“Improved vaccines for the control of ECF in cattle in Africa”

Notes from the ECF Consortium Workshop
Addis Ababa, 9-11 February 2015

Compiled by Peter Ballantyne and Vish Nene

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0900	Opening, welcome, objectives	Vish Nene
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Presentations and posters

All presentations and posters listed are available to project participants in the Yammer network.

Presentations

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1. Re-assessment of p67C Protein and thoughts in Seroneutralization Assay - Anna Lacasta
2. Guava research - Ine de Goeyse
3. Antibodies to define novel candidate sporozoite vaccine antigens - Lucilla Steinaa
4. Genomic based method to dissect bovine antibody responses - Roger Pelle
5. Antibody phage display and recombinant antibody methods for antibody and antigen discovery and mapping antibody responses to p67C - Frederic Fellouse, Valencio Salema and Sachdev Sidhu
6. High-throughput discovery of B-cell epitopes using high-density peptide microarrays - Søren Buus and Morten Nielsen

7a. Improving the ITM production process - George Chaka

7b. ITM Process Improvement at CTTBD, Lilongwe - David Kalenzi

8. Genome re-annotation and identification of putative antigens: 2014 brief review - Joana Silva, Kyle Tretina, Joshua Orvis, Tim Gotia, Roger Pelle, Kemi Abolude, Priti Kumari, Shaikh Iqbal, Richard Bishop, Claudia Daubenberger
9. Genome-wide genetic variation: 2014 Brief review - Joana Silva, Elliott Drábek, Daniel Harris, Joshua Orvis, Kyle Tretina, Tim Gotia, Richard Bishop, Claudia Daubenberger
10. PIM immunization studies - Ine De Goeyse
11. Development of a Tick Challenge Model - Cassandra Olds, Lindsay Fry, Don Knowles and Wendy Brown
12. Characterizing the magnitude, kinetics and functional profile of the protective T-cell response and evaluate Ad5/MVA antigen delivery systems - Tim Connelley

13a. Determining requirements for induction and recall of an effective CD8 T cell response - Ivan Morrison

13b. Identifying new schizont antigens recognised by parasite-specific T cells from immune cattle - Ivan Morrison

14. Selection of T. parva CD4+ T-cell Candidate Antigens and T. parva Immunopathogy - L. Fry, D. Knowles, D. Nelson, I. Morrison, T. Connelley, J. Silva, G. Scoles, C. Olds, and W. Brown
15. Evaluating the Ad5/MVA antigen delivery systems - Nicholas Svitek, Rosemary Saya, Elias Awino, Stephen Munyao, Thomas Njoroge, Sarah Gilbert, Vish Nene, Lucilla Steinaa
16. Bovine Herpesvirus-4 as a delivery platform for Theileria parva antigens - L. Williams, I. Morrison, N. Machugh, W. Brown, L. Fry, D. Knowles and G. Donofrio
17. Use of heat shock proteins for induction of CTL immunity - Charity Muthoni, Benjamin Nzau and Roger Pelle
18. CTL-induction, adjuvants and reagents - Lucilla Steinaa
19. Yeast expression systems and RVC activities - Shan Goh and Dirk Werling
20. Identification of MHCI-associated peptides using LC-MS/MS - Nicola Ternette
21. ECF Project Management Report For the period October 2013–December 2014 - David Kiereini

Posters

- Role of CD4 T cell responses in immunity against the schizont stage of *T. parva* – Charlotte Bell and Ivan Morrison
- Irradiated autologous cells induce cytotoxic CD8 T cell responses but delayed protection – Charlotte Bell and Ivan Morrison
- The role of cross-presenting dendritic cells in priming protective CD8 T cell responses – Charlotte Bell and Ivan Morrison
- Use of a peptide library for antigen screening – Charlotte Bell and Ivan Morrison
- Bovine MHCI typing by Illumina NGS – Tim Connelley and Ivan Morrison
- Tetramer and TCR analysis – Tim Connelley and Ivan Morrison

Background

Due to high mortality and morbidity rates livestock diseases are an impediment to the livelihoods of poor farmers in Africa, who struggle to attain food and nutritional and economic security. Vaccines can alleviate such constraints as they are among the most successful disease interventions invented. The goal of this project is to increase cattle productivity through the development of improved vaccines for the control of East Coast fever (ECF).

ECF caused by the protozoan parasite *Theileria parva* ranks first in tick-borne disease constraints of cattle in sub-Saharan Africa and kills one animal every 30 seconds. It has a devastating impact on pastoralists and smallholder farmers because it can kill within 3-4 weeks of infection. ECF is present in 11 countries where roughly 28 million cattle are at risk, but has the potential to spread with the uncontrolled movement of infected cattle as the distribution of the tick vector and suitable tick habitats is wider than that of the parasite. Over one million cattle die of ECF each year resulting in annual losses exceeding $300 million.

Our goal is to design subunit vaccines for the control of ECF. In phase 1, we proposed a range of key strategic activities in the research to product development continuum to:

- Improve aspects of the current sub-optimal live (infection and treatment method - ITM) ECF vaccine.
- Fill knowledge gaps regarding the qualitative and quantitative aspects of acquired immune responses that mediate immunity to ECF.
- Test the vaccine potential of candidate vaccine antigens and develop a more detailed antigen map.

Outputs from this phase will contribute in the short-term to production of a better quality live vaccine as an interim vaccine solution and provide proof-of-concept for an ECF subunit vaccine aimed at obtaining evidence of protection in 70~80% of animals of defined MHC genotype given a homologous parasite challenge. Success in phase 1 will contribute to our goal of developing a broad-spectrum subunit vaccine for the control of ECF (phase 2).

In order to achieve these objectives we have assembled a team of multi-national experts from the field of ECF research, bovine immunology, parasitology and genomics with essential inputs from a private-public partnership and the private sector. An advisory panel will provide scientific oversight and help evaluate progress and stop-go decisions. This panel will interact with a project management committee to ensure that technical risks are reduced and activities are completed or re-directed in a timely manner, within budget and that maximum advantage is taken of knowledge and learning generated during the project.

ECF activities are also funded by the Normal Borlaug Commemorative Research Initiative (NBCRI) and the CGIAR Research Program on Livestock and Fish.

Objectives of the meeting

1. Review the past 12 months, products, results delivered
2. Plan the next 36 months, products, deliverables
3. Get inputs from the External Advisory Committee and Funding agencies
4. Propose improvements to consortium operation

Session 1 – Introduction

The 2015 Annual ECF Consortium workshop was held at ILRI-Addis Ababa from 9-11 January 2015. The ILRI campus provided an excellent environment for formal workshops and a relaxed atmosphere for post-workshop discussion and debate. This was a second meeting of the Consortium but the first one with the expanded membership due to additional activities sponsored by the supplemental funding from DFID.

The opening session by Vish Nene outlined the objectives of this meeting, to:

1. Review the past 12 months, products, results delivered
2. Plan the next 36 months, products, deliverables
3. Get inputs from the External Advisory Committee and funding agencies
4. Propose improvements to consortium operations.

The presentation reminded Consortium members of the genesis of the project, its structure and management.
As the scope of some activities had changed and new ones added it was necessary to review the Results Framework and working budgets. It was necessary to catch up on the science, discuss problems and tentative solutions, and learn from what worked and did not work during the last 12 months.

