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Vaccinal approach using inactivated vaccine against heartwater and *Ehrlichia ruminantium* genetic diversity

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Context

Ehrlichia ruminantium is an obligate intracellular bacterium transmitted by *Amblyomma* ticks. It is responsible for a fatal disease, cowdriosis or heartwater which affects wild and domestic ruminants. This disease is present in the Caribbean islands (Guadeloupe and Antigua), throughout sub-Saharan Africa and in the Indian Ocean (Madagascar, Comoros, Réunion Island). The economical impact of the disease is important due to loss of animals, decrease of productivity and difficulty to improve the productivity of herds by the introduction of exotic breeds which are particularly susceptible to heartwater. Different ways to control the disease have been followed: control of the vector by acaricide treatment and vaccinal approach. However, the use of acaricides is expensive and polluting and could not be a solution in low input farming systems.

Several vaccines have been developed and tested in controlled conditions and in field trials: attenuated vaccines, recombinant vaccines (DNA and proteins) and inactivated vaccines. Whatever the type of vaccine used, its efficiency is limited mainly due to the genetic and antigenic diversity of *Ehrlichia ruminantium*.

In this context, it seems to be essential to better evaluate the genetic diversity of the strains. Improvement of production of *Ehrlichia ruminantium* antigen at an industrial scale, improvement of storage conditions, identification of the minimal vaccinal dose and field assays using inactivated vaccine were done. A review of these results is presented here.

Genetic diversity of *Ehrlichia ruminantium*

We carried out phylogenetic analyses of *Ehrlichia ruminantium* using sequences of 6 MAP proteins, MAP1, MAP1-2, MAP1-6, MAP1-5, MAP1+1 and MAP1-14. MAP family constitute a cluster of 16 membrane proteins including MAP1 (Major antigenic protein) which is highly polymorphic. These genes coding for these six proteins are located either in the center or at the borders of the *map* gene cluster. Eighty strains were typed using MAP1 and we show that MAP1 is a good genetic marker among Africa, Caribbean islands and Madagascar. Emerging strains have been identified. From 11 to 15 strains were typed using the other MAP and we show that the different *map* paralogs define different genotypes showing divergent evolution within the *map* gene cluster. Moreover, there is no correlation between all MAP genotypes and the geographic origins of the strains. The genetic diversity is conserved at the level of a village, region or continent.

Optimisation of the inactivated vaccine production in industrial conditions

Inactivated vaccine production which is conventionally done in static culture conditions has been successfully adapted to industrial conditions: production of *Ehrlichia ruminantium* antigens at large scale in stirring conditions, optimization of storage buffer and stability of emulsion. In parallel, an important reduction of the efficient antigen dose from 1 mgr to 35μgr has been obtained in vivo. Thus, the cost of the inactivated vaccine decreases at 0.11 euros for one dose. In these conditions, the use of the inactivated vaccine could be an opportunity to control heartwater in low input farming systems as soon as *Ehrlichia ruminantium* diversity is known at the regional level and a local vaccine including a cocktail of strains can be produced to improve its efficiency.

Burkina Faso field trials

For field trials in Burkina Faso (four villages), two successive assays were done on susceptible sheep and genetic diversity of strains detected both in dead control and vaccinated animals was evaluated. We demonstrated that there was a limited protective effect of the Gardel vaccine with 65% of survival rate for the vaccinated group and 49% for the control group whereas during the second year after introduction of a local strain into the inactivated vaccine, the survival rate increased significantly with 72% of survival rate for vaccinated group and 47% for the control group. There was a difference of circulating strains from one year to another and depending on the villages which could explain the difference of protection.

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Conclusions

Inactivated vaccine is still a promising vaccinal approach to control heartwater. The difficulty for vaccine design is to select the appropriate protective strains with the widest protective effect against regional circulating strains. The choice of a cocktail of strains would be an alternative if any reliable genetic marker related to cross-protection could be defined. Several approaches of genotyping using multi locus analysis (MLST/MLVA) of strains in relation to cross -protection data have been tested and could allow identification of markers associated with cross-protection. Furthermore, it is possible that some of these markers could be involved in the protective immune response.

