the field and confirmed *in vivo* by Mhadhbi *et al*.[9]. They caused 4 deaths out of 7 infected cows. In Iran, 7 out of 8 diseased and treated animals died and this resistance is due to a single point mutation in the gene coding for cytochrome b[10]. In each treatment failure, a new Giemsa stained blood smear should be done in order to estimate the viability of the parasite population and conclude if it is a case of buparvaquone resistance or not. The presence of resistant strains represent an emergency and the whole farm should be treated with acaricides in order to eliminate the whole tick population. All positive cattle in the farm should be treated with a theilerical drug at the conventional dose. An acarological survey should be implemented during several tick seasons in order to totally eradicate the tick population.

2.2. Symptomatic treatment

It is of paramount importance; it will depend on the status of the animal determined after a deep clinical examination. Long-acting antibiotics are recommended in order to prevent frequent secondary infections, mainly respiratory tract. Long acting oxytetracycline is indicated since it has certain activity against *Theileria*. Moreover, it is active against *Anaplasma marginale*, a frequent co-pathogen.

In the case of hypotony of the rumen, stimulators of the rumen are indicated since the indigestion can lead to the death of the diseased animal. Finally, the presence of a generalized inflammatory state and high concentrations of proinflammatory cytokines justify the injection of non-steroid anti-inflammatory drugs. Indeed, the concentration of cytokine is proportional to the virulence of the *T. annulata* strain[11].

3. Prevention of tropical theileriosis

Due to the high costs of theilericidal drugs, the high prevalence of carrier state infection and the high costs of treatment, prevention is the best mean to control *T. annulata* infection; it consists of two types of action: (i) control of the vector tick through one or more control options and (ii) vaccination against *T. annulata*. Any of these control options should be implemented based on benefit-cost analyses as it was carried out in Tunisia (Table 1). These control programs could also consider several other "non-veterinary" aspects which can in some contexts make the control program infeasible. Extension programs are lacking in several developing countries despite their importance. They are also lacking in both human and animal health sector. The population does not sufficiently trust on modern medicines. This aspect should be deeply studied by multi-skilled teams (sociologists, epidemiologists, veterinarians, researchers, *etc*.) in order to analyse the epidemiological situation, find the causes of these constraints and suggest and implement a program to solve the problem. A standard extension program should then be established and implemented; its impact should be periodically evaluated. This action should be performed before the implementation of any control program since such constrains can make the program fail despite the investments.

Table 1

Benefit-cost ratios of different control options against *T. annulata* infection[2].

Enzootic state	Roughcasting and smoothing of walls	Vaccination with a live attenuated vaccine	Acaricide control
Enzootic stability	50.37	Calves: 23.7; cows: ND	ND
High enzootic instability	1.62-3.71*	Ratio for the whole farm: 0.20-1.19*	0.32-0.88*

*: Sensitivity analysis giving an interval of values; ND: Not done.

3.1. Vector control

A comprehensive list of the vector species in a region should be established since two sympatric tick species could play as vectors as this is the case in Morocco where Ouhelli and Pandey collected a high number of *H. scupense* (30.8%) and some *Hyalomma lusitanicum* ticks (3.5%)[12]. Correctly realizing this control option will conceptually lead to the eradication of *T. annulata* infection in a farm due to the eradication of its vector, which consists of three types of control options.

3.1.1. Off-host tick stages control

For endophilic ticks, this control option is effective and radical. It leads to the elimination of the *Hyalomma* ticks and then the infection from the farm. The principle of this action is to destroy the shelters of several off-host stages: egg laying females, freshly hatched larvae, hibernating nymphs and freshly moulted adults. This action will consists of roughcasting and smoothing of all the barn walls associated to an excellent cleaning of all the inner and outer barn area by removing the rocks, dugs, trash, *etc*.[13].

This option represents a high financial investment, nevertheless, tropical theileriosis concern the small farmers and they don't have the financial capacity to engage these expenses. Extension program and governmental financial incentives are requested to improve their willingness to pay and compliance to this control option. Veterinarians should explain to the farmers the expected benefits when engaging such high expenses. This control option has the highest benefit-cost ratio in enzootic stability and high enzootic instability states to tropical theileriosis (Table 1). The financial aspects are very important to consider since they can fail the whole control program.

This control option is suitable to control endophilic vector ticks such as *H. scupense* or *Hyalomma anatolicum* but has no effect on exophilic vector ticks such as *Hyalomma lusitanicum*. The last is the exclusive vector of *T. annulata* in Portugal and Spain[13] but is associated to *H. scupense* in Algeria and Morocco[12].

