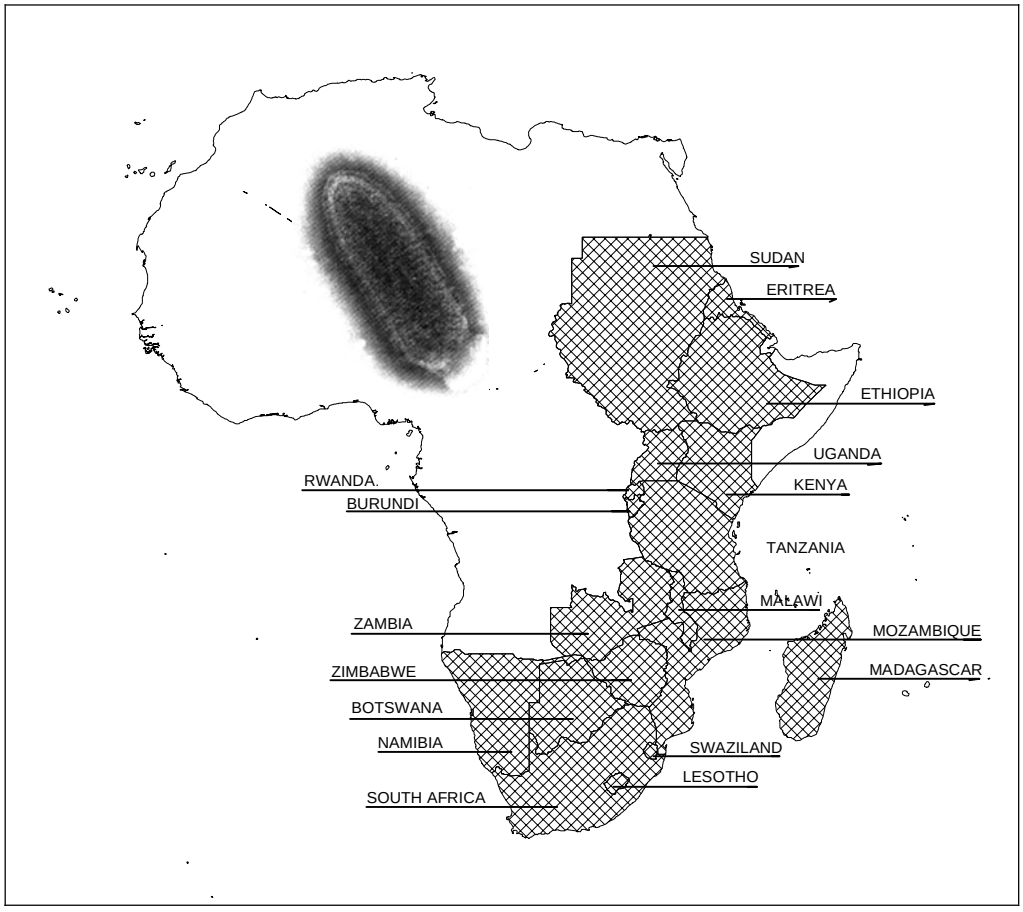
W. H. O.

PROCEEDINGS OF THE SEVENTH SOUTHERN AND EASTERN AFRICAN RABIES GROUP / WORLD HEALTH ORGANIZATION MEETING



EZULWINI, SWAZILAND: 12 - 15 MAY 2003

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PROGRAMME OF THE MEETING

7TH SOUTHERN AND EASTERN AFRICAN RABIES GROUP / WORLD HEALTH ORGANIZATION INTERNATIONAL MEETING

EZULWINI SWAZILAND: 12 TO 15 MAY 2003

Monday 12th May 2003

Arrival of delegates at Lugogo Sun Hotel, Swaziland.

Tuesday 13th May 2003

Opening session: 8:00-10:00

chairperson: A.Wandeler

Welcome	*SEARG committee*
Tribute to Arthur King	*A. Wandeler*
Tribute to George Bishop	*J. Godlonton*
Chairman's report	*R. Dlamini*
Global burden of rabies: where does Africa fit?	*F.X. Meslin*
Guest Speaker:	*Honourable Stella Lukhele (MP) Minister for Agriculture and cooperatives*

Country reports: 10:30-12:30

chairperson: C.T. Sabeta

Botswana	*M. Letshwenyo*
Eritrea	*T. Teclegiorgis*
Kenya	*J. Mwangi*
Malawi	*G. Wanda*
Mozambique	*P. Dias*
Namibia	*F. Mettler*

Country reports: 13:30-15:30

chairperson: R. Winyi Kaboyo

South Africa	*J. Randles*
South Africa	*A. Liebenberg*
Sudan	*Y. Hassan Ali*
Swaziland	*R. Dlamini*
Uganda	*C. Rutebarika*
Zambia	*P. Mijere*
Zimbabwe	*W. Shumba*

SEARG business meeting: 16:00-18:00

chairperson: A. Wandeler

Quo Vadis

The road ahead

Future roles of the WHO and OIE

Proceedings, diagnostics manual and professional guide

Remarks from industry (Chiron etc)

Election of new committee

Cocktail and cultural evening: 19:30-23:00

Wednesday 14th May 2003

Vaccinology and post-exposure prophylaxis: 08:00-10:00

chairperson: L.H. Nel

The frustrations of a rabies telephonist	*J. Godlonton*
Does rabies vaccine history stutter?	*F.X. Meslin*
DNA vaccines against rabies	*N Tordo*
Unraveling the mysteries of cell culture rabies vaccines	*D. Briggs*
New generation poxvirus vaccines for rabies	*J. Weyer*

Challenges of introducing rabies intra-dermal post exposure treatment in Uganda
W. Kaboyo

Experiences with tissue culture rabies vaccine used intradermally in Thailand.
T. Kamoltham

Diagnostics: 11:30-12:30

chairperson: L.H. Nel

European interlaboratory fluorescent antibody test comparison test
F. Cliquet

Molecular diagnostic tools for the rapid strain identification of rabies in humans following exposure associated with travel to rabies endemic countries
A.R. Fooks

A hemi-nested RT-PCR for rabies diagnostic of European bats: its use for field specimen
F. Cliquet

Epidemiology, dog ecology and oral vaccination: 13:30 – 15:00

chairperson: P. Kloeck

From bats to carnivores	*N. Tordo*
Oral vaccination campaigns of dogs against rabies	*A. Vos*

A data-driven assessment of the burden of rabies in the developing world.
D. Knobel

Molecular epidemiology of canid rabies in Zimbabwe and South Africa
C.T. Sabeta

The ecology of dogs in Swaziland: implication for rabies	*R. Dlamini*
The use of monoclonal antibodies for rabies variant typing with some applications to rabies in the SEARG region	*A. Wandeler*

Chiron award presentation: 17:00 – 18:00

Chiron Award: background and objectives	*A. Wandeler*
	A. Liebenberg
Chiron: 2003 Winning project;	*B. Jennings*

Closure of the meeting: 18:00

chair: elected president, SEARG

Closing remarks	*SEARG committee*
Official closure	*Swaziland Minister of Health*

Thursday 15th May 2003

Departure from Swaziland

Opening session

TRIBUTE TO THE LATE ARTHUR KING

A.I. Wandeler[1]

It was a rather unpleasant passage through Heathrow Airport in London this time. There was no Arthur King waiting to shorten the boring wait for the next plane. Arthur, being one-eyed, had a slight handicap. So, exiting from immigration, I usually saw him first, while he was scouting around worriedly not to miss me in the crowd. I knew this was a friend, when his face lit up to a warm welcome when he spotted me.

It is also the first time that I arrived at a SEARG conference destination, that there was no phone ringing less than ten minutes after checking into my room, no deep voice announcing "The Lord is calling", meaning, that you were invited to his room to have a zip from his Glenmorangie bottle and to discuss the most recent changes to the program. That bottle usually emptied rapidly, since so many other SEARG attendants had a zip from it too.

Though Arthur liked to tell stories, he hardly ever talked about his childhood and youth, except that he was about to become a soccer star. I know that he lost his father in World War II during a German bombing raid. His mother passed away shortly after the war on the consequences of the injuries suffered in this attack. I believe Arthur originally worked as a farmhand somewhere in Surrey. Quite incidentally he passed at the Central Veterinary Laboratory in Weybridge, England, and applied for a job, which – to his own surprise – he received.

Arthur King accomplished a truly amazing career in the Central Veterinary Laboratory. He started work there as a Laboratory Attendant in 1952 and climbed through the ranks to become a Principal Scientific Officer. With his many skills he was at the forefront of new technological developments in virology, particularly in cell culture procedures and in fluorescence microscopy. His efforts were originally focused on swine fever virus detection and eradication.

Arthur joined the international rabies community relatively late. We occasionally communicated on the production and specificity of monoclonal antibodies, but had otherwise little contact. This changed. He visited us in Berne, Switzerland, in the early 1980s to observe the distribution of vaccine baits to immunize foxes against rabies. The area we had chosen to show to Arthur was the Val d'Illiez, a small Alpine valley in south-western Switzerland. Though volunteers did the bait distribution in most regions, in this valley the staff of the Swiss Rabies Centre did it in the context of epidemiological field studies. It was a cool spring day, snow was still covering most of the landscape, and on-and-off we had some flurries. We explained our bait distribution strategies, talked about rabies epidemiology, fox ecology and the rodent studies that we conducted in this area. Simon Capt and Heinz Stalder demonstrated the tracking of the foxes that we had radio-collared in this neighbourhood. We noticed that Arthur got increasingly colder and started giving him our pullovers and windbreakers. It didn't help much. Towards the end of the day Arthur was crouched into the back of the car, his jacket on backwards and buried in the garments that we could spare. "The coldest day I have ever lived" came as a comment from pile of textiles. Our friendship was established.

Some years later I informed Arthur that I had received a job offer from Canada and that I would take it. He observed: "You can't have it cold enough. I won't visit you there". I reminded him that he had been in Canada before and that his sufferings there had been of quite a different nature. I remembered his colourful account of how he got forgotten at the Airport in Toronto, how he managed to get to a bus station, how difficult it was to find out how to reach Maple, where his destination, the Research Station of the Ontario Ministry of Natural Resources was. He finally managed to get to a neighbouring town. From there he had to walk to his destination with his humongous one suitcase that he could barely keep from touching the ground. One big suitcase, not two smaller ones, because his supervisor at work had recommended it so. But he also had told about the good times with his Canadian colleagues

[1] Canadian Food Inspection Agency, Ontario Laboratory Fallowfield, WHO Collaborating Center for Control, Pathogenesis and Epidemiology of Rabies in Carnivores – Ottawa – Ontario - CANADA

when he finally succeeded to contact them, and that, to Dave Johnston's horror, he managed to stand up in his canoe in the middle of the Georgian Bay.

Arthur King was interested in rabies virus diversity. He argued that a meaningful classification of Lyssaviruses could be achieved with a proper set of monoclonal antibodies. This topic became the subject of his PhD thesis "Studies of the Antigenic Relationships of Rabies and Rabies-Related Viruses using Anti-Nucleoprotein Monoclonal Antibodies". For these studies he established contacts with foreign rabies laboratories and collected Lyssavirus isolates from all around the World. A particularly good cooperation developed with John Bingham in Harare, Zimbabwe and with Courtney Meredith at the Onderstepoort Veterinary Institute. He visited the Onderstepoort rabies laboratory several times. That is when he discovered his love for Africa. No wonder that he became very involved when Africans created a Southern and Eastern African Rabies Group (SEARG). The initial SEARG meeting was held in Lusaka in 1992 and then every other year in a different place in the region. International Organizations and commercial companies sponsor the meetings. Arthur King and South Africa's late George Bishop became the main solicitors for the group and the principal conference organizers. For the last three years Arthur has been the chairman of the group. As a "rabies ambassador" he visited most of the countries of Southern and Eastern Africa. For SEARG and WHO he explored the state rabies surveillance and control of the region. Though he recounted with great enthusiasm his observations, he also made no secret of his frustrations. It irritated him greatly that he did not succeed to convince international organizations that rabies is a much bigger problem than officially recognized and that resources for alleviating the problem were incorrectly distributed.

