



Photo: ILRI/Kabir Dhanji

DEVELOPING A SUBUNIT VACCINE FOR EAST COAST FEVER

A POTENTIAL ALTERNATIVE SOLUTION TO TACKLING CATTLE'S MOST DAMAGING DISEASE IN AFRICA

KEY MESSAGES

A parasitic disease of cattle, East Coast fever (ECF) kills over one million animals a year in sub-Saharan Africa and is particularly devastating for smallholder farmers.

A live vaccine provides lifelong immunity after a single inoculation, but it is expensive to produce, store and deploy. As a result, scientists are searching for other methods of control.

Extensive research, including the identification of the antibodies and T cells which provide resistance to disease, is helping scientists at ILRI develop a subunit vaccine for ECF.

Once available, the vaccine could dramatically improve cattle health and productivity over a large swathe of Africa.

SUMMARY

Hundreds of millions of households in Africa depend on livestock for their welfare and survival. Not only are they a valuable asset that can be traded in times of hardship, they also provide meat and milk, which can be consumed or sold. Among the diseases which threaten cattle in Africa the most economically damaging is East Coast fever (ECF), caused by the protozoan parasite *Theileria parva* and transmitted to cattle by the brown ear tick. Although a live vaccine provides good immunity, its production and delivery are expensive and time-consuming. Scientists at the International Livestock Research Institute (ILRI) have been developing a subunit vaccine that could be more affordable, easier to produce and safer to deliver to cattle.

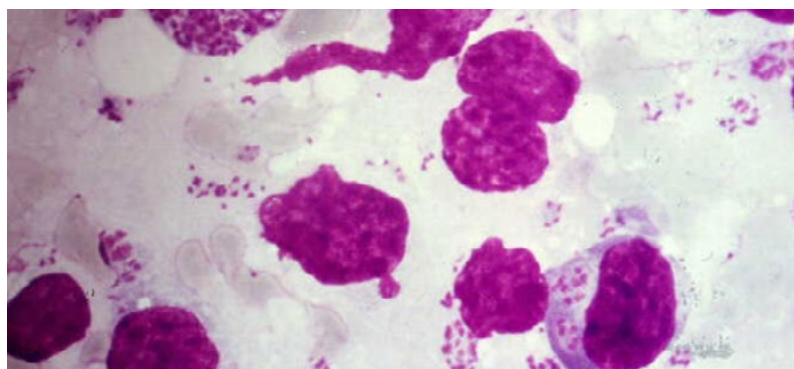


Photo: ILRI

East Coast fever Theileria parva magnified under a microscope. East Coast fever is caused by the protozoan parasite Theileria parva, which ranks as the first in tick-borne disease constraints of cattle in sub-Saharan Africa.

Banner photo: Cattle at the Garissa livestock market in northeastern Kenya, the biggest market of its kind in East Africa. Livestock diseases such as East Coast fever can have devastating effects on livelihoods and national economies.

