Minutes of RATZOOMAN meeting held at the Pest Management Centre, Sokoine University, Morogoro, Tanzania, 16-24 July 2003

The following people were in attendance:-

Lorraine Arntzen, NHLS
Steve Belmain, NRI - technical co-ordinator
Godfrey Chikwenhere, sc to SZ
Mirjam Engelberts, KIT
Herwig Leirs, DPIL and RUCA
Robert Machang'u, SPMC
Adrian Meyer, NRI
Georgies Mgode, SPMC
Martha Mpisaunga, SZ
Rassul Nala, INS
Peter Taylor, sc to NHLS
Solveig Vibe-Petersen, DPIL
Moses Zimba, sc to SZ

Issues that had been marked a priority at the previous meeting were discussed as follows:

Project Finance
Partners reminded that draft financial forms should be completed by all consortium partners by the 1st November 2003 and circulated to the co-ordinator so that any outstanding issues can be resolved. This will ensure a timely submission of the reports at the end of December and help minimise delays in the transfer of the next payment to consortium partners by the EC. **Priority**: Co-ordinator to circulate cost statement forms to all partners by mid-September.

Workpackage 1: Retrospective and prospective investigation of human sera
All partners have identified sources of sera from local clinics and hospitals. There appear to be no problems in gaining access to sera in any of the countries. With respect to patient confidentiality, DC partners must try to get as much patient level information as is possible to assess potential risk factors.

Major and minor project focal areas have been identified in Tanzania, South Africa and Zimbabwe with the information passed to Judith Pender and Ricardo Thompson for GIS activities. Major focal area is identified in Mozambique; however, secondary sites are not fully confirmed. **Priority**: Mozambique to decide whether both secondary sites will be up in Zambezia and Tete Provinces or whether one site should be located closer to the Maputo region to facilitate data collection. Due to excellent mapping data for Quelimane, it may make sense to have both secondary sites in Zambezia using Quelimane (no plague) and Morrumbala (plague endemic) which will facilitate project operation.

Analytical techniques that will be used for the three diseases in human samples has been agreed.

Workpackage 2: Taxonomic identification of rodent species
Data collection protocol agreed. Training in common methodologies of data collection and reporting given in a separate training course (to be summarised separately below). Robin Sauvage of Scientific Pest Control Services in South Africa has locally manufactured snap traps and Sherman traps. These can be supplied to all DC partners quickly and more cost-effectively than suppliers outside of Africa. **Priority**: NHLS to liaise with other DC partners and place a joint order for traps through Robin. Traps should be delivered to all DC partners by mid-September with all trapping lines in place by end of September with data being collected.

Workpackage 3: Isolation of zoonotics from animals
Analytical methods for leptospirosis and plague in animals have been agreed. Training took place on common methodologies (to be summarised separately below). Tools for identifying toxoplasmosis in animals has not been established. **Priority**: All partners to investigate potential methods of toxoplasmosis detection in animals in discussion with local veterinary institutions.

Workpackage 4: Rodent ecology
CMR protocol agreed and training given (to be summarised separately below).

Workpackage 5: Impact of environmental factors
Priority: NRI and KIT need to discuss sampling protocols for water and food analysis and challenge experiments.

Workpackage 6: Socio-economic impact
In conjunction with WP7, NRI staff have visited South Africa and Tanzania. Collaborative agreements with appropriate local institutions have been drafted with activities and questionnaires developed. **Priority**: Visits to Zimbabwe and Mozambique need to be planned as soon as possible.

Workpackage 7: Anthropogenic change
Work as described under WP6

Workpackage 8: GIS
Judith Pender and Ricardo Thompson have developed the basis of the ratzooman GIS and associated databases. Ricardo is currently collecting the available and relevant maps of the region and the project focal areas. **Priority**: Ricardo will need the help of all DC partners to get some map information for your country. This must be done by locals in order to keep the price of the maps down to a minimum. It will be more expensive if Ricardo or Judith make the enquiries. **Priority**: DC countries to locate weather stations nearest to focal localities. **Priority**: DC countries to purchase a GPS (if they don't have one already) for use in increasing accuracy of GIS.

Workpackage 9: Modelling
Priority: John Holt (NRI) to liaise with Stephen Davis (RUCA) over existing rodent population data in Tanzania.

