FSP ACSS
French Embassy
Uganda

Support in epidemiologic methodology and zoonotic risk factors analysis of tuberculosis (*Mycobacterium bovis*, *Mycobacterium tuberculosis*) and brucellosis.

Mission from October the 4th to the 16th.

Eric Etter

CIRAD-EMVT Report n°2004.13 **November 2004**

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FRANCE

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ACCES TO THE DOCUMENT :
CIRAD EMVT Uganda ; Service de Documentation de Baillarguet

AUTHOR ORGANISM :
CIRAD-EMVT

FINANCIAL SUPPORT :
FSP « Agricultural consultation& Sector structuring in Uganda »

RÉFÉRENCE :
Ordre de mission n°30 05 04 469

FOR THE PROFIT OF :
FSP « Agricultural consultation& Sector structuring in Uganda »

TITLE :

Support in epidemiologic methodology and zoonotic risk factors analysis of tuberculosis (*Mycobacterium bovis, Mycobacterium tuberculosis*) and brucellosis.

DATE AND PLACE OF PUBLICATION:
November 2004, Montpellier, France.

CONCERNED COUNTRY:
Uganda

KEY WORDS:
Tuberculosis – Brucellosis - Zoonosis - Epidemiology – Animal health – Animal production - Uganda

Acknowledgements :

I am very grateful to H.E. JB Thiant, French Ambassador, for the interest funded on the milking sector in the region of Mbarara and especially on the implication of the Cirad in public health part of the FSP project with the study of the zoonotic aspect of tuberculosis and brucellosis.

I thank also Mr Alban Bellinguez, coordinator of the FSP project, for the confidence he places in the collaboration with the Cirad-Emvt.

« Last but not least », a special thank you to Patrice Grimaud, Cirad working in Uganda, for his so natural welcoming and for his organisation as faultless as it used to be, and for the implication he has in the job we really made together

Abstract

It was the second mission of Cirad-Emvt in Uganda on the zoonotic problematic for the year 2004. This mission was programmed following the chronogram the Cirad-Emvt proposed after his first mission in March 2004. Its objective was to present to the faculty of Makerere the risk analysis demarche in animal health and to support the beginning of the work of both student studying respectively the implication of animal health in human tuberculosis and brucellosis. These two project (PhD and Master) are part of the 2nd component of project FSP "Agricultural consultation and sector structuring".

Contents

List of the contacts during the mission:

French Embassy in Uganda ; Service de Coopération et d'Action Culturelle (SCAC)

- S.E. Jean-Bernard Thiant Ambassador of France in Uganda

FSP « Concertation agricole et structuration des filières »

- Alban Bellinguez coordinator of the FSP

Mbarara University

- Professor Kayanja Vice chancelor of the University
- Dr Kabakyenga Dean of Mbarara University
- Dr Frederick Byarugaba lecturer in the faculty

Makerere University

- Prof. Nasinyama director of the epidemiology department of the Veterinary Faculty
- Dr Edward Ssekawojwa Master student

NARO-LIRI

- Dr Nakavuma director of the laboratory in Tororo

CIRAD-EMVT

- Dr Patrice Grimaud
- Miss Nelly Grillet French student working on the milk quality in Mbarara

Program of the mission

Tuesday 05th October 04

- ⇒ Meeting with professor Nasinyama
- ⇒ Organisation of the planning with Patrice Grimaud and strategy elaboration on the PhD and Master degree work

Wednesday 06th October 04

- ⇒ Work on the "Risk analysis and Animal Health" presentation
- ⇒ Presentation in the Makerere University
- ⇒ First Meeting with Drs Byarugaba, Ssekawojwa and Kalema-Zikusoka

Thursday 07th October 04

- ⇒ Transport to Tororo
- ⇒ Meeting with Dr Nakavuma
- ⇒ Way back to Kampala

Friday 08th October 04

- ⇒ Work session with Fred Byarugaba : proposal correction
- ⇒ Protocol and sampling procedure

Saturday 09th October 04

- ⇒ Sampling method with Patrice Grimaud

Monday 11th October 04

- ⇒ Transport to Mbarara
- ⇒ Meeting with Mr Dean Dr Kabakyenga in Mbarara University
- ⇒ Work session with miss Grillet
- ⇒ Work session with Fred Byarugaba (bibliography)

Tuesday 12th October 04

- ⇒ Farm visits
- ⇒ Meeting with the Vice-Chancelor of Mbarara University
- ⇒ Way back to Kampala

Wednesday 13th October 04

- ⇒ Work session with Edward

Thursday 14th October 04

- ⇒ Meeting with Gladys
- ⇒ Work session with A. Bellinguez
- ⇒ Work restitution with Prof Nasinyama and Edward

Friday 15th October 04

- ⇒ Restitution to H.E. Mr Ambassador with A. Bellinguez and P. Grimaud

1. Reminder of the Context

Within the framework of its programs of research - development in Uganda within the FSP project "Agricultural Consultation and Sector Structuring ", the Department Emvt (Animal Breeding and Veterinary Medicine in Tropical Countries) of Cirad is interested in the transmissibility of zoonoses to human populations, often weakened by the virus HIV. Because of "Memorandums of Understanding" with two of the universities of the country, the missions of the researcher located in Uganda are to supervise students particularly in the study of two of these zoonoses, tuberculosis and brucellosis, of which Cirad showed strong prevalence within the herds of the district of Mbarara, and who raise problems in public health in Uganda.