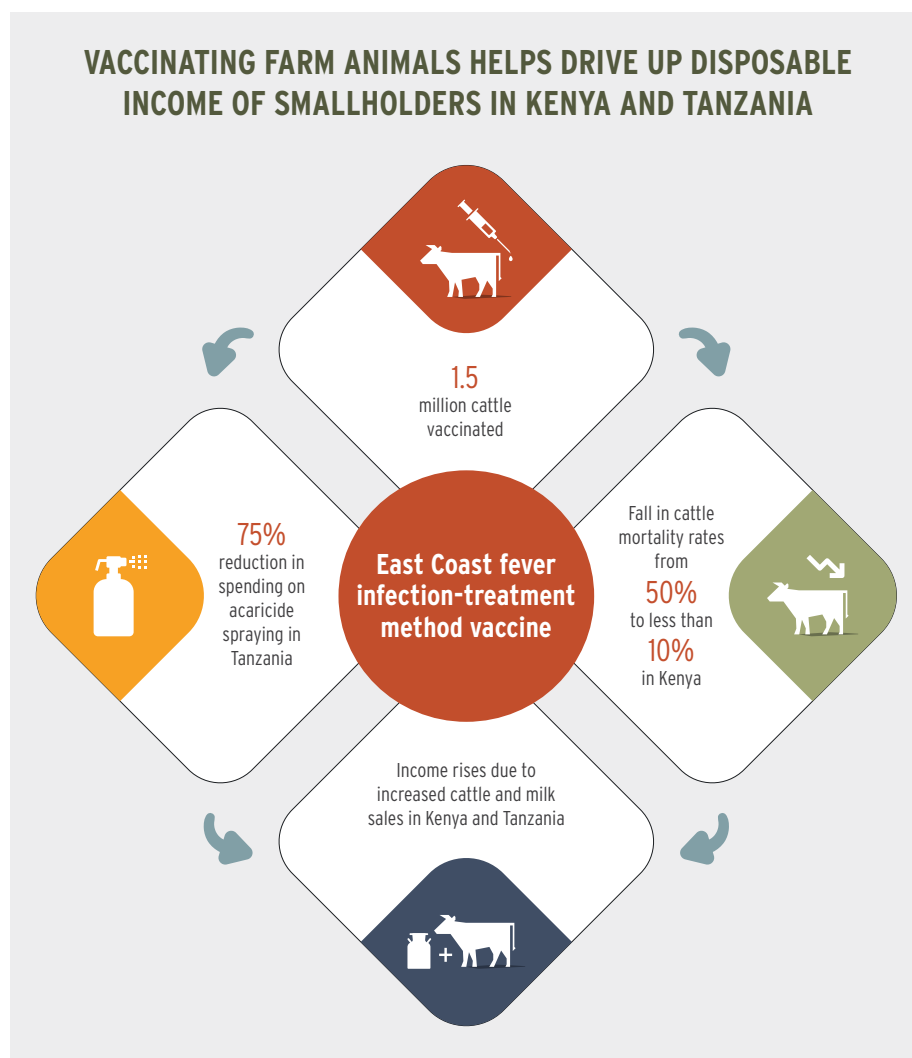
INTRODUCTION

East Coast fever occurs in 12 countries in eastern, central and southern Africa and kills over one million cattle a year. Approximately half the 75 million cattle in this area are at risk of catching the disease. In herds which have not previously been exposed to ECF, over 80% of cattle die within three to four weeks of infection. In 2005, ECF-related deaths led to the loss of some USD 300 million of revenue, representing 44% of the combined value of beef production in Burundi, Kenya and Rwanda that year.

The direct impact of ECF includes the loss of livestock, the stunting of calves, reduced milk production among survivors and the costs of preventing and controlling the disease. Smallholder farmers are often the worst affected, making their households even more vulnerable and food insecure.¹ The disease has also

deterred many farmers from adopting more productive breeds that are less resistant to ECF than indigenous cattle.

ECF was first identified in cattle in the early 1900s, but serious attempts to tackle the disease only began in the 1960s and 1970s. The early work on developing a vaccine led to the creation of the Muguga cocktail, derived from three different isolates of the parasite.² This is delivered to cattle using an infection-and-treatment method (ITM). Cattle are infected with the parasite and simultaneously treated with an antibiotic.³ This is still the main vaccine providing lifelong protection against ECF – it has provided immunity to some 1.5 million cattle – but its manufacture, cold-storage requirements and delivery are complicated and expensive, with the cost of the vaccine being beyond the means of many smallholder farmers.



Impact of the infection-treatment method vaccine against East Coast fever. However, the manufacture, cold storage and administration requirements of the vaccine means it is not easily accessible by smallholder farmers, thus the need to develop alternative solutions.

¹ Marsh, T. L., Yoder, J., Deboch, T., McElwain, T. F., & Palmer, G. H. 2016. Livestock vaccinations translate into increased human capital and school attendance by girls. *Science Advances* 2(12): e1601410.

² Radley, D. E. 1981. *Infection and treatment immunization against theileriosis: Vol. 14*. Current topics in veterinary medicine and animal science. Martinus Nijhoff Publishers.

³ Di Giulio, G., Lynen, G., Morzaria, S., Oura, C. and Bishop, R. 2009. Live immunization against East Coast fever--current status. *Trends in Parasitology* 25(2): 85-92.

DEVELOPING A SUBUNIT VACCINE

Live vaccines like the Muguga cocktail use the entire pathogen to stimulate an immune response similar to the one seen during natural infection. In contrast, subunit vaccines contain components from the pathogen which are selected for their ability to elicit a strong immune response. The risk of side-effects with subunit vaccines is minimal. Production costs are usually lower than those for live vaccines and subunit vaccines do not have the same stringent cold storage requirements. They are therefore easier to deploy and more affordable for smallholder farmers and pastoralists.