While communication between different groups had improved, this remained an issue that could be improved, including how to make better use of the External Advisory Committee. This was the first meeting of the Consortium with the new Senior Program Officer from BMGF, Nick Juleff.

Based on comments from the last Consortium meeting, we decided on a different format for this one, including use of a Facilitator, Peter Ballantyne, which proved to be a great success. The heart of this was reporting and discussing the science in a series of short 5~10 min presentations followed by group discussion, debate and consensus building. Beyond the science, the program also provided self-organized 'workshops' on different topics of shared interest as well as some plenary space to reflect on some overall directions in the consortium.

On the last morning, the program management team met comprising project leaders and members of the External Advisory Committee. The notes and actions from that meeting are included in this report.

Session 2 – Review of 2014 science

During the meeting, teams working on different activities gave brief presentations (5 minutes). These were followed by intensive group discussions and Q&A. Presenters were asked to focus on: Main activities in the past year; Key results and deliverables in the past year; New insights gained; and plans or the coming year.

Here we include notes that were received for some sessions. Here there are no note, please contact the session presenters indicated.

Re-assessment of the sporozoite p67 molecule as a candidate vaccine antigen – Anna Lacasta

A. Why is this piece of research being done and what is its contribution to the challenge 'Developing Improved vaccines for the control of ECF in cattle in Africa'?

Sera from cattle in ECF endemic regions contain sporozoites-neutralizing antibodies that target a surface molecule called p67. This molecule induces weak antibody responses during the ITM, but immunization of cattle with recombinant p67 induces neutralizing antibodies and immunity to ECF in ~50% of vaccinated cattle under laboratory conditions. Interestingly, an 80 amino-acid section called p67C gives the same level of protection as the whole p67.

Unfortunately, the immunity to ECF achieved was not correlated with the titers of antibodies or even the neutralizing activity of those antibodies. We will improve the antibody neutralizing assay to allow determining the neutralizing capacity of the archived sera samples and find a correlation between the immunity achieved and the antibody activity.

B. Briefly, what research was actually done, who by, and what were the key results/insights in 2014?

<u>Re-assessment of p67C</u>
An in vivo experiment was done dividing the animals in three groups. In group 1 (G1) we placed 12 control animals that were not immunize, in group 2 (G2) the 11 animals who received 2 immunizations of 450 µg of p67C mixed with Montanide ISA206 adjuvant (expressed in *E. coli* and purified with Ni^{+} column, 99% purity) and in group 3 (G3) the 11 animals who received three immunizations of the same p67C protein. The immunizations where administered every 4 weeks and three weeks after the last immunization all animals (G1, G2 and G3) where challenged with an LD70 dose of *T. parva* Muguga 3087.

The last results using p67C (10 years ago) were confirmed in this experiment. G3 animals (three immunizations) achieved a ~50% reduction in severity, being the only group with a significant protection achieved (p=0,022). G2 (two immunizations) doesn't have a significant reduction in severity even though the severity was reduced ~30%. With this p67C formulation we cannot reduce the number of doses from three to two, because the protection achieved is not the same.

Sera, PBMCs, whole blood samples were collected every week and stored appropriately. Sera samples were analyzed in an ELISA using p67C recombinant protein as a coating antigen and anti-IgG/M: HRP as a secondary antibody. All sera were serial diluted from 1/11,11 to 1/24300 (3 fold dilution every time). A $CD4^{+}$ proliferation assay was also done with fresh cells using p67C recombinant protein as stimuli.

In both assays (proliferation and ELISA) we observed that the animals in G3 have the highest titers of antibodies and $CD4^+$ cells against p67C recombinant protein in average. This result correlates with the protection achieved in this group. Unfortunately, we were not able to correlate individually.

People involved: Anna Lacasta, Stephen Munyao, Elias Awino, Rosemary Saya, Charity Muthoni, Benjamin Nzau, Thomas Njoroge, Roger Pelle and Lucilla Steinaa.

Seroneutralization assay

The new seroneutralization assay is based on: briefly, mix the sera (different dilutions) and the sporozoites (same batch every time) and incubate for 1 h. After dispense the mix on a PBMCs culture and incubate for 10 days. Finally the read-out of the assay will be done by a cellular ELISA using anti-PIM antibody to detect infected cells (96-well plate).

In order to develop this new seroneutralization assay there was the need to establish the highest dilution where the 100% of the wells are infected. During 2014 this dilution was established in 1/1280 using the *T. parva* Muguga 3087 stabilate #4235.

The anti-PIM (clone ILS32.2) cellular ELISA was confirmed as a good read-out assay using TpM cells as a source of infected cells. It was possible to detect as less as 10 infected cells with this method. It is need to improve a bit the technique but it looks like we are going to implement this method as a final read-out of the new seroneutralization assay.

C. In terms of this piece of research, what are your plans for 2015-2016 (and who will be involved)?

Re-assessment of p67C

- Test all the sera from the re-assessment of p67C expeiment in a p67C recombinant protein ELISA using different isotypes: IgG1, IgG2, IgG and IgM (people involved: Anna Lacasta, Stephen Munyao, Benjamin Nzau and Charity Muthoni).
- Test all the sera in a native p67 ELISA using a capture ELISA and using different isotypes IgG1, IgG2, IgG and IgM (people involved: Anna Lacasta, Stephen Munyao, Benjamin Nzau and Charity Muthoni).
- Test all the interesting sera with the new seroneutralization assay (people involved: Anna Lacasta and Stephen Munyao).
- New formulation with p67C to make it more immunogenic: TMV-p67C, slow-release beads with p67C… (people involved: Anna Lacasta, Stephen Munyao, Charity Muthoni, Thomas Njoroge, Roger Pelle and Lucilla Steinaa).
- Re-assess p67C protein in an LD100 experiment with exotic breeds (Anna Lacasta, Stephen Munyao, Thomas Njoroge, Roger Pelle and Lucilla Steinaa).

Seroneutralization assay

- Look for a bovine T-cell line in the literature and/or immortalize T-cells.
- In parallel work with ConA blast to homogenize the cell culture in the assay.
- Test all the possible positive controls for the assay: AR22.7, AR21.4, 23F, TpM…
- Do the anti-PIM cellular ELISA in a real infection to validate the assay.
- Switch to ILS40 (anti-PIM) antibody to homogenize the reagents with the other members of the Consortium.

D. What were the most significant questions/issues/challenges/feedback that came up in your bus stop, and how are they big addresses (if applicable)?

Re-assessment of p67C

- Look for individual correlation between the protection achieved and the antibody titers or activity:
    - Suggestion from the Bus Stop: Use a different antigen for the ELISA, ideally use the native p67 protein using a capture ELISA.
    - Suggestion from the Bus Stop: maybe there is some reaction against the His-Taq, check it doing the ELISA with another protein containing the His-Taq.
    - Suggestion from the Bus Stop: look at the different isotypes.
- There is no boost in antibody titers or $CD4^+$ proliferation index after the challenge and the antibody titers in general are not high.
    - Suggestion from the Bus Stop: maybe re-challenge the animals to see if then there is a boost.
    - Suggestion from the Bus Stop: look until when is possible to detect antibodies against p67C without challenge or after the challenge.
    - Suggestion from the Bus Stop: reduce the antigen dose, in Malaria field too much antigen is not working.
    - Suggestion from the Bus Stop: try to find a new formulation that makes the p67C protein more immunogenic.
    - Suggestion from the Bus Stop: go for the full length p67 protein now that we have more tools for expression (yeast).
- LD70 dose is problem to find correlations because we have one non-reactor in the control group.
    - Suggestion from the Bus Stop: use an LD100
    - Suggestion from the Bus Stop: re-analyze the data removing the non-reactor animals
- Three are too many immunizations.
    - Suggestion from the Bus Stop: try to find a system that allows fewer immunizations achieving the same level of protection. Maybe the use of BSA particles?
    - Suggestion from the Bus Stop: use of inactivated or dead sporozoites for vaccination.

