INCO-DEV contract number ICA4-CT2002-10056

First Annual Report, January to December 2003.

RatZooMan, Prevention of sanitary risks linked to rodents at the rural/peri-urban interface

http://www.nri.org/ratzooman

Keywords: zoonosis, infectious disease, rodent, peri-urban, epidemiology

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Scientific report

Summary of content

WP1

Collection of human sera has occurred in Tanzania from a number of clinics and hospitals and the samples will be analysed in one batch once further samples have been collected. The other DC partners are finding it difficult to deal with logistical, ethical and bureaucratic issues in obtaining human blood samples. Group discussions with consortium partners have focussed on how best to ensure the project can overcome the problems related to human sera analysis and efforts will be increased to gain access to existing samples. Discussion is under way in South Africa to gain community agreement to collect human blood from households involved in other project activities and will ensure a direct link between the human and animal samples collected for prospective analysis.

WP2

Common protocols have been developed and agreed by the consortium regarding sample processing, labelling, data entry, and preservation. Samples are being identified, where possible, using local expertise. However, samples will be cross referenced by RUCA. Further details of the protocol can be found in the RUCA partner report in the Annex.

WP3

Protocols have been developed and agreed jointly with protocols for WP2. Training has been given to consortium partners in common methodologies for analysis and the types of analysis and tests have been established. Some animal sera samples have been preliminarily screened in South Africa for plague, toxoplasmosis and leptospirosis. Staff in Tanzania have been able to culture leptospires isolated from a number of animal samples for a number of leptospirosis isolates and samples. Detailed results can be found in the partner reports for NHLS and SPMC. Further analysis is expected on samples collected in each of the four countries.

WP4

Protocols have been developed and agreed by the consortium. Training in CMR protocols has been given to all partners involved in the work with particular regard to handling and marking animals and in the operation of specific software used in the data analysis. Further details of the protocol can be found in the DPIL partner report.

WP5

Activities related to the assessment of potential zoonoses contamination through food are under way at NRI using a combination of challenge experiments and food safety protocols such as HACCP to assess contamination risks. Other activities within this work package await the development of the GIS to assess land use changes related to water and human activity.

WP6

Questionnaires were developed by NRI staff and developed further in collaboration with relevant staff identified in South Africa and Tanzania involved in the implementation of questionnaire surveys. Data has been collected and is currently being analysed from two sites with activities planned for two further sites in South Africa and Tanzania. Contacts have been made with potential socio-economic collaborators in Mozambique and Zimbabwe to initiate similar studies to provide comparative data for each country.

WP7

Terms of reference were developed and qualitative studies initiated in South Africa and Tanzania. Guidelines developed and implemented in collaboration with local anthropologists. Data has been collected and is currently being analysed from two sites with activities planned for two further sites in South Africa and Tanzania. Contacts have been made in Mozambique to determine whether relevant anthropological skills exist, however a decision to conduct activities has yet to be made regarding which site would be involved in anthropological work.

WP8

Hardware and software were sourced and purchased Sources of high resolution images were identified and purchased. A framework GIS was designed, programmed and installed on the computer based at the INS and staff were provided with the relevant training on its use. Database fields for the data collected in different work packages was developed and agreed with consortium partners and programmes for insertion into the GIS will be developed.

WP9
Although this work package is not meant to start until month 20, work has already begun in order to ensure that the data collected will be sufficient to develop reasonable models. Epidemiological models for leptospirosis have been discussed between staff at NRI and RUCA with input from staff at KIT. There are many parameters affecting the epidemiology of leptospirosis and decisions were made to limit the model to the most important issues. Therefore, it was proposed that the model focussed on a rodent carrier, where epidemiological modelling can make the greatest contribution. Drivers for the model will be based on:

- Seasonal fluctuations and environmental sources of infection
- Importance of different infection routes
- Interaction between rodent population size, season and transmission routes
- Potential of rodent management interventions on infection rates

WP10
Due to start in month 24

WP11
Due to start in month 26

WP12
Due to start in month 28

WP13
Efforts to advertise and disseminate the project have been made as described under publications.

Problems

The main problem encountered by the project has been the difficulty in sourcing materials for the implementation of activities. Obtaining reagents, analysis kits, traps and other consumables by DC partners took much longer than expected, and this has inadvertently delayed most baseline data collection activities. However, all partners now have the right materials and animal samples are being collected and analysed.

There are difficulties in starting data collection activities with some secondary sites, usually one site in each country. This is partly a problem with logistics of distance and cost to reach sites, but also related to political problems in obtaining local approval for activities to occur. Partners are addressing these issues, and there is still scope to meet the initial protocol guidelines developed in most instances.

Human sera collection has only occurred in Tanzania, and efforts in the other three DC countries must be increased to ensure this activity happens. Partners are finding it difficult to deal with logistical, ethical and bureaucratic issues in obtaining human blood samples. Group discussions have focussed on how best to ensure the project can overcome the problems related to human sera analysis.

Publications

Hyperlinks to the following:

Ratzooman website

Article in International Rodent Research Newsletter

Article appearing in The Mercury, 9 February 2004, Durban, South African newspaper

Outline plans for next year

Activities in the second year are largely similar to those of the first year and should be able to make up for some lost time incurred from the procurement difficulties arising at the beginning of the project. Partners will need to focus on dealing with various logistic, ethical and political problems that affecting data collection in some localities and with regard to human samples. Delays to the start up of baseline data collection mean that some work package activities will need to be extended beyond the time proposed in the original timeline of activities. A revised timeline can be found below.

Revised work package timeline of activities

	Year 1												**Year 2**												**Year 3**											
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
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Management report

Organisation

Project organisation has gone smoothly with most communication among partners relying on email. The technical coordinator has had to remind partners that they must copy communication so that activities and progress can be monitored and to send regular updates of activities and data to work package leaders and the technical coordinator. Generally, cooperation has been very good among partners and their subcontractors.

Meetings

Representatives from each consortium partner, and subcontractors when relevant, have been present at all meetings. Minutes of each meeting can be found in the Annex. A three day inception workshop was held at NRI in March 2003 where each individual work package was discussed and actions were highlighted so that partners were aware of their responsibilities. The main activity was protocol development and agreement on parameters to be collected. Financial and reporting matters were discussed related to the requirements of the EC. The next meeting took place in Tanzania in July 2003 where partners summarised their activities, plans were made for the future and priority actions were described. Tied to this meeting was a training workshop on rodent trapping, sample collection and analysis. This training to all consortium partners was essential to ensure that activities taking place in different countries used the same protocols and that standards and safety measures were ensured. Everyone found the training very useful as they may have previously been doing similar activities in a slightly different way.

Dates of next consortium meetings
Week of 9 February 2004 at INS. Minutes of this meeting can be found in the Annex.
Week of 20 September 2004 at DPIL
Week of 11 April 2004 at KIT
Week of 5 September at SZ
Week of 7 November at NHLS for final project workshop

Exchanges

Staff exchanges have occurred independent of coordination meetings for individual work package activities as follows:

There have been three exchange visits related to WP9, NRI staff visiting RUCA, RUCA staff visiting NRI, and NRI and RUCA staff visiting KIT.

For WP8, NRI staff have visited INS.

For WP7, NRI staff have visited NHLS and subcontractors and SPMC staff and subcontractors.

For WP6, NRI staff have visited NHLS and subcontractors and SPMC staff and subcontractors.

For WP4, 3, 2 and 1, INS, NHLS and SZ staff have visited SPMC.

For WP4 and 2 DPIL and RUCA staff visited SPMC and INS.

Problems

There have been problems with the EC advance to SZ, which is presumably a problem in how the EC has interpreted the organisation of international companies and problems specifically related to how institutions must operate in Zimbabwe to be effective. In short the EC was unable to supply an advance to SZ because of these problems, causing a big delay to activities. The problem has been partially resolved by advances made through their head office, but delays in EC reimbursement are now causing similar problems for activities in Zimbabwe.

There have been the usual problems in partners understanding how to complete E1 forms.

DC partners are very concerned about the EC rules regarding advance payments and are worried how the withholding of payments at the end of the project will affect the delivery of activities as they will not have access to funds through their institutions.

Annexes

Meeting reports

Minutes of RATZOOMAN inception workshop held at the Natural Resources Institute

The following people were in attendance at the meeting:-

Lorraine Arntzen, NHLS
Steve Belmain, NRI - technical co-ordinator
Nan Chalmers, SZ
Philip Davies, NRinternational - financial co-ordinator
John Frean, NHLS
Amy Guyatt, NRI
Rudy Hartskeerl, KIT
John Holt, NRI
Malcolm Iles, NRI
Monica Janowski, NRI
Herwig Leirs, DPIL and RUCA
Robert Machang'u, SPMC
Cammy Marchant, NRI
Martha Mpisaunga, SZ
Rassul Nala, INS
Linda Nicolaides, NRI
Judith Pender, NRI
Guy Poulter, NRI

The Director of NRI, Dr Guy Poulter, gave a brief introduction to NRI and invited consortium partners to contact him in the future to discuss existing activities of NRI or potential new areas for collaboration with NRI. E-mail: R.G.Poulter@gre.ac.uk

Project partners discussed common financial issues and then began to systematically discuss each workpackage and plan the activities in detail for the remaining time available over the next few days of the workshop.

Project Finance
Financial issues were discussed by Philip Davies, Cammy Marchant and Amy Guyatt. Reporting requirements were described. **Priority**: It was agreed that draft financial forms are 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**: It was agreed to investigate the possibility of covering financial shortfalls in advance payments to DC countries, particularly with regard to the final payment after project completion, but this will need to be considered on a case by case basis. **Priority**: DC countries to get subcontractors in order, subcontracts signed up and budgets set. DC partners to inform Steve of names of subcontractors and their indicated budgets so that he can decide whether it is necessary to inform the EC for their approval. This must be done by the 30th April.

Workpackage 1: Retrospective and prospective investigation of human sera
Discussions focussed on three issues: the availability of sera, the analysis techniques to be used, and the collection of samples and data. Historical sera banks are not always common in target countries and some DC partners expressed doubt that significant banked material will be available outside major cities. Other sources of banked sera such as blood banks, other disease screening projects (e.g. malaria, HIV) could also be used, and DC partners will investigate the prospects in their own countries. Access to banked sera, when available, should not be a problem but will require considerable administrative efforts to gain clearance. There will be variable problems associated with the level of information provided with sera samples from different sources. The consortium agreed

that retrospective analysis will largely have to work within the existing systems in each country and try to gain as much information as is possible from existing sera stocks. In most cases banked sera will need to be analysed for the target diseases at location, the implication being that training and finance of appropriate analysis will need to be given by the consortium to appropriate hospital staff. **Priority**: DC partners to identify all sources of sera that could be used for testing and find ways to facilitate their analysis for leptospirosis, toxoplasmosis and plague. A deadline of one month after the meeting (April 18th) to present initial plans was agreed.

Prospective analysis will need to initially rely on hospitals in project focal areas. As many hospitals may not routinely save sera after analysis, DC partners may need to formalise arrangements for hospitals to save sera for a period of time to facilitate their analysis for zoonotics. All DC partners agreed that it would be difficult to gain access to non-clinical sources of sera. However, efforts should be made to fully involve target communities in focal areas through repeated meetings to facilitate the collection of sera in focal areas. **Priority**: DC partners to select one major and two minor focal towns for project activities in each country. Where possible these areas should have active zoonosis and/or some historical data on the presence of zoonotic disease. These focal areas will also be used for all other workpackage activities. **Priority**: DC partners to contact local hospitals in catchment of focal areas for involvement in prospective serological analysis for zoonotic incidence. A deadline of one month after the meeting (April 18th) to present initial plans was agreed.

The types of analytical techniques for each disease were agreed. RAPID tests exist for leptospirosis and toxoplasmosis for human sera. MAT will need to be used for leptospirosis in rodents and other animals and for confirmation of human samples. It is possible that the toxoplasmosis kit can be adapted for use in other animals or that such kits already exist, particularly in cats. For plague, ELISA is available for humans and can be adapted for rodents and other animals. Culturing of leptospires will be necessary where possible. Supplies from rodents will be facilitated from other workpackages. Culturing leptospires from domestic animals poses more problems. For this, access to slaughterhouses was discussed as well as urine collection through catheter and bladder tap or using furosemide to induce urination. **Priority**: NRI to contact Organon Teknika (or BioMerieux) regarding IgM and IgG toxo microelisa kits as well as their lepto DriDot tests and forward on information to consortium partners. **Priority**: DC countries to liaise with each other to establish whether a discount for kits can be obtained by buying for the requirements of all four countries together.

Workpackage 2: Taxonomic identification of rodent species

Discussions focussed on the types of trials and procedures required to collect information about rodents found in rural, peri-urban and urban areas. It was agreed that ten habitats would be selected for trapping in the main focal area in each DC country. To trap in all ten habitats requires 560 traps. Each habitat trap line will be trapped over four nights, meaning that all the habitats can be broken into portions over a number of weeks, thereby reducing the number of traps required by rotating the traps to different places in the focal area. In each habitat trap line, one half of the traps should be Sherman traps, one quarter should be snap traps, and one quarter should be larger cage traps or other indigenously designed traps. The way in which rodents should be collected, labelled and which tissue samples to take was agreed. Surveys will occur every three months during the first 12 months giving four survey times, with the possibility of two further subsequent surveys in year two. The details of the procedures involved will be sent separately to consortium partners. **Priority**: RUCA to circulate data collection protocols to all partners. **Priority**: DC countries to start sourcing and buying traps. Depending on how habitat trapping is rotated in each survey, each country will need roughly 150 Sherman traps, 75 snap traps and 75 other types of traps. Trial management should liaise with WP4 as 200 more Sherman traps are required here.

Workpackage 3: Isolation of zoonotics from animals

For rodents, tissue (liver, kidney, spleen, heart, lung - details to be circulated separately) and blood samples will be taken from animals trapped in WP2. It was not clear whether it would be necessary to culture Toxoplasma and further investigation will be made and whether this would impact on the sample collection protocols. Leptospira will be routinely cultured; however, it was agreed that Yersinia would not be cultured with analysis relying on serological and PCR methods. It was agreed that domestic animal sera should be sampled in the focal areas, particularly, dogs, cats, pigs and goats. Close involvement of the community will be necessary for such sampling to occur. Although difficult, collection of urine from domestic animals remains an option to determine presence of Leptospira. **Priority**: NHLS to search for optimal toxoplasmosis analysis protocols and inform

consortium whether brain tissue should be maintained for culturing or PCR. **Priority**: DC partners to start discussions with focal area communities on the screening of domestic animals.

Workpackage 4: Rodent ecology
The main activity will be to conduct a capture, mark, recapture (CMR) in the main focal area in each DC country. This will be done with a grid of 100 Sherman traps placed in two separate fields on the outskirts of the town where staple crops are grown. The important issue is to ensure that the trapping grid lies half within a cropping area with the other half lying over an uncultivated area (bush or fallow). Captured small mammals will be toe marked and released so it is important that the farmer or community understands and supports the reasons for conducting this trial. Other trials to understand rodent movements between different habitats in the environment involving the use of marked baits and radio telemetry will be discussed at a later time. **Priority**: DPIL to circulate detailed CMR trial protocol. **Priority**: DC partners to identify two sites at main focal area and gain permission for study to run.

Workpackage 5: Impact of environmental factors
Activities are linked with other workpackages under three main areas GIS, anthropology and disease transmission specifically related to water management and land use. GIS will be used to look at broad changes in the DC countries as well as in more detail within the specific localities chosen in each country. Historical images will be used to analyse for changes in land and water usage. Communities within focal areas will be targeted for in-depth surveys to understand changes in cropping patterns, urban growth, water management over time looking for areas of anthropogenic change. It was agreed that point water sources and food within households, markets and stores should be assessed for general rodent contamination as well as presence of leptospires, particularly when samples have been obviously contaminated. The susceptibility of water and food to be contaminated will be investigated and the principles of HACCP applied to the food chain to analyse the critical control points in the food processing systems. pH of water sources, including surface waters as well as their 'quality' to be routinely screened. Social scientists will need to register human activities like swimming, washing, drinking, in different water sources. pH of soil measured and moisture of soil through the seasons (notably at catching times) described. Daily rainfall registered and incorporated into GIS to assess heavy rainfall via swollen rivers. **Priority**: Ricardo Thompson from INS to visit the UK as soon as possible to meet with Judith Pender from NRI to discuss GIS data collection. Ricardo and Judith to agree dates for following meeting in Maputo. **Priority**: DC countries to identify institutions and individuals to be involved in social science studies and liaise with NRI social science staff to prepare visits to DC countries and start surveys. **Priority**: NRI and KIT to work out sampling protocols for water and food analysis and challenge experiments.

Workpackage 6: Socio-economic impact
A number of survey tools, both qualitative and quantitative, will be used to measure the knowledge, attitudes and practice of different members of target communities with regard to the zoonosis and rodents as well as the economic impact the diseases and rodents have upon people's livelihoods. It was agreed that these are best developed in collaboration with local social scientists and with activities in WP5 and WP7. Surveys should concentrate on plague endemic areas of Mozambique and Tanzania to gain an understanding of this disease. Leptospirosis and toxoplasmosis will be studied in all four DC countries. **Priority**: DC countries to identify institutions and individuals to be involved in social science studies and liaise with NRI social science staff to prepare visits to DC countries and start surveys.

Workpackage 7: Anthropogenic change
Human intervention in the environment could increase or reduce the chance of disease spread and/or acquiring zoonosis and qualitative and possibly also quantitative studies are required to understand many of these issues. As above, the survey methods and activities will be developed in collaboration with social scientists in each of the DC countries. **Priority**: DC countries to identify institutions and individuals to be involved in social science studies and liaise with NRI social science staff to prepare visits to DC countries and start surveys. **Priority**: Steve to draft up key issues we believe influence spread of the three key diseases in environment, rodents and people, and descriptions of symptoms of the diseases in key species and in people, and circulate.

Workpackage 8: GIS

Everyone in agreement on the general analysis looking for broad changes over large areas and more detailed information collected for targeted focal areas in each DC country. Current aerial maps could be important for detailed study, but unsure of cost implications to obtain such maps for each focal area. SPOT, SADC and LANDSAT images to be obtained and other sources of map information to be collected. **Priority**: DC countries to investigate costs of local hire of aeroplanes, Judith to make enquiries with collaborators in South Africa on the prospects of aerial map photography. **Priority**: Everyone to liaise with Judith and Ricardo over databases that will be created in other work packages. This is important to iron out issues of importing data into GIS system. **Priority**: Judith and Ricardo to meet ASAP initially in UK to iron out all GIS details. **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
Important to establish which factors are important and their magnitude in order to develop predictive models. Most parameter values are to be covered in previous workpackages. It was highlighted that little information on the infection rate of humans contracting zoonosis would be collected but that this could be estimated as could the infection rate of rodents for which limited data may be available. **Priority**: Everyone to keep John Holt informed of the types of data to be collected and to pass data to him as collected. **Priority**: NRI to liaise with DPIL and SPMC over existing rodent population data in Tanzania.

Workpackage 10: Development of disease management strategies
Little to say about this now as will be built on information collected in earlier stages of the project. Strategies could be very simple and related to minor changes in human practice (e.g. water storage) or related to rodent management by reducing the carrying capacity of the environment or targeted rodent population control measures. Important to collect disease prevalence data before and after strategy implementation to show strategy was effective and to inform and modify predictive model. **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
Role of rodents in disease transmission does not register among DC country governments and aid donors and must be put in context of other human health impacts in DC countries (HIV, malaria). **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 liaising 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
This should happen in early November 2005 to avoid Christmas and give time for workshop results to be incorporated into final project report. **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
Details of ratzooman project will be updated on IPMEurope website:
http://www.nri.org/IPME/rodents.htm
(I've just noticed a few errors on this which should be corrected shortly)
Priority: Everyone to please make links to this site on websites that you and your institutions maintain. Everyone to inform project co-ordinator of information that should be added to ratzooman webpages.

Future co-ordination meetings will be used to discuss opportunities to publish research targeted for different audiences. **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 21 July 2003 at Morogoro, Tanzania. This is more of a training meeting so it is not essential that all EU partners attend. Common methodologies related to rodent trapping, tissue and blood sample taking, preparation of rodent specimens, handling rodents, recording data, etc. will be covered. It makes sense that the key people involved in these activities attend the meeting. You may, therefore, wish to be accompanied by one of your subcontractors, if appropriate, bearing in mind whomever attends this meeting will be required to train others on their return to their respective countries. Also please remember that the week before (14-18 July) the ratzooman meeting is the African Small Mammal Conference in Morogoro where many talks on rodents will be given which you may find useful to attend. The conference website is: http://www.dpil.dk/asms/

Week of 2 February 2004 at Maputo, Mozambique - agreed and confirmed at meeting
Week of 20 September 2004 at Kings Lyngby, Denmark??
Early April 2005 at Harare, Zimbabwe??
Early September 2005 at Amsterdam, the Netherlands??
Early November 2005 at Johannesburg, South Africa for end of project stakeholder workshop

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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 liaising 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.

