

## A SIX-WEEK INSIGHT INTO THE DEVELOPMENT OF A NEW VACCINE FOR EAST COAST FEVER AND LIFE IN KENYA

My six weeks in Kenya were absolutely incredible; mind-expanding in so many ways. I did my best to experience all aspects of the culture while at the same time learning new skills. I also did my best to get out of the lab, spend some time working with vets on the practical side and get out of Nairobi!

The lab work was challenging; not because of the complexity of the tasks that I had to carry out, but because of the need to apply the bench work to the bigger picture of developing a new vaccine, and the even bigger picture of the need for the vaccine.

The International Livestock Research Institute (ILRI) is an international charity, funded by the developed world making affordable and sustainable changes in the developing world in the areas of agriculture and farming (I sound like their PR). I joined the team in lab 6 at the Nairobi Headquarters from 27<sup>th</sup> June until 9<sup>th</sup> August developing a protein vaccine for East Coast Fever (ECF).

### The Need For The Vaccine:

Control of ECF is, at the moment unsatisfactory. It is estimated that it costs the Kenyan farmers US\$ 300 million per year (*Dr E. Taracha, personal communication*). This is due to the inevitable death once a susceptible animal is infected, and loss of productivity during the disease (See appendix 1).

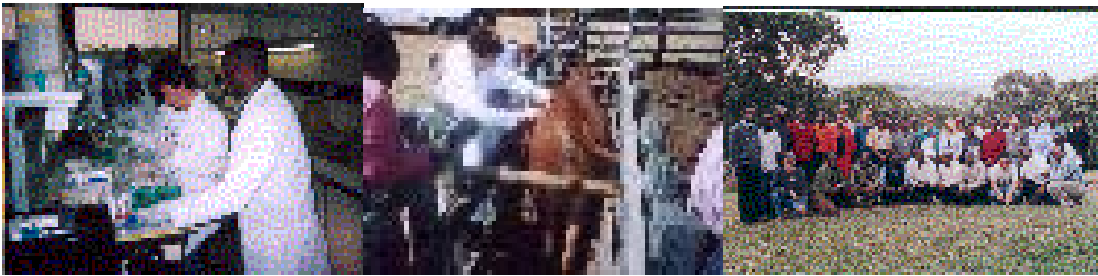
### The Vaccine Development:

Before I arrived, the *T.parva* genome had been sequenced and a cDNA library set up. From this, and serum antibody studies, antigens necessary for causing the immune response in cattle were identified. When I arrived, these antigens had been used to inoculate cattle twice, four weeks apart, and the animals were then challenged by inoculation of the sporozoite. I was to carry out ELISPOT tests, cytotoxicity assays and lymphocyte proliferation assays (see appendix 2) in order to test the efficacy of the identified peptides as immunogens and therefore potential vaccines. In order to carry out these assays, I had to prepare the cells (see appendix 2): transfected fibroblasts and monkey Cos cells, and PBMCs.

I also spent some time with the tick man (he loves his ticks!) dissecting ticks and removing their salivary glands. The ticks were fed on infected animals and then ground to form an innoculum to infect the cattle. I spent some time on the microbiology side too, carrying out PCR of certain sporozoite genes and using electrophoresis to identify which *E.coli* colonies had taken up the gene. These were then sequenced and used by lab 6. I also had some experience of diagnosis; carrying out ELISAs and microscopy of lymph node biopsies.

The result was a vaccine that provided protection for the cattle against the *T.parva muguga* strain of the parasite.

The next step in the vaccine trial is to run a new trial, on newly acquired cattle, which are from the Kuria area of Kenya and from Tanzania and consequently have been exposed to different strains of *T. parva* than those that we were testing in this trial. It is essential that the vaccine protects against all strains of *T.parva*, not just the Muguga strain that is prevalent around Nairobi.