A first mission of an Cirad-Emvt epidemiologist, realized in March 2004, allowed to introduce the work of thesis of an Ugandan researcher, Dr Frederick Byarugaba. This work realized the Mbarara University deals with the link bovine tuberculosis / human tuberculosis. The first part of the work began this summer within the Mbarara hospital in association with NTLP (National Tuberculosis and Leprosis Programme). It concerns the study of the human tuberculosis aetiology, particularly the extra-lung tuberculosis suspected of being of animal origin.

Further to a first working meeting and thanks to the follow-up of the contacts by the Cirad agent in Uganda, a collaboration with the veterinarian faculty of Makerere University began. It focuses ont he supervision of a master degree student who will work on the zoonotic aspect of brucellosis and the consequences on the milk sector in Mbarara district.

Moreover a French student is present in Uganda since May 2004, to realize a study on the milk quality throughout the sector in the Mbarara region. This study focused notably on the milk contamination by the tuberculosis bacillus and by *Brucella* sp.

2. Objectives of the mission (cf Appendix "Termes de référence")

Tuberculosis

TDR 1. Analysis of the first results obtained by Dr Byarugaba

TDR 2. Connection of the works with the National Tuberculosis and Leprosis Programme

TDR 3. Construction of the protocol for the retrospective study. This study will be built on the first results from the TB patients from the Mbarara hospital and the references on the zoonotic aspect of tuberculosis.

Brucellosis

TDR 4. Construction of the study protocol for the Master training on the zoonotic aspect of brucellosis.

Tuberculosis and Brucellosis

TDR 5. Evaluation of the results obtained by Nelly Grillet on the sanitary quality of milk and proposition of a HACCP (Hazard Analysis Critical Control Point) approach. Discussion on the zoonotic impact of brucellosis and tuberculosis on the milk sector.

TDR 6. Feasibility study on the risk analysis on interaction of wildlife with breeding animals in the transmission of both these zoonosis and on the reservoir aspect of wildlife in these diseases.

At the end of the mission:

- Evaluation of the evolution of the PhD on tuberculosis, a projected chronogram will be proposed.
- Work package to carry on this work will be proposed; survey procedures, data collection, sampling protocol and respective roles of each partner and technical, human and money support will be defined.
- Informations will be transmitted to each partners
- Possibility and condition for a training in France (Laboratoire de Bactériologie - Virologie CHU Arnaud de Villeneuve - Montpellier) for Dr Byarugaba will be evaluated
- Milestones, for the Master training, on the zoonotic aspect of Brucellosis in Mbarara district will be elaborated with a beginning of the study in January 2005
- Study of the milk sector using milk quality will be evaluated and the HACCP method will be plan in order to be integrated in the risk analysis of Brucellosis and Tuberculosis.
- Feasibility study on the function of wildlife in the transmission and the persistence of both these diseases will be studied.
- Proposition in formation with implication of the Cirad-Emvt in the organisation or realisation will be specified.

3. Protocol for Bovine Tuberculosis research

Concerning Tuberculosis surveys for Dr Byarugaba PhD, there are several points to mention.

The first study concerns human beings in the Mbarara hospital. People concerned by this study are all new arrived in the Tuberculosis section of the hospital. We have historical data on tuberculosis in Mbarara district showing that a mean of 5% of TB are non pulmonary TB. Literature says that *M. bovis* causes mainly non pulmonary TB. It is also known that in case of *M. bovis* infection, co-infection with the HIV is responsible of pulmonary TB. That is why we have made the hypothesis that the prevalence of *M. bovis* tuberculosis is at least 5% in the tuberculosis sufferer population.

To improve this hypothesis we have chosen to make a survey in this population.

The next steps of the study (animal and countryside survey) will be detailed later (cf. Etter, 2004, Rapport de mission du 23 mars au 1er avril)

3.1. Survey in the TB sufferer population:

We will have a first part in our survey with a sample size around **100** patients from the TB ward. This first sample[1] has been chosen to detect the disease with a prevalence minimum presenting a level of interest. It will allowed us to detect at least 1 bovine TB if the prevalence is higher or equal to 2.95% with an error of 5% or if the prevalence is equal or higher to 4.5% with an error of 1%. If we don't find any bovine TB cases it means that the prevalence of bovine TB in the TB patients' population is smaller than 4.5% with an error of 1% so in such case we have decided not to go on with the survey: bovine TB would not be a public health problem.

Nevertheless we have to keep in mind that the digestive route by ingestion of contaminated milk or meat normally results in the extrapulmonary forms of tuberculosis. Cervical lymphadenopathy, intestinal lesions, chronic skin TB (lupus vulgaris), and other nonpulmonary forms may, however, also be caused by M. tuberculosis. On an other hand, inhaling cough spray from infected cattle (concerning agricultural workers for example) will

[1] For statistical explanations cf. annex 1

be cause of typical pulmonary TB. So, depending of the kind of samples we will use for the analysis, the results should be interpreted with caution.