Minutes of RATZOOMAN meeting held at the National Institute of Health, Maputo, Mozambique, 11-13 February 2004.

The following people were in attendance:-
Lorraine Arntzen, National Health Laboratory Service, South Africa
Steve Belmain, Natural Resources Institute, UK
Aida Cala, National Veterinary Research Institute, Mozambique
Nan Chalmers, Syngenta, Zimbabwe
Godfrey Chikwenhere, Plant Protection Research Institute, Zimbabwe
Venancio Cluiba, National Veterinary Research Institute, Mozambique
Rudy Hartskeerl, Royal Tropical Institute, the Netherlands
Malcolm Iles, Natural Resources Institute, UK
Monica Janowski, Natural Resources Institute, UK
Herwig Leirs, Danish Pest Infestation Laboratory, Denmark and University of Antwerp, Belgium
Robert Machang'u, Sokoine Pest Management Centre, Tanzania
Anabela Manhica, National Veterinary Research Institute, Mozambique
Adrian Meyer, Natural Resources Institute, UK
Rassul Nala, National Institute of Health, Mozambique
Judith Pender, Natural Resources Institute, UK
Malodi Setshedi, National Health Laboratory Service, South Africa
Manuel Sumila, National Institute of Health, Mozambique
Peter Taylor, Durban Natural Science Museum, South Africa

Meeting opened with welcome by the Director of National Institute of Health. Each consortium partner gave a presentation of the activities which took place over the first year of the project, based on their first year technical report.

SB report on NRI with particular reference to activities in WP 5, 6, 7, 9 and 13. MJ elaborated anthropological survey, but interpretation is difficult without other rodent data that is in the process of being collected.

HL report on DPIL with particular reference to WP4 and preparation of CMR protocol for all primary sites and movements of rodents between habitats using marked bait and telemetry studies in Tanzania. It is important that CMR field selection is for two sites that are in the peri-urban interface as near to the urban area as possible. All sites need to collect weather data, which has not yet been sent to HL. Data must be regularly sent to DPIL on a monthly basis, and this will help to correct problems before they are repeated in the next census.

HL report on RUCA with particular reference to WP2 reporting on preparation of protocol and manual for the studies and the taxonomic identification of rodents collected. Not all data that had been collected had been received. Rodents had been trapped in many places but little site information has been provided to interpret the data. Ecological information required to further analysis.

LA report on NHLS. There was delay in sample analysis due to problems in getting kits and reagents ordered and sent to South Africa, but all consumables are now available and analysis is moving along. Preliminary results of serological analysis of rodent samples has not shown any samples plague positive. From Venda 39 samples were tested, from Durban 232 samples, 10 of which were positive for leptospirosis (10%), and 4 samples were positive for toxoplasmosis. Sera from Port Elizabeth showed 63 samples out of 587 to be lepto positive (20%). Rodent sera were analysed with the DriDot test, and RH raised the issue that this test will be unreliable for animal sera, emphasising that MAT must be done to confirm. Human sera have not yet been sourced and there are problems obtaining ethical clearance. The serovars used for lepto MAT need to be approved by RH.

RM on SPMC activities. Human sera were obtained through assistance from on going projects involving HIV and schistosomiasis and from routine malaria and typhoid diagnosis. Sources of the serum specimens included AghaKhan Hospital, Upendo Medical Laboratory and Mikumi Health Centre, with other sera obtained from two referral hospitals. A total of 1702 samples from 5 different locations. Samples will be screened soon. Captured rodent species, trapping from February to December in human residences, preliminary identifications were done to Genus with further taxonomic identification at RUCA. The total number of rodents and shrews captured was 2065 in

Morogoro. First recorded case of Norway rats in Morogoro. Sera and tissues have been collected with opportunistic sampling from Mbeya, Kilimanjaro, Dodoma. Samples have also been collected from domestic animals, i.e. dogs, pigs, cats, sheep and goats in Morogoro. Isolations of lepto from urine and kidneys and samples cultured, isolated 1029 cultures of which 25 were positive for lepto or 2.4% of sample population. RM referred to socio economic data from Lushoto – trouble of funding for social studies, and will have difficulty in funding further socio-economic studies, could probably do studies easily in Morogoro but unlikely to do Masasi.

RH report on KIT. WP1 human sera has not really started except in Tanzania, retrospective analysis, selection of sites has occurred. Culturing is the most important aspect of this work package and must occur. RH raised the question on how do we do this???? WP3 analysis of animals and improved methods for diagnosis of leptospirosis. Isolation can be difficult from kidneys – helps if you centrifuge and concentrate the leptospires with an initial low speed for 5 minute at 150 g and then a high speed spin for 10 min at 6500 g. RH developed new media of bilayer with a bottom layer of charcoal and then a liquid of rabbit sera on top. Some partners will try to use the new media, but agreed to stick with the traditional protocol to ensure comparability with existing data.

RN report on INS. National veterinary research institute has been subcontracted and they had a meeting in August to identify sites and develop the protocols with a second meeting in November with local authorities. Field work started on 1st December. There was real difficulty in sourcing materials and importing them into Mozambique, both traps and reagents; however everything is now happening. Primary site consists of the Maxaquene A, T3 and Tsalala areas of Maputo. It was proposed that a more flexible protocol would be required to obtain data from secondary sites in Central Mozambique because of the costs and difficulties in reaching the area. RN to set up a visit to Morrumbala that will pull in overseas staff for a longer trapping period to happen around May 2004. For GIS, details of study sites have been collected with agreement on the database structures and GIS requirements identified.

GC report on SZ. Rodent trapping and sample collection going well in Mbari, no human sera yet collected. Some problems with one urban site (Hatcliffe) where high numbers of traps went missing and, therefore, had to change the site. Problem of funding raised, as advance is coming from Syngenta headquarters in Switzerland who will not advance any further funds until the EC reimburses their first year expenses. This is effecting their ability to deliver activities.

Various group discussions and resolutions took place regarding the standardisation of data for input to GIS, data tables for WP2 and habitat descriptions, ph water analysis, changes to the socioeconomic questionnaire.

Agreed Actions & Points of Discussion

1. **Data and communication** - Steve will provide common access to all data within the Ratzooman website. To be useful and productive it will be essential that all collaborators provide up-to-date data for inclusion in the database. Everyone must make a greater effort to copy SB in on correspondence and keep him informed of activities!!

2. **Socioeconomic & Anthropological** - It was suggested that it was more appropriate to undertake survey work in Morogoro than other sites in Tanzania to facilitate activities and reduce costs. Firstly there was a great deal more data on rodent populations at Morogoro and secondly much of the work relating to the dynamics of plague at Lushoto had already been undertaken. The importance of undertaking work on the human populations in areas where there were positive indications of disease presence was discussed. The potential to do work on the rodent eating area in Mozambique was discussed and that if appropriate collaborators can be identified the work can occur at the same time as the team visit to Zambezia. Questionnaires circulated for everyone to comment so that Malcolm and Monica can revise as necessary.

3. **Sample collection** - Herwig stressed the need to send data to DPIL and RUCA as soon as possible once collected. This would facilitate more rapid analysis of data. In addition, this early analysis of initial data would identify errors in the data and data collection and recording at an early stage in time for wider correction. Need to trap in open areas outside towns and outside houses but also in peri urban areas. This sample size was currently rather low. Looking for interface between

domestic rodents and rodents around outside of town. In recording trapped rodents it is important that the point at which the rodent was trapped is recorded so that the point can be re-visited at a future date. If a code is used it is important that another data file is set up containing the details relating to the codes. Great care should be taken in recording these data. It is worth setting up a file that will cross check that the data on the record sheet matches to data in the locality file. There is no need to record units in each record, for instance no need to include g when recording in the weight column. It was agreed that further questions be inserted into the record sheets relating to the construction and hygiene standards around the individual houses. Adrian designed these as follows:

DEFINITIONS OF HOUSING CATEGORIES - Proofing
CATEGORY 1 Very poor quality housing - about as bad as it gets! Provides very easy access for rodents.
CATEGORY 2 Poor housing and worse than average. Access for rodents relatively easy.
CATEGORY 3 Better than average. Fairly good housing that provides more limited access for rodents.
CATEGORY 4 Good housing, access for rodents is difficult.

DEFINITIONS OF HOUSING CATEGORIES - Internal Harbourage
CATEGORY 1 Extensive availability of harbourage and cover inside the property. Plenty of places for rodents to hide. Very untidy.
CATEGORY 2 Harbourage and cover available but not in very large quantities. More harbourage than average.
CATEGORY 3 Limited harbourage available. Less harbourage than average.
CATEGORY 4 Almost no places for the rodents to live, a clean and very tidy house.
NB:
1. The presence of an enclosed roof space should be seen as one factor that contributes to the availability of potential harbourage.
2. The presence of a grass roof, because it provides potentially good harbourage, would probably place the house into categories 1 or 2.

DEFINITIONS OF HOUSING CATEGORIES - External Hygiene
NB: All assessments to be taken within 5 meters of the building.
CATEGORY 1 Very extensive availability of harbourage and cover. Generally a very untidy area around the building.
CATEGORY 2 Area is generally untidy and provides cover for rodents and is worse than average, but could be worse.
CATEGORY 3 Some cover but better than average.
CATEGORY 4 Very tidy and clean environment that provides no or almost no cover for rodents.

4. **Leptospirosis Screening** - Rudy confirmed that the DriDot test is not appropriate for animals, but that existing analysis in comparison to MAT may prove useful to show efficacy of DriDot for animal samples.

5. **GIS** - Judith Pender said that partners must do in country searches for large scale digital maps to obtain all roads and streams. Data must be sent to Judith for input as it comes in. Recoding of Longitude and Latitude must be recorded in a standard way.

6. **Sample analysis** – This will be done separately in Zimbabwe, Tanzania and South Africa (with Mozambique samples sent to South Africa for analysis, with the prospect of eventually doing some analysis in Mozambique). Emphasised need to communicate and establish standard analysis protocols.

Next Meetings
Week of 20 September 2004 at DPIL
Week of 11 April 2004 at KIT
Week of 5 September at SZ
Week of 7 November at NHLS

Publications

Hyperlinks to the following:

Article in International Rodent Research Newsletter

Ratzooman website

Article appearing in Durban, South Africa newspaper, The Mercury, 9 February 2004.

Consortium partner reports

NRI

RATZOOMAN
Prevention of sanitary risks linked to rodents at the rural/peri-urban interface

INCO-DEV contract number ICA4-2002-10056

ANNUAL SCIENTIFIC REPORT

Participant:	NRI
Period:	Jan to Dec 2003

Scientific achievements

Modelling activities WP9 - NRI staff visited RUCA staff in November to discuss epidemiological models for leptospirosis. It is proposed that the model is focussed on a rodent carrier, where epidemiological modelling can make the greatest contribution. Drivers for the model will be based on:

- Seasonal fluctuations and environmental sources of infection
- Importance of different infection routes
- Interaction between rodent population size, season and transmission routes
- Potential of rodent management interventions on infection rates

GIS activities WP8 - NRI staff visited INS staff in July to devise a work programme. Hardware and software were sourced and arrangements made with suppliers to purchase the system. Sources of high resolution images were identified to be sourced locally. NRI staff designed and programmed a prototype GIS which was installed on a computer at the INS and provided training on its use.

Anthropological activities WP7 – NRI staff visited: South Africa in June. Fieldwork guidelines developed and implemented in collaboration with local anthropologist. Survey in Mapate and draft reported completed but some problems of interpretation and analysis. Recent visit to Durban to repeat activities. Tanzania in July. Collaborators identified and work carried out between September to December, results currently being analyzed.

Socio-economic activities WP6 – NRI staff visited: South Africa in June. Questionnaire and training material prepared, collaborators identified to carry out survey with 120 households in Mapate. Data now collected and in process of being analysed. Recent visit to Durban to repeat activities. Tanzania in July. Similar questionnaire developed and collaborators identified for survey in Lushoto with 100 households. Data recently received. Contacts have been made with potential socio-economic collaborators in Mozambique and Zimbabwe

Land and water WP5 - Two Food Safety MSc students at NRI have started projects on potential of zoonosis transmission through food. Projects will use a combination of challenge experiments to determine the susceptibility of African food types to contamination by leptospires and other common bacteria and screening prepared food materials for potential contaminants

Scientific problems encountered

None this year.

Publications or presentations

Ratzooman website: http://www.nri.org/ratzooman

Article in newsletter: Rodent Research

Socio-economic WP6 Progress

Overview

Draft documents prepared on strategic approach and on collaboration with other WPs. Close collaboration during the development of methodology and preparatory field visits to South Africa and Tanzania with anthropogenic WP. Some discussion/ exchange of drafts with modelling and GIS WPs. Two field surveys completed, some output drafts prepared and plans well in hand for other surveys.

Specifics

South Africa

1st study area identified, questionnaire and training material prepared, collaborators identified and trained, household survey completed with 120 rural households, data entered on computer spreadsheet. First stage of analysis of data and draft report completed. Secondary analysis to identify socio-economic clusters in hand. Arrangements completed for visit February 2004 to finalise data queries and final survey report.

2nd study area identified (urban, squatter), questionnaire and training material in draft, work programme proposed to Project collaborators. Arrangements completed for visit February 2004 to initiate research.

Tanzania

1st study area identified, questionnaire and training material prepared, collaborators identified and trained, household survey completed with 100 rural households in an endemic plague area. Data entry on computer spreadsheet in hand. Arrangements completed for visit February 2004 for review of data and draft survey report.

2nd study area under consideration (rural, rodent consumption) with Project collaborators. February 2004 visit would initiate research.

Mozambique

Contact with potential collaborators attempted. Urban study area under consideration, questionnaire and training material in preparation. Arrangements completed for visit February 2004 to initiate research.

Zimbabwe

Dialogue established with potential collaborators. Urban study area under consideration, questionnaire and training material in preparation. Arrangements completed for visit February 2004 to initiate research.

Report on the GIS/Remote sensing component WP8.

In July 2003 a visit was made to Maputo and a work programme devised for both Maputo and UK. In addition, during this visit the hardware and software for a computer system in Maputo was sourced and arrangements made with the suppliers for Ricardo Thompson (RT) to purchase the system.

The primary needs of the GIS are for adequate high resolution background topographic data and for remotely sensed images of the study sights for land use change analysis. Sources of such data were identified for Mozambique during the July visit and it was agreed that RT would contact the collaborators in the participating countries to source data for their country. RT was also to source images from the receiving station in South Africa. JP (Judith Pender) subsequently suggested that the budget be divided equally between the countries in the first instance.

The job list agreed is below. I have had no feedback from RT in spite of numerous attempts at contact and do not know the achievements so far. This has severely restricted the work to be carried out by JP in the period under review.

Tasks for Maputo

1. Order hardware and software and install
2. Install prototype GIS

3. Source TM sub-scenes and purchase (for focal sites)
4. Copy TM scenes onto cds and DHL to Pender
5. Source high resolution data for all study sites (SPOT or aerial imagery or existing coverages)
6. Purchase data identified in 5
7. Prepare high resolution background coverages and place in \Rats\GIS\TOPO
8. Add meta details to map data base in Raztzooman.mdb
9. Prepare and distribute topo maps to collaborators for use in the field
10. Send coverages and updated database to Pender
11. Source climatic data for the study sites
12. Input climatic data into data base
13. Source water information
14. Input land use change coverages (see 3,4 above and 2 below)
15. Input rats data
16. Input Socio-economic data
17. Input disease data

Tasks for Pender

1. Continue to source remote sensing and other background data as above
   Continuing – but I would appreciate knowing what has been accomplished by RT
2. Send budget
   Done
3. Analyse the land use change from the TM scenes. Provide coverages for the GIS and send to Maputo. Write report for collaborators.
   Unable to achieve – no data received from Maputo.
4. Obtain database structure from Herwig, incorporate the database structure into Ratzzoman.mdb. Send new database to Maputo.
   Database under construction – further details on structure requested from Herwig, but will be sorted out in Maputo
5. Reply to Herwig
   Done
6. Input rats data
   Future task – will be sorted out after Maputo
7. Structure database for climatic and water resources data
   In hand – design partially complete – Need feedback from collaborators
8. Structure database for disease data
   Need further discussion with collaborators
9. Program extraction of data into GIS
   Future task
10. Up date manual
    Future task
11. Consult the socio economic scientists over relationships between data and GIS
    Meeting held with socio economic scientists
12. Consult with Modellers over use of GIS and database data
    Need more data into GIS
13. Contact Tony Palmer & aerial photography suppliers in RSA
    Done – details passed to RT
14. Send and copy all documentation to Ricardo
    Done
15. Ask AL/Anne about Tanzania data sources used in Lake Tan Gef project
    Done – no suitable data

JP has designed and programmed a prototype GIS in Arcview 3.3. The system has been installed on a computer in INS and a CD provided so that it may be installed in the Ratzooman computer room. A manual of the prototype has also been. The system enables data specific to each survey site to be accessed easily by using a meta database developed in Access 2000. Background data from readily available sources has been added for each country as a start to the GIS (a list of coverages is provided in Appendix 4.). Training was provided to RT on the addition of new data to the GIS as it becomes available.

RT is a busy person and discussions were held on how the volume of work that is going to be generated by the GIS. RT is exploring the available help at the local university and within INS. JP has no further information on the progress of this issue

It was intended that RT visit UK in October or November 2003. It was hoped that by this time some of the data would have been purchased and the land use change started. The visit has not yet taken place

In was also intended that the GIS will be operational be the time of the Ratzooman meeting in Maputo in February 2004, so that partners may explore the possibilities for its future use. The prototype is available but it will not be possible to show the full range of possibilities.

Recommendations

1. It is recommended that JP spend 2 weeks in Maputo in a final effort to try and sort out the GIS component of the project. The meeting will provide plenty of opportunity to meet with the various collaborators and explain what is needed from them for the GIS.

2. The visit by RT should not take place yet – possible dates in April best for JP

Progress on WP 7, Measuring factors of anthropogenic change upon rodent ecology, epidemiology and natural capital

Overview

In planning and in developing methodology there has been close collaboration with Malcolm Iles, responsible for Work Package 6 (Socio-economic impact and livelihood constraints of disease). The intention is that questionnaire surveys set up under WP6 and anthropological field research set up under WP7 will be complementary. Malcolm and I are working together to cover the areas identified as the responsibility of the two packages, rather than taking separate responsibility for them. Once we have the data from our collaborators for each field site, the intention is to look at these together and write a joint report drawing on both the quantitative and qualitative data.

Our selection of sites has been from sites which have been selected for rat-trapping and blood plasma collection by the project. We are not going to be able to cover all of the sites which are being used for this purpose, and we have made a selection from them for data collection under WP 6 and 7. In doing this, we have aimed to ensure that we select sites which cover as many important variables as possible, but in particular: incidence or likely incidence of the diseases covered by the project; human behaviour likely to lead to disease transmission; urban and rural sites.

Due to the more in-depth nature of the anthropological research, and the importance and difficulty of identifying competent local anthropologists to carry out the fieldwork, we may not set up anthropological research in all of the sites where questionnaire surveys are administered. However, to date, in the sites currently being used and those under consideration, we are planning to set up both anthropological field research and questionnaire surveys.

We envisage that the initial stage of data collection under WP6 and 7 may need to be followed up by more in-depth research in the same sites looking at specific areas of human behaviour and perception which are identified as relevant once we are able to look at our initial data from WPs 6 and 7 together with the data from the other WPs which are trapping rodents, collecting rodent blood to look at incidence of the three diseases in question, and collecting human serum, also to look at incidence of the three diseases.

In identifying anthropologists to carry out field research locally, it has been important to ensure that a female anthropologist is involved in carrying out the research in each site, since most of the human behaviour and many of the perceptions which are relevant to the transmission of the diseases (e.g., in particular: hygiene; handling of water; management of children) are the province of women. The ideal is to identify a team of two anthropologists, one male and one female, with the minimum being one female anthropologist.

Sites where research has already started under WP 6 and 7 are Mapate and Lushoto. The questionnaire surveys are complete and the data has been delivered to Malcolm Iles for both sites.

The anthropological field research has been completed and the report should be in hand by the Maputo workshop in February 2004 for Mapate, and the field research should be complete for Lushoto by that time.