The lab work;  
Immunogenicity testing

The field work;  
Stitching up in Kapiti

Lab five and six staff

### The Field Work:

At the beginning, I expressed an interest in getting some practical field experience while I was here. I spent time at the unit on site bleeding animals in order to test their cells in the immunogenicity assays, taking skin biopsies to generate the fibroblasts for transfection, and taking liver biopsies for use in the trypanosomiasis project. I also travelled to Kapiti, to ILRI's 33,000-acre ranch where the herd that provides their experimental animals is kept. Here I assisted with, carried out anaesthesia on and stitched up the removal of a granuloma and the repair of an umbilical hernia. I returned here to carry out some embryo-transfers, in order to propagate a particular MHC haplotype that is known to present one of the potential vaccines. I also visited Naivasha to take some blood samples from some potential trial-candidates (or did we only go there for the good lunch on the way home...?)

## SUMMER PROJECT REPORT, CHARLOTTE JOYCE

### The Down Time:

On my first weekend, I was whisked off to Juja to be initiated into the Hash House Harriers, what a flair setting for my first Hash run; 12Km through savannah against the back drop of mount Kenya, through coffee and maize plantations and along the banks of the Nairobi river. The initiation fines and party were quite an experience... The following weekend I went away with the same guy who took me running, Joseph. We went back to his home manyatta near Kajiado, and I slept a very uncomfortable night on cow hide crawling with bugs in the dark, intolerably smoky atmosphere of his mother's boma. It was an incredible experience and I know how lucky I am to have been. The language barrier was quite a problem, as illustrated by my midnight, mimed conversation with Joseph's mother about going to the toilet... The third weekend was spent with the other students. 16 of us went down to the Masai Mara, where we spent three days enjoying the sounds, scenery, animal-spotting and the famous wildebeest migration, and the nights spent falling off cliffs just metres from the Mara river teeming with hippos and crocs...after a shortened week at work, I was again off to visit a colleague's family, this time in the west of Kenya, at Kisumu on lake Victoria. Kisumu is a small, friendly city, so much nicer than Nairobi, with which I fell immediately in love. The landscape is so different here: green and fertile. We ate the famous Tilapia (only found in lake Victoria) for three meals during my stay. I was surprised by the bread and sausage brought out for breakfast; good job I like fish, eh? It took me until my final weekend in Nairobi to do the tourist traps of the city. I visited the museum, and Karen Blixen's house and got all romantic about the film 'Out Of Africa'. The following day I walked a couple of the Ngong Hills, where Karen Blixen's lover is buried. The Maasai have a legend of the hills being the knuckles of a dead giant. I took a day off from work on my final Tuesday in order to visit Hell's Gate. We cycled the 22km round trip and my rear will vouch for the bumpy 'roads' and poor saddle, not to mention the sand dunes and the drag on my back wheel...it was well worth the pain. The views in this mini grand-canyon are incredible. Some might say I got around a bit in my six weeks here... The week day evenings were spent having barbeques, going to dinner with the other students, and improving my squash skills. Being in Nairobi was quite oppressive at times: no going out of the compound on your own after dark, no taking public transport after dark, no wondering around during the day out of the compound on your own...it got to me a bit, and made me appreciate my freedom back home.



The down-time:  
Lions in the Mara

At the graduation ceremony  
in Kajiado

Milking at the  
Homestead in  
Kajiado

Partying with the other  
students

### The Verdict:

I would thoroughly recommend doing this kind of thing. The skills I have learned in the lab will be directly transferable to any research work that I do in the future, whether in research or while in practice for example, ELISPOT tests are the basis of the new TB test. The surgical skills are the kind of things that I may not have been able to get first-hand experience of in the UK due to differences in formalities in practice; epidurals, collecting and inserting embryos, liver biopsies, hernia stitch-ups.... I also really appreciated the chance to see the country at the weekends. It has been amazing, and I hope to return one day...

All that remains to be said are my thanks: to those that made this possible, whether by financial backing or help with references or by integrating me so well into the lab work and showing me the wonderful country:

Dr Evans Taracha, Dr. Duncan Mwangi, Dr. David Dunne, John Nyanjui, Frederick Onono, Joseph Gesharisha, Dr. David Kihurani, James G, Elias Awino, Menase, Alice and Thomas in lab2, Dr. Josh Slater, Dr. S. Lloyd, BVA, Intervet, Cambridge Commonwealth Trust and UAC of Nigeria, and all in lab 5 and 6...and anyone else I've forgotten...