Seroneutralization assay

- No availability of a Bovine T-cell line:
    - Suggestion from the Bus Stop: try to work with ConA blast
    - Suggestion from the Bus Stop: create it *de novo*, immortalizing the cells transforming them with a specific plasmid construction
- How to make it quantitiative:
    - Suggestion from the Bus Stop: do sporozoite dilutions a part from the sera dilutions
- Need of good positive control:
    - Suggestion from the Bus Stop: use of different monoclonal and/or polyclonal antibodies: AR22.7, AR21.4, 23F, TpM… that we have in house (ILRI)
- Anti-PIM cellular ELISA not tested in a real infection
    - Suggestion from the Bus Stop: do the anti-PIM cellular ELISA in a real infection with the selected cell lines or fresh PBMC as soon as possible to validate the technique
- Anti-PIM used in the assay (ILS32.2) is not the one that the Consortium is using for another assays (ILS40).
    - Suggestion from the Bus Stop: switch to the ILS40 to see if it also works efficiently. If it works just use this one.

Immunization studies with PIM and characterization of sporozoite ligands and host cell receptors that mediate the infection process – Ine de Goeyse

A. why is this piece of research being done and what is its contribution to the challenge 'Developing Improved vaccines for the control of ECF in cattle in Africa'?

The Hepatitis B core antigen (HBcAg) is a self-assembling particle and is an excellent carrier for the presentation of heterologous epitopes. Although well-defined $CD4^+$ T helper epitopes are present, the particle can also act as a T cell independent antigen and immunogenic responses are induced without adjuvant help. Insertions between residues 77 and 78 in the immunodominant loop region induce optimal antibody production, which makes this carrier interesting in the production of an anti-sporozoite vaccine.

B. briefly, what research was actually done, who by, and what were the key results/insights in 2014?

Possible epitopes of PIM were selected and also p67C was chosen to insert in the *HBcAg* gene. Primers were developed at ITM Antwerp and first PCRs were completed. All polypeptides were cloned in a pGEM-T easy vector and sequenced. By the end of the year, HBcAg-p67C was completely finished and sequenced in January 2015. HBcAg-EP1 and HBcAg-EP2 were still under construction.

C. in terms of this piece of research, what are your plans for 2015-2016 (and who will be involved)?

All constructs will be finished and after sequencing, expression will be induced in *E. coli* cultures and checked on SDS-PAGE and Western blot. Expression of the different fusion proteins will be carried out on a large scale and proteins will be purified. After purification, correct folding of the particles will be evaluated by transmission electron microscopy (TEM). After that rabbits will be used to test the immunogenicity of the different particles. All work will be done at ITM Antwerp.

E. what were the most significant questions/issues/challenges/feedback that came up in your bus stop, and how are they big addresses (if applicable)?

Are rabbit trials worthwhile? Maybe it's better to go directly to cattle. The antibody-response of the rabbits will tell nothing about the reaction of the cattle. That's true, but by immunizing just a few rabbits, we know that the inserts are expressed well and patent to be recognized by the immune system. Unless the fact that PIM is not on the sporzoite surface, anti-PIM antibodies might still be able to block off the sporozoite entry in the lymphocytes. One of the leading antigens in the Malaria research is also not present at the surface. We still have no idea about the structure and the function of PIM…

Antibodies to define novel candidate sporozoite vaccine antigens – Lucilla Steinaa

This work was/is done by members of the ILRI ECF team: Anna Lacasta, Lucilla Steinaa, Roger Pelle, Benjamin Nzau, Stephen Munyao, Vish Nene.

To date, P67 is the only discovered antigen to which a humoral immune response has shown efficiency. Using more modern approaches it may be possible to discover other sporozoite antigens involved in the entrance of the parasite which could be possible vaccine candidates.

Nine vaccine candidates have been selected based on orthology with other apicomplexan parasites and one based on CD homology. Expression has been outsourced to GenScript. 4 have been produced and the other 6 are underway. Antisera will be produced and tested in neutralization assay.

Sporozoites were successfully stained and analyzed on the Influx sorter. Costaining with antisera against tick salivary gland material will be attempted and pure sporozoites will be sorted for immunization of mice and cattle for production of antibody libraries, which then will be tested in neutralization assays.

Plan for 2015-2016 is to get the rest of the orthologous molecules produced, generate sera and test in neutralization assay. Sort pure sporozoites, immunize mice and cattle, produce libraries and test in neutralization assay.

Responses at bus stop: It was mentioned, from the Malaria field, that there were additional new orthologous antigens which may be pursued – something we can look at.

Genomic based method to dissect bovine antibody responses

For further information contact Roger Pelle

Recombinant antibodies – Fred Fellouse

A key hurdle in the development of vaccines is the inability to discern protective from non-protective immune responses. Having a set of antibodies to characterize the bovine immune response would help us address this challenge and recognize true protective immune responses. **Goal:** To generate a set of antibodies to characterize the bovine T cell-mediated immune response. **Update:** We have purified two highly relevant bovine cell surface CD antigens and generated recombinant antibodies against these antigens for flow cytometry, immunoprecipitation, and other applications. One antibody will soon be validated at the University of Edinburg. **Next steps:** Identification of other bovine antigens to be targeted for affinity reagent development; generation and validation of novel affinity reagents for bovine immunology applications.

Part of the consortium's strategy is to identify antigens that would enable production of neutralizing antibodies against the sporozoite form of *T. parva*.
Goal: We will be using phage display technology to identify natural and synthetic neutralizing antibodies in order to (1) screen the antigen candidates for their ability to generate neutralizing antibodies (2) generate antibodies that could be used by the consortium as research reagents or vaccine adjuvants. **Update:** The generation of specialized synthetic antibodies under way. **Next steps:** Use the synthetic antibody libraries developed at the University of Toronto to generate neutralizing antibodies; develop and test novel synthetic antibody libraries tailored to targets of interest, in particular novel antigens identified by the "novel sporozoite antigen discovery" focus group.

p67 peptide chips and epitope mapping - Soren Buus

A. why is this piece of research being done and what is its contribution to the challenge 'Developing Improved vaccines for the control of ECF in cattle in Africa'?
We will be using the peptide-chip technology to to a large-scale screening for novel (sporozoite) B cell antigens.

B. briefly, what research was actually done, who by, and what were the key results/insights in 2014?

Nothing has been done on this part of the project in 2014, but we have applied the technology earlier in other project with high success. In particular have we demonstrated how the technology can be used to identify novel antigens for Chagas disease using sera from infected patients.

C. in terms of this piece of research, what are your plans for 2015-2016 (and who will be involved)?

We will define what T.parva proteins to analyze on the chip (other than p67). Define what type of sera to use to screen the chip (sera from ITM vaccinated cattle after a different number of vaccinations?, sera from infected cattle versus sera from infected buffalo?, …). Once these issues have been resolved, we will construct a peptide chip covering the selected proteins, and assay the chip with the selected sera.

