

Evaluation of a Potential Vaccine for East Coast Fever in African Cattle

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Theileria parva is a parasite transmitted to cattle by the brown-ear tick. It is the cause of East Coast Fever (ECF), a deadly disease in African cattle. The ECF research group has been successful in identifying a number of Theileria parva parasite antigens that are targeted by the immune system. These antigens have been tried in cattle as vaccines to induce immunity that can protect the cattle from the disease. These vaccines, however, were unable to induce full protective immunity. As a result, recombinant live attenuated Listeria monocytogenes expressing three defined antigens from Theileria parva was evaluated in this study in the way of a vaccine to determine its potential to induce full protection in cattle against Theileria parva. Listeria monocytogenes has been used to make a vaccine and shown to induce strong immunity in mice and non-human primate animals and is currently being tried as a vaccine carrier against human cancer. Experimental studies using the recombinant Listeria monocytogenes vaccine, however, indicated that the Theileria parva antigens in the vaccine are poorly expressed and recognized by specific immune cells maintained in the lab. The vaccine failed to adequately demonstrate the desired potential of inducing the required immunity (antigen-specific CTL) that can protect the cattle against disease. Research findings from this study will have implications in vaccine formulation in other diseases including cancer where the induction of T-cell immunity is very important.

Background

East Coast Fever (ECF) is a highly fatal disease in cattle that targets and induces uncontrolled multiplication of the cells of the immune system called lymphocytes. It is caused by a brown ear tick called *Rhipicephalus appendiculatus*, which transmits the intracellular apicomplexan parasite, *Theileria parva*. The disease is endemic in twelve countries in eastern, central and southern Africa. In susceptible cattle populations in endemic areas, one cattle succumbs to the disease every thirty seconds, resulting in economic losses of over \$300 million per year. At least 28 million cattle are at risk of the disease, the majority of which are raised under smallholder dairy systems.

Control of ECF mainly involves the use of agrochemicals such as acaricides to control ticks, but this has been hampered by the increasing resistance of ticks to these chemicals. The use of drugs that kill the parasite, such as parvaquone and buparquone, is also employed as an alternative strategy for control of ECF but only before the disease has progressed too far for the drugs to take effect. Drug resistance against the parasite, however, is currently being experienced in northern Africa (Tunisia) where these drugs are heavily used against *Theileria annulata*, a relative of *Theileria parva*. The use of live vaccines, which involves injecting live disease-causing organisms/parasites into the animal and treating the infected animal with long-acting oxy-tetracycline, is the only method of

vaccination available thus far that can give protection to cattle against East Coast Fever. This method, however, is implicated with a number of practical constraints: namely, protection is only associated with the parasite that has been used to immunize the animal, meaning that infection of the animal with the parasite not used in the vaccine would make the animal sick and or die. Secondly, such a vaccine strategy may lead to the emergence of new, and probably more complex, parasite strains that would be difficult to combat once established. Lastly, the vaccine requires a constant supply of a cold chain for preservation of the parasites, making the vaccine extremely expensive for already economically-stressed poor farmers.

In an attempt to improve ECF control, recent research has focused on the development of a sub-unit vaccine that would target both the sporozoite (cells that infect the host) and schizont (cells in dividing form) stages of *Theileria parva* life-cycle. Such vaccines make use of the small components of the parasite that are targeted by the immune system. A sub-unit vaccine therefore promises an integrated approach to the control of ECF, and a number of scientific efforts are currently focusing on developing such a vaccine. Initial attempts at designing a sub-unit vaccine utilized the major sporozoite surface coat protein of *Theileria parva* called p67. Strong immune responses can then be induced in experiments conducted under laboratory conditions. In the field, however, where there

are different populations of the parasite, this kind of vaccine has not resulted in full protection.

The cattle that recover naturally from ECF or those immunized using live vaccines exhibit strong CD8⁺ T cell immune responses against *Theileria parva* in the schizont stage of the life cycle, and a large body of evidence suggests that this subset of immune cells (CD8⁺ T cells) are the dominant protective mechanisms against *Theileria parva*. It has therefore been long postulated that identification of the parasite antigens of the schizont stage that are recognized by the immune system in order to kill the parasite could pave the way for developing an effective vaccine against ECF. Recently, the International Livestock Research Institute (ILRI) was successful in identifying a number of the schizont stage antigens of *Theileria parva* that are targeted by CD8⁺ T cells. Some of these antigens have been used to make vaccines formulated in viruses as carriers of the antigens. These vaccines have produced immunity that reduces the severity of the disease in a few of the affected animals. Such vaccines, however, have failed to induce immunity that can fully protect all the animals once they are sick. Following the sub-optimal performance of these vaccination regimes, in this study, *Listeria monocytogenes* (a bacterium) was proposed as an alternative vaccine carrier to elicit protective immunity in cattle.

Method

This study involved evaluating the vaccine potential of recombinant live attenuated *Listeria monocytogenes* against the parasite *Theileria parva*. Specifically, the project assessed the ability of recombinant *Listeria monocytogenes* to express recombinant antigens from *Theileria parva* that are being recognized by specific CD8⁺ T cells in vitro, vaccinated the animals, and monitored the immune responses induced by the vaccine. Injecting the animals with the parasite causing the disease allowed researchers to determine if such immune responses could protect the animals. Following the screening of the animals for pre-existing immunity to *Listeria monocytogenes*, twelve animals were selected for this study under laboratory conditions.