Her Majesty the Queen of England made Arthur King a member of the Imperial Service Order. He was immensely proud of this accomplishment. But his description of the memorable bestowment ceremony in Buckingham Palace abounds of hilarious comments about the nervousness of other candidates, his own anxiety and the creepy feeling when he discovered an infinite number of Arthurs in the wall-to-wall mirrors of the Palace bathrooms.

Arthur was indeed an extraordinary storyteller. He entertained us for hours with funny anecdotes about his visits to South America and Africa and his tutoring of students from foreign countries. He was a keen observer and quickly noticed unusual and funny aspects of human behaviour. And he also laughed about his own mistakes.

He did visit Canada again, more than once and even in winter. He visited our lab, and we went downtown for sightseeing. He perplexed laboratory technicians and waitresses alike with his thank-you: "Your kindness is only exceeded by your personal beauty and charm." I like to modify Arthur's statement only slightly to describe himself: He was not only intelligent, clever, witty and charming, he also was a personification of kindness and generosity.

TRIBUTE TO THE LATE GEORGE BISHOP

J. Godlonton[1]

George was appointed to the post of permanent secretary of SEARG in 1992 in Lusaka and was an extremely active member of the organising committees of the four subsequent SEARG Congresses held in 1994 in Harare, in 1996 in Zimbabwe, in Nairobi in 1997 and Entebe in 1999 and Malawi in 2001. George's dedication and efficiency played a major role in the success of all these congresses.

Included in aspects concerning his professional career attention was drawn to some of Georges many professional achievements which included:

1. His work in two of his major subjects, the diagnosis and epidemiology of Rabies and Brucellosis.
2. His role as secretary of the National Rabies Advisory Board and the Kwa Zulu Natal Rabies advisory group.
3. His major role as Secretary/Editor of the new South African Rabies Guide for the medical and veterinary professions.
4. His production of educational videos for the public, medical and veterinary professions
5. He wrote fifteen scientific articles for Veterinary and other journals.
6. He wrote five chapters for the textbook "Infections, Diseases of Live Stock in Southern Africa".
7. He attended two WHO meetings in Geneva, delivering papers on Human Rabies post exposure treatment and Oral Vaccination in dogs.
8. He was the South African representative on Rabies at congresses in San Diego and Vietnam
9. He was presented with Honorary Life membership of the South African Veterinary Association in 1988.

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HISTORY.

Born in the Western Cape Province in the little town of Malmsbury on June 13th 1943. His father Dr Burny Bishop a Veterinary moved to P.M.B in 1947 and took up a post at the Natal University as a lecturer in animal diseases. George spent the rest of his life in P.M.B. where he went to school and then to the University of Natal where he completed a degree (BSc) in Microbiology. He joined the staff of Allerton Provincial Veterinary Laboratory in 1968 and remained on the staff until his death.

He completed a Masters degree in Microbiology in 1972. At the time of his death George was the Chief Veterinary Researcher, in charge of the Rabies Unit and the Epidemiology & Training unit at Allerton. George married a farmer's daughter, a Pietermaritzburg trained nurse, they had two sons Michael (an attorney) and David (a doctor).

George's non-working life centered around his home and family primarily and his friends. He relaxed with his carpentry (competent) and his gardening (not competent) he enjoyed trout fishing and was an arid and well-informed follower of cricket and rugby. He went to movies seldom (and fell asleep often). He disliked dancing (it made him dizzy) and snorkelling (it filled his lungs with sea water). He went to bed early and got up earlier and went to work. He always has two tins of tomato juice on an aeroplane trip with salt, pepper, lemon, ice and Worcester sauce stirred not shaken. He never failed to take a

[1] Edendale Hospital - 76 Desmond road – Scottsville - Pieternmaritzburg 3201 - SOUTH AFRICA

bottle of Jack Daniels Whisky on any trip we did - (knock on the door – Jacks here he wants to see you). He smoked far too much and when he tried to stop by changing to a pipe he displayed an amazing dexterity by driving at 130 km/hour filling and lighting his pipe with one hand and pointing at a giraffe in the bushes with the other.

I was with George in the I.C.U. 8 hours before he died, he had regained consciousness, was paralysed on one side had no complaints about himself was concerned about Margaret being on her own and the poor quality of the TV set on which he will have to watch a rugby match the next day.

THE CHAIRMAN'S REPORT

R. X. Dlamini[1]

Honourable Minister of Agriculture and Cooperatives and your entourage, distinguished delegates, sponsors, ladies and gentlemen once again lend me your ears.

I stand in front of you with a sad bright face. I am sad because of the loss of the two esteemed scientists Arthur King and George Bishop. As mentioned in their tribute the two were actually the SEARG executive committee, chairman and secretary respectively for many years and I had really grown fond of them.

My face is bright because I realize the effort and contribution of Arthur, George and others in founding SEARG.

Let me take you back to the inception of the Southern and Eastern African Rabies Group (SEARG)

SEARG was founded at a gathering of rabies scientists, diagnosticians and policy makers in Lusaka, Zambia in 1992.

It was founded as a forum for gathering and disseminating rabies information and its objectives included:

1. To engage the different countries in the group and influence their rabies control policies to evolve taking into account latest information and technology on the disease.
2. To encourage members to conduct adaptive research and use of rabies control strategies used successfully in other parts of the world.
3. To solicit funds to be used in conducting rabies control/eradication projects.
4. To critically analyze rabies control/eradication programs in the Southern and Eastern African Regions and come up with innovative ways of improving such programs.
5. To encourage dialogue between all scientists working on rabies especially between medical and veterinary scientists.

SEARG is the forum that brings together researchers, policy makers/implementers, non governmental organizations and international organizations working on rabies. In fact in 1997 SEARG became permanently linked to WHO such that it is now known as SEARG/WHO.

Membership and presence of well renowned rabiologists in our conferences gives better quality and direction to the organization and its endeavours.

The teamwork and spirit of selflessness and cooperation has enabled SEARG to timeously produce the proceedings for previous conferences, which are a wealth of information on rabies.

Beyond just producing proceedings, SEARG realized the need of harmonizing rabies diagnosis in all its member countries and embarked on a project of producing a diagnostics manual. We are really grateful to Dr Jacques Barrat for patiently and successfully editing the proceedings and manual, both of which will be distributed before the end of this conference.

SEARG is unique in the sense that membership is open to anyone who is interested in the fight against rabies. The focus is and should remain to be the control/eradication of rabies and protection of both human and animal life. However, time has come for SEARG to go beyond the mutual understanding and have a written constitution that will establish it legally and guide its daily activities.

We owe our success to the generous sponsors, especially the recognition, moral and financial support from WHO. We are also greatly indebted to Chiron vaccines for not only financing research through the Chiron vaccine research award but also contributing generously towards the conferences.

[1] PO box 30 - Manzini - SWAZILAND

Amongst other sponsors we would like to mention CDC, Virbac, Fondation Mérieux, IDT, Intervet and Mérial whose financial contributions made this conference possible. Last but not least we would like to thank the Swaziland Government for hosting this conference and sponsoring the local delegates.

May I also take this opportunity to thank Prof. Louis Nel, Dr. Paul Kloeck, the RSA Rabies Advisory Group and many others who contributed in forming the interim committee that organized this conference. I would also like to thank the interim secretary, Claude Sabeta, for rising up to the challenges of fund raising. SEARG is also indebted to the University of Pretoria for managing the conference finances and to Wanda Markotter for all travel arrangements.

I thank you.

OPENING SPEECH

S. Lukhele
Hon. Minister for Agriculture and Cooperatives

Chairperson, Principal Secretary, Director of Veterinary and Livestock Services, distinguished guests and delegates, Ladies and Gentlemen.

I thank you for this opportunity to officiate at the opening ceremony of this historic 8th conference of the Southern and Eastern African Rabies Group (SEARG) in collaboration with the World Health Organisation (WHO). We indeed feel very honoured to be chosen to host this conference.

A very warm welcome to all the delegates and observers present here today. We are conscious that you left your respective countries and organisations to make this conference a success. I am sure you have all come with a single mind and purpose to find lasting and sustainable solutions and strategies in the fight against the rabies scourge in our countries.

THE PROBLEMS.

Amongst the notable zoonotic diseases, rabies still remains one of the most fatal ones. The rabies problem in its various forms has a long history of survival in many of our countries.

It is incredible how the rabies pathogen has succeeded in defying all odds to survive in our environment as observed in the past years in spite of the vaccination efforts exerted against it. Noteworthy and somehow ironic is the fact that man's old companion, the dog, continues to be the main host of the pathogen and is responsible for most of the rabies outbreaks reported in Swaziland.

I noticed that one of the objectives of SEARG is to encourage and facilitate interactive dialogue amongst scientists. Successful containment and eradication of the rabies scourge requires active and continuous collaboration of the medical and veterinary professions, the pharmaceutical industry and other support services. To enhance such collaboration in Swaziland, a zoonotic disease committee shall be formed comprising both veterinary and medical professionals.

The general failure in harmonisation, collaboration and the coordination of all the efforts of stakeholders in member countries can be said the root course responsible for perpetuating the continuous vulnerability of the general public against this zoonotic disease. It is therefore expected that the Zoonotic disease committee will play a vital role here.

It is also noteworthy that in some cases, the problem arises from inappropriate policies failing to enhance and harmonies the activities of the public and the private sector in supporting control strategies against the disease.

1 CHALLENGES.

It is quite obvious therefore that developing countries are still faced with enormous challenges:

1. The cost of diagnosis, surveillance and the vaccination of domestic animals as well as the human victims given the increasing budgetary constraints faced by our government.
2. The monitoring of successful campaigns with the strong support and cooperation of all stakeholders still remains elusive.
3. Emerging disease of economic importance also competing for the scant recourses that most of our governments cannot afford to provide.
4. The apparent warning public interest and funding support in finding lasting solutions to the rabies problem particularly through research.

I am however encouraged to note that Chiron vaccines has now taken the initiative in funding research aimed at reducing the incidence of human rabies in the region.

The serious challenge here is we cannot simply wash away the rabies problem and its challenges. We cannot turn a blind eye neither as the disease may soon grow out of proportion with more animal and human lives lost. No doubt the costs will also grow with the problem when effective prevention and control is far better than cure. It is therefore necessary that clear budget lines for rabies control are made distinctively clear in our national budget.

5. Solutions must also be found in arousing stakeholder interest, support and cooperation. Many members of the public still think of rabies as only a joke and an animal disease because they have not seen the violent and painful death it causes to both animals and men. Awareness campaigns must include audio-visual aids at local and national levels.
6. In many instances reports will say that vaccination coverage is 70 or 90% in a given country. The question is how realistic are our dog census or estimates? What about the many stray dogs that roam our cities and rural areas? Do we have clear identification marks that assure us of vaccinated dogs annually?
7. What about the myths that are quite common in some of our countries that vaccinations render the dogs useless? As a result many people will quite often refuse to cooperate simply because of a mental blockage associated with such myths.
8. How many people will die simply because, in some instances, the medical profession treats rabies only as a veterinary problem instead of a serious zoonotic disease? How many cases of human rabies with loss of life will go unrecorded because medical personnel refuse to handle a dog-bite victim for relief or fear that they too may be exposed through contact or otherwise with such a victim?

Mr. Chairman, ladies and gentleman, I know the list of the rabies challenges that confront us is endless. But these are only a few that I find sometimes worrisome with potentially grave consequences.

I am sure that during your deliberations, you will find some answers to these questions and challenges so that a layperson on these issues can at least find some peace that all is under control.

2 THE OUTCOME.

I am aware that in previous conference you have deliberated extensively on the critical role played by other disease control measures as oral vaccinations in wildlife, the development and application of thermostable rabies vaccines and the privatisation of the delivery of veterinary services against rabies.

I have no doubt that these, in many member countries, have however been helped through participation in previous SEARG meetings to sharpen their knowledge and to improve in rabies control strategies. However, allow me to say that. Let the quality of this conference be measured largely by what the outcome will be in terms of the viability and feasibility of the final conclusions and or solutions that you will recommend.