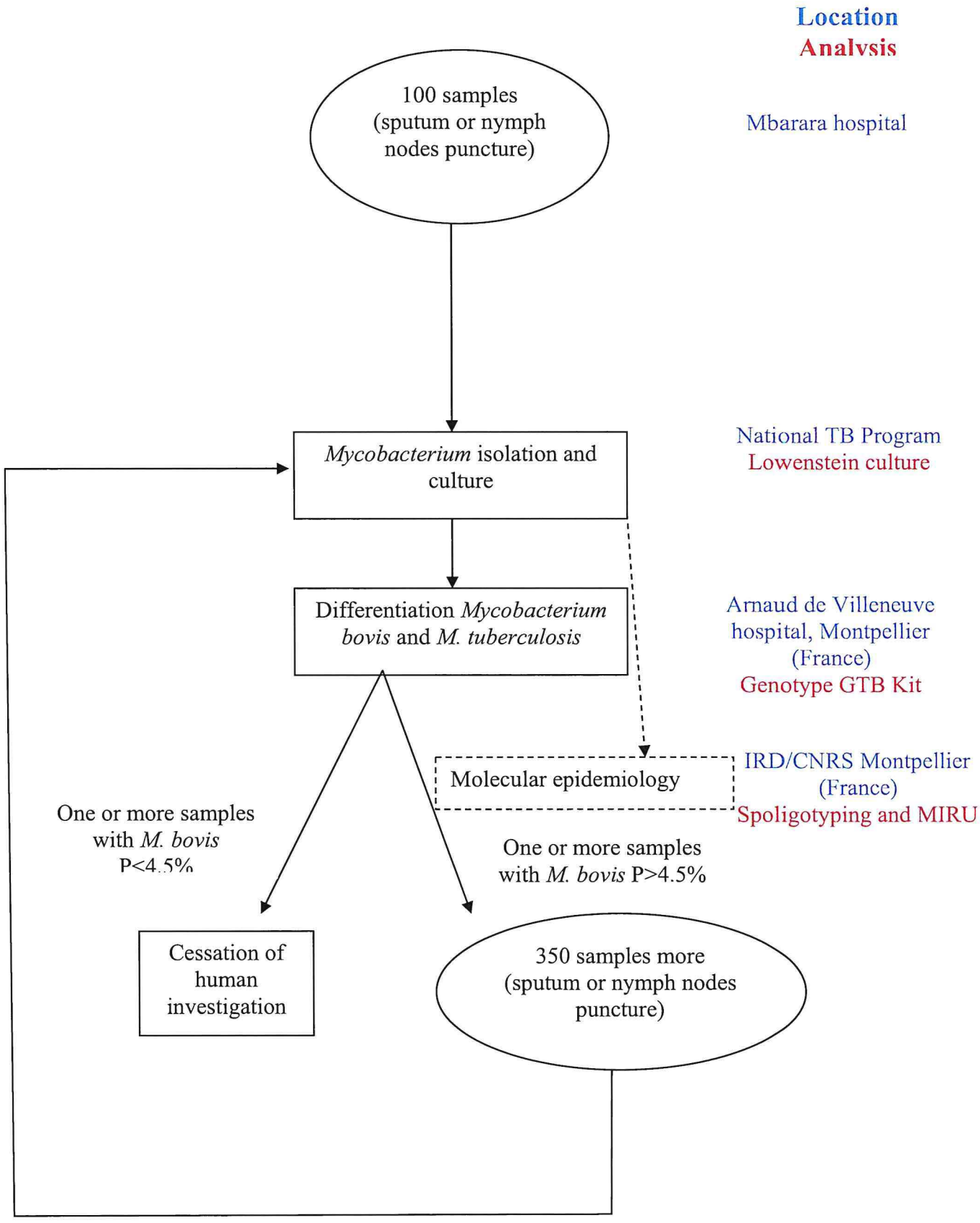
Then after we will take a sample size around **450** to confirm the expected prevalence. It means that if the prevalence we will find is really 5% (it means between 22 and 23 bovine TB cases) the real prevalence in the population of TB patients will be between 3 and 7% (Pa=2%). If the prevalence we will find is higher, for example 10% (with 45 bovine TB cases) our absolute precision will be less accurate: IC[10-2.77 ; 10+2.77]=[7.23% ; 12.77%]

We shall not only analyse the origin of TB but we have also prepared with Dr Byarugaba the qualitative and quantitative questionnaire associated with the samples collection. This questionnaire will collect general information on the patient, his medical history, his work history, his home environment, his use of animal products and his knowledge of disease transmission. The analyse of this questionnaire in regard of the results of the samples analyse will allow us to determine risk factors associated with bovine TB and to quantify them.

This first approach will take place in a larger information collection in order to "feed" our risk analyse model on the zoonotic risk of bovine TB. It will permit to determine which the main scenario is, in the general pathway of the zoonotic risk of bovine TB (cf. infra). These risk factors will be useful in the precise decomposition of these scenarios before attempting to quantify each step of them.