People involved in this are the focus group 3 (and to some extend focus group 2)

D. what were the most significant questions/issues/challenges/feedback that came up in your bus stop, and how are they big addresses (if applicable)?

The most important questions that came up at the Bus stop related to the issues described above (definitions of protein and sera).

Improve the ITM production process - Dirk Geysen

A. why is this piece of research being done and what is its contribution to the challenge 'Developing Improved vaccines for the control of ECF in cattle in Africa'?

- This is an update on the first batch of vaccine production at CTTBD. ITM (Infection and treatment Method) is the only vaccine approach controlling ECF in the field in Kenya, Tanzania and Zambia for the moment. This is based on injecting a titrated dose of live cryopreserved sporozoites under the cover of long acting Terramycine. Efficiency and efficacy in the production process needs to be improved.

B. briefly, what research was actually done, who by, and what were the key results/insights in 2014?

- The first batch at CTTBD has been produced using the ILRI protocol. For the second batch currently under production, glycerol has been replaced by sucrose. This will improve production and facilitate in vitro testing of the vaccine in cell cultures. A change in the pick up protocol resulted in better tick infection rates, and a change in the 2 stage release procedure will reduce the time to release.

C. in terms of this piece of research, what are your plans for 2015-2016 (and who will be involved)?

- Storage of diluent in plastic versus glass bottles to ease distribution problems under field conditions Dose reduction as requested by the field, still using 0,5 ml straws but with diluted stabilate to accommodate 5 or 10 doses.
- New diluent tests to increase viability of sporos after thawing in the field.
- Looking at alternative diluent constituents to reduce price.
- Grinding optimalisation to increase sporozoite yield, and transfer protocol to new grinder equipment (heads and motor).

- Molecular characterization of isolates and field samples using 5 antigen genes and 5 mini sats. This exercise is intended to analyze the epidemiological situation and field strain composition in ECF areas not under Muguga cocktail vaccination.

D. what were the most significant questions/issues/challenges/feedback that came up in your bus stop, and how are they big addresses (if applicable)?

- Possibility of adherence of sporozoites to plastic as experienced in grinding plasmodium sporos.
- Release of new stabilate production protocol as to standardize production of research stabilates by other groups.
- Characterisation of vaccine components.
- Cross immunity tests

Generation of a genome wide locus specific map of genetic variation of T. parva

For further information contact Joana de Silva

Determining sporozoite counts – Ine de Goeyse

A. why is this piece of research being done and what is its contribution to the challenge 'Developing Improved vaccines for the control of ECF in cattle in Africa' ?

A method to quantifiy sporozoites and to distinguish between live and death sporozoites would be really helpful to improve the stabilate production process. The Guava easyCyte 5HPL is a cell counter with fluorescent detection and can discriminate cell particles between 0.19 µm and 1 µm.

B. briefly, what research was actually done, who by, and what were the key results/insights in 2014?

So far, all tests were done on a glucose stabilate of sporozoites. A staining protocol was optimized for monoclonal antibodies directed against sporozoite antigens. Sporozoites can be stained with anti-p67 monoclonal. Different viability dyes were tested: Mitotracker (living cells), PI (dead cells), 7-AAD (dead cells), FDA (living cells), Zombi Green (living cells), but none of them gave the expected results so far.

C. in terms of this piece of research, what are your plans for 2015-2016 (and who will be involved)?

The protocols will be performed on sporozoites extracted from new infected ticks, and compared with the sporozoites from the stabilates. Other dyes will be checked for viability staining. By the end of the year the Guava easyCyte will be shipped to CTTBD in Lilongwe, Malawi, where it will be used in the ITM production process.

D. what were the most significant questions/issues/challenges/feedback that came up in your bus stop, and how are they big addresses (if applicable)?

Maybe the sporozoites have no or low metabolic activity. Are there any biological assays for live/dead staining?

Development of a tick challenge model

For further information contact Glen Scoles

Use a genomics approach to prioritize genes as candidate vaccine antigens

For further information contact Joana de Silva

Characterize the magnitude, kinetics and functional profile of the protective T cell response

For further information contact Tim Connelley

Assess the requirements for induction of a protective T cell response

For further information contact Ivan Morrison

Identify additional CD4 antigens and phenotypic data on ECF - Lindsay Fry

A. why is this piece of research being done and what is its contribution to the challenge 'Developing Improved vaccines for the control of ECF in cattle in Africa'?

- The antigen discovery portion of this work is being done to find CD4+ T cell antigens to include in a recombinant vaccine. This is necessary because, in general, strong CD4+ T cell responses are necessary for adequate priming of CD8+ T cells. The work to characterize the immune response within the actual tissue of acutely infected cattle is being done to broaden our understanding of what happens in the cow during disease – e.g. dissect out appropriate and inappropriate (harmful) aspects of the response. This is being done in the hope that vaccine design will minimize these harmful aspects of this response and to promote a more appropriate, protective response in cattle.

B. briefly, what research was actually done, who by, and what were the key results/insights in 2014?

- Lindsay Fry, Don Knowles, Glen Scoles, Wendy Brown: We established the full T. parva infection cycle in Pullman, WA, and generated infected cattle and ticks, and a sporozoite stabilate.

- Lindsay Fry, Don Knowles, Joana Silva, Wendy Brown: We used in-silico prediction to select 113 candidate T-cell antigens based on predicted conservation and secretion. We ordered peptides that span those antigens (910 peptide pools), to be screened in the upcoming year using T cells from immune cattle.

- Lindsay Fry, Don Knowles, Ivan Morrison: We performed histopathology on tissues from deceased animals and discovered that T. parva causes vasculitis within the lungs, lymph nodes, liver, and spleen, and that this vasculitis is what leads to pulmonary edema and death in cattle with ECF.

- Lindsay Fry, Don Knowles, Wendy Brown: We used immunohistochemistry to begin to characterize the immune response in acutely infected animals. We discovered that pulmonary pathology is likely due to an overly robust IL-17 response, and that acute lesions

are infiltrated by large numbers of immunosuppressive T cells, which may severely exacerbate disease and suppress T cell function.

C. in terms of this piece of research, what are your plans for 2015-2016 (and who will be involved)?

- We will screen the 910 peptide pools using T cells from immune cattle to discover potential CD4+ T cell antigens for inclusion in a vaccine (Lindsay Fry, Don Knowles, Wendy Brown)
- We will finish characterizing the immune response in tissue from acutely infected cattle (Lindsay Fry, Don Knowles, Wendy Brown)

D. what were the most significant questions/issues/challenges/feedback that came up in your bus stop, and how are they big addresses (if applicable)?

- A significant challenge is MHC class I typing of cattle using allele-specific PCR. We have begun work with a collaborator to use deep-sequencing, and plan to evaluate that method throughout the year.

Evaluate Ad5/MVA antigen delivery systems – Nicholas Svitek

Comments:

- Verify if the lower responders are the survivors (weaker response would correlate with survival).
- There might be a short window of time when measuring *ex vivo* cytolytic activity (between 9-12 days).