Workpackage 10: Development of disease management strategies
Priority: Everyone to be aware that this workpackage depends on collecting and analysing data in previous workpackages. It is, therefore, essential to keep to our timeline of activities as closely as possible.

Workpackage 11: Policy issues
Priority: DC countries to inform workpackage leader, SZ, of important contacts in their own countries. For example, people at Ministries or Departments of Health, Health Ministers, organisations strongly active in provision of health services, implementation of disease control, other disease programmes such as malaria, dengue. **Priority**: SZ to carry out background research on current understanding of zoonosis under different DC country policies by liasing with other DC countries. **Priority**: All consortium partners to try to raise the profile of our work and the ratzooman project with appropriate policy makers in their own countries.

Workpackage 12: Stakeholder workshop
Priority: NHLS to think about fixing the dates for this meeting now and compiling a list of the broad types and numbers of each that should attend the meeting (without naming names). **Priority**: Consortium to consider additional funding for attendees' travel and subsistence costs through the CTA or EU. Co-ordinator find out and circulate appropriate applications for consideration.

Workpackage 13: Output dissemination
Priority: All consortium partners to investigate publishing brief summaries of the project in local newspapers, radio, institutional newsletters, etc. within the next two months to raise awareness of the project in their own country.

Dates of next meetings
Week of 9 February 2004 at Maputo, Mozambique
Week of 20 September 2004 at Kings Lyngby, Denmark - **Herwig/Solveig to confirm okay**
Early April 2005 at Amsterdam, the Netherlands - **Rudy/Mirjam to confirm okay**
Early September 2005 at Harare, Zimbabwe
Early November 2005 at Johannesburg, South Africa for end of project stakeholder workshop

Summary of training workshop at Morogoro, 20 to 24 July 2003

Sunday 20th July, DAY 1

In the afternoon we went to Kychangini (Marsh land) just on the border of town to lay traps. We laid three different types of trap:
1: Sherman traps
2: Box traps
3:Big cage traps (Have a Heart)
Each Ratzooman person went with an experienced trapper, so that they could teach us the correct way to bait and set the traps. They also showed us the best places to put the traps and also how to find them the next morning.
The bait we used was a mixture of chicken food and peanut butter. Bait must only be the size of a small marble.
We put out:
30 Shermans
15 Box &
9 Cage traps
Each trap must be set in a line and 10 m apart.

We also went to a set of houses to lay traps. Area Misufini / Sultani. Before the trapping exercise starts, someone has to go to the houses concerned and discuss with the owners if they are agreeable to you putting traps in their houses.
We put out:
5 Shermans
3 Box
1 Cage trap

Monday 21st July, DAY 2

Early on Monday morning we went to the Marsh land to check on the traps that we had laid the afternoon before. Some of the traps had caught rodents. Those traps were picked up and taken back to the lab. New traps were put back in the same place, so that for the 4 trapping nights you always have the same amount of traps that you started off with. The traps in the houses also did not catch too many rodents, but some of the traps had rodents in. These were also taken back to the lab for processing. All cages were marked with the exact location. A form was designed to indicate how many traps / and location of traps. The form indicated how many traps were set out in each area and how many rodents were caught on each night.

BACK AT THE LAB:
Each person had a chance to do the following:
Record on the form the following information:

1. Location of trap
2. Species of rodent caught
3. Date
4. Person responsible for setting/retrieving of traps
5. Anaesthetise the animal
6. Bleed the animal, by orbital puncture or heart puncture.
7. Brush the animal for parasites
8. Collection of parasites
9. Weigh the animal
10. Measure the body & tail length .
11. Attach a permanent label with a plastic tag. Label should only be filled in, in pencil. (very important as pen will come off in ethanol)
12. Dissect the animal.
13. Take out organs needed (sterile conditions)
14. Check to see if it is a male or female. Indicate for females if it is perforated or not. Females check to see if they are pregnant or not, and if so how many foetus are present and which side they are on. Also if the nipples are small or lactating. For male check to see the position of the testes. Check to see if the gybernaculum is visible or not.