3.1.2. Acaricide control

The use of acaricides offers the opportunity to control several tick-borne pathogens such as theileriosis, babesiosis and anaplasmosis. Several molecules belonging to different families could be used depending on national legislation: organophosphates (trichlorfon, phoxim, coumaphos, *etc*.), pyrethroids (deltamethrine, cypermethrine, flumethrine, *etc*.) and formamidines (amitraz)[14]. Acaricides should be used in respect to a strategy depending on the aim (either eradication of the disease or an eradication of the infection from the farm), the enzootic state and the bio-ecology of the tick vector (Table 2). Where *H. scupense* is the vector tick, the acaricide is used with the aim to reduce the tick population by breaking its lifecycle in two positions: adult ticks during the summer season and the immature ticks (larvae and nymphs) during the autumn. This protocol depends on the tick vector phaenology.

Table 2

Periodicity of acaricide application with regard to the aim of the control option[15].

Stage	Aim	Periodicity of acaricide application (days)
Immature stages	Elimination of the ticks form the farm	Duration of its activity against ticks + 15[a]
Adults	Elimination of the ticks from the farm	Duration of its activity against ticks + 8[b]
	Stop *T. annulata* transmission to cattle	Duration of its activity against ticks + 8[b] + 3[c]

[a]: Minimal immature fixation period; [b]: Adults fixation period; [c]: Delay for a tick to be infected by *T. annulata*.

Autumn acaricide application leads to the reduction of adult tick populations during the next summer season. It should be implemented each three weeks with low residual effect of acaricides. Summer treatment targets the adult ticks, which will lead to immediate reduction of *T. annulata* transmission risks. Periodical acaricide application should be adjusted with regard to the residual effect application and the aim of the control option: either annihilate *T. annulata* transmission or eradicate the tick vector (Table 2).

Well carried out, this control option, mainly autumn treatment, reduces effectively the tropical theileriosis transmission risks. In the field, the effectiveness of this control option is limited due to a low observance of the protocol by farmers. Treatment of the walls with acaricides should be banned, on one hand it is harmful to the human and animal health and to environment since it needs big quantities of acaricides, on the other hand, its effectiveness against off-host stages is limited since they enter deeply in the cracks and crevasses and have low metabolic activity during hibernation[10].

3.1.3. Anti-tick vaccines

Conceptually, anti-tick vaccines represent the best tool for control, not only the *T. annulata* infection but also all tick-borne pathogens. A comprehensive review of *H. scupense* was recently published by Gharbi and Darghouth[15]. Since *Rhipicephalus microplus* is a monophasic species, a vaccine against this species was developed and is now commercially available in Australia and Latin America countries. For *H. scupense* species, two vaccine candidates, namely, Bm86 and its ortholog Hd86 (an ortholog isolated from *H. scupense*) showed no activity against adult *H. scupense*. Whilst, Hd86 candidate reduced the number of engorged nymphs (59.19%)[16,17]. This difference might be explained by a Hd86 expression difference between immature and adult stages[18].

3.2. Infection and treatment method

This method is used in the control of East Coast fever (*Theileria parva* infection) by injecting to cattle an infected tick stabilate cocktail (Muguga cocktail) as well as an intramuscularly single dose of long-acting oxytetracycline[19]. The infection induces the installation of a carrier state whilst the antibiotic injection protects the cattle against the installation of symptoms. This method is conceptually possible to be effective against *T. annulata* infection, nevertheless since it causes carrier state infection, it is not suitable for farms in enzootic stability state with very high infection risks (due to high tick burdens) and since other effective options are available, this option is not recommended in the field[20].

3.3. Vaccination against T. annulata

3.3.1. Live attenuated vaccines

Injected subcutaneously, cell lines vaccines confer to animals a solid immunity against homologous strains and a lower protection against heterologous strains[21]. This vaccine is anti-disease and doesn’t block the infection. It has consequently slight effect on the infection epidemiology because the vaccinated cattle remain carriers for several years[22].

The number of passages needed to reach attenuation depends on the parasite strain. For example, the Tunisian strains need between 100 and 300 passages to become inactivated[21]. The protective doses vary between 10^4 and 5×10^5 infected cells[23]. The post-vaccination protection duration depends on the vaccine; it should be more than one year as reported in Tunisia[24]. The vaccine is stored in liquid nitrogen for many years and thawed when needed. This nitrogen conservation represents 30% of the vaccine costs and can decrease the vaccine effectiveness after thawing. This vaccine is actually used in the field in some countries[25].

Attenuation is technically easy but several regional isolates need to be tested to find the best candidate and the following criteria should be respected: (i) adaptation of the attenuation level to the vaccinated animals, (ii) the vaccine should be protected during at least one tropical theileriosis season, (iii) the vaccine should not be transmitted to ticks and (iv) the vaccine should be safe for pregnant cows at least 6 months[23]. In Tunisia, a cell line vaccine, namely, Béja 280 (name of the town where it was isolated) at passage 280 was selected as a candidate[21]. A new delivery system without liquid nitrogen was patented; the vaccine was thawed on request and can be used during the 4 to 6 following days at room temperature with no noticeable decrease of its efficacy.