We are planning to set up anthropological research and questionnaire surveys soon after February 2004 in Durban in S. Africa (Malcolm Iles is planning to work through Peter Taylor there and a local anthropologist has tentatively been identified to carry out anthropological field research) and probably after June/July (when a field visit is planned to the site with the collaborating anthropologist at Sokoine University, Flavianus Magayane) in the south of Tanzania, in the area where they eat rats, using the same site in which other data is being gathered for the project (Masasi?).

Initial discussions have been held with Judith Pender on the incorporation of data into GIS (Work Package 8).

South Africa
A visit was made with Malcolm Iles in June 2003 to Mapate. A local anthropologist was identified and selected to carry out the research - Pfarelo Matshidze, at the University of Venda. Guidelines for fieldwork were drawn up and discussed with her and field visits were made. A contract was set up following this for the field research, funded partly through another project. Fieldwork was carried out between July and October 2003. However the report was delayed; it should be in hand by the time of the Maputo workshop in Feb 2004.

I plan a visit with Malcolm Iles to Durban in February 2004 to plan a questionnaire survey and anthropological field research there. Malcolm will be meeting with Peter Taylor to discuss details of the administration of the questionnaire and I will be meeting with an anthropologist whom I have identified at the University of Durban, Dr. Suzanne Leclerc-Madlala, who specialises in medical anthropology. Malcolm and I will be visiting the field site, if possible together with Dr. Leclerc-Madlala.

I also hope to meet with contacts at radio stations while in Durban; Suzanne Leclerc-Madlala has an interest in communication for development, as I do, and I have asked her to try to set this up through contacts she has. This is with a view to setting up dissemination by radio of health promotion messages identified as important through the project, including the possibility of making short pre-recorded radio programmes.

Tanzania
I made a visit in July 2003, immediately following a visit by Malcolm Iles, to Lushoto. This was made together with two anthropologists identified in Dar es Salaam, Dr. Edmund Kayombo and Ms. Joyce Nyoni (the former specialising in medical anthropology). Joyce later dropped out due to pregnancy; she was worried about plague. The team finally agreed upon was Dr. Kayombo, Dr. Flavianus Magayane of Sokoine University and a female anthropologist whose name I do not yet have (although I have requested it from both Dr. Kayombo and Dr. Magayane several times). The field research began in September 2003 and was scheduled to end by the end of December 2003. I am not yet in receipt of the report on the research, however.

Malcolm Iles and I had planned to make a visit to the south of Tanzania after the Maputo workshop in Feb 2004 with Dr. Magayane, to set up a questionnaire survey and anthropological field research in the project site there (Masasi?). However Dr. Machang'u has advised that it would be better to make this visit in June/July 2004, when travelling will be easier and there will be funds to set up a second field site in Tanzania. This means that the research in this site would be set up to start soon after that.

Mozambique
A contact in Maputo has been identified who may be able to assist in identifying people who can collaborate on anthropological field research in Mozambique; one male anthropologist has been put forward. I will be holding meetings with contacts and potential collaborators before the workshop in Maputo, with a view to the possibility of setting up a field site in Maputo. Due to the difficulty of transport and the likely cost, it is now doubtful that a field site will be set up in the north of the country for WP 6 and 7.

Zimbabwe

A decision as to whether to set up a field site for anthropological field research in Zimbabwe has not yet been made. Discussions at the Maputo workshop will assist in this decision. No anthropological contacts have so far been made in Zimbabwe. Malcolm Iles is visiting Harare after Tanzania in February 2004 and he will do what he can to assist in identification of potential anthropological collaborators.

Modelling work package progress WP9

During the first year of the project, work has started on epidemiological models of leptospirosis. Progress has been made in working towards specification of a model through a series of meetings between Unversity of Antwerp, NRI and KIT. No prior attempt to model this disease has been found in the literature.

It is proposed that the scope of the model be limited to trying to understand the epidemiology of leptospirosis in a rodent carrier. This focus is probably where epidemiological modelling can make the greatest contribution. Although understanding human infection risk factors is the overall goal, humans are a 'dead-end' host for the disease so play no part in the dynamics of the disease in the wider ecosystem. It is a reasonable assumption that leptospirosis infection in one of the major carriers, rodents, is correlated with human infection risk. The nature of the correlation will depend on a plethora of socio-economic factors and these are considered elsewhere in the project.

The model would allow questions to be explored about what drives the disease in a carrier rodent population:

- how seasonal fluctuations in climate affect leptospirosis in the environment and how this has an impact on infection of rodents from environmental sources
- the importance of the three different infection routes in the rodent population: indirect from environmental sources, direct sexual transmission and direct transmammary transmission from nursing mothers to offspring
- how rodent population size interacts with seasonal effects, and modes of transmission, to give infection outcomes in the rodent population
- the potential of rodent management interventions and how they might have an impact on rodent numbers and infection rates in rodents

Technically, the approach will probably be to use the rodent population model developed by Herwig Leirs and colleagues. This is a tried and tested model of the population dynamics of one of the main rodent carriers of Leptospirosis. This model will be extended to include the infection status of each of the three age classes of rodents, juveniles, sub-adults and adults. This age structure will probably need to be retained because modes of transmission differ between age classes. In addition, the model will need to represent the abundance of leptospires in the environment.

The most important type of data needed to test the model will be time series of rodent abundance together with leptospirosis incidence in the rodents. Rainfall data for a corresponding periods and locations would also be useful. It is not possible to obtain any meaningful quantitative estimate of leptospirosis incidence in the environment. As a next step it is hoped to develop the model as far as possible with present knowledge. When data on rat abundance and incidence become available, it will then be possible to determine what missing assumptions need to be made in the model in order to give the outcomes as seen in reality.

Land and water WP5 progress

Two Food Safety MSc students at NRI have started projects on potential of zoonosis transmission through food. Projects will use a combination of challenge experiments to determine the susceptibility of African food types to contamination by leptospires and other common bacteria and screening prepared food materials for potential contaminants. Leptospirosis cultures will need to be shipped from KIT to NRI with appropriate staff training given so that cultures can be maintained for such experiments.

RUCA

RATZOOMAN
Prevention of sanitary risks linked to rodents at the rural/peri-urban interface

INCO-DC contract number ICA4-2001-10125

ANNUAL SCIENTIFIC REPORT

Participant:	RUCA
Period:	Jan 2003-Dec 2003

Scientific achievements

- A detailed protocol for the rodent collecting study (WP2) was elaborated, and standardized data-files were designed for use by all partners.
- A first model concept has been developed for leptospirosis in a rodent population. The model is the first of its kind for this disease.

Scientific problems encountered

- Apart from Tanzania, rodent collections started only late in 2003 in the other countries (and not yet in Mozambique); as a result, only a limited amount of collected material has arrived in Antwerp and is now being investigated for detailed taxonomic investigation.
- Several of aspects of the epidemiology of the infection in rodents is not well documented which will make it more difficult and uncertain to parameterise the model.

Publications or presentations

none

Work Package 3: Economics and effects of control

Objectives

- To ascertain the rodent and insectivore species found among different habitats in SADC communities

Activities

A detailed protocol has been worked out for the collection of rodents from different habitats, and the sampling of relevant tissues from these specimens for taxonomic identification and investigation for the presence of pathogens. The full protocol is attached as an annex to this report.

RUCA has participated actively in the collection of samples from Tanzania. So far, about 2400 specimens have been collected there of which about 1000 have arrived in Antwerp and are being processed for detailed taxonomic identification (using morphometrics and molecular techniques). Results of these analyses are not yet available.

Encountered problems and solutions

For the other countries, collections started late (in September 2003 or later), and from there no material has yet arrived in Antwerp. The South-African material, however, is being investigated at the Natural sciences Museum in Durban, and material from there will only be sent to Antwerp for comparisons or in case of problematic identifications. Trapping efforts are now well underway in all countries, and it is expected that material from Zimbabwe and Mozambique will arrive in Antwerp soon.

Preliminary results

We report here the collection from Tanzania only, since this is the only material in which RUCA so far has been involved. A summary table of specimens trapped for different species at each locality is given on the next table. A considerable diversity has been collected (12 rodent genera, 1 shrew genus) and for several genera there are more than one species, e.g. *Rattus rattus* and *R. norvegicus*, or the presence of at least two species of non-African *Mus* in the peri-domestic environment.

Further analysis is going on for specific identification of all specimens, and for a comparison of the rodent fauna between the different habitats that were samples. In the second year, sampling will be concentrated more on a smaller number of habitats, but repeated every few months in order to be able to incorporate seasonal components into the comparisons.

Locality	Arvicanthis	Cricetomys	Crocidura	Dasymys	Grammomys	Leggada	Lemniscomys	Mastomys	Mus	Praomys	Rattus norvegicus	Rattus rattus	Steatomys	Tatera	(Tom)	Hovedt
Bigwa swamp			21	2	1	1		177								202
Chamwino			2						10			5				17
Chunya								5				1				6
Hilux Hotel								1	1			4				6
Kaumba		4	1						2		6					13
Kichangani		6						13				7				26
Kichangani chini		8									1	4				13
Kichangani swamp								24								24
Kihonda swamp	1		6	2				116	1			5				131
Kilakala			10					20	1	1	1	1	1			35
Kilakala swamp			10					101	2							113
Kipera-Mlali		2										2				4
Kitungwa swamp			4			2		23								29
Kola Hill			2				4	73	1			1		15		96
Kongwa	110															110
Lower Moshi								30								30
Madizini			7						17			29				53
Madizini/Forest		1										2				3
Mafiga		7	7					3	9		2	4				32
Mafisa		15							4		4	26				49
Mafisa B			1					1				8				10
Magadu			6				1	130	1	1		1		1		141
Market									1			2				3
Mawenzi		6	1						9		2	3				21
Mazimbu swamp			1		1			6				1				9
Mindu swamp			6		5			41								52
Misufini			5				1	17	21			25				69
Mji Mpya			10					21	4		2	8				45
Modeko									1			4				5
Morogoro hotel		7										5				12
Msamvu		1	1						12			18				32
Mtoni		6							2			18				26
Mwembesongo		18	37					37	33		4	26		1		156
Nane Nane			7				4	70			2	1		19		103
Ngerengere swamp					1	1		40								42
Ngoto		2						2	62		2	13				81
Ninja		1	1									6				8
Nunge		8										13				21
Railway		2										5				7
Railway quarters		1	1						4		3	2				11
Rock garden												17				17
Sabasaba		14	3					1	65			10				93
Soko Kuu		3						1	45			1				50
Suitani/Misufini									14			13				27
Sultan		1	2						6		1	4				14
Tanesco Swamp			3		2			9	1							15
Town Centre		6	2					1	17		3	8				37
Ujenzi		2							14			12				28
Mlali - Semi Swamp		1					1	84								86
Hovedtotal	111	122	157	4	10	4	11	1047	360	2	33	315	1	36		2213

Work Package 9: Predictive and simulatory modelling

Objectives

- To develop modelling tools that are capable of providing accurate simulations and predictions of when zoonotic diseases may outbreak and to determine particular areas that are or will become increasingly susceptible to zoonosis.

Activities

Work has started on epidemiological models of leptospirosis. Progress has been made in working towards specification of a model through a series of meetings between University of Antwerp, NRI and KIT. No prior attempt to model this disease has been found in the literature.

It is proposed that the scope of the model be limited to trying to understand the epidemiology of leptospirosis in a rodent carrier. This focus is probably where epidemiological modelling can make the greatest contribution. Although understanding human infection risk factors is the overall goal, humans are a 'dead-end' host for the disease so play no part in the dynamics of the disease in the wider ecosystem. It is a reasonable assumption that leptospirosis infection in one of the major carriers, rodents, is correlated with human infection risk. The nature of the correlation will depend on a plethora of socio-economic factors and these are considered elsewhere in the project.

The model would allow questions to be explored about what drives the disease in a carrier rodent population:
how seasonal fluctuations in climate affect leptospirosis in the environment and how this has an impact on infection of rodents from environmental sources
the importance of the three different infection routes in the rodent population: indirect from environmental sources, direct sexual transmission and direct transmammary transmission from nursing mothers to offspring
how rodent population size interacts with seasonal effects, and modes of transmission, to give infection outcomes in the rodent population
the potential of rodent management interventions and how they might have an impact on rodent numbers and infection rates in rodents

Technically, the approach will probably be to use the rodent population model developed by Herwig Leirs and colleagues. This model will be extended to include the infection status of three age classes of rodents, juveniles, sub-adults and adults. This age structure will probably need to be retained because modes of transmission differ between age classes. In addition, the model will need to represent the abundance of leptospires in the environment.

The most important type of data needed to test the model will be time series of rodent abundance together with leptospirosis incidence in the rodents. Rainfall data for a corresponding periods and locations would also be useful. It is not possible to obtain any meaningful quantitative estimate of leptospirosis incidence in the environment. As a next step it is hoped to develop the model as far as possible with present knowledge. When data on rat abundance and incidence become available, it will then be possible to determine what missing assumptions need to be made in the model in order to give the outcomes as seen in reality.

Preliminary results

The current state of the progress under this WP can be summarised as in the notes below, originating from a meeting at KIT, Amsterdam, on 15 January 2004 (15th January 2004 Rudy Hartskeerl (KIT), Stephen Davis (UoA), John Holt (NRI))

Discussion focussed around questions of the biology and epidemiology of Leptospirosis in rodents and the approach the representation of the essential features of the disease in rodents in the form of a mathematical model.

Sexual transmission. It appears that there is a strong likelihood that leptospirosis is sexually transmitted in rodents, and indeed many other hosts. Some sources indicate that sexual transmission is likely to be the main transmission route in rodent disease reservoirs. Clearly this has important implications from a modelling

perspective: assuming that sexual transmission is restricted largely to adults, then adults must be handled as a separate category in the model so that the sexual transmission route can be represented. There is also a variety of evidence, however, that Leptospirosis fails to persist in a rodent population in the absence of infection sources. It is therefore likely that sexual transmission is not usually efficient enough on its own to maintain the disease in a rodent population.

Mother to offspring transmission. Leptospirosis is also known to be transmitted from the mother to the offspring through mother's milk. This can probably occur in all mammals. There is also some possibility that offspring can acquire the disease directed from the mother as a foetus, though transmission through milk is thought to be more common. Though not strictly vertical transmission, it acts in a similar way from a modelling perspective. There are a number of sources that have shown, however, that rat neonatal rodents have passive immunity acquired from mothers that are Leptospirosis carriers. This passive immunity can last for a month or longer. As a result, it may be fairly unlikely that offspring become infected directly from their mothers.

Juveniles are thought to be more susceptible to infection than older individuals, if not protected by passive immunity. Juveniles are also thought to suffer some deleterious effects from the disease but then recover to become carriers. Juveniles are less likely than older individuals to acquire leptospires from environmental sources, as being largely dependent on their mothers, are likely to venture from the nest to a fairly limited extent. For the model, it may be reasonable to assume that juvenile infection from environmental sources is negligible. Thus, the only significant source of juvenile infection might be assumed to be direct from the mother's milk. To represent this transmission route in the model, it would be necessary to have a separate category for the juveniles.

The existence of the three possible transmission routes, sexual, mother's milk as well as non-direct environmental sources, makes it necessary to distinguish rodent age classes having different possibilities of routes of leptospire acquisition. Those age classes used by Herwig, in his rat population dynamics work, seem also to appropriate in this context, i.e. juveniles, sub-adults and adults.

Non-direct infection from environmental sources. Infection of rodents from environmental sources depends on their probability of encountering Leptospires in the environment. Survival of Leptospires in the environment is one important variable. The conditions for Leptospire survival are likely to be better in the wet season when soil moisture and humidity are higher. Clean water is also favourable to Leptospires and in periods of rainfall, water bodies are likely to be fresher and cleaner. Conditions of increased favourability for leptospires are likely to pertain shortly after the onset of rains (though of course, Leptospire concentrations at this time may be low if they have declined during a previous dry period). Further, periods of rainfall are likely to reduce Leptospire concentration in water bodies and therefore the associated probability of rodent infection.

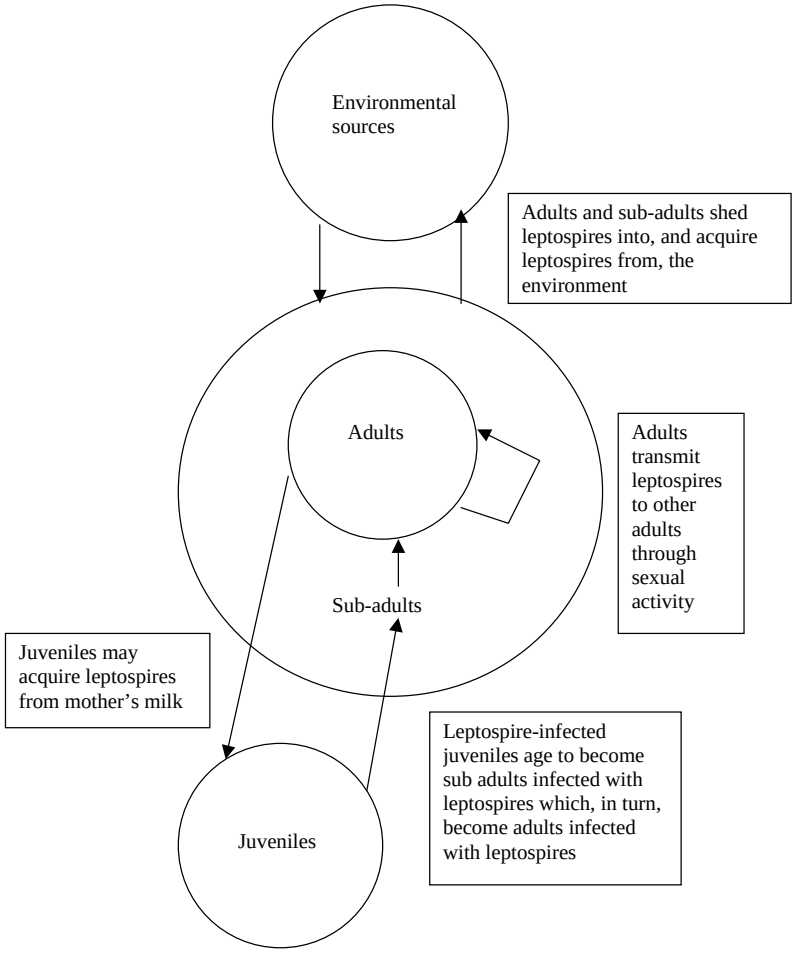
In dry conditions (or in a dry season), leptospire survival is likely to be poor as most rodent urine would probably be shed into unfavourable environments, e.g. dry ground. However, as rodents are likely to concentrate where water exists, greater amounts of leptospires may be shed in and around what water bodies remain. Thus, the concentration of Leptospires in dry-season water bodies could be high, and the corresponding risk of infection of rodents, also high. Survival of leptospires in dry season water bodies may be less than that in the wet season, however, if water quality deteriorates. Temperature, pH and water quality have a large effect on leptospire survival. Whilst in temperate climes, the disease is highly seasonal partly because winter temperatures are unfavourable for leptospires, in the tropics, temperatures are likely to remain favourable throughout the year.

The possible influence of seasonal changes in water abundance on Leptospire infection in rodents is therefore not obvious. In order to investigate the various possibilities in a modelling exercise, it might be most instructive to incorporate a function to represent a continuum of possible responses between rainfall and potential for infection. Thus, rodent infection from the environment might be higher in the wet than the dry season, vice versa, or indeed, if the various processes 'cancel out', give a relatively constant rate of potential infection, independent of season.

Another possible source of variation in leptospires in the environment stems from the intermittent shedding of leptospires by rodent (and other carriers). If there are any seasonally related patterns in leptospire shedding, this would affect the potential for infection in different periods. There is also some possibility that rodent carriers can build up immunity. If this occurred, then it is likely that the affected individuals would cease to be substantial carriers.

The latent period of the disease in rodents (i.e. from acquiring infection to becoming an infectious agent) is typically, probably less than one week. With a relatively short latent period, it is probably acceptable to neglect this and simply have two categories of rodents, healthy and infected (=infectious).