15. Eppendorf tubes should be labelled before you start with the Ratzooman number that is allocated to the rodent. Herwig issued us all with labels, numbers for outside the tubes and also labels for inside the tubes. He also gave us Tag guns to insert the tags for the labels into the rodents.
16. The rodent carcass is closed with metal clips. The carcass is then placed into formalin solution in a bucket. This will later be sent off to Herwig Leirs' lab for identification.

How to do all of the above:

1. Location of trap – this was recorded on the trap, when it was retrieved in the morning.
2. Species of rodent – This we will have to learn how to ID the rodents we are dealing with, but also there will be a backup ID from the carcasses that will be sent to Herwig Leirs' laboratory in Belgium.
3. The date of the traps being lifted.
4. Person's name who was responsible for lifting traps.
5. Anaesthetising of rodents was done by placing the small rodents in a bottle with cotton wool soaked with ether. The larger rodents were placed into a bucket.
6. When the rodents were anaesthetised they were removed from the containers. Bleeding must be done while the animals are still alive, otherwise very little blood will be collected. Orbital bleeding was done by placing a capillary tube into an eppendorf tube and the free end inserted into the edge of the eye socket at the centre of the face. The capillary tube was then rotated and blood would flow down the tube into the tube. Heart puncture was done with a syringe and 23G needle. The heart was punctured and blood was slowly withdrawn. As much blood as possible was withdrawn for the testing procedures. The blood was then transferred into a tube, so that once it was clotted the serum could be extracted. A thick smear was made from a drop of blood. This would be used to do a Giemsa stain on to look for any blood parasites.
7. If the animal was not dead, then it was returned to the ether jar, until it was dead. This would also stun any fleas that were on the rodent. Once the rodent was dead, the body of the rodent was brushed vigorously with a firm brush to dislodge any parasites on the rodent. A deep dish with a white bottom is used, so that you could see any fleas etc. If fleas are present they are sucked up in a tube that is attached to a tube. The tube was also attached to a pipe that has a bulb on the end, when squeezed would create a vacuum. This would draw up any fleas present into the tube. The fleas would then be transferred into a tube with 70% alcohol.
8. The rodent was weighed, by placing it onto a balance.
9. The exact length of the body was taken, this was done by placing the rodent onto the rubber mat and marking with a pin the tip of the nose and the end of the body/beginning of the tail. The tail was also measured in this way. A ruler was used to measure the distance between the pins. The inner ear measurement was taken with a callipers. Measure hind foot length.
10. A label was written out in pencil with the details of the rodent and this was attached to the leg of the rodent with a plastic tag. This is done by inserting a plastic tag into the tagging gun and then push it though the label and the leg. Pull the trigger and the label will be attached to the rodent. The label must be written in pencil, as ink would smudge in the formalin and pencil would not.
11. Pin the rodent onto the black rubber mat and take a sterile pair of forceps and a pair of scissors and cut through the fur and outer skin. The body cavity must be opened up so that you can get to the organs. Care must be taken not to touch the organs with the now un-sterile forceps and scissors. Take a new sterile set of implements and remove the one kidney and place that in a sterile tube for culture of leptospirosis. The other kidney should be put into a tube that contains 70% alcohol – PCR for Leptospirosis. Next take out spleen, liver (tube with 70% ethanol. Lung and heart in a tube to be frozen at –20°C. If the bladder is full of urine, take a syringe and needle and draw out the urine and keep it for Leptospirosis culture. Leptospirosis culture must be done as soon as possible (within 2 hours).
12. Forceps and scissors must be kept in alcohol and flamed before use. After use they are put into a jar with a detergent/disinfectant. They are then washed and returned to the alcohol for re use.
13. Note any abnormalities present in the body.
14. Before you dissect the rodent, after you have weighed it, note if it is male or female. Use the criteria as above.
15. Close the carcass with metal clips.
16. Place the carcass into the formalin solution.
17. ALWAYS WEAR GLOVES AND MASKS AS YOU DO NOT KNOW WHAT DISEASES THESE RODENTS MIGHT BE CARRYING. WEAR A LABORATORY COAT TO PROTECT YOUR CLOTHING.