Questions raised:

- Can we increase/force a Tp1-specific response by using viral vectors expressing stretches of the Tp1 epitope aligned together (poly-Tp1 epitope sequence)?
- What are the other correlates of protection?
- Is the TCR-p-MHC avidity the same in viral vector-vaccinated animals as in ITM-vaccinated animals?
- Can the route of immunization influence the quality of the immune response generated (sub-cutaneous or intra-dermal route would put the antigen in proximity with Langerhans cells)?
- Do the high responding CD8 cells differentiate into a specific function/phenotype (are they mono- or polyfunctional)?
- Why is there no lysis of TpM?
- Can we increase protection by adding CD4 T cells antigens (they are more promiscuous antigens) and B cell antigens?

Suggestions/Ideas:

- Challenge: perform a challenge experiment in cattle vaccinated with p67 and HAd5/MVA-Tp1
- Perform cytotoxicity assay with whole blood cells and spiking with the CTL raised *in vitro* (are there other factors from the blood that we miss in the CTL assay; measuring lysis with all cells around?)
- Add other cell populations in the ELISpot assay (Natural killer cells, etc.).
- Should we perform a third boost and see how it increases responsiveness/protection?

- Compare lung pathology between animals with CD8 and animals without or lower CD8 response (link with Lindsay's work).
- Stimulate CTLs *in vitro* with TpMs inactivated by paraformaldehyde (can it diminish immunosuppression activity of TpMs on CTLs?).
- If performing a tick-challenge, do we have a more natural level of infection and hence a better protection (is the LD100 to strong)?

Evaluate a BHV antigen delivery system

For further information contact Don Knowles

Use of heat shock proteins for induction of CTL immunity

For further information contact Roger Pelle

CTL-induction: adjuvant and reagents – Lucilla Steinaa

This work was done by members of the ILRI ECF team: Lucilla Steinaa, Roger Pelle, Nicholas Svitek, Rosemary Saya, Elias Awino, Stephen Munyao, Vish Nene.

A study was undertaken to elucidate if two A18 BoLA allelic variants (6*01301 and 6*01302) both present the Tp1 antigen. It was mandatory to determine this because presentation of Tp1 has been reported for 6*01301 but not for 6*01302 and the latter is the most prevalent allele in Kenyan cattle and because we are using Tp1 as a model antigen. The conclusion was that there was no difference found between the two alleles regarding presentation of Tp1 for CTL induction so both alleles can be used for vaccine studies.

Further, we know that CTL is important in the immune response against *T. parva* but there is not a good adjuvant/delivery system for induction of CTL in large animals and humans. An adjuvant from Statens Serum Institut (DK) was tested for induction of CTL in cattle when mixed with the Tp1 protein. This adjuvant should supposedly be capable of inducing cellular responses. However, CD8 cells were not positive in IFNγ ELISPOT and tetramer assay and CTL assay were negative.

Plans for 2015 are to test an alpha-virus platform from Harris Vaccines (US) using the Tp1 antigen. This system gives expression in the infected cells but it doesn't produce infectious virions and hence it does not spread. We will also test Silica based nano-beads in collaboration with University of Queensland (AU) in 2015.

Responses at the bus stop were: What other delivery systems will you try? Answer is that apart from these and the Adhu5/MVA we have some other ideas in the pipeline that we will pursue.

Yeast expression systems and the RVC activities – Dirk Werling

A. why is this piece of research being done and what is its contribution to the challenge 'Developing Improved vaccines for the control of ECF in cattle in Africa' ?

Developing new ECF vaccine delivery tools that can be stored at room temperature.

B. briefly, what research was actually done, who by, and what were the key results/insights in 2014?

Dr Shan Goh cloned several Theileria parva proteins in such a way that these can be expressed in the platform delivery system

C. in terms of this piece of research, what are your plans for 2015-2016 (and who will be involved)?

The resulting delivery systems will be tested in vitro for their ability to stimulate a CTL as well as CD4 response. This work will be done by Dr Shan Goh and Daniel Ngugi

D. what were the most significant questions/issues/challenges/feedback that came up in your bus stop, and how are they big addresses (if applicable)?

Everyone thought it is a really great idea – especially if it works.

Session 3 – Workshops

Alongside the science results reported in Session 2, participants formed groups to discuss cross-cutting topics. The aim as to: 1) take stock what is happening in the consortium and 2) identify specific activities or actions for the consortium. As with the science sessions, reports of some sessions are included in these notes.

ECF index and LD70/LD100 challenge – Tim Connelley

The aim of the workshop was to discuss two related but distinct issues

- ECF index – the statistical scoring system used to determine the severity of a response to challenge with T. parva stabilate.
- LD70/100 – the dose of a T. parva stabilate used to challenge immunised animals has either been LD70 (i.e. sufficient to kill 70% of animals) or LD 100 (i.e. sufficient to kill all animals). Which dose is most appropriate for use in forthcoming experiments?

ECF index

- There was general agreement that the Rowlands Index was labour intensive and difficult to implement due to the increased regulatory requirements for limiting the times that animals exhibiting signs of ill health can be maintained under experimental conditions.
- Analysis by Lucilla and Nick using a modified Rowland's scoring system (so-called 'Scotland score') demonstrated that it was conceivable that a more simplified version of the Rowland's index could be established and would prove useful. The 'Scotland score' removed the 'length' parameters in the Rowland's index and focused primarily on the 'first day' scores.
- Data from Nick and Lucilla suggested that using WBC, temperature and schizont parameters it was possible to segregate none/mild, moderate and severe responders by d14.
- Tim suggested that more quantifiable schizont scoring could be achieved by using an IFAT system incorporating anti-schizont antibody and DAPI staining (for nuclear material) to provide a numerical percentage of schizont/bovine nuclei. This staining system also provides a double staining for schizont as DAPI stains schizont nuclei also (as small and punctate clusters of nuclei co-localising with the schizont antibody) and is routinely used at both Roslin and WSU. Additionally IFAT was more rapid to analyse, less technically demanding and more sensitive than giemsa staining. Incorporation of a quantifiable schizont count may also make it a more refined parameter within any modified ECF index scoring system.
- It was agreed that a revised system that reduced the amount of work and gave a score based on measurements taken earlier post-challenge should be devised. To achieve this it would be good to amalgamate recent raw data from ILRI, Roslin and WSU and submit it to statistical analysis. Tim is to initially approach Helen Brown (who generated the 'Scotland score') to see if she is willing to lead this work in cooperation with Jane Pool at ILRI and then seek for the data to be sent from ILRI, WSU and Roslin.

LD70/100

- This discussion was unfortunately curtailed by time limits.
- Lucilla discussed that although there was some preference at ILRI for LD70, its use would require unfeasibly large numbers of animals to compensate for the reduced statistical power.

- Nick showed some data that looked at the effect of stabilate dose and LD values that indicated that small dilution factors (2-4 fold) were sufficient to reduce an LD70 to an LD40.
- Tim and Ivan from Roslin indicated a preference for LD100 as all ITM animals were comfortably protected from this dose. Furthermore, at Roslin the increase in animals required to accommodate an LD70 dose is not practical.
- It was generally concluded that experiments would use an LD100, with perhaps secondary experiments to explore further promising candidates utilising a LD70.

p67 way forward and other sporozoite antigens

For further information contact the organizers – Ana Lacasta and Roger Pelle

Sporozoite purification, quantification and ITM

For further information contact the organizers – Ine and Dirk

Computational resources

For further information contact the organizers – Joana and Morten

Peptide proteomics and BoLA reagents

For further information contact the organizer – Soren Buus

Peptide proteomics - Nicola Ternette

A. why is this piece of research being done and what is its contribution to the challenge 'Developing Improved vaccines for the control of ECF in cattle in Africa'?

