Access to livestock health interventions and products in dairy and cattle value chains in Tanzania

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In Tanzania, one of the main foci of the CGIAR Research Program on Livestock and Fish health flagship, both in its laboratory and field work, was to control East Coast fever (ECF) and contagious bovine pleuropneumonia (CBPP) affecting the smallholder dairy value chain. This brief reviews results from the last five years and draws lessons learned.

East Coast fever challenges

Scientists from the International Livestock Research Institute (ILRI) worked with partners to enhance access of smallholders to the Infection and Treatment Method (ITM) vaccine. The USAID-funded project, Scaling up the delivery of ITM in Tanzania, facilitated access to the treatment along the value chain by helping to establish additional distributors in previously unserved parts of the country. Additional vaccinators have also been trained and various ways of creating awareness among livestock keepers are ongoing. The project is also working with the wider ‘Maziwa Zaidi’ project to develop other delivery models such as through producer organizations, dairy business hubs and cooperatives to link them with distributors or to play a role in the delivery value chain, such as providing cold chain.

Demand

Kidenke, the farmer featured in the box, is fortunate. Less than 5% of farmers, and animals at risk, have access to ITM technology. Despite its existence for the last 30 years, few farmers have heard of it. It has mostly been delivered through pilot programs. The need for specially trained animal health service providers to deliver the vaccine adds to its already high cost (vis-à-vis other vaccines). And given the limited market, the few distributors only cover part of the country. This problem is not limited to Tanzania. Less than 2.5% of the animals at risk in countries suffering from ECF endemics have been vaccinated.

Supply

The other big issue for the ITM vaccine is its availability in the region. Currently the AU-supported Centre for Ticks and Tick-borne diseases (CTTBD) in Malawi is producing the vaccine, following the transfer of the technology by ILRI with support from GALVmed. In Tanzania it is estimated that about two million calves are born in ECF endemic areas every year. Even if only 50% of farmers adopt the technology, Tanzania alone would require at least a million doses per year. Kenya and Uganda could require a similar amount of doses. Can one production site such as the CTTBD cope with such a demand?

Case study: ITM lifted me from poverty

The first impression you get when you meet Nicholas Kidenke is of a man at peace; content, happy, filled with hope about tomorrow. He smiles easily and is ready to tell his story. He first encountered ILRI as a beneficiary of a project to up-scale delivery of the East Coast fever (ECF) vaccine, commonly known as the ‘Infection and Treatment Method’ (ITM). The name comes from the fact that the vaccine infects healthy cattle with specific strains of the causative agent, Theileria parva and simultaneously injects them with a long acting antibiotic. This results in a mild reaction leading to immunity for life.

Kidenke, now a wealthy farmer, attributes his fortune to the effectiveness of ITM. Kidenke had about 800 cattle but started to lose calves, 75% in one year. A veterinarian diagnosed East Coast fever, and advised him to increase the frequency of tick control. But nothing worked. The same veterinarian told him about a recently launched vaccine (ITM). But the nearest supplier was 1800 km away. Kidenke was willing, but the control costing USD 8 per animal was prohibitive.

He sold several bulls and convinced the vaccine supplier to come to Ngara for the cost of the journey and lodgings. Of the 100 animals vaccinated, he only lost 20. The following year, he lost 5, a huge improvement. He had found a solution. But the cost was still prohibitive. He informed his neighbours and together they were able to get their animals vaccinated for the cost of the drug. The group has now become an organization representing the interests of livestock keepers and Kidenke is the secretary.

He has since bought a new farm and has close to 2000 cattle. When the vaccine is available, he gets all his calves vaccinated each year. Kidenke now wishes to invest in improving his animals as he no longer fears ECF. Delighted the ITM project has helped establish a vaccine distributor in Kahama, some 300km from Ngara, the cost of the vaccine is still problematic, and it is difficult to deliver as it requires specially trained personnel who are in short supply in Tanzania.
Dosage
The commercial scale batches of the ECF-ITM vaccine produced by ILRI and CTTBD were supplied in straws containing 32 and 40 doses each, respectively. Each straw must be thawed and diluted immediately prior to use. Once thawed, the vaccine must be used within four hours and any vaccine unused by that time must be discarded. Whilst this is not a major problem when vaccinating large herds in pastoralist settings, use of the vaccine in smallholder settings, where each smallholder may have two or three cattle, can lead to substantial wastage of the vaccine.

Transmission from buffalo
The natural reservoir of *Theileria parva* is the African buffalo. Although the vaccine has proven very effective in cattle infected, via the tick vector, from other cattle, there is conflicting evidence in the scientific literature concerning the ability of vaccinated cattle to withstand challenge from ticks which have fed on buffalo. Prompted by reports of clinical ECF cases in vaccinated cattle on a conservancy where cattle co-graze with buffalo, ILRI scientists undertook a controlled field trial which showed that the vaccine had little effect in protecting cattle against buffalo-derived parasites (Sitt et al. 2015).