3 CONCLUSION.

Mr. Chairman, ladies and gentleman, I am aware that you have a very tight schedule ahead of you. But allow me, in true Swazi culture and tradition, to encourage you all to find time to explore our renowned heritage and cultural sites and sample, our unparallel Swazi hospitality.

In the same vein let me now thank most sincerely, the tireless efforts and work of the SEARG organising committee in making sure that this conference takes place. I am reliably informed that SEARG has no basic source of funds. It is only through the assistance of those who believe in the SEARG vision for the complete control and eradication of the rabies scourge that they have managed so far.

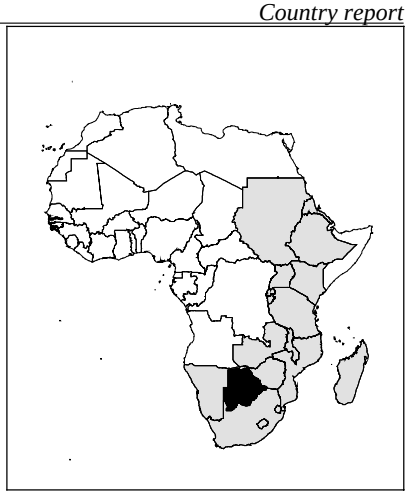
Special gratitude is therefore extended to all those organisations, companies and institutions that made financial contributions towards this conference.

Last but not least I want to thank all the delegates and resource persons who have made this conference possible through their presence and the national organising committee and all who have contributed in different ways to the success of this conference.

Mr. Chairman, it is my singular honour and privilege to declare this 8th conference of SEARG/WHO officially open.

THANK YOU.

Country reports



RABIES IN BOTSWANA

M. Letshwenyo[1]

1 INTRODUCTION.

Rabies is an acute viral infectious disease common to all warm-blooded animals including man due to a rhabdovirus. It is invariably fatal and is usually transmitted by a bite from an infected animal. It occurs in most parts of the world with only a few countries free from the disease and these include Japan, Australia, New Zealand, UK, Iceland, etc…

Rabies manifests itself in most cases by a change in animal behaviour; the friendly dog may become irritable, the wild cat may become affectionate. Wild animals enter villages and even houses apparently without fear. In so called "furious rabies" the animal is restless, runs wildly about and may bite without warning and chew and swallow wood, stones and soil. In "dumb rabies" the animal becomes progressively paralysed but bites if provoked. Inability to swallow causes drooling of saliva that is different in its aetiology from the hydrophobia that may be observed in rabid people. Finally the animal goes into convulsions followed by coma and death.

Rabies is tentatively diagnosed from the field on the basis of history and clinical signs, and confirmed by the Fluorescent Antibody Test (FAT) and Mouse Inoculation Test (MIT) in the laboratory. Animals suspected to have died of rabies but are negative on FAT and further subjected to the MIT.

When a suspect animal is unavailable for sampling (e.g. killed, burned and buried by farmers), but with a history indicative of rabies, a "Rabies Notification" is made and all procedures that normally apply to rabies positive animals are evoked.

2 RABIES IN ANIMALS.

2.1 Epidemiology of animal rabies in Botswana.

There are two interrelated cycles, urban and sylvatic, in which rabies is maintained. The urban cycle is predominantly dependent on domesticated and stray dogs and cats. The sylvatic cycle poses a different problem. Rabies outbreaks have been recorded for ages and seem to centre on certain vectors, usually one, two or more carnivorous species in each particular location. In Botswana the black backed jackal and the mongoose act as the main reservoir in the sylvatic cycle. The domestic animals such as cattle, sheep, goats, dogs and cats due to their close proximity to man are the main risk for transmission of the disease to man.

In Botswana rabies is commonly confirmed in cattle, sheep and goats in 76.21% of total cases, domestic dogs and cats contribute 10.92%, wildlife represent 10.19% and others 2.67%. The disease is endemic in the country and causes economic losses to farmers due to losses in deaths.

[1] Ministry of Agriculture - Department of animal health and production - Private Bag 0032 – Gaborone - BOTSWANA

Of major concern are the human exposures to rabies. These are in the form of bites from dogs, cats and wildlife and also contact with dead food animals at cattle posts where people may handle and even eat meat from atypically rabid animals.

2.2 Temporal distribution of rabies.

Table 1: distribution of rabies cases across species (1997-2003).

Species	1997	1998	1999	2000	2001	2002	2003	TOTAL	Rel. Freq. (%)
Bovine	63	115	55	49	39	28	6	355	43.08
Caprine	50	50	59	33	28	20	2	242	29.37
Ovine	3	1	26	0	0	1	0	31	3.76
Dog	28	24	18	8	2	3	0	83	10.07
Domestic cat	1	2	3	0	1	0	0	7	0.85
Horse	2	1	2	0	0	0	0	5	0.61
Donkey	3	7	3	2	1	1	0	17	2.06
Wildlife	17	27	26	7	3	4	0	84	10.19
TOTAL	167	227	192	99	74	57	8	824	100

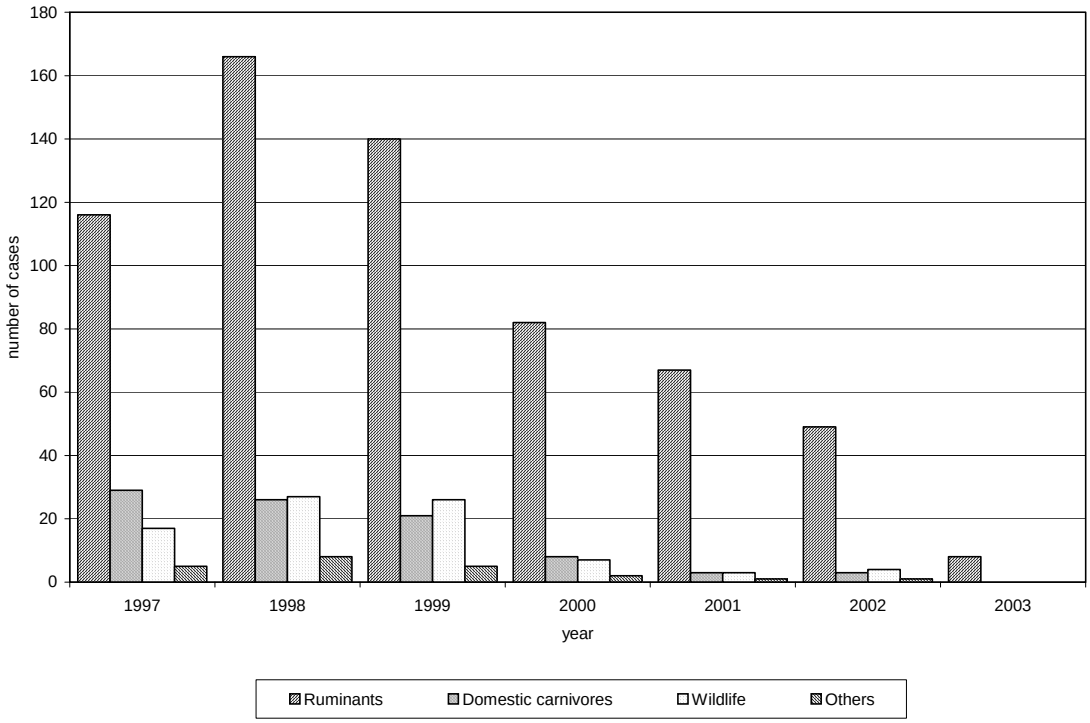
Table 2: distribution of rabies cases across broader species classification (1997-2003).

Species	1997	1998	1999	2000	2001	2002	2003	TOTAL	Rel. Freq. (%)
Ruminants	116	166	140	82	67	49	8	628	76.21
Dom. carnivores	29	26	21	8	3	3	0	90	10.92
Wildlife	17	27	26	7	3	4	0	84	10.19
Others	5	8	5	2	1	1	0	22	2.67
TOTAL	167	227	192	99	74	57	8	824	100

Table 3: rabies cases in wildlife species (1997 - 2003).

Species	
Jackal	64
Mongoose	5
Genet cat	5
Duiker	2
Hyena	2
Bat eared fox	1
Springbok	1
Reedbuck	1
Honey badger	1
Unspecified wildlife	2
TOTAL	84

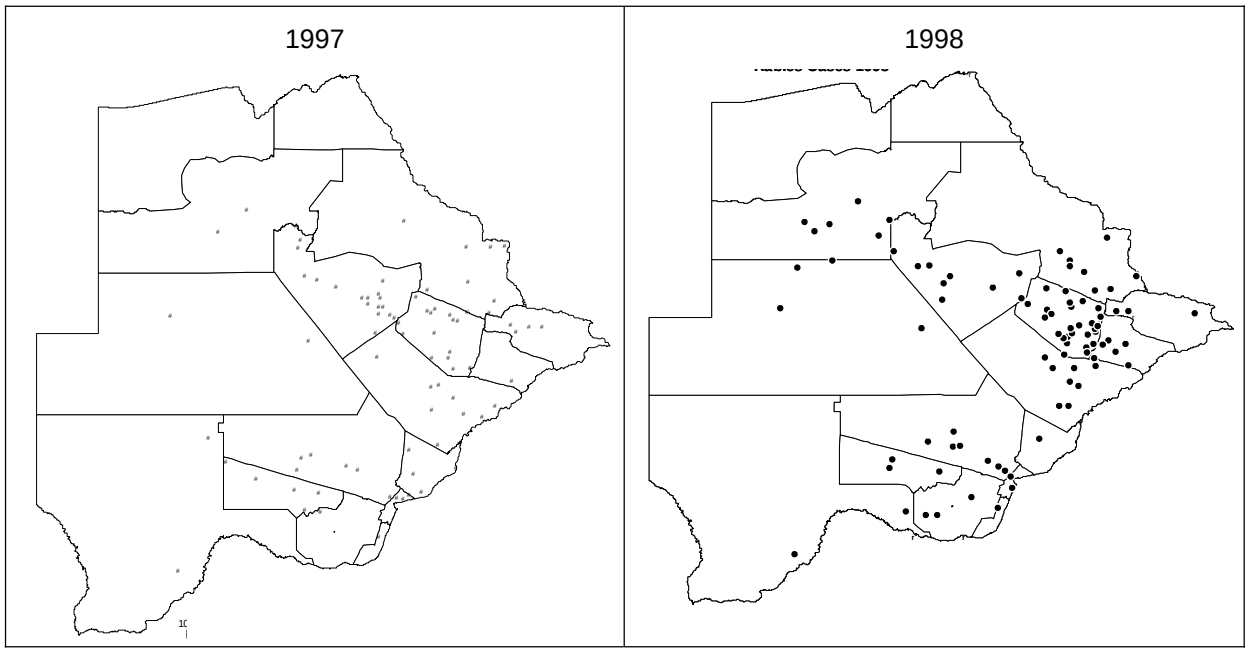
Figure 1: Confirmed rabies cases by group of species from 1997 to 2003.