- The identification of novel T-cell antigens in *T.parva* infection using mass spectrometry provides the basis for the development of new vaccination strategies.

B. briefly, what research was actually done, who by, and what were the key results/insights in 2014?

- In a single proof of concept pilot experiment with Ivan Morrison and Tim Connelley with the help of Joanna da Silva we have demonstrated the ability to perform MHC class I elution of Theileria infected bovine cells, with the identification of >2000 bovine peptides and >50 matching peptides predicted from a 6-reading frame translation of the T. parva genome. In order to control for false-positive identifications, a second control experiment of uninfected cells was performed and analysed under the same conditions. Additionally, further careful evaluation of obtained sequences assisted by BLAST searches (http://www.ncbi.nlm.nih.gov/) was conducted to exclude identifications with high sequence similarities to other mammalian genes.

C. in terms of this piece of research, what are your plans for 2015-2016 (and who will be involved)?

- More samples with different MHC backgrounds will be analysed and regarding control experiments will be optimized. Validation procedures for identified peptide sequences will be implemented. This will include MHC binding studies with Soren Buus, bioinformatical prediction of epitope processing and binding with Morten Nielsen, and analysis of existing T cell responses in infected animals with Tim Connelley.

D. what were the most significant questions/issues/challenges/feedback that came up in your bus stop, and how are they big addresses (if applicable)?

- Analysis of sporozoite proteins directly using mass spectrometry could potentially provide novel antigens for a B cell based vaccination approach as well.

Antigen delivery systems / correlation with CTL

For further information contact the organizers – Ivan Morrison and Lucilla Steinaa

Reagents, protocols, MHC typing, peptides/proteins and viral vectors – Tim Connelley

Reagents

1 - monoclonal anitbodies

- List of available monoclonal antibodies 'in-house' to be made. It was initially decided that this would be done by Tim (Edinburgh), Rosemary and Nicholas (ILRI), Lindsay (WSU) but this may now depend on decisions made by the reagents focus group. There was discussion of generating a comprehensive list of commercially available antibodies including ELISA kits
- Request by Don for list of known anti-p67 antibodies. Anna and Ines to email Don.
- Fred also made reference to the potential cross-reactive potential of antibodies generated in Toronto and that this may be worth having a list available as part of the database being set up by the reagents focus group.

2 – tetramers

- List of currently available MHCI heavy chains and the associated epitopes to be requested from Soren/Nicholas by Tim. This will then be made available through the schizont antigen discovery focus group and also the reagents focus group.

3 – cell-lines

- Ines requested a list of available cell lines. Discussion of what this is to include: Theileria-infected cell lines, CD4/CD8 anti-theileria cell lines, MHC-transfected cell lines. Also discussion of the characteristics that would need to be defined (e.g. for Theileria infected cell lines – MHCI/II type, phenotype (T-cell or B-cell etc.), strain infected with, antigenic variants etc.). Some initial list could be made but may need more discussion as part of the reagents focus group.

4 – sera

- Anna recommended a databank of sera be generated. This may need further consideration from people directly involved in anti-sporozoite immunity work to define the best format this would take and what information would be needed for each sample. Again probably best now referred to the reagents focus group.

5 – Other reagents

- It was decided that primers may be too numerous to form a database for – instead it made be easier if someone needs primers for a specific gen they send a general request out to a 'laboratory techniques' email list to be set up as part of the reagents focus group.

Action points

Location	Person	Action	Deadline 1 – should be done asap 2 – delay until discussed by reagents focus group
Edinburgh	Tim	List of available monoclonals Tetramer list from Soren	2* 1*
ILRI	Anna	Email Don Knowles list of anti-p67 antibodies	1
	Nicholas	List of available monoclonals	2
	Rosemary	List of available monoclonals	2
ITM Antwerp	Ine	Email Don Knowles list of anti-p67 antibodies	1
WSU	Don		
	Lindsay	List of available monoclonals	2
Toronto	Fred	List of monoclonal antibodies that may have cross-reactive potential for cattle	2

Inter-lab collaboration and sharing

For further information contact the organizers – Lottie

Session 4 – SWOT

In plenary, participants discussed, shared and documented the program's strengths, weaknesses, opportunities and threats. The point captured are listed below.

What we like in the consortium

- Exciting science
- Group interactions
- ECF research enthusiasm
- Great technologies
- General organization of the consortium
- Recovering and re-assessing historic data
- Diversity among the team
- Public attention to ECF is rising = more money
- High potential to deliver
- We are getting bioinformatics data to users
- Collaborations targeted to aims

What we need to improve

- Communications - internal especially
- Sharing and harmonizing of reagents/ accessing central resources
- Providing feedback
- Prioritization of approaches; potential problem of too many disparate approaches
- Sharing and shipping can be held back by regulatory issues
- Yammer
- Reporting to ILRI

Ways we can improve

- Monthly sub-group meetings
- Sharing standard operating procedures (SOPs) ...
- Specifying 'go/no-go' milestones
- Publish historical data
- Attend to IP rights
- Set up an e-forum (yammer?)
- More consortium involvement when supplementary funding is proposed or acquired.

Session 5 – Year 1 synthesis, conclusions and recommendations

To synthesize the various presentations and group work, participants re-grouped using a 'world café' method to document conclusions and recommendations emerging from the past year for each consortium objective area. The notes here were transcribed from the group flipcharts.

Objective 1: Improve aspects of the current live infection and treatment method ECF vaccine.

Conclusions

1. CTTBD moved to sucrose in stabilizer for stabilate
2. Still unable to differentiate live vs dead sporozoites
3. ECF ITM vaccine diluent will be moved from glass to plastic vials
4. ECF ITM used as 'gold standard' for investigation of immune response in vaccine/challenge work

Recommendations

1. Investigate use of Roland's index for prediction of clinical outcome post-challenge
    a. Extend broader review for use within consortium
2. Use GUAVA Antwerp for live versus killed differentiation of fresh sporozoites
    a. Go/no-go needed
3. Need to decide how geographic strain data will feed into 'revised' Muguga cocktail formula
4. Investigate improved consistency within Muguga cocktail, eg: remove 1 of 3 in Muguga cocktail
5. Greater interaction with Sanaria to learn from irradiation of malaria sporozoites
6. Investigate salivary gland dissection to increase live sporozoite recovery [Sanaria for freezing investigation]
7. Further search for susceptible continuous cell line for 'invasion assay' for quantification (live versus dead)
    a. Eileen Thacker to check for transformed bovine T cell line with Jim Roth
8. Investigate DNA quantification on stabliate, eg: propidrum monoazide [Bitium] or others
9. Investigate in vitro induction of translation in sporozoites as live versus dead differentiation [Sanaria – plasmodium]
10. Genetic manipulation of sporozoites -> attenuation; over-expression of 'protective antigens'

Objective 2: Induce antibody based immunity by targeting the sporozoite stage of the parasite

Conclusions

1. P67C can impart some protective immunity
2. Neutralisation assay is a priority
3. Ranking of antigens needs to be done by the end of the project [need platform to prioritize]
4. P67C -> + control