Underreporting
Reproductive diseases especially in smallholder dairy systems have probably been underreported. These reproductive diseases could be partly responsible for the long calving intervals reported in smallholder dairy systems together with poor nutrition and poor heat recognition by smallholder dairy keepers. Recently ILRI under the Agricultural Technology Transfer project has started to investigate the importance of reproductive diseases in smallholder dairy systems in Tanzania. The information collected will inform further research into this neglected area. One major constraint has been the lack of easy diagnostic tools for some of the diseases; this area of research could contribute to addressing the issue, especially the need for effective point-of-care tests used by farmers.

ECF achievements
Deploying the vaccine
In 2007/8, ILRI produced the second commercial-scale batch of the ECF-ITM Muguga cocktail vaccine. In recent years, supply of the vaccine to approved distributors became an activity of the flagship. Since 2012, 594,600 doses of the vaccine were distributed, predominantly to Tanzania (85%), Uganda (7%) and Kenya (6%). There were no substantiated reports of vaccine failure from any of the distributors or livestock owners, apart from cattle co-grazing with buffalo (see above). It has been estimated that 594,600 doses of the vaccine protected at least 220,000 cattle from ECF-related deaths, assuming a fatality rate of 40% and 10% in a susceptible herd of pastoral and dairy cattle respectively, in addition to making savings on reduced acaricide use and cost of treatment.

Fewer-dose straws
To overcome the dosage constraint, ILRI demonstrated that the vaccine straws can be thawed, diluted, repackaged and refrozen with minimal loss of viability. An initial experimental production resulted in straws containing 5–8 doses (Patel et al. 2016). With VetAgro Ltd from Tanzania, ILRI showed that the vaccine was safe and effective under both experimental and field conditions.

Characterizing the vaccine
The Muguga cocktail, the most widely used version of the ECF-ITM live vaccine is a mix of three parasite isolates. Obtaining clear knowledge of the composition of the vaccine is important for: quality control of successive batches; studying transmission in field situations; and acquiring a better understanding of its mechanism of action. Scientists in the Flagship contributed to this in two ways. First, using mini- and micro satellite markers they confirmed the existence of 14 different strains (genotypes) of the parasite in the vaccine stablitate (Patel et al. 2011). Second, flagship scientists demonstrated the amount of antigenic variation among the vaccine parasites was relatively limited (Hemmink et al. 2016). The latter observation is important as it was originally thought that the broad protection provided by the multi-isolate Muguga cocktail, compared to single isolates, was due to a greater degree of antigenic variation. This means that possibly a simpler cocktail could also be sufficient to convey protection, and thus make production easier.

Improved manufacturing process
In 2016, a study of the full production process for the Muguga cocktail ECF-ITM by flagship scientists recorded several improvements to the process introduced in the 1996 and 2008 commercial-scale batches produced by ILRI (Patel et al. 2016). This will serve as an important reference document for future production. In addition, the article documented the production and characteristics of the ‘reference’ stablitates made in 2008. These stablitates are intended to be the ‘seed’ stablitates from which future batches of the vaccine will be made.

Stakeholder engagement
With GALVmed, the health flagship convened a workshop in August 2014 of interested parties to discuss the status
and challenges associated with ECF-ITM vaccine. The participants—a range of actors involved in the manufacture and deployment of the vaccine in the affected countries—identified a list of priority items needed to improve the manufacture, distribution and use of the live vaccine (Toye and Ballantyne 2015).

Tolerance to *Theileria parva* infection

A surprising observation from the field trial described above is that the progeny of a particular ILRI sire appeared to be tolerant to buffalo-derived *T. parva* infection. This observation has now been confirmed in subsequent field trials using further progeny of this sire (Toye et al. unpublished data). In a collaboration with the Roslin Institute, flagship scientists are seeking to identify the genetic region responsible for this tolerance. The findings should lead to a genetic marker for breeding tolerant cattle, while identification of the mechanism mediating the tolerance may help the identification of new vaccine or therapeutic targets.

Developing a next-generation vaccine

In addition to the need for a liquid nitrogen cold chain to deliver the ITM, the vaccine’s cost and the complexity of producing it, the use of live parasites for vaccination increases the risk of spreading the parasite to non-immune animals from vaccinated animals.

Flagship scientists are thus working on 2 strategies to discover a cheaper and safer sub-unit vaccine:

- **The first is to gain a greater understanding of the immune mechanisations elicited during the ITM vaccination**, since ITM induces a strong cytotoxic T lymphocyte (CTL) response and, part of the immunity conferred needed in a subunit vaccine.