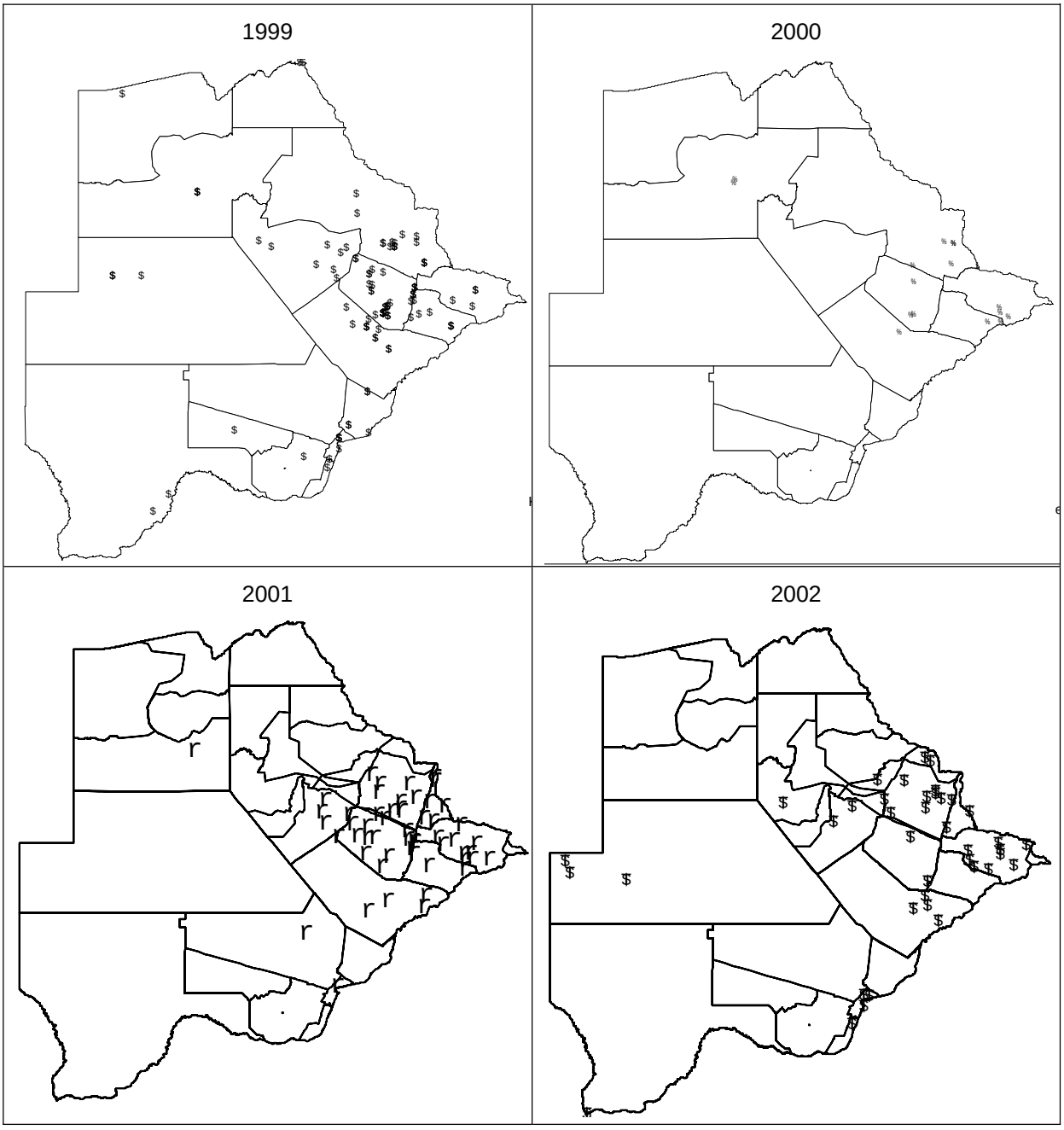


2.3 Spatial distribution of rabies.

The disease occurs throughout the country, following the distribution geographical distribution of hosts.

Figure 2: Spatial distribution of rabies 1997 – 2003.

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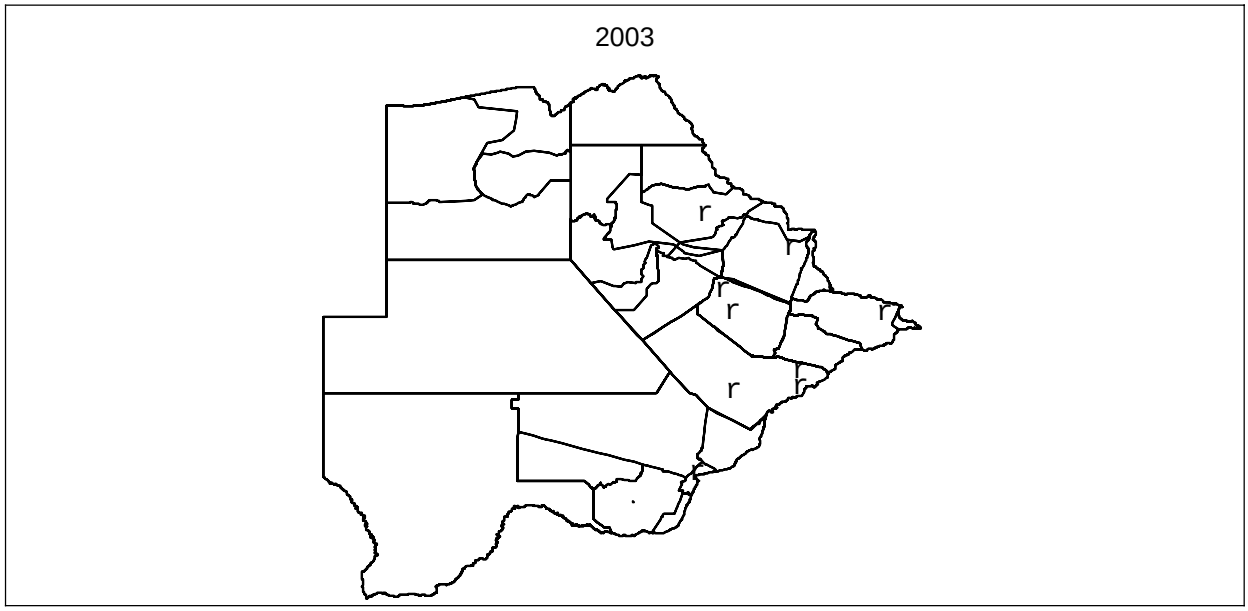
2000
2001
2002



3 RABIES CONTROL IN BOTSWANA.

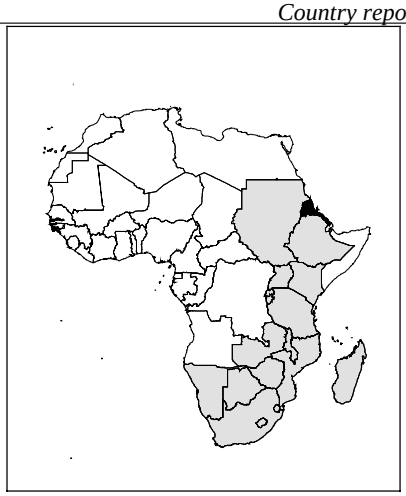
Due to the public health and economic importance of rabies, the government of Botswana has in place a rabies control program to prevent the spread of the disease between man and animals. The following activities are carried out for this purpose:

The government protects the public by carrying out:

- Vaccinations for all dogs and cats above the age of three months for rabies and every 12 months thereafter. This is compulsory and done at no cost to pet-owners.
- Diagnostic services of the disease in animals and man.
- Public education about the disease
- Population control of the reservoir in the wild
- Undertaking movement control of dogs and cats and hence permits are required for all the movements.
- Destroying all stray dogs and cats during "tie up" order declared following each annual vaccination campaign
- Destroying stray and unvaccinated dogs.

4 CONCLUSION.

Rabies is a fatal zoonotic disease and as such, it requires to be tackled from both the animal and human health fronts. The current trend in Botswana where the disease occurrence in food animals is very high is worrisome and calls for more applied research to understand its epidemiology: given the relatively low and declining prevalence in domestic carnivores (urban reservoirs), is it being spread to food animals by wildlife reservoirs or not?, if it is, what intervention measures can be deployed?- oral vaccines for wildlife?…



RABIES IN ERITREA

T. Tekleghiorghis[1], T. Yosief[1]

1 INTRODUCTION.

Eritrea is strategically located in the north-eastern part of the continent. It is bounded by the Red Sea on the east with about 1000 km of coastline, by the Sudan on the west and north, by Ethiopia on the south, and by Djibouti on its south-eastern extreme. It has an area of about 124500 km^2 and a population estimated between 3 and 3.5 million, which is growing at about 2.9% per annum.

The country is administratively divided into six regions. Population density is high in the central highlands but low in the lowlands.

The Veterinary Services Division carries out all animal health activities in the country.

2 ANIMAL RABIES.

Rabies is among the most important zoonotic diseases (brucellosis, tuberculosis, anthrax, echinococcosis, etc.,) in the country. Rabies is endemic in the urban and peri-urban areas where it is maintained in a dog-to-dog cycle, hence is of the urban type. Dog rabies is a serious problem, marked by significant animal and human mortality. Dogs are kept to protect the owner and his property. To some extent there could be a limited role of wild carnivores in maintaining rabies virus infection but this has not been studied.

2.1 Methods of diagnosis.

Fluorescent antibody test (FAT) had been used as a routine test in the Central Veterinary Laboratory (CVL) from 1996 to 2001 using the Centocor FITC conjugate. Mouse inoculation test had been also used for negative results. Standard operating procedures are essentially similar to those described by D. J. Dean *et al.* and H. Koprowski, (1996) Laboratory Techniques in Rabies, WHO, Geneva. Since 2002 there was no suitable conjugate and FAT tests were not used as the Centocor FITC conjugates are out of product.

Table 4: number of rabies cases in domestic animals in Eritrea between 2001 and 2002.

Species	2001	2002
Dogs	15	23
Cattle	15	63
Equine	11	41
Sheep and goats	-	70
TOTAL	41	197

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2.2 Rabies control strategies.

In Eritrea the dog population is estimated to be about 50000.

The following rabies control strategies are practiced in the country:

- Stray dog elimination
- Immunisation of dogs, cats and other domestic animals at risk
- Laboratory diagnosis to confirm clinical cases
- Public awareness to cooperate in the control programmes.

The control strategy is divided into two:

a) **Urban rabies:** dogs in the cities and towns will be registered, vaccinated and tagged; stray dogs are also eliminated. Dog owners are charged for the vaccine.

b) **Rural Rabies:** disease surveillance, vaccination and dog elimination programmes are practiced. Cost recovery policy has been introduced, hence farmers are charged for cost of vaccines in the control programmes.

Table 5: vaccinated and destroyed dogs between 2001 and 2002.

Year	Vaccinated dogs	Destroyed dogs
2001	6051	753
2002	919	1803
TOTAL	6970	2556

The type of vaccine being used in animals is Rabisin. The pattern of dog-mediated rabies cases is increasing as vaccine costs has been increased and especially dog owners in the urban and rural areas are reluctant to vaccinate their dogs. The liaison between the medics and veterinarians concerning rabies disease is very loose. The situation of human rabies is not clearly known. There are human rabies cases in most of the regions of the country, but not properly reported. Most human rabies cases are underreported as patients leave hospitals or health centres after they develop clinical signs of the disease.

Table 6: number of reported exposures in humans bitten by rabid dogs between 1998 and 2002.

Year	Reported exposure	Rabies cases	Deaths*
1998	1488	48	Unknown
1999	906	16	Unknown
2000	701	20	1
2001	1368	19	3
2002	1212	8	1
Total	5675	111	5

* Underreported

Source: Ministry of Health

The human diploid cell culture vaccine (IMOVAX, Pasteur Mérieux) is used in pre-exposure vaccination and post-exposure treatment for human beings.

3 FUNDING.

No earmarked budget has been allocated for rabies control. All vaccines, diagnostic reagents and other expense costs are covered from the Government budget on a cost recovery base. There is an urgent need for an input by the World Health Organization (WHO) and other Funding Organizations for the success in the eradication programmes of rabies in the country.

ACKNOWLEDGEMENT.

To the SEARG Organizing Group and the Ministry of Agriculture of Eritrea for organizing this meeting.