The cattle used in this experiment experienced no or low responses to *Listeria monocytogenes*, in addition to being naïve to other diseases such as East Coast Fever, anaplasmosis, and babesiosis. All the animals were immunized on the first day, whereby the test animals received the recombinant *Listeria monocytogenes* (5×10^{10} cfu/ml) carrying *Theileria parva* antigens, while the control animals received *Listeria monocytogenes* (5×10^{10} cfu/ml) devoid of antigens. Both the test and control animals were injected intravenously (i.v.).

After twenty eight days, all the animals were immunized again as before but received ten times more than the first immunization dose (5×10^{11} cfu/ml). Three weeks later

(on day 49), all the cattle were infected with a potentially lethal dose of the disease-causing parasite, *Theileria parva* sporozoites. This step was used to determine if the vaccinated animals can be protected if they are exposed to the disease after vaccination. In both cases following immunization, monitoring of the immune responses by assessing the trends using purified CD8⁺ T cells and whole peripheral blood mononuclear cells (PBMC) was conducted using in vitro assays as well as performing white blood cell and platelet counts. The ability of induced CD8⁺ T cells to kill parasite infected cells in vitro was also assessed using Cytotoxic T Lymphocyte (CTL) assays. Different parameters were also used to measure how severely the animals reacted to the disease (East Coast Fever reaction index) following infection with a lethal dose of the parasite.

Results

Using highly sensitive in vitro assays, it was demonstrated that *Theileria parva* antigens in the recombinant *Listeria monocytogenes* were weakly expressed and recognized by specific immune cells that have been cultured in the lab called CD8⁺ T cells. After the animals were immunized using recombinant live attenuated *Listeria monocytogenes*, the specific immune responses induced by the antigens in the vaccine were only in a proportion of the animals. This was detected in two of the three antigens used in the vaccine. However, there were strong immune responses to the *L. monocytogenes* antigen, called listerio-lysin-O (LLO), following immunization. While such responses are seen to be due to CD8⁺ T cells of the immune system in small animal models, like mice, the responses were confined to CD4⁺ T cells in cattle.

Although it was possible to induce immune responses in a number of animals, this vaccine formulation failed to demonstrate adequate potential to induce protective immunity when the cattle were infected with a lethal dose of the *Theileria parva* parasite. There were three survivors after the animals were infected, two that had been vaccinated and one from the control group. Of the two vaccinated animals that survived, one animal demonstrated strong immune responses after vaccination, while the other animal did not show any detectable immune responses. In the blood of the two vaccinated animals it was not possible to detect the presence of immune cells that have been shown to kill the *Theileria parva* parasite. These cells are called cytotoxic T lymphocytes (CTL). The survival of one control animal and lack of strong supporting evidence from the data made it difficult to conclude that the vaccine formulated in *Listeria monocytogenes* was involved in the protection of the two survivors.

In conclusion, the study failed to convincingly demonstrate that recombinant *Listeria monocytogenes* was expressing recombinant antigens that were effectively presented to

CD8⁺ T cells in vitro. The data obtained in one of the experiments was not reproducible. Following vaccination, the induced immune responses were only detected in a few of the animals, and the majority of the animals were not protected.

Due to the failure of recombinant live attenuated *Listeria monocytogenes* to sufficiently express the *Theileria parva* antigens recognized by specific immune cells (CD8⁺ T cells) in vitro and the inability to effectively induce strong immune responses in immunized animals and provide protection, research in the following areas is recommended:

1. Improving the robustness of *Listeria* for greater protective immunity: Although *Listeria monocytogenes* has shown potential to induce immune responses in cattle following immunization, additional work is needed to make *Listeria* a more robust solution to induce protective immunity.
2. Modifying the injectable dose: Given that the recombinant *L. monocytogenes* vaccine was poorly expressing the antigens, future work should focus on modifying the injectable dose that correlates with body weight.
3. Formulating a vaccine construct to induce stronger immune responses at lower doses.
4. Changing the route/mode of vaccine administration by targeting routes that can induce strong immunity, such as the sub-cutaneous route, whereby material is inserted into the fatty tissue just below the skin.

Practical Implications

The data obtained from this study will contribute to efforts in vaccine development against *Theileria parva* in cattle. The findings will give guidelines regarding the choice of antigens, antigen preparation, dose and mode of vaccine administration that, ultimately, will be useful for maintaining a healthier population of cattle and thus, providing a better economic opportunity for smallholders.

Findings will be equally critical to vaccine development against human cancer where *Listeria monocytogenes* is currently being employed as a platform for vaccine formulation and the induction of T cell immunity is critical. Research conclusions will thus provide excellent information of a *Listeria*-based vaccine in large order animals, such as cattle, that may be extrapolated to livestock management and human health.

Further Reading

Brockstedt D. G., A. G. Martin, L. L. Meredith, S. B. Keith, G. Yi, L. William, L. Weiqun, N.C. David, A. P. Daniel, and W. D. Thomas, Jr. 2004. "Listeria-based cancer vaccines that segregate immunogenicity from toxicity." *Proceedings of the National Academy of Sciences* 101 (38): 13832– 13837.

Graham S. P., P. Roger, H. Yoshikazu, M. M. Duncan et al. 2006. "*Theileria parva* candidate vaccine antigens recognized by immune bovine cytotoxic T lymphocytes." *Proceedings of the National Academy of Sciences* 103(9): 3286–3291.



Daniel Kerage checks on the proliferative status and integrity of Theileria parva-infected lymphoblasts.
Photo by Victor Riitho.

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