Mirjam Engelberts (KIT) taught how to culture the kidneys and urine for Leptospirosis. She had made up Fletchers medium in glass tubes. Each tube contained 6 ml medium. The medium is kept in the fridge and allowed to come to ambient temperature before use. Use a sterile pestle and mortar and crush the fresh kidney. Add a small amount of sterile PBS to this mixture. Take a sterile pipette and transfer +- 0.5 ml of this mixture to the tube of Fletchers medium, but flaming the top of the media tube when you open it and again after you have added the kidney mixture. For culture from blood add 1,2 or 3 drops of freshly taken blood to the tube of Fletchers medium. For urine, add 5 drops (not more than 0.5 ml) to a tube of Fletchers medium. The Fletchers medium tubes must then be incubated at 30°C (not in direct sunlight).

On Monday afternoon the traps were set for the capture, mark, release study as follows:
Decide on area that is suitable for capture release studies. Have a long piece of rope that has been marked off at 5m intervals. One person must stand at mark A1 and another person takes the rope and walks down the row eg, A2, A3, A4, A5, A6, A7, A8, A9, A10 – place a painted brick at each mark. From A1 take the rope and go along the row in a 90° angle. Put a painted brick at each place eg, B1, C1, D1, E1, F1, G1, H1, I1, J1. Then go to the mark A10 with your rope and measure were B10 should be. Place a marked brick there, now take the rope from B1 and join up with B10. Place marked bricks at each 5m mark. Do the same with all the rows until you have a grid that looks like this:

A1	B1	C1	D1	E1	F1	G1	H1	I1	J1
A2	B2	C2	D2	E2	F2	G2	H2	I2	J2
A3	B3	C3	D3	E3	F3	G3	H3	I3	J3
A4	B4	C4	D4	E4	F4	G4	H4	I4	J4
A5	B5	C5	D5	E5	F5	G5	H5	I5	J5
A6	B6	C6	D6	E6	F6	G6	H6	I6	J6
A7	B7	C7	D7	E7	F7	G7	H7	I7	J7
A8	B8	C8	D8	E8	F8	G8	H8	I8	J8
A9	B9	C9	D9	E9	F9	G9	H9	I9	J9
A10	B10	C10	D10	E10	F10	G10	H10	I10	J10

Once you have all the bricks in place, then in the afternoon place a Sherman trap at each brick.

Tuesday 22nd July, DAY 3

First thing in the morning go to the CMR field (grid field) and pick up any traps that have rodents in them. Replace with new traps. Take the full traps back to the lab for CMR study. Also go to the marsh area and the houses and collect the traps from there and take back to the lab.

The traps from the marsh and houses, deal with the rodents as above.
The capture and release rodents, deal with as follows:

1. Take the rodent out of the cage by placing it in a bag. Weigh the rodent in the bag, that has been previously weighed.
2. Enter the Grid co-ordinates onto the computer.
3. Record the weight on the computer programme.
4. Carefully take the rodent out of the bag and hold it behind the head, so that it does not bite, note the sex. If it is female, note if it is pregnant, if the vagina is perforated or not and also if the nipples are clearly visible (lactating) or small. If it is a male, note if the testes are visible or abdominal. Enter this information on the computer program and it will come up with a toe clipping code that you must use. E.g. 25.
5. Toe clipping is done by holding the animal on its back and looking at the diagram working out which toes to clip. Take a pair of forceps and hold the toes that is going to be clipped. Then hold it

with the pair of scissors and change the forceps to the top of the toe and cut off the first digit of the toe. This must be put into a sterile tube with a special label that has been pre-printed. Ethanol must be added to the tube. This tube must be kept at room temperature and sent to Prof Herwig Leirs for DNA testing. After this procedure the rodent is put back into the cage and returned to the field to the exact spot that it was taken from. This process is repeated for 4 nights. Any new rodents that are caught, go though the same process, but any repeat caught rodents are still recorded on the computer programme.

Wednesday 23rd July, DAY 4

The above processes went on each day, collecting and processing rodents from the marsh land, houses and grid.

On the Wednesday, Lorraine Arntzen (NHLS) taught the ratzooman delegates how to sensitise an ELISA plate, in preparation for the next day.

Thursday 24th July, DAY 5

The above processes went on each day, collecting and processing rodents from the marsh land, houses and grid.

On the Thursday, we did the Competitive Blocking Plague ELISA for the detection of antibody against F1. It worked well and everyone who tried the ELISA were very capable and managed well.