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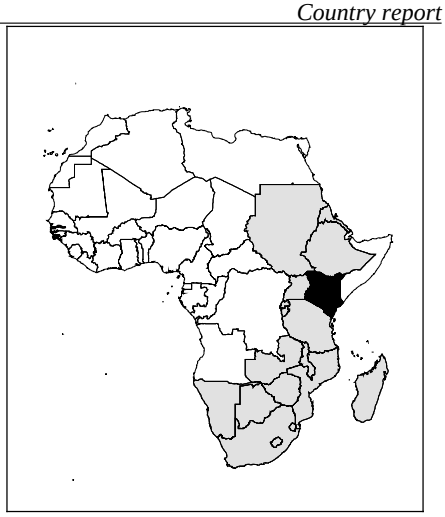


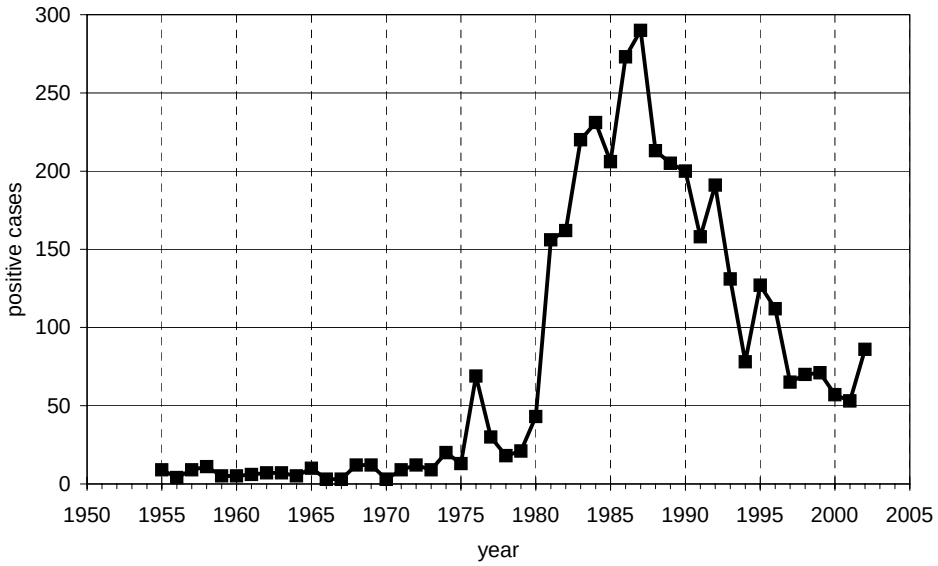
STATUS OF RABIES IN KENYA 1998 - 2002

Macharia M.J.[1], Ombacho, K.M.[2], Kasiiti J.L.[1], Mbugua, H.C.W.[1] and Gacheru, S.G.[1]

1 INTRODUCTION.

The first report of rabies in Kenya was in South Nyanza in 1902, but the first confirmed case was in 1912 in a dog which was previously involved in a fight with a jackal in the outskirts of Nairobi (Hudson, 1944). Rabies epizootics were reported in the 1930s and 1940s. Usage of locally manufactured Low Egg Passage (LEP) dog vaccine in mid-1950s and 1960s, virtually eliminated the disease by 1973 (Figure 3). However, two outbreaks in Taita/Taveta (1974) and Transmara (1979) districts led to wide-spread distribution of rabies within the country (Kariuki, 1998). The increase in rabies incidence after 1980 should be interpreted cautiously since it may be confounded by factors such as an increased level of surveillance and improved diagnostic capabilities (Perry, 1992). The decreased incidence after 1987 could also be influenced by several factors, which include improved application of control strategies or a deteriorating level of surveillance. The disease is currently endemic in all administrative districts. It is a "notifiable disease" (Chong, 1993) and majority of the cases have been reported from dogs (Binepal *et.al.*, 1992).

Figure 3: positive rabies diagnoses between 1955 and 2002.



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2 DIAGNOSIS.

The diagnosis of rabies is done on the basis of clinical signs observed in animals and man. Laboratory confirmation is done at the Central Veterinary Laboratories at Kabete and at the Regional Veterinary Investigation Laboratories at Mariakani using brain material. Laboratory tests used are the Fluorescent Antibody Test (F.A.T.) and Mice Inoculation Test (M.I.T.) as described by King (1995). M.I.T. is carried out for all samples negative on F.A.T. Live rabies suspect animals are kept under isolation in specially built kennels for observation.

3 HUMAN RABIES.

The epidemiology of human rabies in Kenya is not clear. Human cases are diagnosed on the basis of history of a dog bite and the presenting symptoms. The last suspect human rabies case which was referred to the laboratory for confirmation was in 2001, and before this, in 1996. Also, the number of unconfirmed clinical cases cannot be estimated accurately. However, the number of laboratory confirmed human rabies cases prior to 1997 are as shown in Table 7.

Table 7: positive human rabies cases - Central Veterinary Laboratories, Kabete, 1991-1996

Year	Cases
1991	45
1992	31
1993	49
1994	1
1995	1
1996	1

Some work done at the Nairobi Hospital in 1988 estimated some 200 fatal human rabies cases per annum. In a dog ecology study in Machakos district between 1982 and 1987, Kitala *et.al.* (1993) found a human dog bite prevalence of 40 per 100000 people in a year. In a population where the dog:person ratio was 1:9.6, unprovoked bites were 50% (21% household dogs; 29% unknown dogs). WHO studies have estimated Kenya as having about 11 cases per million people. With Kenya's total human population estimated in 1999 to be 30 million, the above implies that the risk of human rabies is great in Kenya.

Mwingi district, with a human population of 303829 (1999 census), is among the districts with high reported incidence of human-animal bite cases. In 2002, this district reported 401 cases of human-animal bites, 132 of which were in males, 269 in females and 180 in youngsters aged below 18 years of age. 390 (97.3%) of these cases were dog bites. It therefore seems that females are more susceptible to animal bites than men, probably since culturally, there is close association of women in this community with domestic animals. They also fetch water in rivers far from home and look for firewood far in the bush, thus becoming more predisposed to dangers. In this district, communities in the north and who have a nomadic way of life are more prone to animal bites. There are more cases reported during dry months than during wet months, probably because, during the wet months, people are busy in their shambas and this limits the time spent with animals. 98 (24.4%) patients did not complete their full dose of treatment and were classified as defaulters. Defaulters mostly cite un-affordability of the anti-rabies vaccine as the reason for absconding. 4 deaths were recorded, 3 in the district hospital and 1 while being attended by a traditional herbalist.

4 ANIMAL RABIES.

Rabies remains endemic in all districts of Kenya. During the last five years (1998 – 2002), 560 samples were submitted to the laboratories and some 337 (60.2 %) were confirmed positive. This translates into an average of 67.4 positive cases being diagnosed each year, down from the annual average of 82 positive cases reported previously (Macharia *et al.*, 2001). The number of confirmed cases during this period shows a wide geographical distribution (Figure 5) and a wide range of affected species (Table 8).

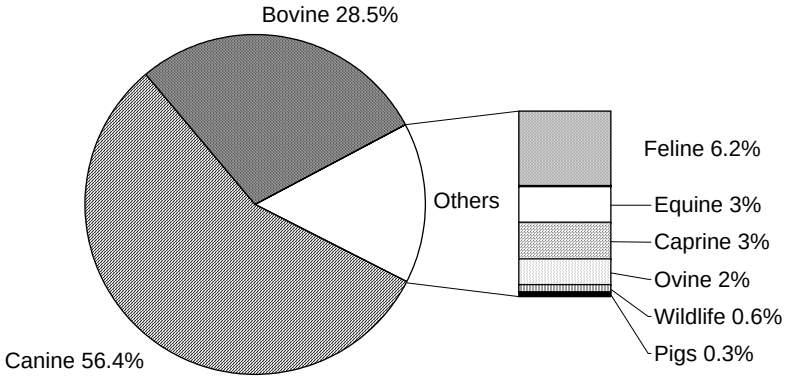
Table 8: positive cases according to species, 1998 – 2002

	1998		1999		2000		2001		2002		TOTALS	
	Pos	**Total**	**Pos**	**Total**	**Pos**	**Total**	**Pos**	**Total**	**Pos**	**Total**	**Pos**	**Total**
Dogs	42	50	41	55	28	61	27	56	52	76	190	298
Cattle	16	19	19	24	18	24	19	28	24	31	96	126
Cats	7	9	4	6	5	5	3	7	2	3	21	30
Equine	1	3	4	4	2	3	1	2	2	4	10	16
Goats	1	2	1	2	2	2	3	5	3	3	10	14
Sheep	2	2	0	0	2	2	0	2	3	5	7	11
Wildlife	0	4	2	3	0	2	0	12	0	41	2	62
Pigs	1	1	0	0	0	1	0	0	0	0	1	2
Human	0	0	0	0	0	0	0	1	0	0	0	1
TOTAL	70	90	71	94	57	100	53	113	86	163	337	560

Pos = Positive cases

The disease is most prevalent in dogs (56.4%), followed by cattle (28.5%), cats (6.2%), equines (mainly donkeys – 3.0%), goats (3.0%), sheep (2.1%), wildlife (0.6%) and pigs (0.3%) in decreasing order, the dog being the primary source and vector (see Figure 4). The wildlife involved are mongoose, squirrel, wild dog, hyena, wild cat, civet cat, hedgehog, honey badger, hare, mice, bat, leopard, lion, monkey, wild fox and jackal.

Figure 4: positive rabies cases by species, 1998 - 2002.



Ruminant rabies was concentrated in Central, Rift Valley (Nakuru, Uasin Gishu, Kericho districts) and Nairobi Provinces (Figure 5). Analysis of bovine cases of rabies indicated an incubation period of 18 days (range 12-28) from the time the animal was bitten by a rabid dog. Once the clinical signs were exhibited the disease lasted 6 days (range 2-14) before the animal died.

In the year 2002, a total of 163 cases were submitted for diagnosis as compared to 113 cases in 2001, 100 cases in 2000, 94 cases in 1999 and 90 cases in 1998 (Table 8). In 2002, positive canine cases were 52 out of a total of 76. This was followed by bovine (24/31 cases), ovine (3/5 cases), caprine (3/3 cases), feline (2/4) and 2 positive cases from equine species. Nairobi (Figure 5) reported the highest number of positive cases (37/58) followed by Nakuru (15/58), Kiambu (8/12), Nyeri (5/6) and Machakos districts (4/5).

In 2001, positive canine cases were 27 out of a total of 56 (Table 8). This was followed by bovine (19/28 cases), feline (3/7 cases), caprine (3/5 cases) and 1 positive case from equine species. Nairobi reported the highest number of positive cases (19/42) followed by Kiambu and Nakuru districts (6 cases each) and Uasin Gishu district (4/12).

Figure 5: districts with confirmed rabies cases for 2001 and 2002.

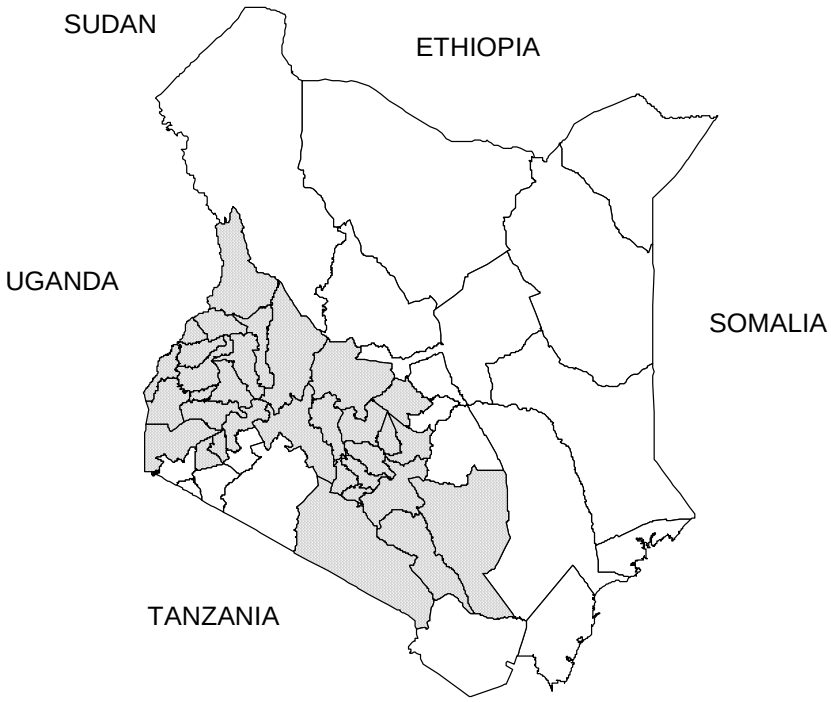


Table 9 shows that, in 2001, Regional Veterinary Investigation Laboratories submitted the most samples for rabies diagnosis (26/113) followed by Government veterinarians (20/113) and the Kenya Society for the Protection and Care of Animals (KSPCA – 15/113). However, most of the samples submitted by government veterinarians (60.0%) turned out to be positive (Figure 6) possibly indicating a better recognition of the clinical signs of rabies. They were followed by Regional Veterinary Investigation Laboratories (53.8%) and KSPCA (53.3%).