A prerequisite to developing a vaccine, either live or subunit, is an understanding of the immune factors and parasite antigens which help cattle develop immunity. The ITM vaccine demonstrates that it is technically possible to vaccinate cattle, and this provides a strong justification for developing subunit vaccines. Research by

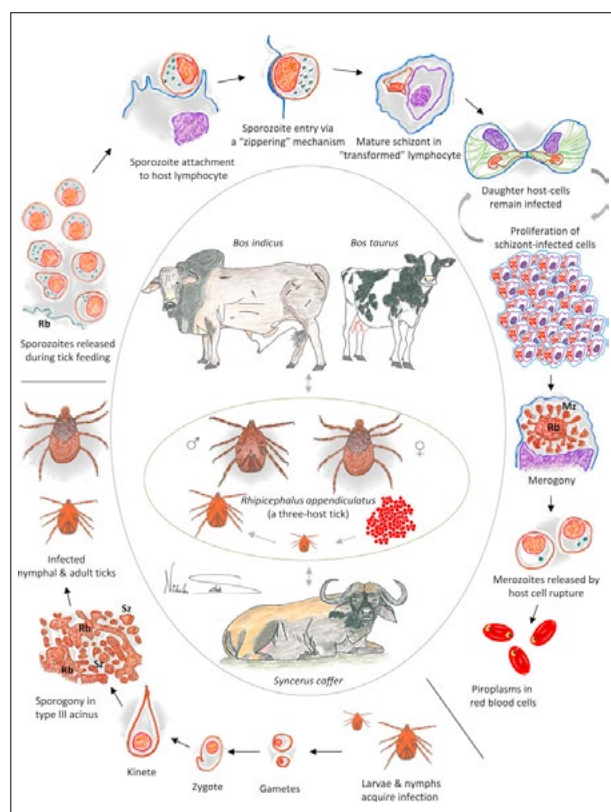
scientists at ILRI and the International Laboratory for Research on Animal Diseases (ILRAD) and its collaborators led to the identification of two types of immune response that contribute to immunity to ECF, one dependent on T-cells, the other on antibodies.

Research on the ITM vaccine led to the identification of a cytotoxic CD8⁺ T-cell response that kills schizont-infected cells.⁴ This is the major mechanism mediating immunity to ECF.⁵ In addition, cattle from ECF endemic areas were found to contain antibodies that neutralize the infectivity of the sporozoite stage of the parasite.⁶ This means that antigens derived from the parasite are candidates for developing subunit vaccines, as they can respectively elicit cytotoxic CD8⁺ T-cells and antibodies. These two types of response are also the subject of intense research in developing vaccines for malaria, HIV and Tuberculosis (TB).

SEARCHING FOR THE RIGHT ANTIGENS AND METHODS OF IMMUNIZATION

One challenge when tackling ECF lies in the complex nature of the parasite. *T. parva* has approximately 4000 genes⁷, compared to fewer than 20 for an RNA virus like SARS-CoV-2. This makes the search for candidate vaccine antigens a demanding exercise. In addition, we know that the ITM vaccine induces strain-specific immunity, indicating that a broad-spectrum subunit vaccine will be dependent on the use of antigens that are common between different strains, or require the judicious use of a cocktail of antigens to mimic the immunity imparted by the Muguga cocktail. Nevertheless, several antigens – the targets of sporozoite-neutralizing antibodies and schizont antigens targeted by T cells from cattle immunized by ITM – have been identified.

The quality of immune responses generated, and thus the level of immunity to ECF, varies with different methods of immunization, so the second challenge lies in identifying methods of immunization that induce robust sporozoite and schizont killing activity. In the general area of vaccinology, it is harder to induce CD8⁺ T-cells responses than it is to induce antibody responses. Hence, considerable effort has gone into defining antigens and assessing immunization methods.



This figure illustrates the different life cycle stages of the parasite as it cycles through the mammalian and tick host. Originally published in [dx.doi.org/10.1016/j.ttbdis.2016.02.001](https://doi.org/10.1016/j.ttbdis.2016.02.001).

⁴ Pearson, T. W., Lundin, L. B., Dolan, T. T. and Stagg, D. A. 1979. Cell-mediated immunity to Theileria-transformed cell lines. *Nature* 281(5733): 678-80.