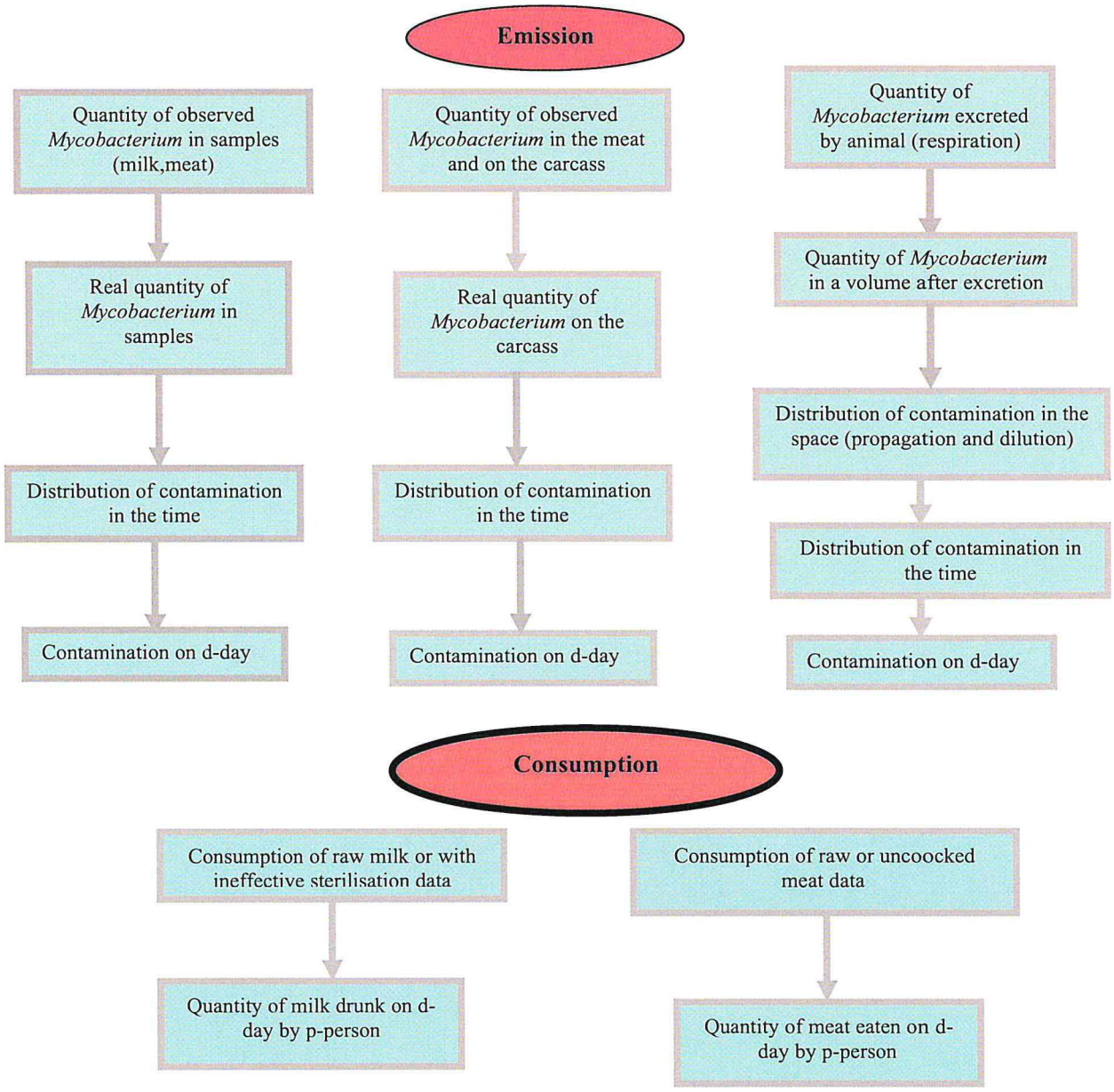
The study of the impact of Mycobacterium bovis on human beings takes place in the "Effect" part of the pathway in the box "Infection risk for p-person on d-day". Thereafter we have to determine the proportion of disease in the infected population reminding that with only sputum samples we approach better the pulmonary tuberculosis and so the breath route of infection.