Table 9: source of samples for laboratory diagnosis, 1998 – 2002.

Source	1998		1999		2000		2001		2002	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
District Veterinary Office	17	0	11	4	10	8	12	8	16	9
R.V.I.L.	18	7	11	4	19	7	14	12	20	4
K.S.P.C.A	9	3	26	6	15	11	8	7	25	13
Farmers	15	4	15	3	7	8	10	4	9	5
University of Nairobi	5	0	1	0	1	0	6	8	2	1
Private veterinarians	5	3	7	6	5	8	3	9	13	4
Researchers	1	2	0	0	0	0	0	10	0	40
Police	0	1	0	0	0	1	0	0	0	0
Public Health Dept.	0	0	0	0	0	0	0	1	1	1
Kenya Wildlife Service	0	0	0	0	0	0	0	1	0	0
TOTAL	70	20	71	23	57	43	53	60	86	77

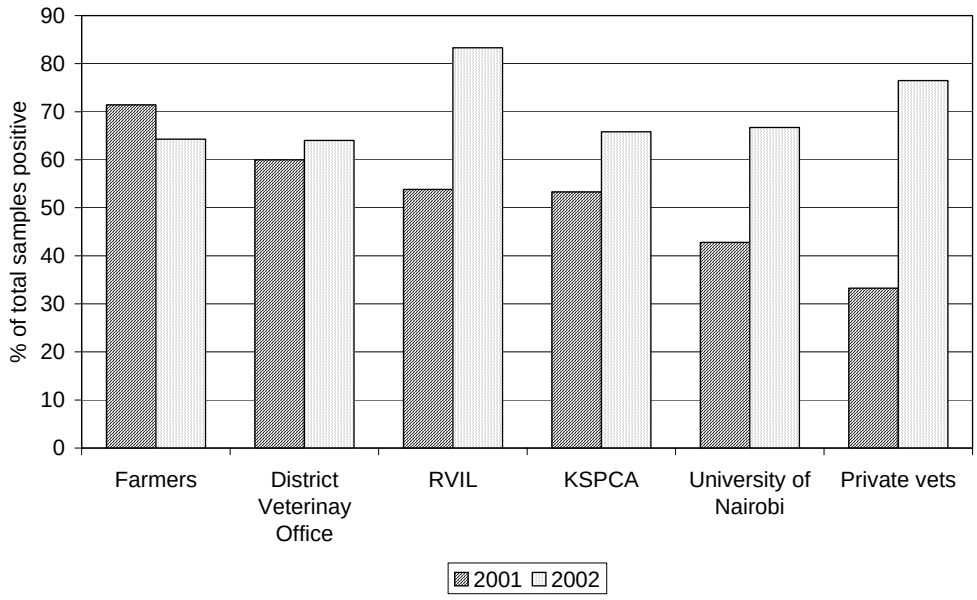
R.V.I.L = Regional Veterinary Investigation Laboratory
K.S.P.C.A. = Kenya Society for Protection and Care of Animals
Pos = Positive
Neg = Negative

In 2002, the Kenya Society for the Protection and Care of Animals (KSPCA – Table 9) submitted the most samples for rabies diagnosis (38/163) followed by the Government-employed veterinarians (25/163) and the Regional Veterinary Investigation Laboratories (24/163). However, most of the samples submitted by Regional Veterinary Investigation Laboratories (RVIL-83.3%) turned out to be positive (Figure 6). They were followed by private veterinarians (76.5%), University of Nairobi (66.7%) and KSPCA (65.8%). In both 2001 and 2002, most of the cases indicated as submitted by farmers were actually submitted by veterinarians who recorded them in the owner's names or they were ownerless dogs found dead but recorded using the names of the people who sent them to the laboratories.

The details of results of previous years is covered in reports published in the Proceedings of Southern and Eastern African Rabies Group (SEARG) meetings held in Zambia, South Africa, Zimbabwe, Kenya, Uganda and Malawi.

During the period 1989-1995, 2257 samples were submitted with a positivity of 46.9% and an average of 322 cases per year, while the period 1996-2002 had 794 cases submitted with 65.1% positivity and an average of 113 cases per year. A comparison between these two periods shows that, in the 1996 - 2002 period, there was a more thorough field clinical appraisal as to various differential diagnoses before samples were dispatched for confirmation.

Figure 6: Rabies cases according to source.



5 RABIES CONTROL.

Animal rabies control in Kenya is carried out mainly by the Department of Veterinary Services and is governed by Animal Diseases Act (Cap 364) and Rabies Act (Cap 365) of the laws of Kenya. Private Practitioners, Kenya Veterinary Association, Kenya Society for the Protection and Care of Animals (KSPCA) and some Town Councils are involved in some control activities. Ideally, the control of rabies is done by vaccination of dogs, cats and domestic animals. The vaccination of dogs and reduction of their numbers through castration, spaying, etc... of stray dogs is the most common activity to reduce the disease spread and prevent possible transmission to humans. The overall benefit is lower than expected due to low vaccination coverage (33%), the high turnover rate for dogs (2.1 litters/bitch/year at 5.2 puppies per litter) and low funding of control measures. The actual animal vaccination figures have been declining since 1999, in proportion to the decreasing funding for rabies control. There is, therefore, an urgent need to increase financing of all rabies control activities, including vaccinations, diagnosis, epidemio-surveillance and sero-monitoring activities.

Rabies is controlled in humans through pre-exposure prophylaxis in high-risk groups and post-exposure vaccination and use of Human Rabies Immuno Globulins (HRIG). The Department of Public Health, Ministry of Health, records a usage of approximately 10000 doses of human anti-rabies vaccine annually for post-exposure immunisation of humans (Table 10). There is little usage of HRIG (Table 11), most probably due to lack of public awareness.

Table 10: annual usage of Government-procured human anti-rabies vaccine.

Year	Vaccine Name	Vaccine Type	No. of doses used
1998	HDCV	Tissue culture	4000
1999	Verorab	Tissue culture	10000
2000	Verorab	Tissue culture	20000
2001	Verorab	Tissue culture	10000
2002	Verorab	Tissue culture	10000

Table 11: annual usage of Human Rabies Immuno Globulins (HRIG)

Year	Importer	No. of doses used
1998	Rhone Poulenc	120 x 5ml
1999	-	-
2000	Aventis	40 x 5ml
2001	-	-
2002	-	-

6 CONCLUSION AND RECOMMENDATIONS.

Since rabies is widespread, the whole country is regarded as a rabies endemic area for the purposes of control. However, prevalence and incidence rates in both animals and humans are unknown. Domestic dogs remain the principal vectors for rabies. Reduction of dog rabies will greatly reduce human rabies. However, due to the unknown human incidence of the disease, rabies is ranked lower (and funded poorly) than most zoonoses of economic public health importance. Rabies control has not been very successful due to decreasing financial resources at the disposal of the Department of Veterinary Services and other veterinary service providers. The situation is further confounded by the generous presence of wildlife in Kenya and whose contribution to the rabies cycle in Kenya is not well documented. According to Rupprecht *et al.*, (1995), the epidemiology of rabies in the United States has changed substantially during the last half century, as the source of the disease has changed from domesticated animals to wildlife, mostly due to the human attraction to the recreational and economic resources provided by wildlife. This has contributed to the reemergence of rabies as a major zoonosis in the USA.

The following measures will need to be put in place to enhance the control of rabies in Kenya:

1) Studies on dog and wildlife ecology relevant to rabies spread should be carried out country-wide to provide epidemiological data on dog population and dog-human-wildlife contacts for use during vaccination campaigns. Field studies have shown that areas of high dog population densities with poor dog supervision are associated with high rabies incidence. This calls for:
    - Reduction of dog numbers through spaying, castration, etc…
    - Responsible dog ownership awareness creation
    - Dog confinement/restraint to reduce dog contacts with wildlife and man
2) Improvement of the vaccination coverage for dogs and wildlife.
3) Determination of the actual incidence of rabies in animals and especially in humans. This will require the strengthening of the liaison between the medical and veterinary professions for purposes of rabies diagnosis, surveillance and control. According to Kaboyo (1999), the strongest motivation for rabies control will come from the magnitude of the rabies burden not in animals, but in the human population.
4) Provision of adequate financial capacity to enhance the enforcement of legislation and regulations in the Rabies Act, the Rabies Ordinance, the Public Health Act and the Animal Diseases Act as it relates to Rabies Control Areas, the Compulsory Rabies Vaccination Areas, quarantine, etc…
5) Scaling up community awareness and their active participation in rabies control activities and strategies.

ACKNOWLEDGEMENTS.

To the late Drs. Arthur King and George Bishop for selflessly keeping the rabies awareness campaign and the flame of rabies control worldwide burning. To the interim SEARG committee members for rising to the occasion and ensuring that the Seventh Biennial Conference is held in a timely manner even under the difficult circumstances following the untimely passing on of Drs. Arthur King and George Bishop. To the Southern and Eastern African Rabies Group (SEARG) who made it possible for Dr. Joseph M. Macharia and Mr. Kepha M. Ombacho to participate in this conference. To the Director of Veterinary Services, Kenya, and the Director of Medical Services, Kenya, who allowed free access and use of data and records available in their respective departments. To the Director of Veterinary Services and the Director of Medical Services for granting permission for this paper to be published.

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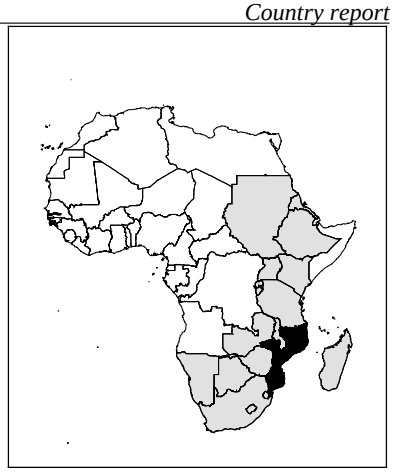
RABIES IN MOZAMBIQUE

M.P.R. Travassos Dias[1] and F. Rodrigues[2]



Rabies continues affecting humans and animals in Mozambique, as a result of low economic and logistical capacity from the veterinary authorities for control of the disease.

Rabies has been endemic in Mozambique since 1908 and constitutes a serious public health problem, with dog rabies accounting for over 88% of confirmed cases (Dias,1995).

1 DIAGNOSIS AND REPORTING.

Animal rabies is diagnosed at the Central Veterinary Laboratory (CVL) in Maputo and at 3 Provincial Veterinary Laboratories (PVLs), located in the south, centre and north of Mozambique. While Sellers staining, Fluorescent Antibody Test and Mouse Inoculation are performed at the CVL, only Sellers method is done at the 3 PVLs. These laboratories also send brain specimens periodically to the CVL for further confirmation of their results and the remaining 6 PVLs also dispatch samples of suspected rabies cases to the CVL.

Human rabies cases are also reported monthly to an epidemiology unit at the Ministry of Health. At the provincial capitals, the main hospitals gather information of people bitten by animals, collecting data on age groups, species of animals responsible for the bites and ownership of these animals.

As a contribution to improve coordination between the human health and veterinary authorities, a form for registration of people bitten by animals has been proposed. This form contains not only data of the patient, but also relevant information regarding the animal and a copy of the form is passed to the veterinary authorities. These would fill in data on the animal being observed, allowing a flow of information, at the end of the observation period, so that correct decisions are made regarding medical assistance.

2 HUMAN AND ANIMAL RABIES.

Table 12 reports the numbers of human and animal rabies cases from the period 1998 - 2002: the numbers suggest that many animal cases go unrecorded, as these should be much above the number of human cases.

Table 12: Human and animal rabies cases in Mozambique in the period 1998 - 2002.

	1998	1999	2000	2001	2002	Total
Human rabies	25	22	26	33	29	135
Animal rabies	24	31	17	22	33	127

There are reports of dogs that are killed by people in rural communities, whenever they show signs of aggressiveness and other clinical manifestations of the disease and no samples are collected from

[1] National Institute of Veterinary Research – CP 1922 – Maputo - MOZAMBIQUE

[2] National Directorate of Livestock – Maputo - MOZAMBIQUE

these cases. This behaviour reveals the inexistence of local resources to assist bitten people and to prevent their infection from rabies.