As a first step, laboratory reagents needed to be developed to enable the assessment of the antigen-specific CTL immune response in cattle immunized with *T. parva*, or with recombinant CTL antigens of the parasite. These reagents are recombinant major histocompatibility (MHC) class I molecules, known as bovine leukocyte antigen (BoLA) class I molecules in cattle. A library of 15 different BoLA molecules, covering the major BoLA molecules present in Holstein/Friesian cattle of Kenya were expressed, purified, and, since eight CTL lines expressing the correct BoLA class I type were available, eight of them were tested in vitro with this bank of CTL lines from *T. parva* immune animals. These reagents have allowed us to characterize more specifically, by flow cytometry, the phenotype of CTL elicited towards the known immunodominant *T. parva* antigen Tp1. Moreover, the use of these BoLA molecules, in conjunction with the use of an immunoinformatics (reverse immunology) platform, allowed us to identify the exact minimal CTL epitope of a known *T. parva* antigen (Tp2) as well as a new BoLA specificity of a known CTL epitope.

- **The second is to identify antigens that could be included in a subunit vaccine.** It is already known that ITM-immunized animals develop CTL that kill parasite-infected cells, and that some antigens of the parasite induce that response; Tp1 antigen being one and p67 another (the latter being the major surface protein on the parasite at the sporozoite stage).

Flagship scientists tested several methods to deliver the Tp1 antigen, the p67 antigen or both antigen together in groups of 13 to 15 cattle, to see if the target of 70% level of protection could be reached. Upon infecting the three immunized groups of cattle with an LD100 infecting dose of the parasite, 33% were shown to be immune in the p67C only group, 27% were immune in the Tp1 only group, and 46% of the animals in the combined p67C & Tp1 antigens group were immune. Though the 70% target level of protection has not been achieved, it is clear that combining antigens can increase immunity levels. Repeat experiments are planned using an LD70 infecting dose which is more representative of what is happening in the field.

Contagious bovine pleuropneumonia

CBPP, another major cattle disease, is caused by *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*). The existing vaccine, a live attenuated vaccine, confers limited protection and causes a reaction discouraging farmers from presenting animals for vaccination. But perhaps the biggest constraint to delivery is its short duration of protection; it requires re-vaccination within a year. The available diagnostics are good at herd level, not at an individual cow level, thus limiting the control of the disease.

Flagship scientists have taken two approaches to the development of an improved vaccine:

**The first is to study host-pathogen interactions**, specifically trying to understand the immunological responses to broaden the basic knowledge of the disease, and identify immune targets for subunit vaccines. In efforts to understand the underlying immunological mechanisms of CBPP, scientists have characterized the core surface proteome (Krasteva et al. 2014). They also examined antigen specific antibody responses towards 65 surface proteins for up to 232 days post infection, and observed that high antibody titres did not protect against formation of Mycoplasma containing sequestra in the lungs of infected cattle (Schieck et al. 2014).

**The second is to employ state-of-the-art synthetic genomics tools** to engineer *Mycoplasma mycoides* subsp. *capri* to explore the function of specific genes/molecular structures of the bacteria.

Synthetic biology tools have been established at ILRI, such as genome transplantation for *Mycoplasma mycoides* subsp. *capri* (*Mmc*) which infects goats and is the closest relative of *Mmm*, the causative agent of CBPP. Flagship scientists generated a mutant strain which lacks the terminal gene in the galactofuranose polysaccharide synthesis pathway. This mutant lacks galactofuranose polysaccharide coating the surface of the bacteria. In vitro, this mutant showed increased cell membrane permeability, enhanced adhesion to host cells and increased resistance to serum killing activity compared to the wild type (Schieck et al. 2016). Preliminary data show that the galactofuranose polysaccharide is a virulence factor and the mutant is a promising vaccine strain for Mmc and this knowledge will inform the vaccine development for CBPP.

Computational modelling has shown that improved diagnostic tools will also be crucial to eliminate CBPP (Ssematimba et al. 2015).
Lessons learned

- Production of ITM has several uncertainties it would be less risky if there were more than one producer of the vaccine. Given the disease spreads from South Sudan to Mozambique and given the logistically complexities of transportation, it would also seem that having the production in more than one geographic region would help.
- It is important that ILRI continue to engage long after products have been developed and transferred to producers. The question is perhaps how long this should continue. It is clear that some of the impacts may not have been realised unless ILRI continues to engage with the relevant partners to ensure the product goes to scale.
- In hindsight perhaps, ILRI should have spent more research effort in improving the ITM technology. For instance more effort should have been put into research to produce a smaller dose package. This would probably enhance adoption of the technology among smallholders.
- To form hypotheses and theories and test them, more basic knowledge on mycoplasma and the interaction of different species with their hosts (e.g. ruminants) is needed. Existing infection models are also sub-optimal and need to be improved. More samples are needed from the field and more opportunities need to be created for discussion with epidemiologists about prevalence and transmission to inform lab work.

References

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