Non-carrier hosts. Hosts that develop disease symptoms as a result of Leptospire infection are unlikely to be important carriers because they shed Leptospires for a relatively short period prior to either recovering or succumbing to the disease. Thus, for modelling purposes it was proposed to model the disease in a rat population as an example of what might happen in a rodent carrier. The link between infection in the carrier and that in the human population clearly depends on a great many socioeconomic and anthropological factors, but as humans are a 'dead-end' host for the disease, it is expected that infection (and abundance) in the main carrier would indicate the potential risk for humans.
Diagram summarising leptospirosis transmission routes in a rodent population



RATZOOMAN
WORKPACKAGE 2

TAXONOMIC IDENTIFICATION OF RODENT SPECIES FOUND IN RURAL AND PERI-URBAN HABITATS

GUIDELINES FOR COLLECTING SMALL MAMMALS

In order to allow full and standardised use of the small mammal specimens that will be collected during the RATZOOMAN project, the following guidelines are proposed. Not following them will make specimen processing and data analysis much more difficult or even impossible.
Technical procedures are extensively described in the different appendices. In case of questions or problems, please contact RUCA (Herwig Leirs) immediately in one of the following ways;

email: herwig.leirs@ua.ac.be
fax: +32-32 18 04 74
phone: +32-32 18 04 69
postal address: Dept. Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium

GENERAL OVERVIEW

The small mammal trapping provides the basic material for a number of work packages (WP2, WP3, WP4) and the results of the latter WP are important for WP8, WP9, WP10.
Collected specimens should provide information about:

species composition of the small mammal fauna
small mammal abundance
small mammal demographic information
small mammal reservoir status for specific pathogens
geographical, habitat-linked and seasonal variation of the above

It is important that all trapping is carried out in a standardised way, providing all the information that is necessary. This means that arrangements must be made about the following:

identification of trapping sites: which cities, which habitats in a city, how many replicates
identification of trapping periods
how to trap small mammals: traps, baits, trapping systems
how to record captures
labelling of captured specimens
which characteristics to record for each specimen (internal, external)
sampling procedures for blood and organ samples
storage of samples and specimens
shipment of samples and specimens

PREPARATIONS

Selection of trapping sites

In each RATZOOMAN partner country, select 3 localities where traps will be placed:

one major focal area (small or medium sized town, with a clear urban-rural interface): in this focal area, repeated trapping sessions will be organised, so it is important that this area is easily accessible, has comfortable lab facilities etc...

two minor focal areas (from where human data on prevalence are available, i.e. linked also to data that are collected for WP1)

Within each locality, select the following trapping sites, belonging to 10 habitat types (if present; if some of these habitat types are absent, you may replace them with other common or rodent-infested habitats in the locality where you are working). Use maps, aerial pictures or satellite images to help you in locating the sites (see 2.2. below). Make sure to obtain the necessary permits and inform the community well about the purposes of your work. Since trapping will be repeated several times, ensure that it will be possible to work in the same sites during a period of two years.

site	traps each
2 market areas	20
5 houses without gardens (if present) (these may include apartment buildings)	5 indoors, 5 outdoors (<5 m)
5 houses with gardens	5 indoors, 5 outdoors
2 commercial grain stores/mills	5 indoors, 5 outdoors
1 slaughterhouse	10
3 open areas (abandoned land, field, rubbish tip)	20 each
20 poor quality houses	5 indoors, 5 outdoors
5 rural houses	5 indoors, 5 outdoors
3 fields outside town	20
3 (semi-)natural vegetation and fallow	20
TOTAL: 31 sites	600 traps

Within each site, try to identify subhabitats, e.g. in houses, subhabitats exist in gardens, outside sheds, basements, attics, storage rooms). Identify possible trapping sites in the different subhabitats. Preferentially, select sites with known rodent presence.

In open areas, fields and natural or fallow vegetation, place the traps in one or more trapping lines with 10 m between consecutive traps.

Description of trapping sites

Try to find detailed map of your localities (e.g. topographic maps at 1/10.000-1/50.000). Urban city maps are useful for the inner parts of the town. Try to find out if reasonably recent aerial pictures of the area exist. Contact Judith Pender at NRI to obtain SPOT-images of the area if aerial pictures are not available. Find out if there are existing GIS data for your localities.

Within each locality, give each trapping site a unique code. Use a code that is easy to remember because you will need it in the field when checking traps. Use e.g. the name of the house owner, the street, the name of the market,. if needed in combination with a serial number.

Describe sites verbally and draw a map of the situation. If possible, take some pictures (and label them appropriately). Make a rough description of the habitat (% ground cover, vegetation height, type of vegetation, e.g. grass, bushes, crop, river bank...) Mention explicitly the humidity of the soil: dry, mud, presence of water puddles,... Locate the exact position of each site with GPS or by indicating on a map.

Trapping calendar

Trapping will be organised at regular intervals, with trappings sessions of four consecutive nights each. Indicate the session dates on a calendar, and take them into account when organising other activities.

major focal area: 4 sessions, 3 months apart during a first 12-month period. Afterwards, 2 more sessions, period to be decided after preliminary analysis of the first data

minor focal areas: for each area, two sessions in two different seasons, to be organised somewhere during the first two years

Within an area, all trapping for the same session should preferably be simultaneous. However, in order to reduce the number of traps needed, a session can be spread over up to three consecutive weeks. (Note, however, that the cost of having people in the field during additional weeks in each session may be higher than purchasing additional traps).

Traps and bait

In each habitat type, use different types of traps since these may yield different species of small mammals. As a rule of thumb, use the following division:

50% Sherman live traps: preferably use Large Folding Aluminium Treadle and Doors Galvanized Trap (3 x 3.5 x 9" Body .025 Aluminium Treadles and Doors 30 ga. galv. .56 lb), cost about 14 € per trap (more information at http://www.shermantraps.com/). If you need to order traps, contact Herwig since we may be able to get a better deal when ordering a larger number of traps.

25 % snap traps: any kind, but large enough to also take rats

25 % other trap types, e.g. local traps (like funnel traps) or large cage traps for larger mammals

The proportions of different trap types may be changed according to the local conditions.

Bait: use your usual bait if you are happy with it. If not, use a mixture of peanut butter and maize bran or rolled oats. When trapping in town, it sometimes useful to add some dried fish, sardine oil or the like. When trapping for Cricetomys (giant pouched rats) use fresh fruit or green maize as a bait.

Decide who will collect, process and transport the specimens

Identify responsible scientists/technicians who will be in charge of the trapping and processing of the specimens. Make sure that they aware of the objectives of the study and all trapping and processing procedures. Decide how many assistants or occasional local field attendants should join the team. Those that will handle traps and small mammals must receive basic training in the trapping, handling, and processing of the trapped specimens, as well as safety and decontamination procedures.

Check infrastructure and equipment

Identify laboratory space where captured specimens can be centralised and processed and samples can be stored until transfer to your own lab. The lab should be freely accessible to your team but not to the public and it is advisable to carry out all work away from spectators. Check ventilation, availability of water and, if needed, electricity.

Check all necessary equipment, especially labels, autopsy equipment, vials and chemicals for storing samples, before starting the trapping session.

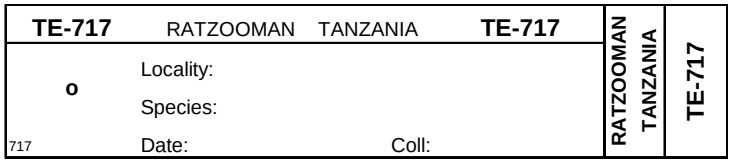
Consider all the logistic requirements for trapping equipment and supplies, specimen handling and transport to the laboratory (timing, route, transit temperature requirements, shipping procedures, and documentation like export permits etc.), and decontamination procedures in advance. In addition arrange transport, accommodation (if necessary), and protection of the team, and secure lines of communication (phone, email).

Biosafety and decontamination procedures

As there is always a risk that trapped animals are infected with some kind of infectious agent, strict safety and decontamination measures must be followed. To protect the specimen collector and colleagues common safety precautions should be followed and each animal should be handled as infectious. Protective equipment (gloves, mask) should be worn and local regulations about safe work practices followed to reduce exposure to potentially infective material. A first aid kit is essential, and should be readily accessible at the site of specimen collection.

Disinfection of work areas and decontamination of spills of blood or infectious body fluids is readily achieved by chemical disinfection with chlorine based solutions. Sharps and spoiled glass slides should be discarded directly into a puncture-resistant disposal container, which is then incinerated. Basic safety precautions are listed in appendix 2. Make sure that all workers understand the need for protection and understand the safety measures that are applied.
Live animals stored for some time before processing or transport should always be kept outside under a shelter or in a well ventilated area.

PROCESSING TRAPPED ANIMALS
Overview
In general, the following steps will be followed when processing captured animals. Clearly, some steps (e.g. anaesthesia, collecting ectoparasites, taking blood,...) make no sense with animals that died in the trap.
record trapping site for each trapped animal
label each specimen
anaesthetise animal
take blood sample
collect ectoparasites
fill out collection form with external characteristics and measurements
dissect and take tissue samples
close animal and preserve
In a few cases, it will be necessary to make karyotypes; this is not a standard procedure and requires a separate protocol. However, also for these animals, all the above steps are needed.
We now discuss these eight steps in more detail.
Record trapping site for each trapped animal
Make sure not to mix specimens from different trapping sites. Before leaving the trapping site, indicate the trapping site's code on the trap with removable ink or pencil. If the animal is dead, the label can possibly already be fixed to the animal in the field, but the easiest is to put the animals in a labelled plastic bag. It is not necessary to indicate the individual trap's number.
Live traps are taken as such to the lab, and the animal will be removed in the lab. Replace the empty place in the field with a new trap before leaving, otherwise you have to return with a trap later. Dead animals can be placed in a plastic bag (write the site code on the bag) and replace the trap. Check bait in all traps, also those that were not successful.
Label each specimen
Each specimen must be assigned a unique identification number by the collection team. This unique identification number should be present on all data-forms, records, slides, vials etc and used as a common reference for any given individual.
Pre-printed, pre-numbered labels for the small mammal specimens will be provided by RUCA. Labels are printed on water-resistant paper. Do not prepare your own labels, but inform RUCA well in time if you need additional ones. They look as follows:



The specimen number is pre-printed on the label. It should be written also on the back of the label, IN PENCIL.
Other information to record on the label is:
Locality: the name of the capture locality, i.e. the name of the town (not your own trapping site code, but a name that can be found on maps etc.)
Species: your preliminary field identification of the small mammal; in most cases this will probably only be the genus name; if you do not know it, simply leave it blank
Date: the date of collection as dd/mm/yyyy
Coll: the name of the collector, i.e. the person who was responsible for the capture and who can be contacted later for questions about the capture, the site, etc... Usually this will be the name of the scientists in charge of the trapping session
Any other information can be added on the back of the label. Note that all information must be written with a (soft) PENCIL: information written in ballpoint, marker pens, ... will fade when the specimen is put in water or alcohol.

The label should be fixed to the (preferably) left hind leg of the specimen. Punch a hole where indicated on the label, then fix the label through the thigh muscle with a plastic tag. Alternatively, use a short piece of thin rope, fix the label to it with a noose and firmly tie the rope to the hind leg just above the heel so that it cannot slip off.
Note that the labels come printed on sheets with 30 labels each, starting with the lowest number in the corner to the lower right and going up per column. This makes it easier to cut of the labels in the right sequence. The left margin of the sheet mentions the label numbers on the sheet and can afterwards be kept as a record that these numbers have been used. Cut the labels with paper scissors, do not use dissection scissors as they will soon go blunt. Make sure to punch a hole in the label, rather than simply putting your needle through it, since that may cause tearing.

Anaesthetise animal

The easiest way to anaesthetise the animals in the field is to use a liquid like ether, chloroform or halothane. Find out what is easiest to obtain and what the relevant local regulations are. Use a large glass jar with a thick layer of cotton wool at the bottom; poor a small amount of the anaesthetic inside and place the animal inside; lock the jar with a lid. Observe the animal until it becomes unconscious, then quickly take it out and start processing it. If the animal wakes up during processing, replace it in the jar.
It is often not necessary to add new anaesthetic for each individual animal.

Take blood sample

he blood sample needs to be large enough for all tests. This requires blood taken from the heart or from the retro-orbital sinus. In case of heart puncture, you will need syringes and needles; taking blood from the retro-orbital sinus requires heparinised glass capillary tubes. In both cases, the blood must be collected in a labelled eppendorf tube and left to stand at room temperature until the serum is separated. Afterwards, the serum is pipetted into TWO new labelled eppendorf-tubes, on with about 300 µl of serum, the other one with rest. The first one is used for the standard tests in Ratzooman, the rest is frozen at -20°C for later use in confirmation or additional tests.
The last part of the blood in the needle or capillary can be used to make a blood smear on a labelled glass slide and dried to the air.
If the animal is dead and no more blood can be taken, then during the dissection the whole heart must be removed and stored in the eppendorf tube.

Collect ectoparasites

Immediately after blood sampling, the ectoparasites must be removed. Do this in a large pot (white or light colour). Brush of the animal's fur vigorously, then pick up the ectoparasites with an entomological pooter (a sucking device) or a wet fine paintbrush. Store all ectoparasites in a labelled eppendorf tube with alcohol.

Fill out collection form with external characteristics and measurements

An example of the removal capture form to be used is given in appendix 5. Such a form has to be filled for each trapping night at each locality.
Be sure to use always the same number of traps and trapping configuration during each trapping session. If there occurred something which disturbed the trapping, note this very clearly (f.i. 21 traps stolen during night 3, bush fire prevented trapping on lines A-C). Always indicate how many traps were closed without a catch (f.i. after a heavy rain).
A written form should be made for each trap night and afterwards introduce the data into the computer file (excel file). After each trapping session (4 nights) send the data as an attached file to me by email.
The following data must be entered for the full sheet:
Collector: the person who was actually doing the trapping
Date: dd/mm/yyyy (this is the date when you collect the rodents)
Locality: the town or municipality where captures were made
Weather: describe the weather during the night you were trapping (rain, cloudy or not, full moon,....).
and detailed information must be provided for every capture:
Species: fill in the scientific name of the species caught. If you are not sure only indicate the genus name. You will get feedback after confirmation.
Label-number: The number from the specimen label. All material which is collected from the rodent (f.i. stomach contents, tissue samples) should be marked with the same label-number.
Site: code for the trapping site
Trap: trap type
Sex: M=male; F=female. Indicate the sex only when you are sure of a correct identification. Determination of the sex is not always straightforward for some species especially when they are still very young. We will check for this when the animals arrive in Europe.
Sexual condition: the following possibilities can occur:
Males:
testis: abdominal (A) or scrotal (S)
swelling of the cauda epididymis visible (V) or not (N)

So for example for a sexually active male with clearly visible swelling of the epididymis you should fill in SV

Females:
vagina: closed (C) or perforated (P)
nipples: small (S) or lactating (L)
pregnant: Yes (Y) or No (N).

In some species after copulation they have a kind of plug in the vagina opening which might prevent sperm competition, indicate this in the remarks if observed.

Measurements: Measurements are best taken by the same person. At the start, do check the accuracy of your measurements by taking each measure on the same animal 10 times and calculate the variance which may not exceed 5%.
Weight: record the weight in g. Some will use a electronic balance while other will use Pesola spring balances. When animals are wet indicate this clearly in the remarks. Snap trapped animals are often partly eaten by all kind of other animals (ants, shrews, slugs ….), do also note this in the remarks if it is the case.
Total length: body and tail length in mm, use decimals for small species.
Tail length: length of tail in mm from the bending point (anus) up to the tail-tip. If a tail is broken (which quite often occurs) take also this measurement but note it in the remarks.
Length of the left hind foot (0.1 mm): do not include the nail in this measurement.
Ear length (0.1 mm): from the basis of the ear (insertion curve) to the ear tip.
For the latter two measurements, you will need callipers with fine points. For the other measurements, a normal ruler will do
Samples taken: If samples have been taken, indicate the appropriate abbreviations:

Karyology:	KA
Blood smear	BS
Blood serum	BL

Tissue samples:
Liver – LI
Kidney – KI
Lung/Heart - LU
Spleen - SP
Stomach - ST
Ectoparasites - EP

Remarks: See items before. Please note all information which you think is relevant (parasites, wounds, ….).
Dissect and take tissue samples
Sterile sampling
Dissections must be done in a sterile way. Do this as follows:
place the animal on its back on a dissection mat
wet the fur on the belly with some alcohol or a watery disinfectant
cut through the skin and open the abdominal and thoracic cavity, using a single set of scissors and forceps; after each animal, rinse these scissors in alcohol or a watery disinfectant and keep them there until the next dissection
take a fresh pair of small scissors and forceps; dip them in alcohol and flame them; use only these instruments for taking out samples
disinfect this set of scissors/forceps in alcohol or a watery disinfectant; clean it with soap and water; rinse with water; keep clean until the next dissection and flame with alcohol just before use (the easiest is to have at least 5 sets of scissors/forceps ready and only clean them after each batch of animals)
if the scissors or forceps would touch something else than the tissues that you are dissecting, then put them away for cleaning and continue with a new set
Take care of your dissection kit. Only use the scissors for dissections, not for cutting other items or paper since they will easily go blunt. Because of safety reasons, do not use scalpels when dissecting.
Samples to be taken and storage
Take the following samples and store appropriately. Remember that due to high temperatures animals start to decompose very fast once dead, Therefore quick processing is essential; if an animal is partly rotten, then indicate that on the form but still take the samples where possible.

Karyotype: upon request
Blood sampling: heart puncture or from orbital sinus
blood smear: dry

serum: 2 aliquots (300 mic, rest): -20°C
Tissue samples
Liver: in 96% ethanol
Kidney: one in 96% ethanol, one fresh to culture
Heart + Lung: in -20 freezer
Spleen: in 96% ethanol
Ectoparasites: brushing, in ethanol
Endoparasites: in ethanol when obvious
...
Label all the samples with the same ID-number as the animal. Separate small sticky labels will be provided to stick on vials, glass slides etc. When material is stored in vials with alcohol, do not only place sticky labels on the vial or write on the vial. Instead, use a pre-printed insert label (provided) or cut a small piece of ordinary paper, write the specimen number on it IN PENCIL and place that piece of paper inside the vial in the alcohol together with the sample.
Store the samples appropriately, in the freezer if indicated. If in doubt about storage conditions, contact the Work package manager.

Preservation of carcasses
Close the dissection cut so that internal organs do not come out. Preferably use Michel wound clips (7.5 mm), one or maximum two clips per animal; if you run out of clips, you can use paper staples but these will corrode in the formalin. You can also close the abdomen with a needle and a thread, but this is very time consuming.
Ensure that all carcasses are labelled with a well attached label and that the used ink is waterproof (e.g. pencil).
Small animals (<40 g can simply be thrown in the formalin solution); in larger animals, inject formalin in the stomach and in large muscles (shoulders, neck, thighs); inject formalin in the brain in large skulls (make sure not to damage the skull itself).
Leave carcasses at least three days, preferably longer, in the formalin, but not more than three months; if necessary, transfer them to alcohol
Prepare the formalin solution as described in the annex.
Packaging and labelling collections for transport.
Detailed packaging, documentation, and handling requirements for the international transport of biological materials are contained in the regulations of the International Air Transport Association (IATA) and in documentation of the International Health Regulations. Reference to the most recent regulations and guidelines is required, as they are subject to periodic review and modification. In addition to these international requirements, any additional requirements established by national authorities and commercial carriers must also be followed.
Address labels on outer packages should display the sender and laboratory name with complete addresses and telephone numbers for both the sender and receiver. Documentation should also contain specimen details (number, type), appropriate biohazard labels, and the storage temperature requirements. Copies of letters, forms, permits, airway bills and other identifying/shipping documents for the receiving laboratory should be placed together in a plastic bag and taped onto the outer transport packaging. The transport service must also receive a copy of these documents. The receiving laboratory should receive a copy of these documents in advance and the airway-bill number should be transferred immediately once known.
Transport of specimens
Before transport, the collection team should notify the receiving laboratory of all shipping and specimen details in advance of specimen arrival. In many cases, initial surface transportation of specimens from the field site to transport facilities may be required prior to shipment to processing laboratories. If international transport is necessary, authorization to import the specimens should be organized by the laboratory, which should also inform the sender of receipt or non-receipt of the specimens.

Specimens may be sent by mail in conformance with all relevant international, national, and commercial carrier requirements. Contact with the postal authorities should be established prior to the collection of samples to ensure their ability to transport the materials and to verify understanding of the shipping requirements.
For international transport by passenger aircraft, the total quantity of specimens that may be transported in one package is 4 kg or 4 litres. There are now several commercial organizations specializing in transport of biological materials. They will provide details of the specimen packaging and documentation requirements, so if it has been decided to employ their services it is advisable to contact them early in the course of an investigation.
The receiving laboratory should record the date and time when the specimens were received, name and initials of the person receiving the specimens, and a record of specimen quality. The investigation team should receive a line listing form or computer data file with the unique identification number and relevant information for each specimen.