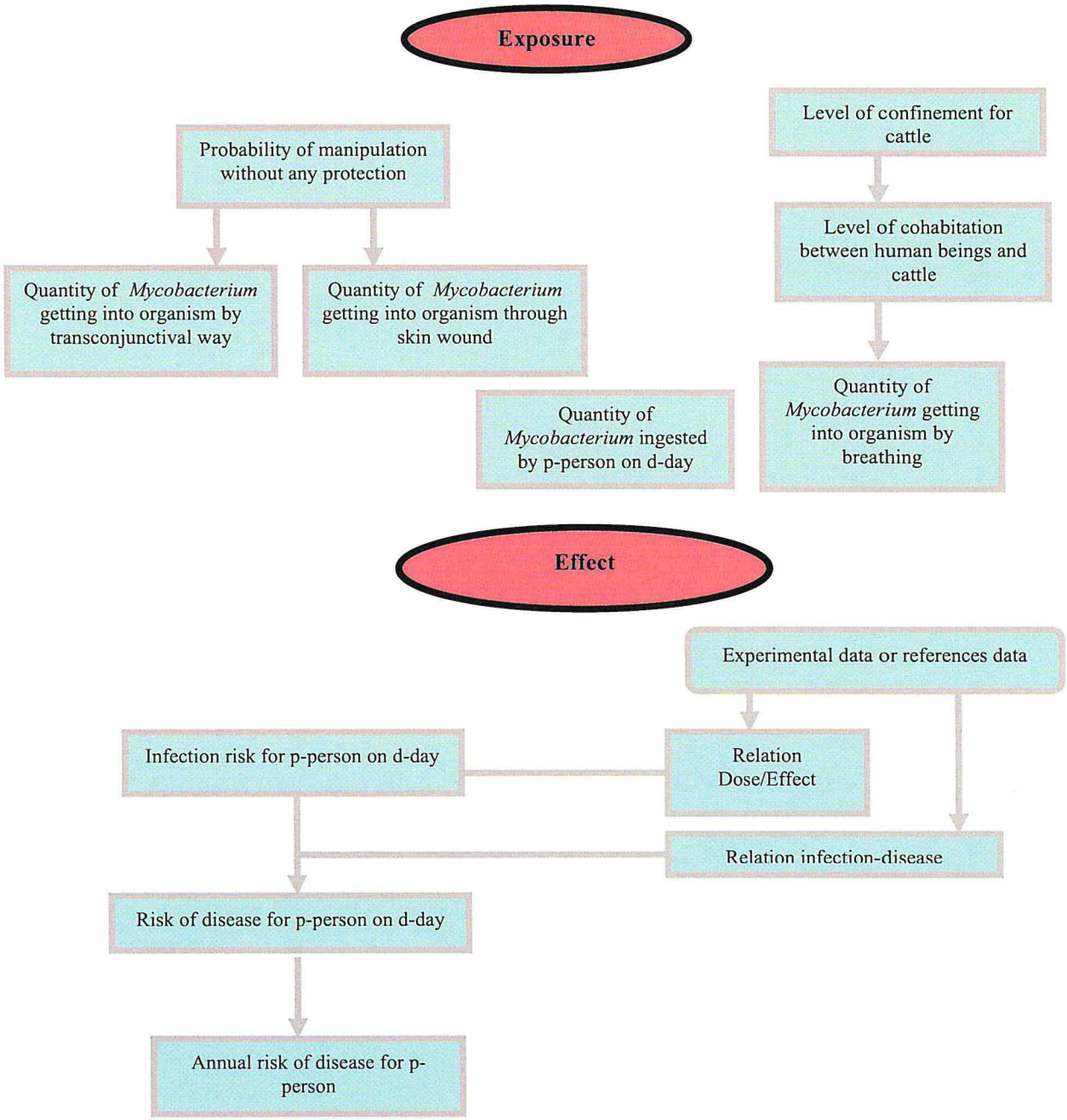
The study of Nelly Grillet takes place in the "Emission" part of the pathway. The results could give us a first view of the contamination by the digestive route. Her original approach with the survey on the distribution of milk from Mbarara to Kampala allows to assess the hazard due to *M. bovis* along the commodity chain, in particular in the first steps of the chain.


Location
Analysis
100 samples (sputum or nymph nodes puncture)
Mbarara hospital
Mycobacterium isolation and culture
National TB Program
Lowenstein culture
Differentiation Mycobacterium bovis and M. tuberculosis
Arnaud de Villeneuve hospital, Montpellier (France)
Genotype GTB Kit
Molecular epidemiology
IRD/CNRS Montpellier (France)
Spoligotyping and MIRU
One or more samples with M. bovis P<4.5%
One or more samples with M. bovis P>4.5%
Cessation of human investigation
350 samples more (sputum or nymph nodes puncture)

Proposition for the zoonotic risk analysis of Tuberculosis

Presentation of the pathway for different modules of the transmission from animals to human beings




Exposure
Probability of manipulation without any protection
Level of confinement for cattle
Level of cohabitation between human beings and cattle
Quantity of *Mycobacterium* getting into organism by transconjunctival way
Quantity of *Mycobacterium* getting into organism through skin wound
Quantity of *Mycobacterium* ingested by p-person on d-day
Quantity of *Mycobacterium* getting into organism by breathing
Effect
Experimental data or references data
Infection risk for p-person on d-day
Relation Dose/Effect
Relation infection-disease
Risk of disease for p-person on d-day
Annual risk of disease for p-person

4. Protocol for Brucellosis Study

4.1. Objective:

4.1.1. Risk factors analysis by comparison between herds free of brucellosis with infected farms

4.1.2. Assessment of agro-ecological environment as risk factor

4.1.3. Brucellosis risk assessment in raw milk consumers in Kampala

4.2. Protocol:

The district will be stratified according to the 3 agro-ecological zones (Dabusti and Vancauteren 1999). Three counties (Ibanda, Kashari and Nyabusozi) representing the agricultural, agro-pastoral and pastoral zones respectively will be selected for further sampling. Subsequent sampling within each of the three counties will be done separately using a two-step cluster sampling technique.