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Nutritional strategies to control gastrointestinal parasitism in small ruminants

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Traditional control of gastrointestinal parasitism with drugs in ruminants is no longer sustainable. This is because there is an increasing development of gastrointestinal parasite resistance to anthelminic drugs, and the development of this resistance is so rapid and widespread that the efficacy of this mode of chemical control is dramatically challenged. For all these reasons it is now widely accepted that there is a need to develop alternative or complementary methods to control gastrointestinal parasites in ruminant systems and these methods need to be sustainable. The focus of this paper was to consider one, relatively short term such strategy, that has the potential to influence the ability of small ruminants to cope with gastrointestinal parasites: *host nutrition*.

Host nutrition is able to affect gastrointestinal parasitism through various routes. Since one of the main consequences of gastrointestinal parasites is to draw upon host resources, such as loss of plasma, blood, or tissue damage, any increase in nutrient resources will improve the productivity of the host, once the requirements for the above parasite consequences have been met. This amelioration of the detrimental effects of the parasitic infection through nutrition is called *host resilience*. Hosts also attempt to regulate their parasite populations through the actions of their immune system. This function, called *resistance,* manifests as the ability of the host to decrease establishment of parasites in the gastrointestinal tract, delay worm growth, reduce female worm fertility and egg excretion and finally expel existing adult worm populations. Like all bodily functions host immune response requires resources for its functioning and therefore it is to be expected that host nutrition would affect the extent of resistance. The influence of host nutrition on resilience and resistance can be seen as the *indirect* effects of nutrition upon parasite populations. Although there are several nutrients that can potentially enhance host resilience and resistance through nutrition, the majority of the focus has been on supplementation with (metabolisable) protein (MP). This is because this nutrient is often scarce for growing and reproducing small ruminants, due to e.g. consumption of low-quality forages or restricted feeding, at times of high MP requirements for growth and periparturient reproductive functions. The magnitude of these effects would be dependent on the degree of nutrient (MP) scarcity. As such, it would be expected that the largest benefit from protein supplementation would be expected in animals with high nutrient requirements, e.g. multiple-rearing ewes or goats in a relatively poor body condition and in growing lambs or kids with a high growth potential.

However, it has also been shown that host nutrition can have *direct* effects upon parasite populations. The ingestion of specific food compounds or their metabolites may have such direct consequences. Plant secondary metabolites (PSM) fulfil this role since their properties appear to be detrimental on both adult and mature forms of parasites under *in vitro* conditions. Whether and how consumption of forages high in PSM (bioactive forages) is able to affect gastrointestinal parasitism *in vivo* continues to be the subject of scientific investigation. It is likely that the effects of PSM consumption on the host as well as on the parasite need to be taken into account when considering such an approach. As well as having antiparasitic effects, PSM also have negative effects against the host when they are consumed; such effects are usually referred to as anti-nutritional ones. The consumption of PSM-rich plants *per se* by ruminant herbivores can result in reduced intake, weight loss, toxicity and death. In order for parasitized ruminants to benefit from the anthelmintic properties of bioactive plants, the antiparasitic effects should outweigh the anti-nutritional consequences on the performance of the parasitized host. The latter will depend on the severity of the consequences to the host and to the parasite. It will also depend on the duration of exposure to such forages. Parasitized hosts might be able to tolerate short-term negative consequences (e.g. toxicity) if they can attain long-term benefits (e.g. parasite reduction). Consequently, short-term exposure may be one option for including PSM in parasite management schemes. In addition to the PSM route, the excessive consumption or the lack of certain nutrients may alter conditions in the gut environment from beneficial to detrimental and even toxic for parasite survival. Gastrointestinal nematodes identify and select specific locations to reside within the host. It can thus be envisaged that by also influencing the ability of incoming parasites to identify their niche, host nutrition may inhibit their abilities to feed and reproduce. Although these principles have been applied to the control of parasites in non-ruminant animals, they have yet to be applied in ruminant ones.

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