⁵ McKeever, D. J., Taracha, E. L., Innes, E. L., MacHugh, N. D., Awino, E., Goddeeris, B. M. and Morrison, W. I. 1994. Adoptive transfer of immunity to Theileria parva in the CD8⁺ fraction of responding efferent lymph. *Proceedings of the National Academy of Sciences of the United States of America* 91(5): 1959-63.

⁶ Musoke, A. J., Nantulya, V. M., Buscher, G., Masake, R. A. and Otim, B. 1982. Bovine immune response to Theileria parva: neutralizing antibodies to sporozoites. *Immunology* 45(4): 663-68.

⁷ Gardner, M. J., Bishop, R., Shah, T., de Villiers, E. P., Carlton, J. M., Hall, N., Ren, Q., Paulsen, I. T., Pain, A., Berriman, M., Wilson, R. J. M., Sato, S., Ralph, S. A., Mann, D. J., Xiong, Z., Shallom, S. J., Weidman, J., Jiang, L., Lynn, J., and Nene, V. 2005. Genome sequence of Theileria parva, a bovine pathogen that transforms lymphocytes. *Science* 309(5731): 134-37.

STATUS OF EXPERIMENTAL SUBUNIT VACCINES

Significant advances have been made in the identification and characterisation of antigens which can be used in a vaccine.⁸ This entailed the development of assays for antigen identification, exploring different immunization methods and the development of immunological assays to measure immune responses in cattle.

Two antigens of interest for the development of a subunit vaccine directed against the sporozoite cycle stage of the parasite were identified several years ago.⁹ However, only the p67 antigen, the major surface antigen, has given consistent immunity to ECF, varying in efficacy from 20% to 70% in laboratory studies, depending on the different forms of recombinant p67 used and the method of immunization.¹⁰ Unfortunately, it has not been possible to express stable recombinant versions of p67 that mimic the native sporozoite protein. Recent experiments have focused on a portion of p67, known as p67C, as it has been easier to work with and achieves the same level of protection in vaccinated animals.

Five different p67C nanoparticle-based antigens combined with the ISA206VG adjuvant have been tested. Immunization with a combination of two of these resulted in 50% immunity and required three doses of antigen. A vaccine target product profile requires an immunization method of two doses and an efficacy of at least 70%, so more research is required to improve the efficacy of sporozoite vaccine antigens. In this context, eight new candidate sporozoite antigens have been discovered using a genome mining approach, but these need further testing on cattle. It is possible that a robust sporozoite vaccine requires a cocktail of antigens.



Photo ILRI

Microscopic view of a tick from ILRI's tick laboratory in Nairobi, Kenya.



Photo ILRI/David White

Scientists dissect infected ticks in ILRI's tick Laboratory.

Considerable effort has also been expended on developing a vaccine which can stimulate a lethal response from the CD8⁺ T cells at the schizont stage of the parasite life cycle. This is the *modus operandi* of the live ITM vaccine. Using different assays, a total of ten schizont antigens have been identified and nine different immunization methods have been tested. In one early experiment to develop a schizont subunit vaccine, 19 of the 24 cattle immunized with five parasite antigens showed readily detectable CD8⁺ T cell responses, but in only four of the animals did this response lead to the killing of infected cells.¹¹ Recently, the use of just one antigen – Tp1 – conferred immunity on approximately 30% of the animals when used in a dose which killed 100% of the unvaccinated control group. The antigen was deployed in a prime-boost regimen, where cattle were immunised with Tp1-recombinant human adenovirus boosted with Tp1-recombinant Modified Ankara Virus.¹² Much remains to be done to identify more potent methods of immunization and/or combination of antigens.

Experiments exploring immunization of p67C with the Tp1 antigen reveal an increased rate of survival when compared with the use of p67 alone at 100% lethal dose. This was done using the two corresponding immunization regimen, indicating that a combination of the two arms of the immune system, the cytotoxic T-cells and the antibodies, can enhance the protective effect. Combination approaches may also help when developing a broad-spectrum subunit vaccine. What remains to be uncovered is the identity and number of sporozoite and/or schizont antigens that will be required.

⁸ Nene, V. and Morrison, W. I. 2016. Approaches to vaccination against *Theileria parva* and *Theileria annulata*. *Parasite Immunology* 38(12): 724-34.