In Uganda counties are divided into parishes, sub-parishes and then local administrative zones. It is assumed that there is no significant variation in the disease distribution between sub-parishes and local administrative zones; therefore further sampling will be done with local administrative zones as primary sampling units. Between 30 – 50 % of all local administrative zones will be selected randomly from a prepared sampling frame of local administrative zones, the number will depend on total number of zones in each county, their accessibility and available logistics. A sampling frame of cattle and/or goat farmers will be constructed (used as a proxy for cattle and/or goat herds) with the help of the Local Council 1 chairpersons or local guides.

4.2.1. Sampling for cattle

Since we wish to study risk factors associated with Brucella seropositivity, we shall first do a comparison between two groups, one of disease positive herds against another of disease negative herds. Secondly the 3 agro-ecological zones will also be investigated as risk factors for the Brucella seropositivity. In order to do a statistical mean comparison of the two groups with parametric tests we need groups of size equal or greater than 30 herds. According to

statistical calculation[2], 79 herds are needed to be able to use parametric tests to compare the results of the survey between brucellosis positive and brucellosis negative herds.

However for us to compare the results of the 3 agro-ecological zones we shall require at least 30 herds from each zone bringing the total number of herds to 90. This number is also sufficient for the comparison of the two brucellosis positive and brucellosis negative groups. To take care of the losses and denials the number of cattle herds to be sampled will be increased to **95**. Sampling within the herd will be done using the formula for detecting presence of disease (cf. supra) at 15% individual prevalence (Faye et al., 2004) and 5% of accepted error we will have 19 animals to take in each farm. In farm with cheptel smaller than 190 animals we still take 19 animals and in farms with less than 19 animals we will take all of them. At the end in our sampling for cattle we will have more or less about **1805 samples**. Each blood sample will be analyzed in Rose Bengal test. In case of suspect results, the samples of the animals belonging to the suspected herd will be analyzed with Elisa test.

4.2.2. Sampling for goats

According to the previous study done in Nyabusozi county, the prevalence of brucellosis was recorded as 10% and 43% at the individual and herd levels respectively (Kabagambe et al., 2001), the same survey reported the average herd size of 8-9 goats. The total goat population for Mbarara District is 165,000 (Production Dept. annual report, 2003). Using the formula for determining disease prevalence (cf. supra), at 95%, CI., and 5% (10%) absolute precision the total number of goats needed would be 138 (35) while for the herds it would require 377 (94). However because of the enormous costs involved in sampling large herds we shall use an absolute precision of 10% for calculating the sample size for the goats giving us 94 herds. It mean that if the herd prevalence we shall find is 43% we will have a CI=[33%-53%].

On the other hand for us to be able to compare the results of the survey between the brucellosis positive herds and brucellosis negative herds using parametric tests we shall need at least 30 herds for each of the two groups. According us a maximal error of 5% and assuming a herd prevalence of 43%, then solving the equation (1) $p(X+ \geq 30) > 95\%$, X+ = nb of brucellosis positive herds, we will have a sample size of 87 herds and solving the

[2] cf. annex 2

equation (2) p (X- ≥ 30), X- = brucellosis negative herds , we will find n = 64 herds. So we will keep the higher number of n=87 herds

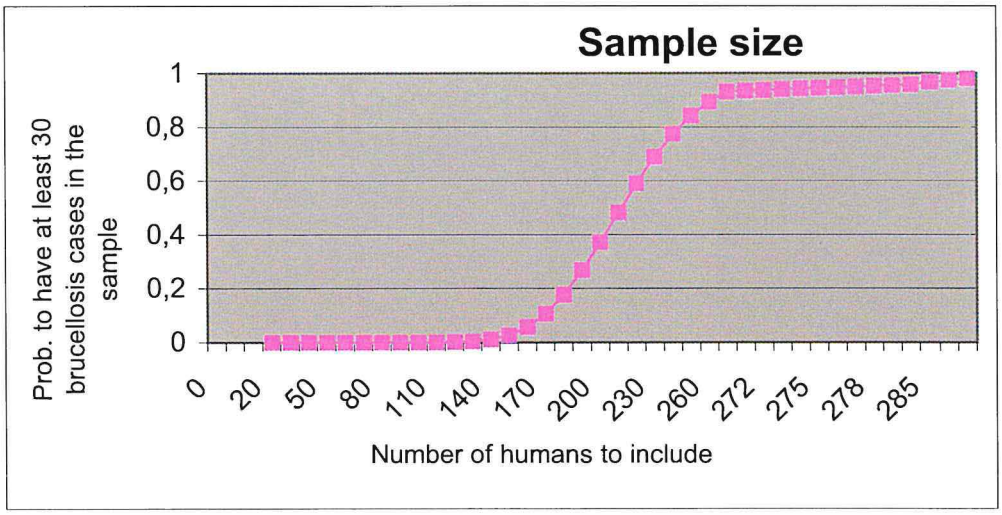
Further more in order for us to compare the results of the 3 agro-ecological zones then we shall need at least 30 herds for each zone bringing the total to 90 herds.