Appendix 1. Basic safety precautions

Use latex gloves when taking and handling specimens. Dispose gloves after each working session. Do not attempt to clean and reuse gloves as this may promote the spread of pathogens.
Wear protective clothing (gown, coat or apron) when collecting tissue samples from freshly dissected animals.
Discard used needles directly into sharps box, without recapping them.
Work areas and surfaces should be organized and disinfected with 1% household bleach daily. Use 10% bleach to clean up spills after wiping the surface clean. Personnel carrying out cleaning or decontamination should wear a protective coat and thick rubber gloves.
Contaminated non-disposable equipment or materials should be soaked in 1% household bleach for 5 minutes.
Heavily soiled disposable items should be soaked in 10% household bleach before incineration or disposal.
All animal cages containing animals should be disinfected at least once a week (10% bleach solution followed by thorough rinsing with water) or immediately after animals are removed from the cage.
Contents from cages should be transferred to the open and burned on a regular basis.
If there is any indication that stored animals are sick, kill them immediately, take samples exercising safety precautions and fixate them in formalin. Disinfect all materials the animals came in contact with.

Appendix 2. Materials for collecting and processing rodents

Latex disposable gloves
Disposable sterile lancets
Glass slides, cover slips, slide box
Filter paper
Fixatives such as methanol, ethanol (absolute 96%), formalin
Vacutainer, sterile screw-cap tubes (or cryotubes if indicated)
Labels and indelible marker pen, pencils
Eppendorf tubes of different sizes
Pesola balances (50g,100g, 500g, 2000g)
Tweezers and wound clips (7,5x1,75mm) to close dissected animals
Bleach solution for disinfection
Needles to fix animals for dissection
At least six dissection kits

Appendix 3. Fixation of animals

A. Storage of tissue samples

- cut tissues (muscle, liver, spleen, heart) of freshly killed animals in small pieces (grain size).
- clean accurately scissors or other instruments before working on another animal
- place the tissues in a small tube (2-10 cc) and add ethanol (96%) at a ratio of 1: 10; preferably use non-denatured ethanol.
- label the tubes with PENCIL and not ink (in case alcohol leaks out during transport it would destroy the notes on the label); unless pre-printed adhesive etiquettes (that can withstand alcohol leakage!) are used, it is best to use a small piece of paper on which the following information is written in PENCIL:
- specimen number (an individual code, the same one as used for the label that goes as with the carcass specimen in formalin, so that results from specimen and tissues later can be linked)
- species (if identified)
- date, collection locality (possibly with latitude and longitude), collector.
- shake the tubes gently so that alcohol penetrates between the tissue pieces; repeat after 1 and 2 hours
- it would be desirable substituting the alcohol after 24 hours.

Make sure that the rest of the specimen is properly labelled and fixed in ethanol; tissue samples that cannot be linked to a formalin-carcass are useless!

Instead of ethanol, another fixation fluid (DETS) can be used, but then clearly indicate this. With ethanol or DETS, the samples can be kept at room temperature or in the fridge. Alternatively, the tissues can also be frozen without any fixation fluid, but then they must stay frozen at all times.

Protocol for preparing tissue storage solution (DETs)

End-volume (ml)	50	100	200	250	500	1000	Final conc.	Order
Tris (g)	0.6	1.2	2.4	3	6	12	0.10 M	1
EDTA (g)	4.5	9	18	22.5	45	90	0.25 M	2
NaCl (g)	10	20	40	50	100	200	3.4 M	3
H20 (demi-water, ml)	12	24	48	60	120	240		4
DMSO (ml)	10	20	40	50	100	200		5
1 M HCl (ml)	2.8	5.6	11.2	14	28	56	pH 7.5	6

Mix the salts and fluids in the order as mentioned in the table. Then add demineralised water until the end-volume is reached (if the samples are meant for leptospirosis analysis, the water should be filtrated through a 0.22 um Millipore filter to reduce addition of saprophytic leptospires). After this, dissolve the salts by stirring. This can take some time. Not all salt will dissolve, some salt will settle at the bottom. Do not remove this salt. It guarantees that the solution is NaCl-saturated. At the end check the volume again. Adjust it to the end-volume with demi-water (volume will change during dissolving of the salts). Preferably, make the DETs a week before the samples are stored. This again is to guarantee that the solution is NaCl-saturated. When storing tissue-samples into small tubes the solid salt is not to be transferred.

DMSO will facilitate transfer of all solvents and solved chemicals through the skin. Although the chemicals in DETs have very low toxicity, wear gloves at all times.

B. Formalin-fixation of whole body specimens

Formalin solution preparation

For 30 l Formaline-solution, use: 1200 g paraformaldehyde powder
270g $NaCO_3$
30 g soap (do not add more than 30 g!)
add 30 l reasonably clean water

The paraformaldehyde powder may be replaced by 3 l concentrated (40%) aqueous formaldehyde-solution if that is more easily available (note "formalin" is used as a common name for a 40% formaldehyde solution; a 10% formalin solution is a tenfold dilution of the 40% formaldehyde solution).

Fixation of carcasses

- if the carcasses were dissected, close the cut so that internal organs do not come out
- if carcasses were not dissected, make a small opening in the abdomen so that the formalin can easily enter the body cavity
- ensure that all carcasses are labelled with a well attached label; the label and the ink shall be water proof (use e.g. pencil)
- small animals (<40 g can simply be thrown in the formalin solution); in larger animals, inject formalin with a syringe in the thoracic cavity (unless that was opened during dissection), the stomach and in large muscles (shoulders, neck, thighs); inject formalin in the brain in large skulls (make sure not to damage the skull itself)
- leave carcasses at least three days, preferably longer, in the formalin
- never leave carcasses in formalin for more than three months, because they will become too rigid to handle; if necessary, transfer them to alcohol (see below)

Sending formalin-fixed specimens

- drain the formalin from the carcasses; only send carcasses that have been fixed in formalin for at least 72 hours;
- fill up zip-lock bags with the carcasses; make sure that the amount of formalin fluid in the bags is minimal; lock the bags carefully;
- put a double large plastic bag (type trash bag) in the drum and fill the inner bag with the zip-lock bags with carcasses; close the inner bag tightly without too much air inside; close the outer bag tightly;
- fill up the space above the bags with other, not sharp, luggage or with any filling material (crumpled newspapers, polystyrene filling material, wood-wool...)
- put a shipping list on top, mentioning the specimen number, tentative identification, date and locality for each specimen ; add a letter signed by a vet, indicating that the specimens are not infectious (example attached);
- close the lid and screw on the sealing ring
- label the drum with the correct address (add also phone number, fax number and email address)
- contact the addressee in advance to arrange for the specimens being picked up from the airport or received from the courier service

Transferring material to alcohol

- about 2-3 months after fixation in formalin, take out the animals
- rinse animals for two days in water (put them in a bucket and let water run continuously in the bucket)
- put the animals per genus, and within each genus grouped per tag number, in jars filled with 70 % ethanol ; after two weeks, refresh the alcohol
- label the jars appropriately ; store them, well closed, in a dark, dry and preferably cool place
- check the jars periodically to make sure that the alcohol has not evaporated ; if necessary, refill

send to:

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Belgium

telephone: +32 3 2653469
email: herwig.leirs@ua.ac.be

SCIENTIFIC MATERIAL - NO COMMERCIAL VALUE

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SCIENTIFIC MATERIAL - NO COMMERCIAL VALUE

Example of a letter which you should have when you carry animal specimens with you on a flight to Europe.

ATTENTION: THIS FORM IS NOT APPROPRIATE FOR SENDING POTENTIALLY BIOHAZARDOUS MATERIALS!

EU-Ratzooman project
SUA Pest Management Centre
Sokoine University of Agriculture
POB 3110 Morogoro
Tanzania

TO WHOM IT MAY CONCERN

This is to certify that Dr XXX is carrying to Belgium a scientific collection of 645 small vertebrates, for identification at the University of Antwerp. The transport of this material is part of a scientific research project which is carried out with support from the European Commission (Proposal N° ICA4-CT-2002-10056).

The specimens do not contain any infectious agents as they have been fixed in formalin or disinfected in 96% ethanol for at least 72 hours. To the best of our knowledge, the collection does not contain any specimens of endangered or protected species.

Please accord Dr……. your kind assistance.

Prof .Dr. Robert Machang'u
Leader Pest Management Centre

Dr. YYY
Veterinary Pathologist

Morogoro, 29 June 2001

Appendix 4 Removal capture form

RATZOOMAN REMOVAL-FORM TANZANIA

Collector: Date: (dd/mm/yy): Wheather:

Locality

Species	label-nr	Site	Trap	Sex	Sex.Cond.	Weight	Total L.	Tail L.	Hind foot	Ear L.	Samples alc.

Males: Testes **A**bdominal or **S**crotal ; Swelling of the epididymis **N**on-visible or **V**isible; Females: Vagina **C**losed or **P**erforated ; **S**mall nipples or **L**actating ; Pregnant (**N**o or **Y**es)
Samples in alcohol : Liver, Spleen, **K**idney,... Samples in -20: Blood (Serum), **H**eart/**L**ung,...

DPIL

RATZOOMAN
Prevention of sanitary risks linked to rodents at the rural/peri-urban interface

INCO-DC contract number ICA4-2001-10125

ANNUAL SCIENTIFIC REPORT

Participant:	DPIL
Period:	Jan 2003-Dec 2003

Scientific achievements

- A detailed protocol for the Capture-Mark-Recapture study (WP4) was elaborated, designated software adapted for the Ratzooman project.
- A pilot study with marked baits was undertaken to investigate the movements of rodents from focal points in Morogoro, Tanzania; the results indicate that animals who feed at these sites can be found several hundred meters away from the feeding site.

Scientific problems encountered

- Since CMR-data are only available to a very limited extent, no data analysis has been performed yet.
- The results from the marked bait study are surprising and need to be corroborated.

Publications or presentations

Work Package 4: Rodent ecology in rural/peri-urban Africa

Objectives

To establish rodent population dynamics for the major rodent and small mammal species identified in targeted areas of the SADC
To understand the interactions among different small mammal communities in these areas
To analyse the roles of the different species identified in WP 2 in relation to human populations and zoonosis
To discover potential factors influencing small mammal species prevalence

Activities

4.1. Capture-Mark-Recapture study

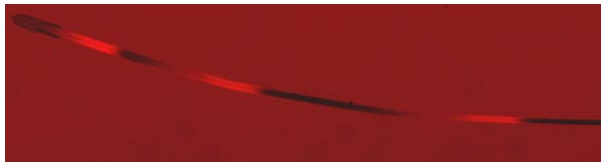
For the capture-mark-recaptures study, a full protocol was worked out and appropriate software for the input of data was modified in order to accommodate the Ratzooman CMR-work. Briefly, in each of the involved African countries, a replicated CMR-study was to be set up in two fields at the peri-urban/rural interface in the main focal site (the locality where the Ratzooman studies are concentrated in each country). Monthly trapping sessions were planned during which animals would be marked and release. See the appendix for a full detail of the protocol.

In Morogoro, Tanzania, SUA continued an already ongoing CMR study in a field-fallow mosaic in order to provide a background for the interpretation of the other studies.

The CMR-data needed to be sent by the African partners to DPIL, in order to be analysed and compared, but since CMR trappings started very late (or not yet) in all countries, no analysis was carried out yet.

4.2. Movements of rodents in towns

In order to study rodents' movements in towns, two methods were selected. Telemetry provides detailed information at the individual level, but is limited to a small number of individuals that can be followed. As a first approach, we therefore chose for a bait marker technique. For this technique, baits (based on peanut butter and maize scraps) were mixed with 0.15 % Rhodamin B. After ingestion by the rodents, they attempt to clear this compound from their body by depositing it in inert tissues, mainly fur. Since rodent whiskers grow very fast, they are a prime site for deposition of excretory products. As can be seen in the fluorescent microscope picture below, the deposition of the Rhodamin B in the whiskers is so fast, that different periods of feeding (here three sessions in consecutive weeks) can be recognized in the whiskers.



In Morogoro, Tanzania, 9 focal sites were selected as places where rodents (or pathogens) from rural areas may be imported in town environments (markets, grain stores and mills, slaughterhouse and butchers). At each of these sites, coloured baits were provided for a short period. Afterwards, trapping sessions were organised at different distances from these foci, in order to verify how far from the foci animals would still show evidence that they had been feeding at the foci. This work was carried out as part of an M.Sc.-thesis (N. Willekens).

Encountered problems and solutions

Delays in the delivery of traps caused field work (not only for WP4) to be considerably delayed in most countries. As a result, the CMR-studies were carried out only partially in Tanzania, started very late (September) in South Africa and not before the end of the year in Zimbabwe and Mozambique. Therefore, it has not been possible to carry out any CMR-analysis. Measures are now taken so that, latest in February 2004, the CMR-studies will be going on in all African countries as scheduled. This means that it will still be possible to carry out almost two full years of CMR-work within the project, but it also means that analyses will only be finished

after the project has come to an end. This means that modelling work later in the project will be hampered by an incomplete understanding of the demography and that models and control strategies may have to be revised several times when new data are coming in.

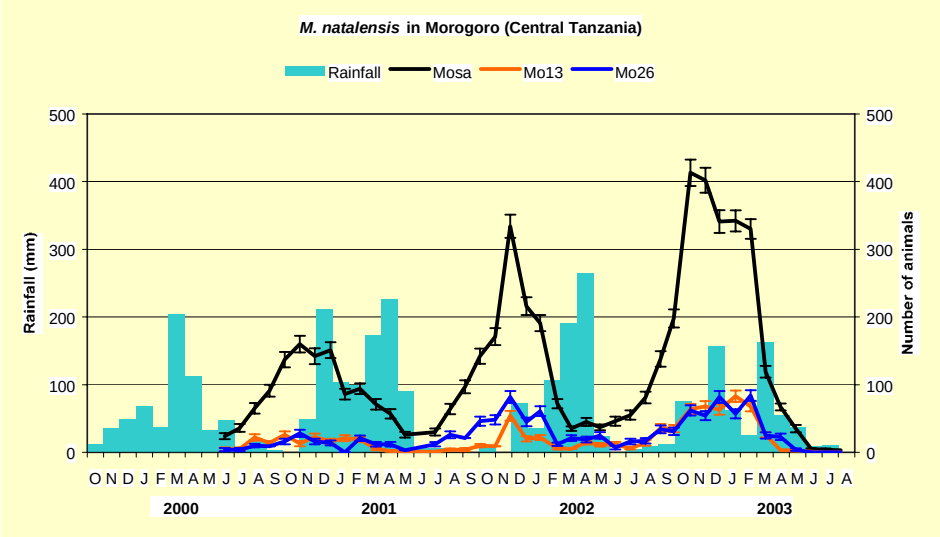
Preliminary results

4.1. Capture-Mark-Recapture study

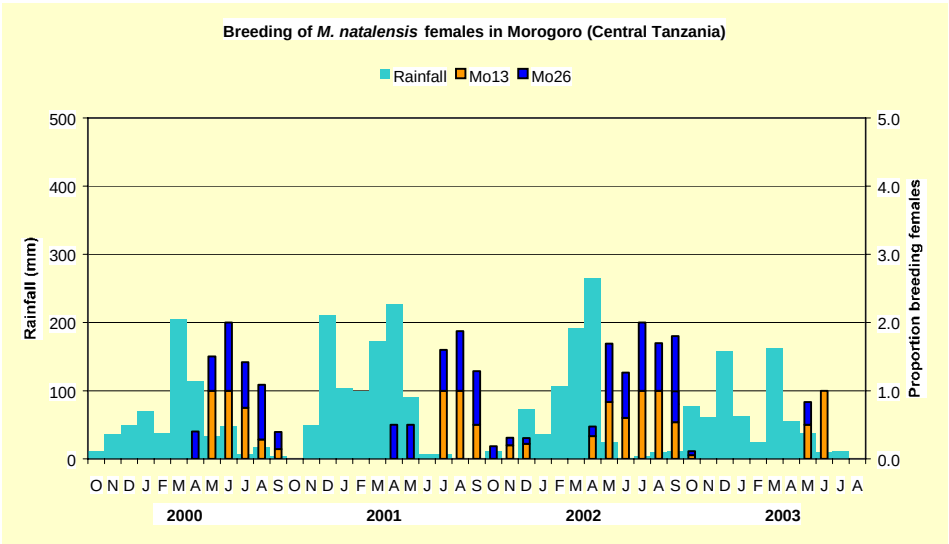
As indicated above, CMR trappings started very late (or not yet) in all countries and therefore, no analysis was carried out yet.

Tanzania:	background CMR-study as planned CMR-studies at peri-urban interface now to start in February 2004
South Africa:	CMR-studies started at two sites in September 2003; so far 3 monthly sessions with, due to very low densities, a total 32 captures only
Mozambique:	CMR-studies to start in February 2004
Zimbabwe:	CMR-studies started in January 2004

The below figures therefore only present fairly raw data from the ongoing background study in Morogoro. The only purpose of including them here is to illustrate what kind of data can be expected from the CMR-studies once data will be arriving.



4.2.



The uptake of coloured baits was very successful. Consecutive trapping around the foci yielded high proportions of animals with clear signs of marked bait consumption. The table below gives a summary of the results, pooled for all species.

	0m	10-60m	60-110m	110-160m	160-210m	>250m
Central market	2/5	5/8	32/68	-	-	-
Mwembesongo market	8/9	8/8	14/21	3/4	-	-
Saba Saba market	1/5	9/15	8/8	5/12	3/13	6/12
Misufini grain mill	0/0	1/6	5/8	13/14	0/2	-
Msamvu grain mill	0/0	3/3	2/3	3/6	3/8	-
Tanesco grain store	4/4	49/73	10/14	9/10	-	-
Shamwino butcher	1/1	7/10	3/5	-	-	-
Madizini butcher	3/4	11/20	18/29	-	-	-
Central slaughterhouse	4/4	7/10	5/9	1/2	-	-

These results are rather surprising, since they imply that distances covered by rodents for foraging are considerable and larger than generally assumed. The high proportions of bait-positive animals even at large distances of more than 200m suggest that the foci are a very important centre where rodents from a large perimeter possibly can have contact with each other. It is worth mentioning that 8 of the 12 animals trapped >250m from Saba market were house mice, and 5 of these were bait-positive. While such foraging distances are expected for *Rattus* sp., they are very surprising for house mice. This pilot study therefore certainly needs to be replicated to see whether these results can be corroborated.

RATZOOMAN
WORKPACKAGE 4

RODENT ECOLOGY IN RURAL/PERI-URBAN AFRICA

GUIDELINES FOR SETTING UP THE CAPTURE-MARK-RELEASE STUDY

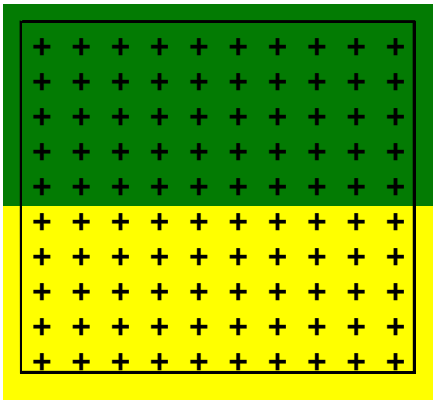
The Capture-Mark-Release I(CMR) studies will provide information about the population ecology of the studied rodents. The resulting insights will form a core for the design of population models and, based on that, models about the risk of disease transmission.
In order to allow full and standardised use of the data that will be collected during the RATZOOMAN project, and in order to allow comparisons between different countries, the following guidelines are proposed. Not following them will make data analysis much more difficult or even impossible.
Technical procedures are extensively described in the different appendices. In case of questions or problems, please contact DPIL (Solveig Vibe-Petersen or Herwig Leirs) immediately in one of the following ways;

email: s.v-petersen@ssl.dk and h.leirs@ssl.dk
fax: +45-45931155
phone: +45-45974301 (Solveig) or +45-21231651 (Herwig)
postal address: Danish Pest Infestation Lab, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark

Select research areas

In the major focal area (see also Work Package 2), locate two sites at the border of the town where you can place study grids of 1 ha (100 x 100 m). Make sure that the fields are far enough from each other in order not to interact with each other (> 1 km, preferably more) and that they are at least 300 m from sites where removal trapping (WP2) is carried out. The vegetation on the plots should be fallow and must not be marginal wasteland. The best is to use an area that recently has been an agricultural field. Avoid being near fields where rodenticides are applied. Make sure that you can use the areas during an uninterrupted period of two full years. Indicate the grids' position on a map/aerial picture/SPOT image of the area.

Each study grid should be laid out on a square grid of 10x10 trapping stations, 10 m between each. Half of it should be fallow, the other half should be peridomestic (near a house) or a field close to human habitation. The field part should be managed according to normal local practices (with regard to e.g. ploughing and planting). It is easiest to labour the field yourself; if it is done by a local farmer, arrangements must be made to avoid rodent poisoning (you should think about damage compensation), maintenance, ownership of the crop, responsibilities for weeding, fertilising,…



Mark the trapping stations with a permanent tag, for example a painted brick. Give each trapping station a permanent co-ordinate number, i.e. from 1A to 10A, to 1J to 10J and indicate that co-ordinate on the mark so that it can be read easily in the field. One Sherman trap will be placed next to each mark.