3 MEDICAL ASSISTANCE TO BITTEN PEOPLE.

Data collected from the Health authorities in Maputo city show that there is an average of 108 people bitten by animals each month in Maputo, 16.25% of which receive post-exposure treatment (Table 13). Similar data are collected in each of the 10 provinces of the country.

The age groups that seem to be more subjected to animal bites are the 5-9 and the 20-49 years groups (Table 14).

If we extrapolate these figures for the whole year, the estimate number of people bitten by animals would be 1300 in Maputo city alone, of which about 211 persons would require post-exposure treatment.

Table 13: Persons bitten by animals in Maputo city and proportion of persons that receive post-exposure treatment. Mean monthly data.

	Period 1992-1994	Period Jan- March 2003
Bitten persons	74	108.6
Post-exposure treatment	13	17.6
% of post-exposure treatment	18	16.25

Table 14: Age groups of people bitten by animals in Maputo city in the period January-March 2003

Age group	Nr. of persons
0 - 4	14
5 - 9	53
10 - 14	34
15 - 19	23
20 - 49	170
> 49	29
N.D.	3
TOTAL	326

N.D. Not determined
All the 326 persons except one were bitten by dogs.

From all the dogs that have bitten people in Maputo, in the last 3 months period, 73.8% were owned dogs (Table 15). This indicates that most owners allow their dogs to roam free at least during part of the day or at night. The number of people exposed to suspected rabid animals is high throughout the country. Despite the existence of much more prevalent diseases, as AIDS, malaria and tuberculosis, more resources should be invested into the rabies problem (Wandeler, 1997).

To overcome the great need of public awareness towards rabies control, an instruction leaflet was prepared in 2002, and a colouring booklet for children was submitted for appreciation by the provincial veterinary officers.

Table 15: Dogs responsible for bitting people in Maputo city - period January- March 2003.

Owned	Not owned	% of owned dogs
240	85	73.8

4 MEASURES FOR THE CONTROL OF RABIES

Annual vaccination campaigns are carried out free of charge, in the main cities and at some districts, where the prevalence of the disease is higher. Central point vaccination strategy is used in urban areas, whereas in rural areas diptanks and crushpens constitute vaccination points. In Maputo, veterinary students participate in the annual campaigns.

In 2002, a total of 45562 dogs and cats were vaccinated.

The actual information reveals that measures for the control of rabies in Mozambique have to be strengthened, in several aspects, including public awareness, increased vaccination coverage, coor-

dination between health and veterinary authorities, as well as education, and reporting. Although the vaccination coverage is not known, it is certainly very low, taking in consideration the dog to human estimates of some countries in the region. Therefore, the annual vaccinations done yearly throughout the country have hardly any impact on the disease status (Rodrigues, 1997).

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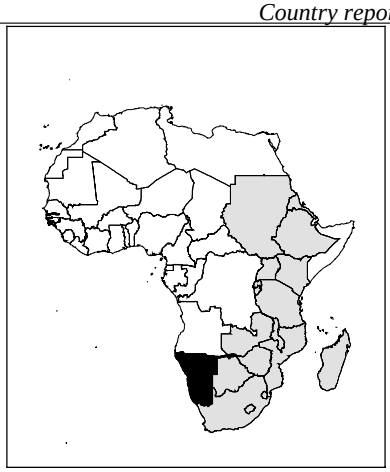


SOME ASPECTS OF THE RABIES EPIZOOTIC IN NAMIBIA

F. Mettler[1]

1 GENERAL SITUATION IN THE NORTHERN COMMUNAL LAND.

From the socio-economic structure Namibia is divided in a highly populated rural part in the north of the country and a much larger commercial part consisting of large farms with a low population density.

A recent study (Sorin and Mvula, 2001) done on dog-human relationship in part of the north of the Namibia showed following results:

- 79% of the homesteads have dogs with an average of 1.3 dogs per homestead, resulting in an estimated 115000 animals.
- Most people have the dogs for protection only, but 33% have them in addition as a source of meat. In the poor areas this percentage is even much higher.
- 97% of all dogs are unrestricted during day and night resulting in 110000 dogs, most of them roaming in search for food, what makes it difficult to distinguish them from stray dogs.
- Six to ten human rabies cases occur in this region per year, with a majority of children under 12 years. They are less afraid especially of young dogs and may not report minor injuries.
- Despite an annual vaccination campaign only 12% of the dogs are - according to this study - estimated to be vaccinated. A number that stays in contrast to the official estimated number of around 35%.
- Reasons why owned dogs are not vaccinated:

  1) 40% of the owners indicated, that they did not know where to go - a point that has to be improved.

  2) 20% said, that the dog was too young, now or during the vaccination campaign.

  3) Some other answers were: did not take the time or dog too aggressive to be handled, or veterinary staff not showing up at meeting point such as crushpen, etc.
- The fact that so many dogs are too young to be vaccinated during the annual campaign indicates that the mean age of dogs is rather low and life expectancy is short. The mean age of the dogs is, according to the study, indeed below 2 years. Among the various reasons, one is that dogs get little veterinary attention – what means no other vaccinations and no treatment of infectious or parasitic diseases. Since many owners have indicated to keep the dogs also for food, it is obviously quite common that people are killing and eating a dog because it is ill.
- Various measures such as wide-spread information, co-operation with communities and especially with schools and hospitals are aimed to improve the situation, however, the high turnover of the dog population makes it difficult with an annual vaccination campaign to achieve the minimum recommended rate of 50% of dogs vaccinated.

[1] Central Veterinary Laboratory – Windhoek - NAMIBIA

The number of confirmed canine rabies (Table 16) shows that there is a clear increase in the northern communal land over the last 10 years.

Since the canine brains sent to the Central Veterinary Laboratory in Windhoek are mostly of dogs, which have bitten people, these numbers indicate a real worsening of the situation. And the number of rabid cats additionally supports this trend. The increase of positive cases in livestock is a sequel of this development.

Table 16: confirmed rabies cases in the northern communal land.

	1993	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**
Canine	15	7	17	18	23	32	30	49	59	47
Feline	1	-	1	1	-	1	1	6	10	7
Jackal	-	-	-	1	-	-	1	-	-	-
Bovine	1	4	2	2	-	2	4	14	21	15
Ovine / Caprine	2	-	1	2	-	-	-	4	7	4

2 GENERAL SITUATION IN THE COMMERCIAL FARMING LAND.

In contrast to the north with its urban rabies situation, there is a sylvatic situation in the commercial areas with the jackal (*Canis mesomelas*) as main vector. A clear correlation can be recorded between the number of confirmed rabid jackals and the number of infected bovines (Table 17). The marked fluctuation of the number of rabies cases has a climatic explanation. After rainy seasons with under average rainfall the animals will increasingly meet at water holes, what leads to more occasions for contact and biting between the jackals and between jackals and livestock. Therefore the figures are further increasing towards the end of the dry season. The number of rabid dogs, however, is in the commercial farming area constant over the years and therefore has no influence on rabies in livestock.

Table 17: confirmed rabies cases in the commercial land.

	1993	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**
Canine	3	5	8	8	12	2	5	9	11	3
Feline	2	2	3	1	2	3	1	1		
Jackal	13	13	39	34	10	22	20	10	13	14
Other wild carnivores	2	6	7	10	4	5	7	6	2	
Bovine	27	41	83	59	20	49	33	18	20	35
Ovine/Caprine	7	14	17	4	5	8	11	11	7	14
Kudu *reported*	-/5	-/7 *1*	-/9	-/12 *1*	-/5	-/6 *10*	16/29 *33*	1/6 *4*	3/4 *3*	17/38 *144*

3 THE SITUATION OF KUDU RABIES.

3.1 History.

A first localised outbreak of rabies in kudus (*Tragelaphus strepsiceros*) occurred on a farm in Windhoek district in 1975. But the beginning of a large epizootic among these antelopes took place in February 1977 south of Okahandja. During the following years the disease spread an average of 40 to 60 km per year among the kudu population through most of the central and northern part of the country, except that for 2 years the spreading in easterly direction was prevented by a game fence. The highest number of confirmed cases of kudus was reported in 1980 with a second peak in 1982. Afterwards a continuous decrease was noted. In 1985, 8 years after the first outbreak, the epizootic subsided after causing an estimated loss of 30000 to 50000 antelopes. At the time of the outbreak the land was overpopulated with an estimated kudu population of 1 kudu per 40 ha.

3.2 Present situation.

After many years with no confirmed kudu rabies a localized outbreak occurred 1999 south of Waterberg in the Otjiwarongo district with 12 confirmed cases on one farm and on two neighbouring farms 2 cases each.

After isolated rabies cases in the following two years mainly in the Okahandja district, 17 kudus were confirmed positive in 2002 in the Okahandja and Karibib district. And in the current year already 15 animals from the same region have been tested positive. However, according to farmers and veterinarians many hundred kudus have died or have been killed with rabies-like symptoms. And all farmers questioned have responded that there is an overpopulation of kudus on their farms.

The present situation, which shows many similar features to the epizootic 16 years ago, makes it most likely that we may stand at the beginning of another detrimental rabies epizootic in kudus.

3.3 Some biological facts of the kudu.

Kudus are usually forming small herds, which averages 5 animals and rarely exceed a dozen. These groups stay close together, they move together and they feed together. Bulls often range over great distances – especially during the rut (June, July) – and make contact with other social groups.

Kudus are browsers and in Namibia they are largely dependent on the leaves of Acacia trees. It has been shown that the sharp thorns of these trees can inflict wounds in the oral cavity.

Since there was no observed increase of vectors such as jackals, an oral transmission of rabies from kudu to kudu has been assumed, especially also because they are feeding close to each other and may have contact with saliva of infected animals also by grooming. Experimentally it has been proven that kudus are susceptible for an oral transmission of the rabies virus (Barnard *et al.*, 1982).

During favourable years with good rainy seasons the number of kudus may increase considerably. However, if this time is followed by dry years the food gets scarce, what forces the animal to feed even in closer contact. Such conditions with an overpopulation of the antelopes and with following dry years happened in the outbreak of 1977 and are also now present. Farmers in the vicinity of outbreaks should be advised to reduce the number of their kudus to stop the disease spreading.

The main symptoms of rabid kudus are:

- loss of fear, they do not flee when approached, visit buildings
- moderate to copious salivation
- ataxia, swaying gait followed by paralysis
- some animals behave aggressive

Interesting is the fact that during a kudu rabies outbreak many animals obviously with rabies-like symptoms, which are submitted to rabies testing, proved to be negative. In a study performed by Barnard and Hassel (1981) 27 of 80 kudus, which showed rabies-like symptoms were tested negative. 15% of them had increased salivation and 25% showed docility, many of them visited buildings. No answer for this behaviour could be given.

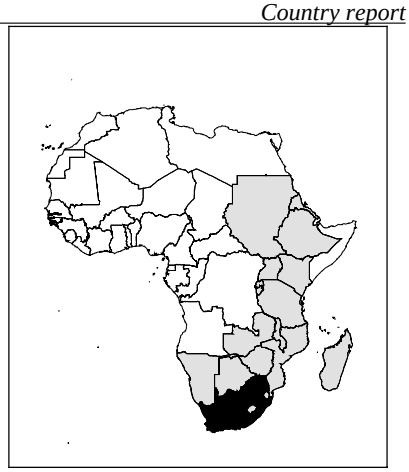
3.4 Chronic tannin poisoning.

Our results revealed that around half of the submitted kudu brains were tested negative. In one case some additional organs were brought in, which showed a severe loss of condition of the animal, despite a full rumen. This created the idea of tannin poisoning in some of the animals, since various factors favourable for a rabies epizootic in kudus are also favourable for chronic tannin poisoning and may even produce similar symptoms:

- acacias contain high levels of condensed tannins (proanthocyanidins)

- the acacias produce more tannin during browsing
- browsing of trees rich in tannin is increased in dry years with an overpopulation of kudus
- condensed tannins in high concentration decreases the absorption of various nutrients especially amino acids (loss of condition, docility)
- salivary glycoproteins are binding tannins (increased salivation)

The hypothesis of enhanced kudu mortality through tannin poisoning in the time of kudu rabies, however, has to be proved.