## Appendices to summer project report, Charlotte Joyce

### Appendix 1: the need for a vaccine

ECF is caused by *Theileria parva*, a protozoan. It infects lymphocytes and subverts their cell signalling system, causing the cells to transform, multiply and metastasize (*Dobbelaere, Fernandez and Schultzer, 2000*). Death occurs due to the metastasis of cells to the lungs where cytokines and physical damage cause the endothelium to be damaged and pulmonary oedema to follow (*Dr. D. Mwangi, personal communication*).

Recovery leads to permanent immunity, as long as it is boosted by continued challenge, and this is the basis of treatment at the moment. Animals are infected and then treated with tetracyclines to kill the sporozoites, but allowing a solid immunity to develop. The problems with this are the cost of the drugs, the vet and the follow up treatment and the high rate of failures. Tick control is also in place, but is costly (pour-ons cost US\$ 28.85/adult/year in Kenya in 1998 (*McLoed and Krisjansen, 1999*)). It is necessary to control ticks to prevent the infection of highly-productive exotic species such as Friesians, but this means that exposure of the resistant breeds falls below that required to maintain their immunity.

### Appendix 2: The process of developing a vaccine

The Overall Aim: To identify antigens produced by *T.parva* sporozoites that stimulate the immune response to infection in cattle. These antigens could then be produced either in the form of *T. parva* DNA expressed by *E.coli* vectors or as peptides. When inoculated into the cow, the presented peptide should stimulate the bovine CTLs to monoclally expand and kill the infected cells, while producing memory cells for protection against a subsequent challenge. The immunity would be boosted continually by the presence of the tick vector and the injection of the sporozoite and its immunogenic antigens. When challenged by the immunogenic antigens of the sporozoite, the memory T cells will recognise the antigen and CTLs specific to that antigen will kill any infected cells that express it.

#### The Testing Protocol:

- 1) Immunisation by route 1 and adjuvant 1 —————> immunogenicity assays
- 2) Immunisation by route 2 and adjuvant 2 —————> immunogenicity assays
- 3) Challenge with virulent *T.parva*
- 4) Compare proliferation assays, ELISPOT and restimulation results with previous results

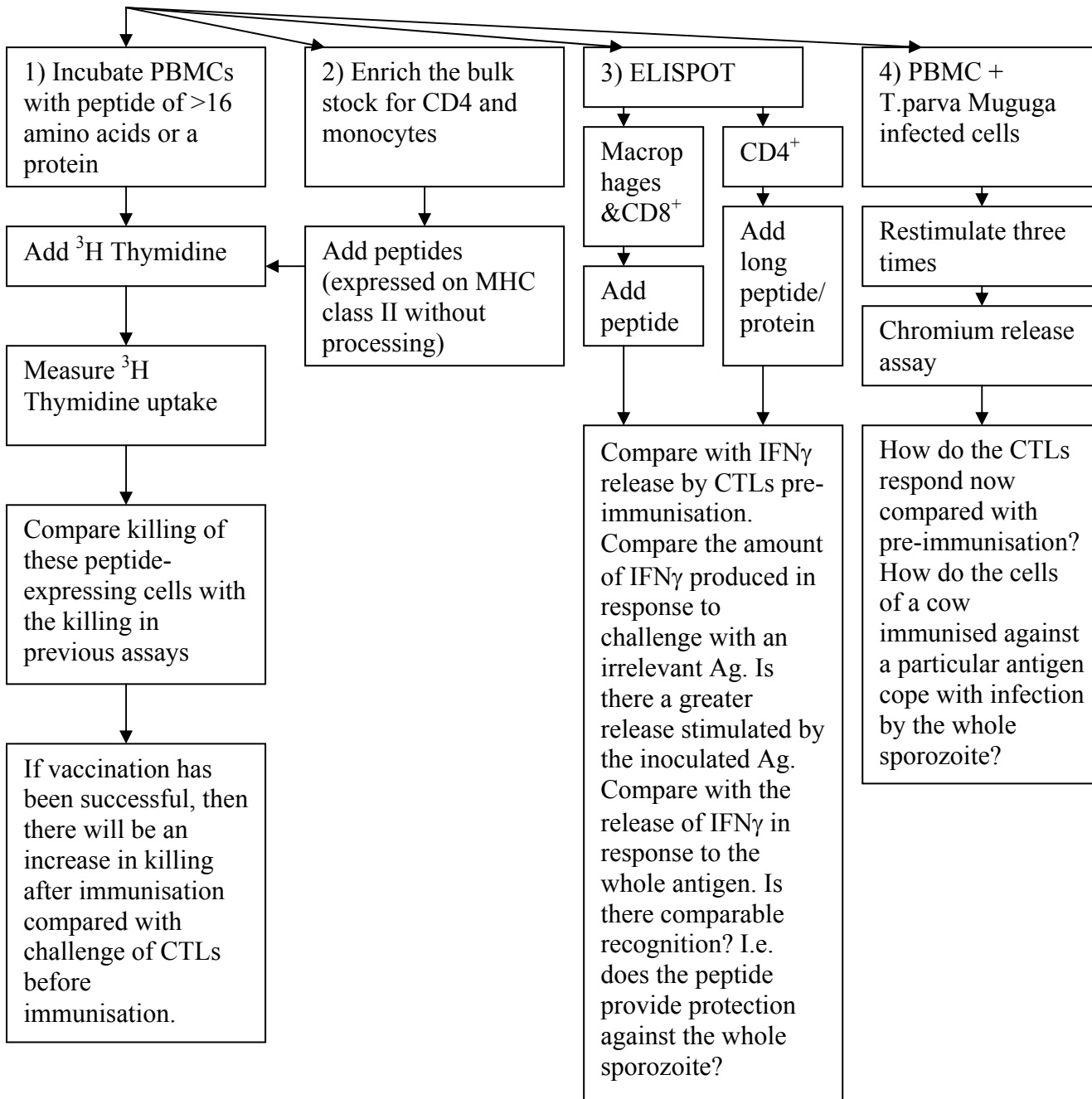
If the vaccine has been successful, then killing by the CTLs after vaccination should be more pronounced and more effective than the killing of the CTLs collected prior to vaccination. I.e. there would be an increase in CD4, CD8 and lymphocyte responses.