Equipment checklist

The following is needed:

250 Sherman traps (100 per area + 50 spares)
bricks or other tags to permanently mark the trapping stations
nylon bags for removing rats from traps
balance (1g or 0.1 g)
sharp small dissection scissors for clipping toes
eppendorf tubes to store toe clippings
alcohol for toe clippings
paper+pencil for labels in eppendorf tubes
PC for storing data
forms to record capture data (see appendix)
transport to/from field
field “laboratory” (i.e. a place conveniently located near the field, where you can process the animals; how “near” depends on the transport facilities)

Trapping

Each monthly trapping session lasts three nights.

A trapping calendar must be made so that it is clear right from the start when CMR-trapping should take place. Trapping should be done in both fields simultaneously if number of traps allow, otherwise both grids may be trapped in consecutive weeks. Trapping in each grid must be repeated every fourth week (i.e. one week of trapping, then three weeks without trapping in the grid, then again trapping). The scheme should be followed as tightly as possible. The trapping normally takes place from Monday to Thursday; a few days (3) earlier or later in the week is allowed if it cannot be avoided, but the three trapping nights should follow each other.

Traps are placed in the afternoon, well before dark, baited with a mixture of peanut butter and milled maize (or your own other usual bait, if suitable for Sherman traps).

Traps are collected in the morning, always latest before 9 am, and taken to a field laboratory.

Rats are marked with toe clipping and data about weight and reproductive status are recorded in CMRINPUT (manual attached, the program will be explained at the training meeting in Morogoro). The data are also recorded on a paper sheet (example attached). The CMRINPUT-manual also describes the toe clipping procedure.
Rats are released as soon as possible at the exact trapping station where they were trapped. Rats that died in the trap or during the treatment are preserved in formaline following the standard procedure from WP4 (including labelling with a standard label, taking samples etc.); for these rats the tag number is also recorded in the CMRINPUT program.
After each trapping session, data are sent per email to DPIL (s.v-petersen@ssl.dk) where we check for any problems and suggest how to correct them.
An annex to this protocol stresses some points that often cause problems in CMR-studies.
Collect weather data
For each study area, collect weather data (daily precipitation, max temp, min temp). The weather station should be close to the study area (<20 km) and in any case have the same kind of weather as the study area (e.g. not be situated at the other side of a mountain).
Verify the following before the start of the study:
how reliable are the data?
is it reliable that data will be available throughout the project period
will you have to pay for the data? how much? fixed price?
do you get the data on paper or as a computer file?

APPENDIX: CMR TRAPPING FORM

CMR Grid: Date :

Name : Traplines : # traps/station : Wheather during the night:

Sp.	Toe clipping code	Trap	Weight	males	females	Remarks

Testes **A**bdominal or **S**crotal ; Swollen epididimys **N**on-visible or **V**isible

Vagina **C**losed or **P**erforated ; **S**mall nipples or **L**actating ; **P**regnant (**N**o or **Y**es)

ANNEX: CMR-TRAPPING: COMMON POINTS FOR ATTENTION

TRAP PLACEMENT
Do not change the trapping calendar without consulting the responsible researcher
Traps are placed in the afternoon and baited fresh every day.
Leave the lab with enough traps, but take a number of spares.
Traps with holes must not be used.
Check the sensitivity of each trap when placing it.
Trap entrance must be level with the ground.

TRAP PICKUP
Traps are checked early in the morning (all animals must be collected before 9 a.m.)
Write the trap station on the trap with a marker pen (and the grid if more than one grid trapped that day)
Traps are handled carefully, not to disturb the animals too much.
Open traps need not be picked up (unless risk of theft is high), but must be closed (and then reopened and rebaited in the afternoon).

ANIMAL HANDLING IN THE LAB
Order the traps per line, handle animals per line; start with different lines every day (this is particularly important when you trap many animals so that different animals spend the longest time in the trap every day)
Animals must be handled gently.
Only the upper one phalanx of a toe is to be clipped.
Put toe clips of an individual stored in an eppendorf tube with alcohol (ethanol not containing ether).
Put paper label with toe clipping code written in pencil in the tube; remember to write also the series number on the label; at the back of the paper strip, write the grid name and the date.
Check sex carefully! Look at each characteristic separately.
Check capture history carefully with regard to sex.
when deciding to assign a B-code, write the B at the end of the toe clipping code, with a space in between

ANIMAL RELEASE
Release animals as soon as a group is ready, do not wait with all animals until the end.
Release animals gently at the trapping site.
If an animal is dead at release, take it back to the lab, use CMRINPUT, type the toe clipping code, change "TAG" to the collection label number and add as remark "DEAD AT RELEASE". Then take samples and store the animal in formalin.

ADMINISTRATION
Keep forms chronologically in a ledger for each study.
Daily, copy the data to a diskette when quitting CMRINPUT and email the files to DPIL
Two files must be sent: the data file with the date in the name, e.g. cm020301.txt and cmrtraps.txt (see CMRINPUT manual for more explanation about these files)

TRAP MAINTENANCE
After each session, traps are cleaned (use water only, no soap as that may leave an dour that repels the rats) and written trap numbers are removed
broken traps are repaired, doors with holes are repaired or replaced

KIT

INCO-DEV RATZOOMAN

Contract # ICA4-CT-2002-10056

Partner 3, Department of Biomedical Research, KIT (Koninklijk Instituut v.d. Tropen/Royal Tropical Institute

Report for January to December 2003

Work package 1: Retrospective and prospective investigation of human sera for the presence of antibodies against Leptospira, Toxoplasma, and Yersinia antigens

This WP was due to start at the beginning of 2003. A plan was formulated for this study at the inception meeting in UK in March 2003. The availability of sera, the analysis techniques to be used, and the collection of samples and data were discussed with the following outcomes: (1) For the retrospective analyses, DC partners should identify all sources of sera that could be used for testing and find ways to facilitate their analysis for leptospirosis, toxoplasmosis and plague. (2) DC partners would select one major and two minor focal towns for project activities in each country, also to be used for all other work package activities. DC partners would contact local hospitals in focal areas for involvement in prospective serological analysis. (3) Tests: Rapid tests exist for leptospirosis and toxoplasmosis on human sera. MAT will be used for leptospirosis in rodents and other animals and for confirmation of human samples. The Toxoplasmosis kit can possibly be adapted for use in other animals. PCR could serve as an alternative. For plague, ELISA is available for humans and can be adapted for rodents and other animals. Culturing of leptospires will be done where possible.

Work package 3: Analysis of rodents, insectivores and domestic animals for the presence of leptospirosis, toxoplasmosis and plague
Leptospira will be routinely cultured, Advice on serology and culturing is/was given.

Work package 4 : Rodent ecology in rural/peri-urban Africa
Participation in the discussions only.

Work package 5: Impact of water management and land use strategies upon rodent zoonotics

KIT supplied/supplies all information on leptospirosis (dynamics) that may be required for GIS (WP 8) and modelling (WP 9) purposes and for optimal sampling from environment and food

Work package 11: Analysis of policy issues
The start month of this work package is month 26.

Work package 13 : Output dissemination and project co-ordination
Rudy Hartskeerl of KIT visited the inception meeting in March 2003.
In July 2003, staff of KIT (Mrs. Mirjam Engelberts) visited the African Small Mammal Conference in Morogoro, Tanzania and the connected interim meeting for RATZOOMAN. Mrs Engelberts was involved in the presentation of practical aspects of leptospirosis during the adjacent training meeting for learning tests and techniques related diagnosis (MAT, rapid assays, culturing e.a.) and methods to rodent trapping, tissue and blood sample taking, preparation of rodent specimens, handling rodents, recording data, etc

Rudy Hartskeerl, December 2003

SPMC

PROJECT ANNUAL PROGRESS REPORT (Jan.-Dec. 2003)

Project title: Prevention of Sanitary Risks linked Rodents at the Rural/Periurban Interface (RATZOOMAN). Contract No ICA4-CT2002-10056.

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1.0. REPORT SUMMARY

The project activities effectively took off in the middle of February, 2003 following the disbursement of the first instalment of funds. Activities carried out addressed certain work packages (WP) generally according to the plan charted out at the project's semi annual progress report meeting held in Morogoro, July 2003.

The project study areas established were Morogoro urban/periurban, which constituted the major study area, and Lushoto rural/periurban as one of the minor study areas. The other proposed minor study area, namely, Lindi and Mtwara could not be covered this year.
The work packages addressed include:
WP1: Retrospective and prospective investigations of human sera for zoonotics
WP2: Taxonomic identification of rodent species in the study areas
WP3:Isolation of zoonotics from rodents and domestic animals from the study areas
WP6: Socio-economic impact of rodent transmitted disease (plague)
WP7: Socio-anthropogenic change upon rodent ecology

Generally speaking the activities were performed satisfactorily, given that most of these shall continue into the second year of the project. No serious technical problems have been encountered. However, the budgetary demands for the socio-economic and socio-anthropogenic components have proven to be way above those initially anticipated. Future activities within WPs 6 and 7 necessitate a serious review, especially if the other minor study area is to be investigated.
Future activities shall include:
Coverage of the second minor area of study.
Serum analyses of sera obtained through WPs 1,2 and 3 in 2003.
Further characterization of isolated pathogens and their rodent vectors.
Population dynamics of rodents in the major and minor areas of study.

2.0. DETAILED REPORT OF PROJECT ACTIVITIES

2.1.Retrospective and prospective investigation of human sera for zoonotics.

Human sera were obtained from ongoing research activities e.g. HIV, schistosomiasis screening, and from routine diagnostic procedures e.g. malaria, typhoid or PUOs in Morogoro and from referral hospital serum banks elsewhere. Wherever possible the clinical-epidemiological information related to the donor of the serum was recorded, however, without disclosing the identity of the donors. These sera shall subsequently be screened for antibodies to *Leptospira spp*, *Toxoplasma spp* and where appropriate also *Yersinia spp*. The amounts and origin of the serum samples were as shown in Table 1.

Table 1: -Human serum collections by locality from May to September 2003

Region	**Locality**	**No of Samples**
Morogoro	Aga Khan Hospital	336
Morogoro	Upendo Medical Laboratory	325
Morogoro	Mikumi Health Centre	290
Kilimanjaro	KCMC - Referral hospital	352
Dar Es Salaam	MUCHS - Referral hospital	399
TOTAL		**1702**

2.2. Identification of predominant rodent and shrew species in the study areas (WP 2)

Rodent and shrews were trapped between February and December, 2003 using procedures described by Leirs and others. During the Months of February, March, July and August trapping was done weekly, while for the remaining months trapping was done once per month. Areas sampled included human residences, peridomestic sites, home gardens and fallow lands in the vicinity of human settlements primarily in the Morogoro area. A limited number of rodents from other areas were kindly provided by other researchers during their trapping expeditions outside Morogoro. Identification of the captured rodents was preliminarily done to the genus/species levels at SUA. Partner 3 (RUCA) shall carry in-depth taxonomic analyses to confirm the preliminary findings. Rodent and shrew captures in Urban and periurban Morogoro were as shown in Table 2

Table 2: Rodent and Shrews captured in urban and periurban Morogoro (February to December 2003)

Species	**Months of collection**											
	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	**Tot.**
Mastomys	213	32	-	-	-	183	328	177	40	10	29	**1012**
Crocidura	59	17	4	-	4	13	16	22	-	4	5	**144**
Rattus rattus	104	14	37	38	22	29	21	7	30	10	1	**313**
Rattus Norv.	10	4	5	-	2	6	2	4	-	-	-	**33**
Mus	154	111	20	15	17	24	21	4	2	-	-	**368**
Cricetomys	1	20	23	9	18	21	5	9	14	1	-	**121**
Tatera	5	3	-	-	-	3	12	4	2	3	3	**38**
Lemnisco	5	3	-	-	-	-	3	-	-	-	-	**11**
Grammo.	-	-	-	-	-	-	5	1	1	2	1	**10**
Leggada	-	-	-	-	-	3	1	1	1	1	2	**9**
Dasymis	-	-	-	-	-	-	2	2	-	-	-	**4**
Praomys	2	-	-	-	-	-	-	-	-	-	-	**2**
Steatomys	1	-	-	-	-	-	-	-	-	-	-	**1**
Arvicanthis	-	-	-	-	-	-	1	-	-	-	-	**1**
Total	**554**	**204**	**89**	**62**	**63**	**290**	**422**	**227**	**88**	**28**	**38**	**2065**

2.3. Collection of rodent and shrew sera for zoonotic screening

Subsequent to trapping (WP 2) sera from the captured rodents and shrews were taken for future analyses of zoonotic infections. The total amounts of sera and their origin were as shown in Table 3

Table 3: Rodents sera collections by locality

Period	**Region**	**Locality/District**	**No of Samples**
Feb. - Dec.	Morogoro	Urban & peri urban	1347
May	Mbeya	Chang'ombe - Chunya	5
June	Kilimanjaro	Mabogini – Lower Moshi	26
June & July	Dodoma	Ihanda - Kongwa	73
TOTAL			**1451**

2.4.Collection of sera from domestic animals
A total of 300 serum samples, each, were collected from of dogs, cats, pigs and small ruminants (goats and sheep). These animals were from urban and periurban Morogoro. The sera have been stored for ulterior analyses

2.5.Isolation of zoonotic agents from rodents and shrew specimens (WP3)
Primary isolation of leptospira spp. was attempted from most urinary bladders and kidney tissues of the rodents and shrews obtained in WP2 (Table 4).
Specimens were inoculated into Fletcher's medium and incubated at room temperature under a regular weekly screening for bacterial growth over a maximum period of 4 months.
Spirochetal isolates were obtained from *Cricetomys spp.*, *Mastomys spp*, and *Crocidura spp*

Table 4: Leptospira isolates from rodents and shrews

Host	Total cultures	Total positive	% Positive	Locality
Mastomys	5	-	-	Mbeya
Mastomys	29	-	-	Kilimanjaro
Mastomys	410	7	1.71	Morogoro
Crocidura	77	10	12.99	Morogoro
Cricetomys	173	8	4.62	Morogoro
Rattus spp	190	-	-	Morogoro
Mus	99	-	-	Morogoro
Arvicanthis	22	-	-	Dodoma
Leggada	5	-	-	Morogoro
Tatera	1	-	-	Morogoro
Lemniscomys	2	-	-	Morogoro
Grammomys	11	-	-	Morogoro
Dasymys	4	-	-	Morogoro
Steatomys	1	-	-	Morogoro
Total	1029	25	2.43	

Preliminary characterizations of these isolates into serogroups and serovars have started and shall continue in 2004.

2.6. Socio-economic and socio - anthropogenic studies in Lushoto district
This study focused on villages in the plague endemic areas of rural Lushoto District in Tanga Region, and shall be reported by Partner No1 (NRI)

3.0.GENERAL COMMENTS
The activities carried out went on smoothly after some initial period of preliminaries. Technical problems encountered were minimal and collaboration with other partners was good. It is yet to be decided on the most appropriate serological procedure to be used for toxoplasmosis analyses of the human and animal serum samples collected.

The engagement of a research assistant dedicated to this project has proven to be very valuable. Additional assistance was through students (MSc) and A/ O levels) who spent some of their vacation time in tapping rats and in the preparation of specimens. It is anticipated that the exposure of these young persons to the project idea shall stimulate interest in related research in the future.

A manuscript titled "**Rodent and shrews as carriers of potentially zoonotic spirochetes in rural and periurban Tanzania"** is under preparation, to be published in the East African Journal of Public Health.

The single major constraint experienced was in connection with the relatively small budget allocated for subcontracting. The subcontracting costs for the socio anthropogenic component alone far exceeded the entire budget allocated for this item. As a result the re are outstanding payment to the consultant, which can only be disbursed from the next instalment. The socio-economic study component could only be possible through payments made from other line items (travel, personnel). Consequently training of technical staff could not be carried out this year as anticipated.

4.0.FUTURE ACTIVITIES

Future activities shall complement the preliminary works carried out so far, namely:

Extension of WPs 1,2 and 3 activities in the second minor area of study.

Serological analyses of human, rodent and domestic animal sera for rodent transmitted zoonotic infections (Leptospirosis, toxoplasmosis, plague?)

Further identification of the rodents and shrews predominant in the study areas.

Characterization of the leptospira isolates from the small mammals.

Population dynamics of rodents (CMR) in rural, urban and periurban areas of Morogoro and Lushoto districts.

NHLS

Prevention of sanitary risks linked to rodents at the rural / peri-urban interface – RATZOOMAN

PROGRESS REPORT, YEAR 1 (2003)

PRIME CONTRACTOR:

SA NATIONAL HEALTH LABORATORY SERVICE
(John Frean, Lorraine Arntzen, Malodi Setshedi, Chantel le Roux)

INSTITUTIONAL SUBCONTRACTORS:

PPRI/ARC, Pretoria
(Frikkie Kirsten, Emil von Maltitz, Fanie Phanuel S Malebana)

Natural Science Museum, Durban
(Dr Peter Taylor)

INDIVIDUAL SUBCONTRACTORS: Pfarelo Matshidze, Takalani Thakani (Thohoyando)

Prevention of sanitary risks linked to rodents at the rural / peri-urban interface – RATZOOMAN

WORKPACKAGES 2 & 4

OBJECTIVES:
Measurement of disease prevalence for the three major diseases, plague, leptospirosis and toxoplasmosis. Host ranges will be investigated, and the infection dynamics within the host populations and from the hosts to humans will be studied.

SAMPLING AREAS : Mapate, Durban, Port Elizabeth

Mapate (information courtesy of PPRI)

Trapping Location:
Mapate village, Vhembe district, Limpopo Province
Located in the western foothills of Soutpansberg mountain near Thohoyandou.
Vegetation according to Acocks (1984) is North-eastern mountain sourveld.
Height 650-700 m above sea-level.
Summer rainfall (900-1050mm annual)
Approximately 22° 59 S 30° 21 E
Population 1000 households, in a peri-urban set-up
Village consists mainly of subsistence small-scale farmers. Major crop is maize, produced for home consumption. Other crops include groundnuts, sweet-potato, pumpkin, vegetables (tomatoes, cabbage, onions etc) and fruit such as mango orchards and paw-paws (papaja). Fields are usually inter-cropped maize-pumpkin. Some farmers have livestock (cattle and goats) and some have chickens in the homestead, but grazing area is very restricted. The staple crop is either stored on the cob in a granary (mud and pole, thatched roof) or shelled in bags in the home. Due to drought over the past 3 years, crop yields have decreased, and fields near the village have been taken over by buildings (new homesteads) and very little natural vegetation or bush is left as almost every piece of land is used for crops. Abandoned and fallow land is scarce or non-existent.
Rainfall is higher in this sub-tropical area than in the rest of the province, normal period of highest rainfall is November to January

A Crop post-harvest PRA survey was conducted in this village in 2000.

Trapping sites:
Habitat types:
Market areas. None. None in village or nearby, no shops sell organic produce
Houses with gardens 5 (code HG 1-5)
Houses without gardens 5 (code HG 6-10) (the term "with or without gardens" is relative)
Mill. One only (consists of shop with a hammer-mill in outbuilding) (code MM)
Butcher (trapping in butchery as well as "slaughterhouse", very irregular slaughtering (code MB)
Poor houses. Term relative, but picked selected poorer houses than in 2 and 3
(code HP1-11) could not arrange 20 as not all villagers cooperative in long term, high number of traps difficult to manage in the number of hours of a day.
Rural houses. Houses on edge of town as none are "very rural" (code HR 1-5)
Open areas - abandoned land is very scarce. (code FA 1-3)
Fields outside town (code FC 1-3)
Semi-natural vegetation (code FB 1-3)
A GPS grid-reference was recorded for each site.

Traps are placed inside and outside buildings (sub-habitats)
In fields etc, traps are placed in possible trapping lines

Traps:
Two types used:
Kness snap back-breaker and the sherman-style as provided (50% of each at each site). No other traps available or traditionally used

Bait; usually peanut-butter mixed with some oats, or maize kernels and cold maize "pap" which is available in households.

Trappers:
Village trappers have been trained during a week long course in Pretoria. Training was given by Lorraine Arntzen (NHLS), Chris Chimimba (Univ Pretoria including staff of Transvaal museum) and Art Booman (Mpumalanga Dept Health). Further training on the use and setting of traps was conducted by PPRI. Trapping assistants are young post-school villagers under strict supervision. Trials are managed by PPRI.

Laboratory:
Use of Univ of Venda for Science and Technology laboratory for normal collections. CMR trial data collecting and processing is conducted in the field.