Consequently the 94 herds are sufficient to investigate the herd and individual prevalences as well as studying the comparison between the results of the brucellosis positive and brucellosis negative herds and the comparison between the agro-ecological zones. To take care of losses and denials we shall sample **99 herds**. Because of the small size of the goat herds all the goats in selected herds will be included in the study. Eligible animals for sampling will include unvaccinated animals (cattle and goats) over 6 months of age.

At the end the maximal total **number of goats** to sample will not be higher than **1000**.

4.2.3. Sampling for humans

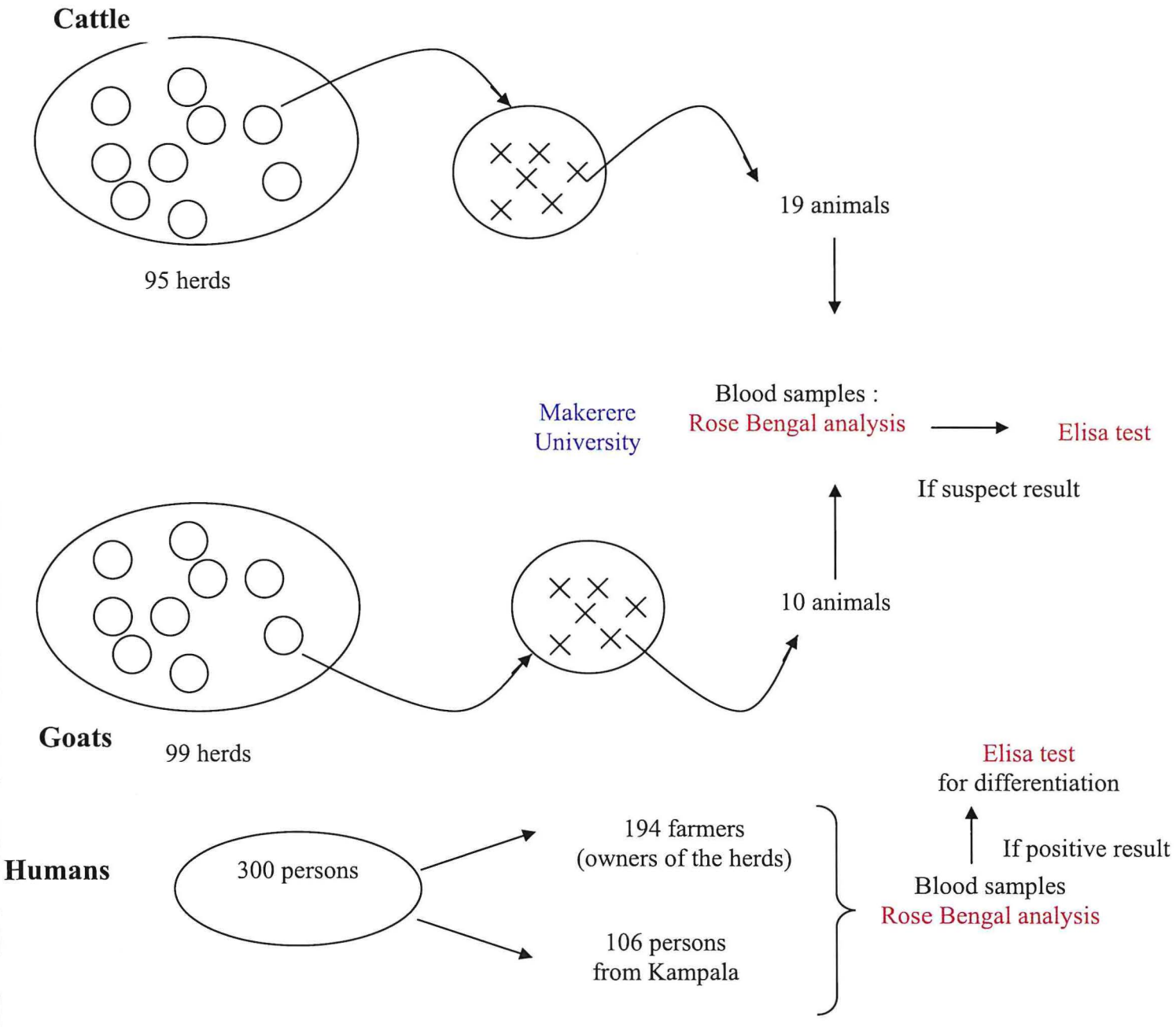
In order to do our statistical analysis we need to compare at least 30 cases vs 30 people without brucellosis. We use the same formula of a cumulative binomial distribution as we used for cattle sampling. The national human brucellosis prevalence is estimated from 14 to 40 % (source?). So to evaluate the consumers' risks assuming a prevalence of brucellosis in the unselected hospital patients' population of 14% (Kalema-Zikusoka, 2004) we need a sample size equal or higher than 277 people. Because of the uncertainty on this prevalence and on the possibility of deletion in the selected persons we shall choose a sample size of **300 persons**. These all people include the 194 one's samples in the farms where we will do the cattle and goats survey (or just the 99 if we just consider the cattle survey). The other ones will be collected in Kampala.