3.3.2. Perspectives and optimisation of vaccines against T. annulata

Despite their effectiveness, cell culture vaccines are facing several problems: the risk of accidental contamination by other pathogens during the production process, the use of a cold chain during the distribution of the vaccine and the presence of a virulence reversion risk[22].

Researches have been carried out in order to identify protective antigens that could be used as recombinant vaccines. Several antigens have been identified as vaccine candidates[26]. Nevertheless, to date only two antigens showed partial protection, namely, SPorozoite AntiGen antigen 1 (SPAG-1) and *T. annulata* merozoite (TAMs).

SPAG-1 is a protein expressed by sporozoites[27]. Vaccination with this antigen showed a partial protection against both homologous and heterologous strains[26,28,29]. The best protection was obtained by injecting the SPAG-1 antigen with an adjuvant (RWL). Indeed, 3 out of 6 animals survived (12 control animals died). Moreover, the vaccinated animals showed a significant increase in both pre-patent and the incubations periods.

TAMs antigen is expressed in *T. annulata* merozoites and erythrocyte stages[30]. The injection of recombinant TAMs associated with an adjuvant (immunostimulatory complex) conferred a relative protection against homologous strains[28].

3.3.3. Association of antigens

Boulter *et al.*[28] immunised animals by associating TAMs and SPAG antigens and Darghouth *et al.*[26] immunised animals by associating Montanide-SPAG-1 with cell culture vaccine. This association induced a dramatic improvement of the protection against a heterologous challenge.

Table 3

Control measures against tropical theileriosis depending the enzootic state[23].

Enzootic state	Acaricides against adults	Acaricides against larvae and nymphs	Immunisation with a cell live attenuated vaccine
Enzootic stability state	Animals of more than one tick season: during the peak activity	Prohibited except for eradication	Calves and newly introduced animals
Low enzootic instability state	During peak activity	Prohibited except for eradication	Animals to 3rd tick season and newly introduced animals
High enzootic instability state	During the activity season	Indicated	Indicated

3.3.4. DNA vaccines

d'Oliveira *et al*. showed that immunization with plasmids DNA coding for TAMs gene conferred a partial immunisation against homologous challenge[31].

3.3.5. Prime boost immunisation

The prime boost approach conferred a significant protection of animals vaccinated with a mix of two antigens (TAMs and SPAG)[29].

4. Strategy of *T. annulata* infection control

Control of tropical theileriosis should be implemented as an integrated approach: (i) integrate at least two different control options (veterinarians should offer a basket of control options and not only one, with regard to the benefit-control options and different non-veterinarian criteria), (ii) it should target the eradication of the infection and not the disease since the highest proportion of losses is due to carrier animals[2], (iii) consider each enzootic state when choosing the control option (Table 3), (iv) integrate an extension program since farmers may have important confusions in the infection transmission, (v) this approach should be participative so that the farmers adopt the control program.

5. Conclusion

Tropical theileriosis is a major tick-borne disease of cattle affecting many countries in Europe, Africa and Asia. It causes high losses by affecting different production types: milk, meat and reproduction and even death. The epidemiology of this protozoan infection is complicated since it involves three partners: ticks, the protozoan and the cattle. The control needs an excellent knowledge of local epidemiological feature and the adoption of an ecopathological approach. This control program should be integrated to a progressive barns upgrading for regions where the vector is endophilic[24]. This program should progressively lead to the vector eradication, improve the breeding condition and also contribute to control several other health problems. In this context, the live-attenuated vaccine associated to acaricides should represent an accompanying option conferring a protection of exposed animals, mainly when shifting from one enzootic state to another due to the reduction of tick population. When vaccinating animals with live-attenuated vaccines a cost-benefit analysis should be carried out in order to guide the vaccine development by giving a baseline values. Moreover, in order to improve the delivery system of liquid nitrogen stored vaccines more research on vaccines against tropical theileriosis is needed.

The paucity of financial resources should be considered by the decision makers and the farmers. In order to optimise the use of financial resources, cost-benefit analyses should be performed. This analysis will allow (i) ranking the costs of different animal health problems (it is an important issue since tropical theileriosis will rank the top among other health problems, which will provide a strong argument to animal health decision makers to implement specific control programs), (ii) choosing the best control option among many others (it will depend on the country and a beneficial control option can become not beneficial after some years or in a different socio-economic context). In the beginning of the control implementation, animal health decision makers can implement highly efficient control programs with low beneficial returns in order to decrease the infection biomass in a given region or population. Some education programs should be carried out, showing the farmers the benefits of implementing a precise control program. At the end of a control option, the decision makers can implement non-beneficial options in order to achieve the eradication of the infection from a farm, a region or a whole country.

Conflict of interest statement

We declare that we have no conflict of interest.

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