⁹ V. N., Nene, V., Iams, K. P., Gobright, E., Gobright, E., Musoke, A. J. and Musoke, A. J. 1992. Characterisation of the gene encoding a candidate vaccine antigen of *Theileria parva* sporozoites. *Molecular and Biochemical Parasitology*, 51(1), 17-27.) and PIM (Toye, P. G., Metzelaar, M. J., Wijngaard, P. L., Nene, V., Iams, K., Roose, J., Nyanjui, J. K., Gobright, E., Musoke, A. J. and Clevers, H. C. 1995. Characterization of the gene encoding the polymorphic immunodominant molecule, a neutralizing antigen of *Theileria parva*. *Journal of Immunology* 155(3): 1370-81.

¹⁰ Nene, V., Kiara, H., Lacasta, A., Pelle, R., Svitek, N. and Steinaa, L. 2016. The biology of *Theileria parva* and control of East Coast fever - Current status and future trends. *Ticks and Tick-Borne Diseases* 7(4): 549-64.

¹¹ Graham, S. P., Pelle, R., Honda, Y., Mwangi, D. M., Tonukari, N. J., Yamage, M., Glew, E. J., de Villiers, E. P., Shah, T., Bishop, R., Abuya, E., Awino, E., Gachanja, J., Luyai, A. E., Mbwika, F., Muthiani, A. M., Ndegwa, D. M., Njehira, M., Nyanjui, J. K. and Taracha, E. L. 2006. *Theileria parva* candidate vaccine antigens recognized by immune bovine cytotoxic T lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America* 103(9): 3286-91.

¹² Svitek, N., Saya, R., Awino, E., Munyao, S., Muriuki, R., Njoroge, T., Pellé, R., Ndiwa, N., Poole, J., Gilbert, S., Nene, V. and Steinaa, L. 2018. An Ad/MVA vectored *Theileria parva* antigen induces schizont-specific CD8⁺ central memory T cells and confers partial protection against a lethal challenge. *NPJ Vaccines* 3(35).

NEXT STEPS

Being able to predict protection based on immune parameters eases the task of testing different antigens and delivery systems. Considerable effort has been put into identifying immune correlates, especially after immunizing animals against the sporozoite stage of the parasite, and targeting the generation of protective antibodies. Different assays have been developed based on the quantification of antibodies present in serum and an assessment of their quality. The analysis of several hundred archived samples is showing very promising results.

A Feed the Future Innovation Lab for Animal Health project on ECF https://pdf.usaid.gov/pdf_docs/PA00Z41T.pdf will use these assays for antigen selection prior to animal experiments. The antigens will be delivered in nanoparticle form to increase potency and stability. The project will also develop new in vitro assays and introduce new nanotechnology platforms to be used for ECF and other diseases where an antibody-based subunit vaccine would be useful.

Regarding induction of cytotoxic T cells to schizont antigens, there are two main things to consider. The first is choosing the right antigens and second is determining whether one or several antigens are needed to kill the infected cells. The many *T. parva* strains in the field and the diversity of tissue-type antigens in cattle complicates the process of selecting antigens. A particular parasite antigen may be good in one calf but not in another and a comprehensive study would be required to select the best antigens, involving screening of many immunized cattle with different antigens for their reaction to all the 4000 genes from *T. parva*. Nevertheless, this could be done, followed by selection of the most “popular” antigens.



Photo ILRI/Lwitiko Mwakuluka

A trainee administers the East Coast fever infect and treatment vaccine (ITM) to a cow in Mbeya, Tanzania. The subunit vaccine work has been informed by years of experience in developing and administering the ITM vaccine.

The other important consideration involves choosing a suitable vaccination system for induction of cytotoxic T cells in large animals, which is at the same time easy to perform and cheap to produce. If many antigens are needed it may be necessary to identify T cell epitopes to reduce the amount of antigens that need to be inserted into the vaccine..



Photo ILRI/Kabir Dhanji

Cattle for sale at the Garissa livestock market in northeastern Kenya.

CONCLUSION

Substantial progress has been made on developing a subunit vaccine for ECF, but there is some way to go before one will be available. Technically the easiest pathway is to explore whether a combination of p67 and new sporozoite antigens could do the trick. Different cheap and easy-to-produce formulations and delivery systems would need to be tested to obtain adequate and long-lasting immune responses. The cellular immunity is far more complicated to work with and requires a substantial amount of research and funding to select the antigens and identify a way to deliver them.

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