Recommendations

1. Improve the delivery of p67C to reduce to 2 injections only
2. Develop functional assay to predict protection -> down select new antigens

3. P67 assay to correlate Ig response with protection
4. Generate RNA sequence plus protein profiles of sporozoites
5. Common platform (adjuvants) to evaluate new antigens, also CTL
6. Find a cell line per NIT
7. Select antigens for the chips
8. Generate a hyperimmune sera (multiple animals)
9. TSG antigen: tick saliva = protein antigens
10. Choose 1-2 platforms for B cell Ap2(Z?) antigen identification

Objective 3: Induce T-cell mediated immunity by targeting the schizont stage of the parasite

Conclusions

1. We are not convinced about the direct correlation between CTL on its own as an indicator of protection but it may still be an important parameter
2. Viral prime-boost system induces CN8 resistance but not CTL effector function against parasite-infected cells
3. Delivery systems tested so far do not give an adequate protection
4. The wrong antigens or wrong delivery systems? MUCH DEBATE
    - ❑ Majority conclusion: viral delivery system appears defective in its ability to generate a response similar to ITM immunization
5. Need for further work to refine the functional profile of responses induced by vaccine constructs and the responses to challenge in comparison to those induced by ITM immunization
6. Standardization of antigens to take forward in the antigen delivery systems to be tested

Recommendations

1. Compare the response induced by ITM/Cell line vaccination (and compare these) with those induced by 'vaccines' and recall of responses at challenge
2. Balance between testing delivery systems and understanding fundamental immune parameters of protection
3. Screening for new antigens
4. Compare in different strains (conserved, non-conserved
5. 5 Evaluation of ALL potential delivery systems by end of 2015
6. Neutralisation assay to finish

Objective 4: Apply evolutionary and comparative pathogen genomics to ECF vaccinology

Conclusions

1. Downstream approaches benefitted from re-annotation
2. Transcriptional analysis of Sp2 material; other life-cycle stages. Also, buffalo vs cattle analysis
3. Establish data sharing platform

Recommendations

1. How are 'strains' identified – need a system?
2. How are 'strains' to be selected – for sequencing?
3. 'Strains' are defined as what? Genetic differentiation

4. 12-15 cloned parasites X buffalo [Kenya, rift valley fever, SAfr] ->2mb?
5. [all these already in ECF bible]

6. Comparison between buffalo and bovine infection
7. Transcriptome for parasite and host
8. Data sharing platform [announce when website is up]

9. List of Theileria specific genes
10. Look for protein families of antigenic significance

11. RNA seq exp. Infected buffalo vs. T. parva-infected cattle.
12. Measure sequential samples pre-infection, during infecyion [DrLN, Contra LN, immune response genes.
13. Compare IR genes {or whole transcriptonomics) in normal buffalo vs. cattle
14. Naïve calves? OR?

15. Make sure one common Muguga cloe is used in all labs
16. Transmission blocking -> why the piroplasm of buffalo is not transmissable

Points from the cross-cutting workshops

Conclusions

1. LD70/LD100 ECF index
2. p67 should it proceed
3. Sporozoite purification
4. Comp. genome database / proteomics
5. CTL assay / antigen delivery systems
6. Reagents and protocols
7. Make more immunogenic p67c and delivery vehicles (systems)

Recommendations

1. Re-analyze data with statistician and come up with standards in LD70/LD100 ECF index

2. Viral vectors + p67/p67c
3. Prioritize the identification of new B c ell antigens
4. Produce fragments of all the part of p67 (better than the full length?)
5. Make stable full-length
6. Making multivalent p67 will make it better? TMV
7. Identification of tick antigen proteins by using immune sera for animals immunized with sporozoite tick delivery glands
8. p67, should it be pursued?

9. Viability and infectivity of sporozoites – sp. purification

10. EUPathDB -> eukaryotic pathogen database (insert data)
11. Adding sporozoites geneomic/transcriptomic data
12. Transcriptomics of tick slavery glands
13. Computational/genomic database

14. HHC-peptides validation [binding prediction, binding assay, T cell response?] Proteomics

15. What is the gold standard of CTL measurement?
16. CD8/CD4 RNA sequencing
17. Profile of response in ITM immunized animals -> what time point?
18. CTL assay/antigen delivery system

19. Using standardized SOP to compare, side by side
20. Using a commercial company to make it easily available
21. Reagents sharing
22. Protocols
23. Using synthetic phage display technology to fast rack generation of new antigens (?)

Session 6 – Results framework

This was reviewed
Several changes were proposed and adopted

Session 7 – Vision of success

Exercise

1. Looking forward one year from now
2. What does success look like? For you as an individual, for the consortium as a whole?
3. What are the common elements - of the community, of the platform - that MUST be in place for you, for us, to deliver the success?

The information below was captured from group discussions – not validated nor prioritized

Visions of success for 2015

Antigens

- Identify new CD4 and CD8 antigens
- Identify sporozoite candidate vaccine antigens by mass-spec
- Identification of best antigen delivery system for protective CD8 and T cells
- New T cell antigen identified
- Yes or no on BHV-4
- produce enough antigens for vaccine
- new sporozoite antigens identified and validated
- immune parameters that correlate with success or failure of schizont antigen immunization
- Improved antigen delivery system for schizont based immunity
- One (10) new T and CTL and B cell antigens discovered, including low polymer

Proof of Concept (PoC)

- Proof of concept P67/TP1 = results
- Improvement of the P67 vaccine
- Define the determinants of success/failure
- Further progress toward vaccine proof of concept

New developments

- efficient data delivery (flow cytometry/fluorescent microscopy)
- understand the biology/mechanism of the bovine antibody repertoire
- new vaccine candidates identified
- New whole genome assemblies
- reliable polymorphism estimate
- the right cell line for the sporozoite assay

Existing ITM vaccine

- good uptake of the ITM vaccine in the field
- improved ITM vaccine

Common elements needed for success

Teamwork

- Happy consortium
- Effective group interactions and discussions
- Offering and receiving critical evaluation and feedback
- Functional focus groups

Working together

- Sharing reagents

Standards developed and adopted

- Standard operating procedures
- Standardization in assay/reagents/read-outs
- Standardized neutralization assay = permissive cell line
- Standardized clinical model -> clinical end-points

Budgets

- Efficient budget burn rate

Session 8 – PMC-EAC discussion

Program Management Committee and External Advisory Council members held a side discussion. The agenda proposed was:

1. EAC Feedback on the work so far
2. EAC terms of reference
3. Administrations and budgets update
4. Structure to manage the consortium
    - ❑ meetings
5. Tapping into other advice

Main conclusions and actions

1. Make the PMC a real management-oriented group. Draft a TOR for the PMC (more management and strategy/oversight focus). Action: Vish Nene.
2. Establish science 'focus groups' to drive work on different cross-cutting issues and support delivery of objectives. Identify list of focus groups and leaders. Action: Flesh out the concept during the meeting. Then Vish Nene to follow up.
    - science focus
    - trust-based
    - small (5 people)
    - once a year face to face convening
    - involve outside expertise if needed
    - with a TOR
    - facilitate communications
    - meet virtually (as needed, might be often)
    - perhaps time-bound set up for a specific need
    - report to annual consortium meeting
    - perhaps organize a monthly virtual meetup (in rotation) for any interested folks
    - document work and share to PMC and report to Vish
3. Ask GALVmed to work on some upcoming policy and regulatory issues. Action: Vish Nene.
4. Circulate revised EAC TOR. Action: Vish Nene and EAC members.
5. Explore ways to improve management oversight and delivery and accountability for objective 'work packages' - or similar. Action: Vish Nene.
6. Agree a calendar of meetings and frequency.