The immunogenicity assays: Record pre-immunisation levels of PBMCs, CD8, CD4 and macrophages. Before vaccination: make TpM infected cell lines and skin fibroblasts from each cow to use as stimulators of the immune mediator cells.

The immunogenicity assay, continued:

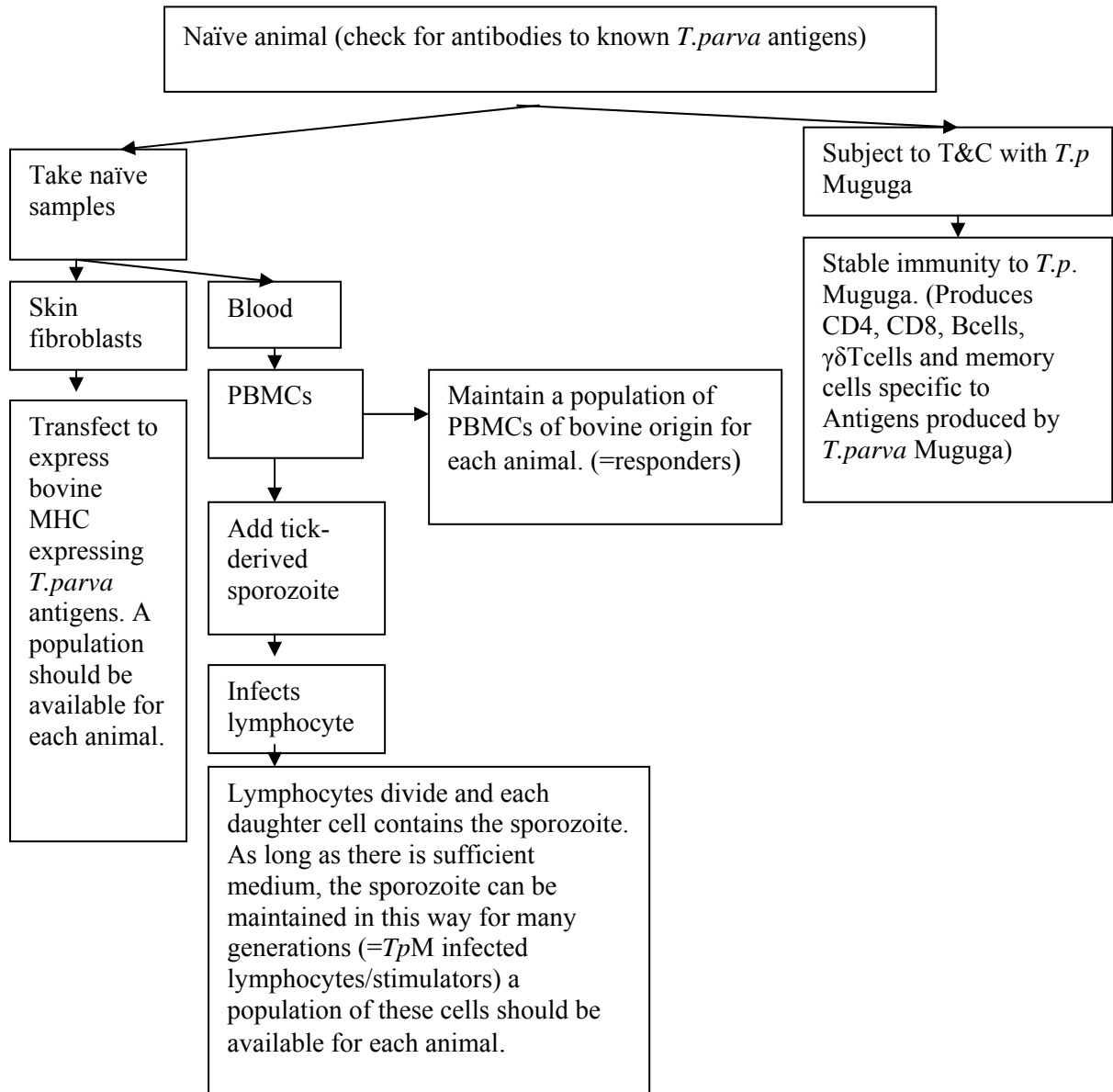
Animals are vaccinated with one of five *T. parva* antigens

Take blood sample



Stages Involved In the Immunogenicity Assay

1) Generation of *T.parva* Muguga-Specific CTL s



2) Immunisation of cattle: Cattle are immunised by infection with the tick borne sporozoites and simultaneously treatment with long-acting tetracyclines. Subsequently, animals develop a solid immunity to *T.parva* challenge. Solid immunity is maintained by repeated exposure to the infected ticks in the field.

3) Preparation of PBMCs: Mix 15ml of blood with 20ml of Ficoll-paque plus and centrifuge. This will divide the blood into four bands. a) plasma; b) lymphocytes, monocytes and platelets; c) ficoll; and d) granulocytes and red cells. The layer containing lymphocytes, monocytes and platelets should be removed and washed. More anticoagulant is added and the tube is centrifuged again.

4) Do the immune system cells recognise the vaccinated antigen?

a) ELISPOT assay: A measure of IFN $\gamma$  production by CTLs as a measure of their recognition of the antigen against which the animal was immunised. When a CTL recognises its target (antigen plus MHC) it releases IFN $\gamma$ . This can be measured using labelled antibodies and the amount released can be quantified.

Antojen	Monoclonal antibody
CD3	MM1a
CD4	IL-A11 IL-A12
CD8	IL-A51 IL-A105
$\gamma\delta$	GB21a
B lymphocytes	IL-A30
monocytes	IL-A24

Method: take PBMCs and add IL-A105. Incubate and then add goat anti-mouse antibodies bound to magnetic micro beads. Use a magnet to separate out the CD8<sup>+</sup> Tcells from PBMCs. Separate out monocytes. To the CD8 cells and monocytes, add *T.parva* antigen peptides. Incubate to allow recognition of the peptide and release of IFN $\gamma$  and wash. Use rabbit anti-bovine IFN $\gamma$  antibodies to detect released IFN $\gamma$ . Use anti-rabbit antibodies tagged with APS and then add the substrate to quantify the amount of IFN $\gamma$  produced using an ELISPOT reader.