Trapping Calendar;
Normal trapping, 4 nights:
1st quarter: 1-5 September 2003
2nd quarter: 13-16 January 2004
3rd proposed for mid April

Work package 4.

CMR trapping.
3-nights, monthly (last week of month)
Two crop fields >1ha, with fallow and crop parts.
Rain very late this year and most farmers thus ploughed/planted very late. Natural vegetation around demarcated fields are also cleared (was cleared in October/November) with fields ploughed and planted in November/December.

CMR field 2 (Walter's Zippo field) is alongside a rivulet on a level terrain. Field ploughed and planted in November, and fallow part of trial was cleared (grass cut) at same time by owner. In the trapping grid, the northern part (grid A11-A16 and J11-J15) lies in the fallow part, while A/J 17-20 is the maize field. Weeding of crop field was done early January by owner.

CMR field 1 (Chief's field) was a 1.5 ha fallow field for a couple of years previous.
Grid is A1-10, J1-10.
Owner agreed to plant 1 ha and fallow 0.5 ha, but in January ploughed the whole area including surrounding bush.
The question is; do we continue trapping in this field (only one rodent trapped in 4 months) or start in a new crop/fallow field?

Trapping calendar
24-26 September 2003
29-31 October 2003
3. 2-4 December 2003
4. 26-29 January 2004

Weather data:
ARC Institute Soil, Climate and Water have a long-term weather station managed by LPDAE nearby (30km). (Data was promised, have not received yet)

[CMR and normal trapping data (as recorded on data sheets)

B. Durban
Summary of collection sites

LOCALITY (Durban)	**Site or house**	**Rats caught?**	Type of site
No. 933 Umgeni Road	Dee's Tavern	x	buildings (shops)
No. 49 West Street		x	restaurant buildings
Hostel Merebank		x	hostel
Pine Street		x	buildings (flats and shops)
N o. 486 Point Road		x	buildings (town- flats)
No. 50 West Street		x	buildings (town- flats)
Browns Road, Point		x	buildings near the harbour
Mackeurtan Road, DBN. N		x	Supermarket
No. 58 West Street		x	Shops
Pickering Street		x	buildings near the beach
Warwick Ave	Poultry market	x	Poultry market
View Street	Shacks	x	Shacks
Malandela Road, nr intersection with KwaMashu Rd	Containers with foodstuffs; taxi rank	x	Containers
Maydon Wharf/Parker Road	Road verges	x	Harbour wharf
L-shed (rice shed), Quayside Rd, Harbour	Inside rice shed	x	Harbour (rice storage)
Russell & St George, Albert Park		x	Supermarket
Cato Crest - Area 6	Mhlongo cc0506	x	Informal shacks
Cato Crest - Area 2	Kwamakhanyile Tuck Shop cc1514	x	Informal shacks
Cato Crest	Cllr Chamane		Formal house nr shacks
Cato Crest - Area 2	T. Ngcongo - cc1548	x	Informal shacks
Cato Crest - Area 6	TR Mbewana (Committee) cc0229		Informal shacks
Dalton Hostel, Congella	Beer Hall	x	Skin merchant
Cato Crest	Zona Madiya cc 1329	x	Informal shacks
Cato Crest	Nyathi/Mzobe/Mwandla cc1549		Informal shacks
Cato Crest	Sbongile Ndlovu cc1331		Informal shacks
Hammarsdale WP56			Sugar Farm
Hammarsdale WP55			Sugar Farm
Hammarsdale WP54			Sugar Farm
Hammarsdale WP53			Sugar Farm
Hammarsdale WP52			Sugar Farm
Hammarsdale WP51			Sugar Farm

C. Port Elizabeth

Serum samples received were taken in course of routine trapping activities, by local authority environmental health officers. No information regarding the sites of collection is known.

RESULTS

Rodent samples tested:

AREA	Venda			Durban			Port Elizabeth		
TEST	Plague	Lepto	Toxo	Plague	Lepto	Toxo	Plague	Lepto	Toxo
NO. RECEIVED	39	39	39	232	232	232	587	587	587
NO. TESTED	35 (4 insuf)	Not yet tested	39	210 (21 insuf)	188	128	Not yet tested	376	Not yet tested
NO. POSITIVE (%)	0		3 (7.69)	0	10 (5.31)	4 (3.12)		63 (20.12)	

SUMMARY OF RESEARCH TO DATE

It is interesting to note from our initial testing, that there appears to be a reasonably high prevalence of leptospirosis and toxoplasmosis in the samples tested. We would have to carry out a larger number of tests before we could make any prediction as to the prevalence value in the rodent populations.

The sample collection got off to a slow start, but seems to be running much better now.
The second minor site for testing is Coega in the Port Elizabeth area and they are just sending rodent sera.. This is in line with the normal routine plague surveillance testing.
Coega Development Corporation and the Nelson Mandela Metro have still not come to an agreement about the project, thus delaying the collection of other samples from the rodents.

We had delays in obtaining kits and reagents, but now have that under control and we are getting through the testing much faster.

Prepared by:
Lorraine Arntzen

SZ

RATZOOMAN PROJECT ZIMBABWE

ANNUAL TECHNICAL REPORT: YEAR ONE, 2003

RATZOOMAN PROJECT ZIMBABWE

The Ratzooman Project Zimbabwe is an EU sponsored project with Syngenta Zimbabwe as the Project Partner represented by Mrs Martha Mpisaunga and Mrs Nan Chalmers. There are two Subcontractors to the project namely; Dr. Godfrey Pasurayi Chikwenhere PhD, Agricultural Entomologist with Plant Protection Research Institute, Government of Zimbabwe and Mr. Moses Zimba MSc, Medical Entomologist with Harare City Health, Local Authority.

A number of line items ranging from planning meetings, making contacts with Blood Transfusion (Ministry of Health), University of Zimbabwe (Biological Sciences), Department of Veterinary services and Plant Protection Research Institute (Ministry of Agriculture). Further activities included the procurement of project equipment and consumables and data collection. All these activities took place from January 2003 and continued through to January 2004. In this report, the Ratzooman Project related work has been presented in Phases (Phase I to IV).

1. Phase I (January- March 2003)

The Phase I part of the project was dominated by planning meetings and contacts. Planning meetings were necessary to enable the team to plan and discuss criteria for study areas. Contacts with relevant authorities was established to ensure the availability of human blood in case of the Blood Transfusion and laboratory facilities from the University of Zimbabwe and blood from domesticated animals (Veterinary Services). The team also embarked on a desk study on the history of rodent/plague outbreaks as presented below;

Brief History of Rodent/ Plague Outbreaks in Zimbabwe- a case of a desk study

Rodents

Rodent outbreaks in Zimbabwe have been characterized by land use systems, which are associated with reduced biodiversity coupled by environments conducive for rodent population build-ups. The documented brief history of rodent outbreaks in Zimbabwe backdates from 1912 with subsequent ones in 1920, 1932 and 1956. Further outbreaks took place between 1967 and 68 while subsequent ones occurred in 1975/76, 1983, 1993/94 and 1994/95 (Weinman, 1975 and Chivinge at.el, 1996).

The 1993/94-crop production was adversely affected by rodent outbreaks that severely reduced crop stands early in the season. During that period, rodents significantly contributed to the food deficit in some southern parts of Zimbabwe more specially on irrigated crops.

The most recent outbreaks took place between 1996/97 which affected areas were the southern parts of the country whereby most farmers had to replant 4-5 times and that cropping season resulted in poor stands and reduced yields.

The trend of rodent outbreaks in Zimbabwe had been occurring after a period of drought (Epstein and Chikwenhere, 1994).

The number of rodent species in Zimbabwe is above 30 and those of economic importance are the giant rat (*Cricetomys gambianus*), house mouse (*Mus musculus*), black rat (*Rattus rattus*), multimammate mouse (*Praomys= Mastomys natalensis*) and the velvet rat (*Aethomys chyrophilus*) (Smithers, 1975).

Bubonic Plague

Rodents and their population dynamics have characterized the occurrence and subsequent spread of bubonic plague among both rodents and humans, whereby man is considered an accidental host. Bubonic plague disease is caused by the bacterium, *Yersinia pestis*, which is a primary disease of rodents and is transmitted, from infested rodent by an insect flea, *Xenopsylla cheopis* (Rothschild).

Historically, the bubonic plague decimated the entire civilizations. In the 1300's the plague became known as the "Black Death" after it had killed approximately 20 – 30 million people in Europe. The Great Plague of London in 1665- 66 killed 70 000 people out of the estimated population of 500 000 (Smithers, 1975). In the mid-1800s, the plague killed 12 million Chinese.

The history of the Bubonic Plague in Zimbabwe backdates the 1970s. The disease is likely to have been present in the country much earlier but no records were available until 1972 when the first outbreak occurred. Sporadic plague outbreaks have occurred over the last thirty years and mainly were recorded from September through to March. A large major epidemic was recorded in September 1974 and in March 1975 in Hwange, Lupane and Nkayi and followed by yet another one in January1982 in Lupane (Anonymous, 1994).

In 1993, four cases of suspected plague were report in Nkayi but were not confirmed bacteriologically and serologically. In January 1994 a case of suspected plague was reported in Lupane.

The most recent and major plague outbreaks occurred between September and October 1994 with 37 confirmed cases (11 deaths) (Annex 1.1). The majority of the cases were children aged between 3 and 15 years and the most affected was Nkayi District with over 95% of the cases reported in Matebeleland North Province of Zimbabwe.

Relationship between Climate Changes and Rodent/ Plague Outbreaks

In Zimbabwe, rodent population exploded as a consequence of climate variability, when heavy and then lighter rains came in 1993 and 1994, on heels of six prolonged drought (Epstein and Chikwenhere, 1994). When the rains came, rodents found themselves in a world where avian and land predators were virtually absent. In general, rodent outbreaks occur after a period of drought which time natural control agents such as diseases, competition and predators such as snakes, owls, falcons, hawks, cats and jackals, are killed or reduced significantly in numbers. Shortage of alternative food at the time of drought could also affect the number of natural enemies.

Moreover, because so many draft animals (cattle and donkeys) succumbed, there was little tillage of the land and the underground burrows in which rodents live went largely undisturbed. Soon after an initial successful harvest in 1993, rodents decimated the maize crop in Zimbabwe. As a result, in October 1994 human plague broke out in the country and further plague outbreaks were reported in Malawi and Mozambique. Subsequently, in same year (1994) a rodent-borne virus took the lives of 81 elephants in South Africa's Kruger National Park (Epstein, 1997).

In recent years, there has been an increase in Plague outbreaks in the Southern African Region and the noted ones were in Botswana and Tanzania in 1989 and in 1991 in Namibia. In 1994, apart from Zimbabwe, Mozambique, Malawi and Zambia also experienced similar plague outbreaks and these were reported soon after widespread rodent outbreaks.

Rodent outbreaks are now an annual occurrence but the biology and ecology of the pest and causes of outbreaks thus including subsequently plague outbreaks are not well understood.

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2. Phase II (April- June 2004)

Visits and site selection were the major project activities during the phase under review. Mbare, Chitungwiza, Nkayi and Hatcliffe were selected as study areas. Hwange area was dropped because the distance from Harare and Nkayi was identified and the area is known as a plague area (Annex 1.1). The coordinates of the study areas have since mailed to the overall Ratzooman Project Coordinator.

3. Phase III (July- September 2003)

Chitungwiza site was dropped because of trap vandalism and the community was not comfortable when discussed the implementation of WP4. Instead Hatcliffe replaced Chitungwiza. The advantage of Hatcliffe is that it's a Government Agricultural Farm situated in the Great Harare near the High Density Suburb of Hatcliffe in the Borrowdale area. The study area is fenced and both WP 2 and WP4 are being undertaken there with the cooperation of the community and farm authorities.

4. Phase IV (October- January 2004)

As an example some of the minutes of meeting held at Syngenta Zimbabwe offices on 26 Nuffield Road, Harare in October 2003.

4.1The meeting was a follow-up from the one took place on October 13, 2003.

The purpose of the meeting was to get feed back on assigned activities as well as updates in regards to proper execution of the project.
Agreed Points to note were as follows;

The next meeting was scheduled for Monday 03 November 2003 and the agreed venue continued to be Syngenta Zimbabwe Offices. The meeting will be centred on two letters from Dr Steve Belmain, the overall Ratzooman Project Co-ordinator. The areas to covered consist of need to visit the website; **http://www.nri.ratzooman** and to make comments for UK parliament debate on Ratzooman Project.
Discussion on Financial Report Writing
Trip to Nkayi to meet the Vet District Personnel to discuss future blood sampling from domesticated animals in that area.
Write conformation letters to Blood Transfusion and University of Zimbabwe, Department of Biological Sciences to start as per specified project activities.

Action on Lorraine's letter in regards to procurement of Test Knits and Culture Media, visit to **www.leptonet.net** and write to Fort Collins in the USA.

Further discussions during the meeting covered on the procurement of Equipment and Consumables an assignment that was previously given to Mr Zimba on 13 October 2003. The list items to be purchased under the Ratzooman Project are shown on the attached table.

It was agreed that 20 cage traps need to be purchased by the project but in the meantime the team can go ahead using borrowed ones from City Health.

The issue of two laptop computers for the project subcontractors was stayed because of budgetary limitations.

Mr. Zimba to continue looking for the needles and clippers, while Mrs Mpsisaunga and Dr Chikwenhere will go and purchase other remaining items (Annex 2.1).

4.2 Rodent Trapping, Blood sampling and organ preservation work

Field and laboratory was covered during the fourth phase as shown on Annexes 4.1 and 4.2. Data was mailed as per request and more data will continue generated depending on the availability of more resources. Rodent trapping was undertaken in Mbare and Hatcliffe. A total 97 rodents were worked on under WP2 but the number trapped was more than 120 rodents. Mortalities were experienced under laboratory before the animals were worked. One rodent died due parasite as shown on the picture below

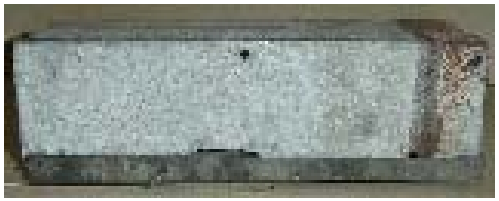
RODENT DUE TO PARASITES A DAY AFTER CAUGHT IN SHERMAN TRAP IN MBARE

Annex 1-1 Details of rodent/plague outbreaks in Zimbabwe since 1912

Year	Party of country	Affected crop area	Damage to crop	Plague cases	*Plague related deaths*
1912	-	-	-		
1920	-	-	-		
1932	-	-	-		
1956	-	-	-		
1967-68	-	-	-		
1972				1	-
1974-75	Hwange, Lupane Nkayi	-	-	-	-
1975					
1975-76	Triangle in the Lowveld	10 000 ha	30% on sugar cane		
1982	Lupane	-	-	-	-
1983	Plumtree	400 ha	Sorghum		
1993-94	Country wide rodent outbreaks	5415ha -	80% on maize	41- Nkayi 1- Lupane	11 -

Annex 2.1: Equipment and Consumables- Ratzooman Project Zimbabwe

Item	Quantity	Remarks	Responsible
Harvard traps	300	Done	
Cages traps	20	Done	
Laptop-Computers	2	Stayed	
Formalin	60litres	Done	
Ethanol	2.5litres	Done	
Chloroform Ether	2.5litres	Done	
Alcohol	10litres	Done	
Tubes 1.5 ml	1000	Pending	MZ
Screw-cap tubes	1000	Pending	
Syringes 5 ml	1000	Done	
Needles	1000	Pending	MZ
Dissection Scissors	10	Done	
Forceps	10	Done	
Fixing needles	30	Done	MZ
Gloves	200	Done	
Masks	200	Done	
Dust Coats	4	Pending	MM
Rulers	4	Done	MM
Clippers	2	Done	MZ
Buckets	6	Done	
Large white pots	3	Pending	MM/GC
Wire brush	10	Pending	GC/MM
Horse tail brush	10	Pending	GC/MM
Electronic balance	2	Pending	UZ
Linen bags	5	Pending	MM
Microscopic lens	2000	Pending	GC/UZ
Cover slides	10000	Pending	GC/UZ
Large Glass jars	2	Pending	GC/MM
Cotton wool	5 kg	Done	MM/GC
H. glass cap tubes	1 000	Pending	MM/GC
House bleach 1%	10litres	Done	MM/GC
House bleach 10%	10litres	Pending	MM/GC
Thick rubber gloves	20	Done	GC



SHERMAN TRAP USED IN ZIMBABWE FOR RATZOOMAN PROJECT

Annex 4.1
RATZOOMAN PROJECT REMOVAL TRAPPING - FORM

Collector: MZ/GC Date (03 /12/2003- 06/ 12 2003) Weather: Dry and Sunny

Locality: Mbare

Species	Label nr	Site	Trap	Sex	Second	Weight	Head body	Tail L.	Hind foot	Ear L.	Samples alc	Samples-20	Remarks
R rattus	Z1-1	Mbare	S	F	PLY	146.3	175mm	186mm	34mm	22mm	LSK	BHL	Pregnant L3
R rattus	Z1-2	Mbare	S	M	SV	101.62	159mm	183mm	33mm	21mm	LSK	BHL	
R rattus	Z1-3	Mbare	S	M	A	32.75	116mm	141mm	26mm	17mm	LSK	BHL	
R rattus	Z1-4	Mbare	S	M	AN	28.69	115mm	131mm	25mm	16mm	LSK	BHL	
R rattus	Z1-5	Mbare	C	F	LPN	109.28	170mm	195mm	32mm	25mm	LSK	BHL	
R rattus	Z1-6	Mbare	C	M	VS	145.1	175mm	195mm	33mm	25mm	LSK	BHL	
R rattus	Z1-7	Mbare	C	M	SV	109.13	172mm	180mm	32mm	25mm	LSK	BHL	
R rattus	Z1-8	Mbare	C	F	CSN	108.01	172mm	193mm	33mm	21mm	LSK	BHL	Pregnant L1
R rattus	Z1-9	Mbare	C	F	PSY	86.69	144mm	166mm	33mm	19mm	LSK	BHL	Pregnant L1
R rattus	Z1-10	Mbare	C	F	PSY	118.11	165mm	195mm	34mm	22mm	LSK	BHL	Pregnant LI (12 fleas)
R rattus	Z1-11	Mbare	C	F	CSN	100.85	170mm	178mm	32mm	23mm	LSK	BHL	
R rattus	Z1-12	Mbare	C	F	PSY	99.99	165mm	160mm	33mm	24mm	LSK	BHL	Pregnant L1 (Tip of tail cut off)
R rattus	Z1-13	Mbare	C	F	LPN	108.9	163mm	185mm	34mm	22mm	LSK	BHL	
R rattus	Z1-14	Mbare	C	M	SV	99.49	156mm	170mm	34mm	22mm	LSK	BHL	