Each blood sample will be analyzed in Rose Bengal test; for positive people, a complementary test (Elisa test) will be done to determine the nature of the agent (*B. abortus* or *B. melitensis*).

4.2.4. Sampling for buffaloes in Lake Mburo Park.

A random sample of 150 female buffaloes will be selected for the study. On top of sera samples data will be corrected on their location, age and any overt clinical signs recorded.



5. Planning :

	PLANNED
	FINISHED

Annexes

ANNEXE1

Sample size to confirm a prevalence

In this whole paragraph we assumed that population size (N) is much more important than sample size (n) i.e. N>10*n. We will choose a sample size which can allow us to confirm the bovine TB prevalence we were expecting. We have to make an other hypothesis on the distribution of the prevalence between several samples. We will accept to model it with a normal distribution. So the prevalence we will find in our sample will vary depending of the sample size. So we will have to give a confidence interval with this prevalence:

$CI = [p - 1.96\sigma; p + 1.96\sigma]$ with σ the standard deviation of the prevalence and 1.96 because of the normal distribution and the accepted error of 5% (with an accepted error of 1% we should take 2.56).

We will call 1.96σ the absolute precision. The sample size will now depend on this absolute precision we will choose.

Indeed $\sigma = \sqrt{\frac{p(1-p)}{n}}$ (standard deviation for a proportion) so

$n = \frac{1.96^2 * p(1-p)}{Pa^2}$ with Pa the absolute precision

Now with the table 1 it is possible to choose the sample size function of the expected prevalence and the absolute precision we need.

Table 1 : Sample size to estimate prevalence

Sample size (with the acceptation of an error of 5% on the confidence interval, and a population size (N) respecting n/N<10))

		Expected Prevalence (%)									
		1	2	3	4	5	6	10	20	30	40
	1	380	753	1118	1475	1825	2167	3457	6147	8067	9220
	2		188	279	369	456	542	864	1537	2017	2305
	3			124	164	203	241	384	683	896	1024
	4				92	114	135	216	384	504	576
	5					73	87	138	246	323	369
	6						60	96	171	224	256
	7							71	125	165	188
	8							54	96	126	144
	9							43	76	100	114
	10							35	61	81	92
Absolute precision (%)	11								51	67	76
	12								43	56	64
	13								36	48	55
	14								31	41	47
	15								27	36	41
	16								24	32	36

Expected prevalence (p)
Confidence interval (CI)
Absolute precision (Pa)
Relative precision (Pr)

CI=p+/-Pa
Pr= Pa/p
Example : with p=5% if the Pa=1% it means that CI is [0.04-0.06] and Pr=20%

Sample size to detect disease

In this whole paragraph we assumed that population size (N) is much more important than sample size (n) i.e. N>10*n. If we just want to detect the disease we will need an other sample size. It means that with this sample we must found at least one bovine TB to confirm that the prevalence is equal or superior to 5%. The size of the sample we need also depends on error accepted (mainly 5%)

The equation given the sample size n is:

$n = \frac{Log\alpha}{Log(1-p)}$ with α the higher risk we accept to make a mistake (conclusion will be given with the accepted error of α) and p the prevalence minimal this sample size allowed to detect.

Table 2: sample size to detect a disease

Sample size to detect at least 1 case (with α =error level accepted it means the probability to detect 0 case)

Prevalence(*100)	α =0,05	α =0,01
0,1	2995	4603
0,2	1497	2301
0,4	748	1149
0,5	598	919
1	299	459
2	149	228
3	99	152
4	74	113
5	59	90
6	49	75
7	42	64
8	36	56
9	32	49
10	29	44
11	26	40
12	24	37
13	22	34
14	20	31
15	19	29
20	14	21

ANNEXE 2

Sample size to find at least n animals with brucellosis (example with n=30)

In order to do a statistical mean comparison of the two groups with parametric tests we need groups of size equal or greater than 30 herds. Assuming N > 10n (N = population of cattle in Mbarara District, n = sample size), we accept a maximal error of 5%. So to determine our sample size we need (1) p ($X^+ \geq 30$) $\geq$ 95%, X^+ = no of brucellosis positive herds, (2) p ($X^- \geq 30$) $\geq$ 95%, X^- = no of brucellosis negative herds. Because of the population size the distribution of these probabilities is a binomial distribution. So the formula for cumulative distribution is

$$p(X^+ > x) = \sum_{i=0}^{x} \binom{n}{i} p^i (1-p)^{n-i}$$ with p the herd prevalence and x=29 in our case

So assuming the herd prevalence of brucellosis to be 53% (Faye et al., 2004), using 5% for the maximal error, solving the equation (1) the calculation gives n $\geq$ 69 herds, for the equation (2), the calculation gives n = 79. Therefore 79 herds are needed to be able to use parametric tests to compare the results of the survey between brucellosis positive and brucellosis negative herds.