Action: Establish focus groups

Below is a transcript of the notes taken by each group. Vish elaborated on some of the scope notes. It is expected that as groups meet, these scope notes will evolve.

Group 1: Infectivity assay of sporozoites

convened by Ine

Scope: Improving the current in vitro infectivity assay

Actions:

1. Find a plasmid and transform a cell line (please clarify what is meant)
    a. conA cell lines –CD4, CD8, WC1 – adapt them to lower amounts of IL2 and conA
    b. date: June 2015
2. Test the different stabilates for the chosen cell lines
    a. date: Dec 2015
3. Cell based ELISA
    a. improve assay by March 2015
    b. ILRI and Antwerp work in parallel
4. Work on dyes? – try dual labels? If not work, just establish particle counts?

Group 2: Immune responses to p67

Convened by Vish
(called sp p67 epitope specificity during the Results Framework discussion)

Scope: To define immune correlates with protection induced by p67C

Current status: 2 doses p67C + ISA206 does not protect; 3 doses gives ~50% protection. How do we improve this?

Actions:

1. Define isotypes, avidity, and ?? – to p67C (and native p67?)
2. Determine neutralizing titers
3. Map epitope specificity of p67C sera from immune and susceptible cattle (increase composition of peptide chip to proteome)
4. Survey of BCR repertoire (sample at peak response; purify with p67C?)
5. Make recombinant antibodies and determine specificity and function

Need to complete contract with Atreca; share reagents; design experiments.

Group 3: Sporozoite antigen discovery

convened by Lucilla

Scope: To define new antigens that induce sporozoite neutralizing antibodies

Actions:

1. Identify members of the Focus group
2. Convene meetings – weekly for first 3 months, decide on later intervals
3. Update of detailed activities
4. Identification of bottlenecks to get people started

5. Time table of detailed activities
6. What protein on the peptide chip
7. Which sera to use on the chip
8. Plan procedure for mice antibody library

Group 4: Reagents – communal resources

convened by Lotte

Scope: To collate and catalog existing resources, new resources as generated, a list of resources required.

Actions:

1. Discussion within Consortium to define new needs.
2. Collate lab protocols
3. Reagents database
    a. mAbs, hybridomas
    b. Tetramers, BoLA
    c. Cell lines (TpM, CD8, CD4)
    d. Stabilates (of ?)
    e. Recombinant proteins
    f. Recombinant cytokines
4. Shipping information (forms etc?)
5. Link to BoLA database
6. Link to genomics database
7. April 2015
    a. Preliminary database available
    b. Meeting of group to provide feedback before release to Consortium
8. Representatives from each site: Chairs – Lotte & Lindsay
    a. ILRI – Anna
    b. Edinburgh – Lotte
    c. WSU – Lindsay
    d. Oxford - ?
    e. UoT – Fred
    f. UoC – Soren
    g. IGS – Joana
    h. Antwerp – Ine

Group 5: Schizont antigen discovery

convened by Tim

Scope

The scope of the focus group needs to consider that the current work funded in the consortium is using both traditional antigen discovery methods (T-cell screening of peptide libraries) and more novel methods (MHCI elution and algorithm based). It is important that for maximum output potential there is adequate communication and coordination of the activities between the groups primarily performing 'traditional' methods (ILRI, WSU and ROSLIN) and those primarily involved in the 'novel methods' (Oxford, Buenos Aires, Copenhagen and Maryland). In addition to a strong effort at coordination the scope should include

- Selection of antigens for traditional screening
- MHC haplotypes to focus on

- Methodologies to use for screening and how to best sharing techniques and if necessary harmonise
- Reagents required for screening (MHC transfected cell lines, mAb for read out systems, ipp etc. , peptide resources…..)

Agenda

There was a strong feeling that it was necessary for several of the issues raised during the discussion to be dealt with urgently. Most pressing were:

1 – MHC haplotypes on which the 'novel' techniques should focus. Due to a desire to i) rapidly generate MHC tetramers that could then be deployed in ongoing experiments and ii) use the pMHCI data to support the NetpanMHC algorithm for the MHC haplotypes we are focused on, that work at Copenhagen and Oxford will stall unless the MHC haplotypes/alleles to focus on aren't defined imminently.

2 – Transfer of techniques used for screening – to ensure harmonisation of work and avoid unnecessary difficulties in establishing techniques that may already be working in other labs (e.g. interest from Nicholas for details of the LDH assay being used at ROSLIN and Wendy about different IFNG read-out systems available).

IT WAS THEREFORE DECIDED TO INITIATE THE FIRST SKYPE MEETING IN THE FIRST WEEK OF MARCH. HOW TO PROCEED FROM THERE WILL DEPEND ON PROGRESS AND NECESSITY.

Group 6: T. parva genome resources

convened by Joana

Scope: Provide reference and comparative genome sequence data to support current and future activities in ECF.

Actions:

1. Identify parasite isolates for WGS in order to generate polymorphism estimates (improve ITM?)
    a. Joana to generate spreadsheet to log isolates and metadata
    b. Over next 2~3 weeks, relevant parties find what they have and fill out spreadsheet
    c. In one month – conference call for final selection to sequence
2. Identify life-cycle stages for RNAseq
    a. Sporozoites/sporoblasts of Muguga and Chitongo
3. Establish best route to get material to IGS
    a. Probably through ILRI as there is existing permission for this

Group 7: Antigen delivery systems

convened by Don

Scope: To define the best methods to induce protective anti-sporozoite and anti-schizont immune responses

Actions:

1. List of systems
2. Adjuvants
3. Measurement and standards
4. Distribution of vectors
5. Share stocks – QA on stocks
6. Share data
7. Recommendation on use

Group 8: Proof-of concept

Convened by Ivan

Scope: To define and standardize the parameters to differentiate immune for susceptible cattle to sporozoite needle challenge (?)

Actions:

1. Generic
    a. Define sporozoite challenge – stock and dose
    b. ECF index to measure disease severity
    c. Define protection/non-protection
2. Schizont
    a. Choice of antigens/BoLA haplotypes for testing delivery systems
    b. Standardize protocols for immunological monitoring
    c. Other samples to archive
3. Sporozoite
    a. ???

Action: Calendar of meetings

1. Whole consortium once a year face to face. Next meeting week of 1-3 February 2016

2. PMC meeting 4 times a year, once face to face, 3 time virtual

3. Monthly science meet-ups, virtual, led by focus groups or others, rotational

4. Several small focus group face to face convenings, as needed, on different topics

5. EAC meeting twice a year, once virtual, once face to face, invited to join any others as needed/interested

Action: Communications

The focus groups and the more regular meetings are intended to improve internal communications across the consortium

In addition, work is needed on:

- The Yammer network – as a means to regular sharing and updating on consortium activities

- The ILVAC website – as a vehicle to report to the wider world on consortium activities and results

- The 'Genomics' package website. Action: Joana (branding needs to be checked)

- An email exchange platform (?) to facilitate more practical and hands-on exchanges among the ECF 'tech' people

Annex 1: Participants

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