R rattus	Z1-15	Mbare	C	M	VS	70.92	147mm	173mm	33mm	19mm	LSK	BHL	
Rhabdomys	ZI-16	Mbare	C	F	PSY	26.85	105mm	75mm	20mm	20mm	LSK	BHL	Pregnant R1
R rattus	ZI-17	Mbare	C	M	SV	121.32	165mm	180mm	34mm	23mm	LSK	BHL	
Praomys	ZI-18	Mbare	S	F	CSN	36.09	130mm	110mm	20mm	16mm	LSK	BHL	
Praomys	ZI-19	Mbare	S	F	PSN	33.88	127mm	102mm	22mm	18mm	LSK	BHL	
Praomys	ZI-20	Mbare	S	F	CSN	37.02	121mm	100mm	21mm	14mm	LSK	BHL	
Praomys	ZI-21	Mbare	S	M	SV	61.85	135mm	117mm	24mm	17mm	LSK	BHL	
Praomys	ZI-22	Mbare	S	M	SV	49.19	143mm	106mm	23mm	19mm	LSK	BHL	
Praomys	ZI-23	Mbare	S	F	PSN	24.85	108mm	97mm	21mm	16mm	LSK	BHL	
Praomys	ZI-24	Mbare	S	M	AV	58.3	136mm	113mm	23mm	22mm	LSK	BHL	
Praomys	ZI-25	Mbare	S	F	PSN	29.25	115mm	99mm	22mm	18mm	LSK	BHL	
Praomys	ZI-26	Mbare	S	M	AN	26.62	113mm	101mm	22mm	21mm	LSK	BHL	
Praomys	ZI-27	Mbare	S	M	AN	51.57	126mm	96mm	24mm	21mm	LSK	BHL	
Praomys	ZI-28	Mbare	S	M	SV	33.86	100mm	92mm	21mm	16mm	LSK	BHL	8 fleas
Praomys	ZI-29	Mbare	S	M	SV	54.15	122mm	100mm	22mm	18mm	LSK	BHL	10 fleas
Praomys	ZI-30	Mbare	S	M	AN	63.96	134mm	102mm	25mm	19mm	LSK	BHL	14 fleas
Praomys	ZI-31	Mbare	S	F	SCN	33.55	104mm	92mm	20mm	14mm	LSK	BHL	3 fleas
Praomys	ZI-32	Mbare	S	F	PSN	54.56	125mm	111mm	20mm	16mm	LSK	BHL	13 fleas (L1)
Praomys	ZI-33	Mbare	S	M	SV	67.65	136mm	105mm	21mm	19mm	LSK	BHL	9 fleas
Praomys	ZI-34	Mbare	S	F	PSN	37.11	104mm	80mm	20mm	15mm	LSK	BHL	3 fleas
Praomys	ZI-35	Mbare	S	F	CLN	52.07	123mm	98mm	22mm	18mm	LSK	BHL	8 fleas L1
Praomys	ZI-36	Mbare	S	F	CLN	49.75	116mm	107mm	21mm	17mm	LSK	BHL	4 fleas (L1)
Praomys	ZI-37	Mbare	S	M	SV	46.27	121mm	102mm	22mm	18mm	LSK	BHL	8 fleas
Praomys	ZI-38	Mbare	S	M	SV	55.78	124mm	100mm	23mm	16mm	LSK	BHL	9 fleas
Praomys	ZI-39	Mbare	S	M	AN	48.17	129mm	103mm	23mm	22mm	LSK	BHL	4 fleas
Praomys	ZI-40	Mbare	S	M	SV	58.82	134mm	101mm	24mm	20mm	LSK	BHL	4 fleas
Praomys	ZI-41	Mbare	S	M	SV	54.57	130mm	105mm	23mm	19mm	LSK	BHL	9 fleas
Praomys	ZI-42	Mbare	S	M	NA	44.13	129mm	99mm	22mm	18mm	LSK	BHL	6 fleas
Praomys	ZI-43	Mbare	S	F	CLN	39.14	104mm	91mm	22mm	19mm	LSK	BHL	7 fleas
Praomys	ZI-44	Mbare	S	F	PSY	57.68	120mm	94mm	23mm	19mm	LSK	BHL	12 fleas pregnant L9
Praomys	ZI-45	Mbare	S	F	PSN	31.19	115mm	92mm	21mm	18mm	LSK	BHL	11 fleas
Praomys	ZI-46	Mbare	S	M	SV	68.07	139mm	107mm	24mm	20mm	LSK	BHL	20 fleas

RATZOOMAN PROJECT REMOVAL TRAPPING- FORM

Collector: GC/MZ Date(07/01/2004 - 10/01/2004 Weather: Wet and Sunny

Locality: Hatcliffe

Species	Label nr	Site	Trap	Sex	Sex cond	Weight	Headbody	Tail L.	Hind foot	Ear L.	Samples alc	Samples-20	Remarks
Praomys	ZI-47	Hatcliffe	S	M	SV	51.45	122mm	104mm	23mm	19mm	LSK	BHL	
Praomys	ZI-48	Hatcliffe	S	M	SV	58.18	125mm	115mm	23mm	15mm	LSK	BHL	2 fleas
Praomys	ZI-49	Hatcliffe	S	M	SV	52.09	125mm	110mm	21mm	20mm	LSK	BHL	
Praomys	ZI-50	Hatcliffe	S	F	CSN	41.85	123mm	65mm	22mm	19mm	LSK	BHL	2 fleas (tail tip cut off)
Praomys	ZI-51	Hatcliffe	S	M	SN	49.53	126mm	111mm	23mm	21mm	LSK	HL	6 fleas
Praomys	ZI-52	Hatcliffe	S	F	CSN	34.77	119mm	105mm	22mm	18mmm	LSK	BHL	5 fleas
Praomys	ZI-53	Hatcliffe	S	M	SV	49.37	132mm	111mm	23mm	19mm	LSK	BHL	4 fleas
Praomys	ZI-54	Hatcliffe	S	M	SV	81.53	145mm	148mm	30mm	18mm	LSK	BHL	5 fleas
Praomys	ZI-55	Hatcliffe	S	M	SN	59.42	140mm	122mm	24mm	22mm	LSK	BHL	3 fleas
Praomys	ZI-56	Hatcliffe	S	F	CSN	41.69	120mm	105mm	23mm	20mm	LSK	BHL	3 fleas
Praomys	ZI-57	Hatcliffe	S	M	SV	60.02	146mm	111mm	23mm	17mm	LSK	BHL	4 fleas
Praomys	ZI-58	Hatcliffe	S	M	AN	45.88	130mm	104mm	23mm	18mm	LSK	BHL	3 fleas
Praomys	ZI-59	Hatcliffe	S	M	SN	68.65	130mm	103mm	23mm	22mm	LSK	BHL	2 fleas
Praomys	ZI-60	Hatcliffe	S	M	SV	62.8	139mm	112mm	22mm	19mm	LSK	BHL	
Praomys	ZI-61	Hatcliffe	S	F	CSN	39	110 mm	99.06 mm	19 mm	20 mm	LSK	BHL	2fleas
Praomys	ZI-62	Hatcliffe	S	F	SCN	33.26	112 mm	105 mm	21 mm	19 mm	LSK	BHL	
Praomys	ZI-63	Hatcliffe	S	F	SCY	31.19	106 mm	102 mm	21 mm	18 mm	LSK	BHL	2 fleas Preg L3
Lemniscomys	ZI-64	Hatcliffe	S	F	PLY	61.85	113 mm	50 mm	20 mm	21 mm	LSK	BHL	
Praomys	ZI-65	Hatcliffe	S	M	SV	46.32	118 mm	95 mm	20 mm	19 mm	LSK	BHL	Preg L3
Saccostomus	Z-66	Hatcliffe	S	F	PSN	12.05	85 mm	45 mm	9 mm	16 mm	LSK	BHL	5 Fleas
Praomys	ZI-67	Hatcliffe	S	F	SCY	30.26	105 mm	99 mm	21 mm	19 mm	LSK	BHL	2 Fleas

Praomys	ZI-68	Hatcliffe	S	M	SV	60.41	137 mm	96 mm	23 mm	19 mm	LSK	BHL	2 Fleas Preg L1
Praomys	ZI-69	Hatcliffe	S	M	AN	46.45	122 mm	109 mm	21 mm	18 mm	LSK	BHL	1 flea
Praomys	ZI-70	Hatcliffe	S	M	SV	50.84	127 mm	103 mm	23 mm	19 mm	LSK	BHL	6 fleas
Lemniscomys	ZI-71	Hatcliffe	S	F	PSN	58.58	135 mm	139 mm	29 mm	17 mm	LSK	BHL	1 flea
Praomys	ZI-72	Hatcliffe	S	F	CSN	32.93	106 mm	94 mm	21 mm	16 mm	LSK	BHL	
Praomys	ZI-73	Hatcliffe	S	M	SV	59.77	124 mm	104 mm	20 mm	17 mm	LSK	BHL	
Praomys	ZI-74	Hatcliffe	S	M	SV	45.56	119 mm	91 mm	22 mm	17 mm	LSK	BHL	1 tick
Praomys	ZI-75	Hatcliffe	S	M	SV	58.49	123 mm	110 mm	22 mm	18 mm	LSK	BHL	
Praomys	ZI-76	Hatcliffe	S	M	SV	50.64	126 mm	96 mm	22 mm	19 mm	LSK	BHL	3 fleas
Praomys	ZI-77	Hatcliffe	S	F	CSN	31.49	116 mm	85 mm	21 mm	14 mm	LSK	BHL	
Praomys	ZI-78	Hatcliffe	S	M	SV	31.97	124 mm	91 mm	21 mm	14 mm	LSK	BHL	
Praomys	ZI-79	Hatcliffe	S	M	SV	54.3	130 mm	104 mm	23 mm	19 mm	LSK	BHL	
R.rattus	ZI-80	Hatcliffe	S	F	PLN	116.52	173 mm	180 mm	32 mm	24 mm	LSK	BHL	
R.rattus	ZI-81	Hatcliffe	C	M	AV	81.76	151 mm	164 mm	21 mm	21 mm	LSK	BHL	
R.rattus	ZI-82	Hatcliffe	C	F	PLN	124.96	160 mm	181 mm	24 mm	24 mm	LSK	BHL	
R.rattus	ZI-83	Hatcliffe	C	M	SV	165.43	198 mm	172 mm	26 mm	26 mm	LSK	BHL	
Praomys	Z1-84	Hatcliffe	S	M	SV	61.54	138 mm	112 mm	19 mm	19 mm	LSK	BHL	3 fleas
R rattus	Z1-85	Hatcliffe	S	F	CSN	38.86	123 mm	146 mm	20 mm	20 mm	LSK	BHL	2 fleas
Praomys	Z1-86	Hatcliffe	S	F	CSN	36.2	112 mm	94 mm	19 mm	19 mm	LSK	BHL	
Praomys	Z1-87	Hatcliffe	S	M	SV	43.57	125 mm	96 mm	18 mm	18 mm	LSK	BHL	
Praomys	Z1-88	Hatcliffe	S	F	CSN	25.61	110 mm	97 mm	18 mm	18 mm	LSK	BHL	
Praomys	Z1-89	Hatcliffe	S	M	AN	18.86	95 mm	85 mm	18 mm	18 mm	LSK	BHL	1 flea
Praomys	Z1-90	Hatcliffe	S	M	SV	51.35	128 mm	104 mm	20 mm	20 mm	LSK	HL	2 fleas
R.rattus	Z1-91	Hatcliffe	S	F	CSN	19.01	95 mm	103 mm	16 mm	16 mm	LSK	HL	

R.rattus	Z1-92	Hatcliffe	S	M	AV	20.35	98 mm	102 mm	20 mm	20 mm	LSK	BHL	2 fleas
Praomys	Z1-93	Hatcliffe	S	M	SV	51.51	124 mm	100 mm	19 mm	19 mm	LSK	BHL	
Praomys	Z1-94	Hatcliffe	S	F	CLN	33.44	112 mm	102 mm	18 mm	18 mm	LSK	BHL	1 flea
Praomys	Z1-95	Hatcliffe	S	M	SV	37.11	99 mm	83 mm	19 mm	19 mm	LSK	BHL	2 fleas
Praomys	Z1-96	Hatcliffe	S	M	SV	34.15	103 mm	100 mm	19 mm	19 mm	LSK	BHL	1 flea
R.rattus	Z1-97	Hatcliffe	S	M	AV	17.2	98 mm	100 mm	17 mm	17 mm	LSK	BHL	

Data Collectors

GC= Godfrey Chikwenhere

CAGE TRAP USED IN ZIMBABWE FOR RATZOOMAN PROJECT

HARVARD TRAP USED IN ZIMBABWE FOR THE RATZOOMAN PROJECT

Annex 4.2

CMR

Name: Godfrey Chikwenhere and Moses Zimba Grid: Hat 1 Traplines Date: 26 Jan 204

Weather during the night: Cloudy and Humid

Sp	Toe Clipping code	Trap	Weight	Males	Females	Trap Code	Remarks

MN	115	S	69.29	SV		8F	
MN	116	S	56.85		CSN	10A	
MN	117	S	48.91		CSN	1L	fleas seen
XX	118	S	65.98	SV		5G	Short tailed Rhabdomys
MN	1110	S	45.36		PSY	1H	pregnant L2
MN	1120	S	46.06	SV		2D	
MN	1130	S	53.83	SV		1G	fleas present
MN	1140	S	102.29		CSN	3C	
MN	1150	S	56.33		PSY	10C	pregnant L4
XX	1160	S	114.84	SV		10D	
XX	1170	S	62.19	AN		10J	
MN	1180	S	41.31		PSN	3A	
MN	1190	S	109.61	SV		7D	swollen feet hind
MN	11100	S	66.46	AV		3D	fleas present
MN	125	S	53.77	SV		2G	fleas present
XX	126	S	117.99		PLN	9C	Dapi

Name: Godfrey Chikwenhere and Moses Zimba | Grid: Hat 1 | Date: 27 Jan 204

Traplines | Weather during the night: Cloudy and Humid

Sp	Toe Clipping code	Trap	Weight	Males	Females	Trap Code	Remarks
MN	127	S	41.3		CSN	1D	
MN	128	S	50.4	SV		1B	
MN	1210	S	105.3		CLY	4E	
MN	1220	S	93.4		PSN	3C	
XX	1230	S	110.3		CLN	9C	
MN	1240	S	51.2	SV		9B	
MN	1250	S	61.2	SV		2J	
XX	1260	S	93.5	SV		6C	
MN	1270	S	52.8		CSY	8F	
MN	1280	S	49.8	AV		6I	
MN	1290	S	61.2		PLY	10D	
MN	12100	S	67.3	AV		10F	

Name: Godfrey Chikwenhere and Moses Zimba | Grid: Hat 1 Traplines | Date: 28 Jan 204 | Weather during the night: Cloudy and Humid

Sp	Toe Clipping code	Trap	Weight	Males	Females	Trap Code	Remarks
MN	135	S	52.6	SV		10G	
XX	136	S	72.8	SV		8D	
MN	137	S	58.4	SN		8B	
MN	138	S	52.7		CSN	9B	
MN	1310	S	56.3	SV		8J	
XX	1320	S	56.8	AN		9B	
MN	1330	S	50.2	SN		2J	

Recapture Data

Name: Godfrey Chikwenhere and Moses Zimba | Grid: Hat 1 Traplines | Date: 27 Jan 204 | Weather during the night: Cloudy and Humid

Sp	Toe Clipping code	Trap	Weight	Males	Females	Trap Code	Remarks
XX	118	S	65.98	SV		5G	Short tailed Rhabdomys
MN	1140	S	102.29		CSN	2B	
XX	1170	S	62.19	AN		10J	
MN	1180	S	41.31		PSN	4B	
MN	125	S	53.77	SV		3F	fleas present

Recapture Data

Name: Godfrey Chikwenhere and Moses Zimba | Grid: Hat 1 Traplines | Date: 28 Jan 2004 | Weather during the night: Cloudy and Humid

Sp	Toe Clipping code	Trap	Weight	Males	Females	Trap Code	Remarks
MN	1240	S	51.2	SV		9C	
MN	1250	S	61.2	SV		3B	
XX	1260	S	93.5	SV		4G	

MN	1270	S	52.8		CSY	8D	fleas present
MN	1280	S	49.8	AV		6I	

CMR

Name: Godfrey Chikwenhere and Moses Zimba				Grid: Hat 2		Date: 29 Jan 2004	
				Traplines		Trap	Weather during the night: Cloudy and Humid
Sp	Toe Clipping code	Trap	Weight	Males	Females	Code	Remarks
MN	1340	S	41.3		CSN	5A	Fleas present
MN	1350	S	52.3		CSY	5D	L2
MN	1360	S	44.5	SV		10A	
MN	1370	S	65.2	SV		3H	
MN	1380	S	50.4		CSN	8D	fleas present
MN	1390	S	55	SV		4H	

CMR

Name: Godfrey Chikwenhere and Moses Zimba				Grid: Hat 2		Date: 30 Jan 2004	
				Traplines		Trap	Weather during the night: Cloudy and Humid
Sp	Toe Clipping code	Trap	Weight	Males	Females	Code	Remarks
MN	13100	S	61.8	SV		5D	fleas
MN	145	S	70.3	AV		5A	fleas
MN	146	S	55.7		PSY	9A	fleas L1

CMR

Name: Godfrey Chikwenhere and Moses Zimba				Grid: Hat 2		Date: 31 Jan 2004	
				Traplines		Trap	Weather during the night: Cloudy and Humid
Sp	Toe Clipping code	Trap	Weight	Males	Females	Code	Remarks
MN	147	S	65.4	AN		8A	fleas present
MN	148	S	65.7		PLN	1F	
MN	1410	S	59.2			3J	fleas present

Recapture Data

Name: Godfrey Chikwenhere and Moses Zimba				Grid: Hat 2		Date: 30 Jan 2004	

Traplines

Weather during the night: Cloudy and Humid

Sp	Toe Clipping code	Trap	Weight	Males	Females	Trap Code	Remarks
MN	1350	S	54.9		CSY	3B	L2
MN	1360	S	44.6	SV		8B	fleas present
MN	1380	S	50.2		CSN	1E	fleas present

Recapture Data

Name: Godfrey Chikwenhere and Moses Zimba

Grid: Hat 2

Date: 31 Jan 2004

Traplines

Weather during the night: Cloudy and Humid

Sp	Toe Clipping code	Trap	Weight	Males	Females	Trap Code	Remarks
MN	1340	S	41.3		CSN	3B	Fleas present
MN	1360	S	44.5	SV		8C	
MN	1380	S	50.2		CSN	3G	fleas present, swollen front left leg
MN	1390	S	65.3	SV		4I	
MN	145	S	61.3	AV		9E	
MN	146	S	63.4		PSY	8B	

INS

RATZOOMAN PROJECT

TECHNICAL REPORT

National Institute of Health (INS), made first meeting with INIVE (National Veterinary Research Institute) as the institution for technical support for field activities. The main objective was to discuss ways to work together. INS has a sub-contracted the institution for the " RATZOOMAN PROJECT".

An agreement was achieved and a team was immediately set up in INIVE to carry out the project. Project protocol, site definition and mechanisms of collaboration were discussed in a 2nd meeting between the two institutions. The team was informed about the methodology to be used in the project and site identification, definition of timetable for training and the final timetable for data collection. The team leader defined clear definition of responsibilities for all members of the group.

Contacts with local authorities at the site took place for communities' awareness and initial training was made in November 2003 involving the 2 institutions. The team selected sites as following:

-Primary Site- Maputo city, which include (urban, per urban and rural sites.

-Secondary Sites that include. Zambézia (Morrumbala) and Tete (Mutarara).

The team made rat trapping in primary sites. In this site we have included market areas, houses with garden and without garden, commercial grain stores, slaughterhouse poor quality houses, rural houses, and an area for capture-release and recapture.

In parallel, contacts with an South African company (Suppa Kill) were made in order to get rat traps, because in Mozambique there is not any company manufacturing rat traps. From Suppa Kill we have purchased 150 small rattraps, and 100 medium rattraps

We had some other traps locally made, not for a manufacturing company, but made for a single person in a number of 50 traps.

Ongoing contacts with World Vision in preparation of field activities in the secondary site in Zambézia and Mutarara.

Activities in the field

The team decided to wait for Professor Herwig, which come days earlier to attend the meeting in Maputo, and start on 6th of February 2004. We have considered the first day as day 0, because we could not set up all the traps we were supposed to. The communities even the awareness made while we were defining ours sites, the did not take it as a serious work. So after viewing the rattraps they realize that were something to benefit them as well. We visited Tsalala, Matadouro and T3 and set up the rattraps there.

The following days we put traps in the chosen sites.

Tsalala 11 rural houses
T3 22 house in total with garden and without garden, 9 with poor conditions

Mercado T3 9-inside small stall, because the rattraps were not safe outside small stall.

The commercial grain stores/mills were identified, but we did not work there in day one because it was a weekend
Matadouro Municipal (Slaughterhouse) 1- we placed their rattraps.

Total number of captured rats:

Locality	Species	Total
Matadouro	R. norvegicus	18
Tsalala	Mus	5
T3 (houses)	Mus	30
T3	Mastomys	1
Mercado T3	Mus	3

Total number of rat traps placed per day and number of captured rats/day

Local	kind of rattrap	Quantity	Nº of rats captured
Tsalala	Locally made	5	-
	Sherman	15	-
	Small (Sherman)	31	5
T3	Locally made	5	-
	Medium (Sherman	40	-
	Small (Sherman)	56	31
Matadouro	Locally made	10	18
	Medium (Sherman)	4	-
	Small (Sherman)	10	-
Mercado T3	Medium (Sherman)	8	-
	Small (Sherman	18	3

Main Constraints

The delay on starting the fieldwork was related with the delay of getting traps from South African Company. Put together all the team and train them.

Delay on getting invoices to by reagents, and other necessary material for fieldwork.

The medium size of rat traps purchased in Suppa Kill Company are not working at all. They did not capture any rat while we are doing trapping.
Contacts are being made, in order to have them fixed, back in South Africa, or to have somebody coming down to Maputo and fix it.

FUTURE ACTIVITIES

Ongoing activities in Maputo, in the areas that were not covered.

For CMR the activities are going to take place in the last week of February.

Mechanisms are being made to collaborate with the Ministry of Health in the Provinces in order to make things easier, because we are founding difficulties on getting agreements with the World Vision.

Fieldwork activities for the secondary sites are planned to start in March, and the team decided to do it twice a year, because of logistic situation.