Result: There was a significant increase in release of IFN $\gamma$  when cows were inoculated with certain antigens (the identification of these antigens is commercially sensitive)

b) Cytotoxicity Assay (Killing Assay)

set-up:

	Autologous TPM		Blasts or SF	Pep1 in Blasts or SF	Pep2	Pep3	Pep4	Pep5	Pep 6	Pep 7
100 $\mu$ l Effector: 50 $\mu$ l Target										
80:1										
40:1										
20:1										
10:1										
No effector	medium	Tween20								

Method: Incubate target cells from the experimental animal (taken before vaccination) with <sup>51</sup>Chromium. These target cells should be transformed to express certain peptides. Incubate these cells with effector cells (CTLs from the vaccinated animal). After four hours, take a sample of the cells and use a Geiger counter to measure the release of <sup>51</sup>Chromium. Tween 20 lyses all the target cells, and so represents a maximum release of <sup>51</sup>Chromium. Recognition of the target cell by the effector CTLs results in lysis of the target cell and release of Chromium into the supernatant.

Result: The cells immunogenicity assays carried out after vaccination revealed a population of cells more cytotoxic to the cells expressing antigen and, indeed those infected with the whole sporozoite.

c) Indirect immunofluorescence assay:

Method to determine the proportion of CD3<sup>+</sup> and CD8<sup>+</sup> T cells: Take PBMCs and incubate with a 1<sup>o</sup>Antibody; anti-CD8 IL-A51 (murine IgG1 vs bovine CD8). Wash and then incubate with a second 1<sup>o</sup> antibody; anti-CD3 MM1a (murine IgM vs bovine CD3. After washing this, incubate with 2<sup>o</sup> antibodies tagged with FITC (green); goat, anti-mouse IgG1, and then with a 2<sup>o</sup> antibody that binds to mouse IgM, tagged with PE (red). Use FACS flow cytometry to detect the proportions of cells of these cell lines.

Result: There was an expansion of the CD8<sup>+</sup> CD3<sup>+</sup> Tcell population. This indicated that immunisation causes an increase in cytotoxic cells.

d) Proliferation assay:

Method: Incubate PBMCs with irradiated TpM infected cells from the same animal for 1 week. Harvest the live cells (using Tryptan blue) and resuspend at the correct concentration with fresh TpM infected cells. Repeat this process twice and harvest the PBMCs that have been stimulated by the presentation of *T. parva* antigens. Incubate these PBMCs with a *T. parva* Muguga antigen and then add <sup>3</sup>H Thymidine. Thymidine is a nucleic acid that will be taken up by metabolically active cells. The activity of a cell is therefore quantified by its uptake of <sup>3</sup>H Thymidine.

Result: when a stimulation index was calculated, taking into account the stimulation of PBMCs by irrelevant antigens, there was significantly more proliferation of PBMCs after vaccination than before.

Overall Results of the immunogenicity assays: During this vaccine trial, two antigens of *T.parva* Muguga were identified that made significant differences to the immune status of the vaccinated cows, when assessed by the above four methods (a-d).

5) What Happens Now?

Now that certain antigens have been identified that can stimulate a significant immune response *in vitro* when challenged with the *T. parva* Muguga strain, the next step will be to see if the vaccine protects animals from other strains of the disease. Due to 20 years of passaging, the *T.parva* Muguga stock is fairly homogeneous, whereas other strains such as *T.parva* Marikebuni is much more heterogeneous (McKeever, D J *et al*, 1999). This means that animals protected from *T.parva* Marikebuni are also protected from T.p Muguga, whereas the opposite is not true. In order to develop the vaccine and test its protection against other strains of *T.parva*, the lab has acquired 10 Zebu cattle from the Kuria district of Kenya, and from Tanzania. These animals have been exposed to and will therefore be protected from different strains of *T.parva*. The vaccine should be able to protect from multiple strains of *T. parva* if it is going to be commercially viable. The test of this, initially, is to see if the cells of these animals, immune to foreign strains of *T. parva*, will recognise and be activated against cells expressing the potential vaccine antigens. These *in vitro* experiments will be carried out alongside *in vivo* experiments where naïve cattle are vaccinated with the potential vaccine and then inoculated with various different strains of *T.parva*.

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## Appendices to summer project report, Charlotte Joyce

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