PROVINCIAL REPORT: KWAZULU NATAL, SOUTH AFRICA

J Randles[1]

The Province of KwaZulu Natal is one of nine provinces in South Africa and lies to the north east of the country. It is bounded by Swaziland and Mozambique to the North, the Eastern Cape Province to the South, Lesotho and the Drakensberg mountains and the Free State to the West, and the Indian Ocean to the East.

Figure 7: KwaZulu Natal province.

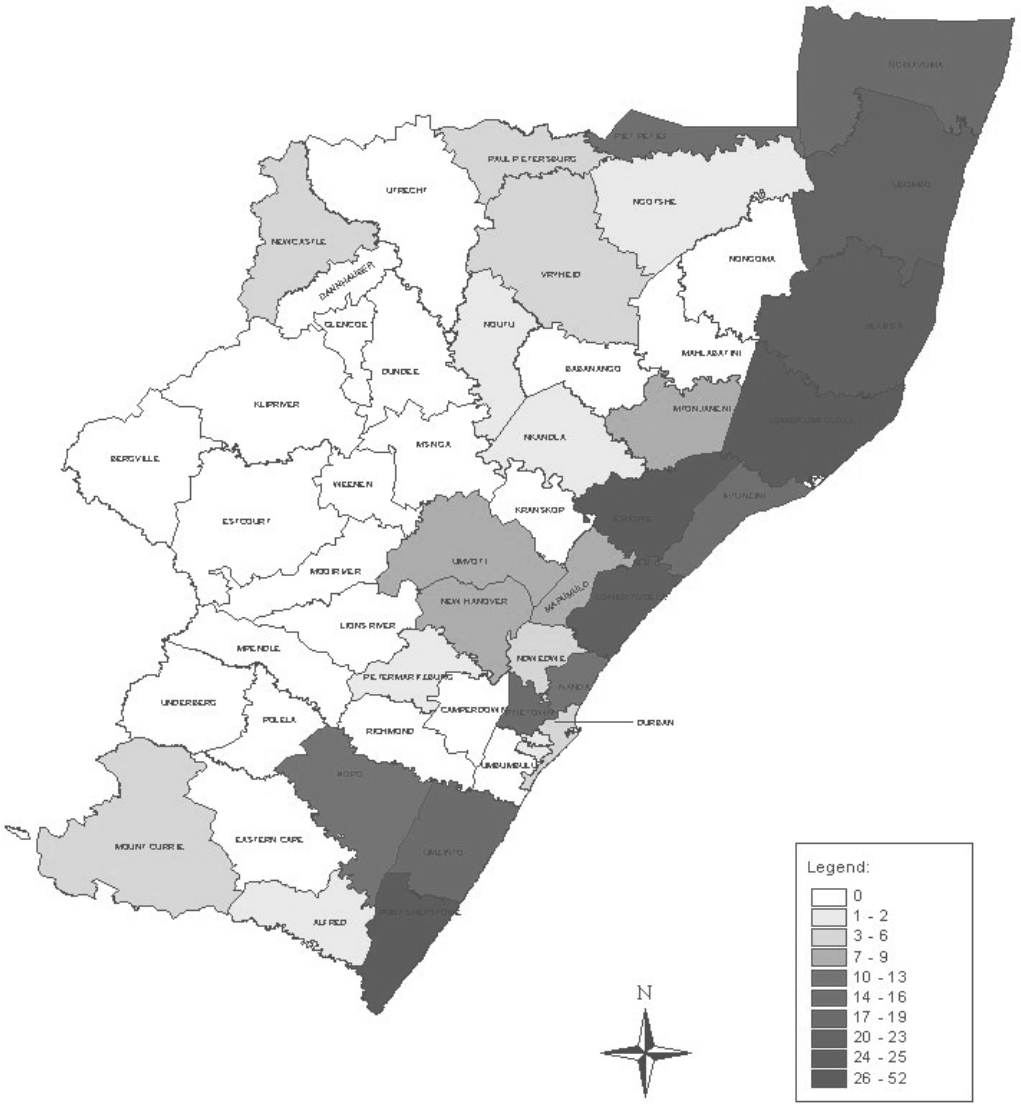
The epidemiology of rabies in KwaZulu Natal differs from that in the other provinces in South Africa in that in KwaZulu Natal the disease is dog-driven. Strictly speaking, rabies is not endemic to KwaZulu Natal. The first outbreak of rabies in dogs in KwaZulu Natal occurred in 1961 and lasted until 1968 when it was brought under control by extensive vaccination and strict control of movement of dogs into and out of the province. Rabies was not diagnosed again in the province until 1976 when it broke out again in northern KwaZulu Natal and spread progressively southwards, reaching the Transkei region by 1987. In spite of the now concerted efforts of the Directorate of Veterinary services, rabies has not yet been brought fully under control in the province.

The number of canine rabies cases diagnosed annually in KwaZulu Natal increased from 1976 with peaks at 3-4 year intervals, each greater than the one before and reaching a zenith of 374 cases in 1995. Twenty-three humans, mostly children, are known to have died of rabies in the province that year.

[1] Allerton provincial veterinary laboratory – Pietermaritzburg- Kwazulu Natal - SOUTH AFRICA

Subsequently there has been some change in the approach to rabies control in the province and a downward trend in rabies cases followed, lasting until 1997 when only 127 dog cases were diagnosed in the province. Since then, there has once more been a steady upward trend and a total of 200 and 196 dog rabies cases were diagnosed in 2001 and 2002 respectively.

Figure 8: rabies in KwaZulu Natal in 2001 – 2002.

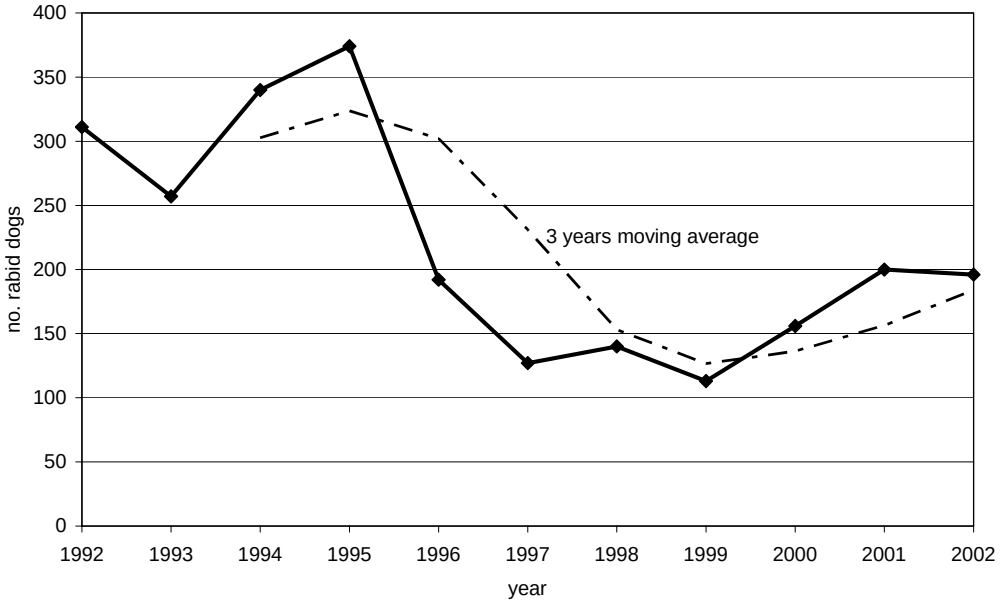


There was an outbreak of foot and mouth disease in KwaZulu Natal in September 2000 that was finally brought under control in November 2000 but surveillance work continued for a further 2 years. Over this period of time the efforts of field staff were focused on foot and mouth disease control and surveillance to the detriment of rabies control in certain areas.

Table 18: rabies prevalence in dogs and humans, and number of dogs vaccinated from 1992 to 2002

	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Dogs	311	257	340	374	192	127	140	113	156	200	196
Other species	33	27	42	35	26	17	10	8	15	26	29
Total animals	344	284	382	409	218	144	150	121	171	226	225
Dog vaccinations	NA	NA	NA	359218	337671	330753	364396	334652	316418	308982	362495
Humans	22	21	18	29	12	3	5	4	8	8	8

Figure 9: dog rabies trend in KwaZulu Natal.



The reasons why rabies appears to have become firmly established in KwaZulu Natal are not entirely clear and are probably multiple. Since 1976, social and political circumstances have changed dramatically in South Africa and KwaZulu Natal and have contributed in part to the spread of the disease in KwaZulu Natal. However, these factors alone do not explain why the disease has spread as it has in the province, mainly down the coastal areas and only to a lesser extent inland. Factors that have probably influenced the spread of rabies in the province and affected its control either positively or negatively include the following:

- Political
  - Political unrest prior to 1994
  - Change of government (1994)
  - Subsequent restructuring of Veterinary Services under one umbrella. Previously there were separate services for Natal and KwaZulu. The opportunity to apply coordinated disease strategies then became more feasible.
- Social
  - Faction fighting between rival clans that prevented access to affected areas in order to vaccinate dogs
  - Population increases, human and dogs
  - Population movement from rural areas into towns and growth of informal settlements with uncontrolled dog populations
  - Increased mobility of people (and their dogs)
- Personnel and provisioning
  - Staff shortages (veterinarians and animal health technicians) and/or lack of appropriately trained staff
  - Shortage of (suitable) vehicles
  - Budget constraints (vaccine, mileage, etc)
- Public apathy
  - Political change
  - Economic depression

- Poverty, hunger and disease
- Political violence, intimidation, civil unrest, faction fighting, crime
- Ignorance
- Complacency

In addition:

- Public awareness of rabies was increased through schools (video material) and the media (TV and newspapers)
- The Directorate began to work more closely with the Department of Health and efforts were made to alert health workers to the dangers and proper treatment of dog bite victims
- Rabies "hot spots" were identified so that problem dog populations could be targeted for vaccination
- The timing of dog inoculation campaigns was synchronized throughout KwaZulu Natal
- More effective vaccination strategies were applied according to the circumstances of the people in the affected areas (house to house, or street to street, or road to road "mass" vaccination campaigns as opposed to "rabies clinics" at traditional spots)

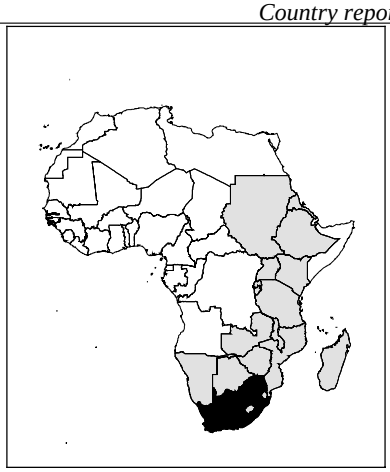
Building up and maintaining an epidemiological database is important in order to be able to measure the efficacy of rabies control in an area and to "tailor" vaccination campaigns if necessary. Important "key performance indicators" are the number of dog rabies cases and dog vaccinations done in a particular area over a specific period of time, and the number of human rabies cases.

It is necessary to examine these parameters on the basis of functional units, for example magisterial districts or State vet areas, in order to gain a more accurate reflection of the true relationship between the number of dogs vaccinated and the number of dog (and human) rabies cases diagnosed.

Rabies outbreaks in the province tend to form clusters on the map. The location of these clusters varies over time. This gives the impression of a scenario in which an outbreak occurs and spreads, dogs in the area are vaccinated, the outbreak is contained but then another outbreak "flares up" elsewhere.

There is clearly a need for strategic and tactical planning of rabies vaccination campaigns in districts in KZN where rabies occurs and for overall coordination of these campaigns if we are to succeed in eradicating rabies in the province as a whole. Education of all dog owners regarding this disease and the need for their dogs to be vaccinated against rabies, improvement of the infrastructure in remote rural areas and good functional cooperation between the Department of Health and the Directorate of Veterinary Services on an ongoing basis are also essential.

RABIES IN SOUTH AFRICA

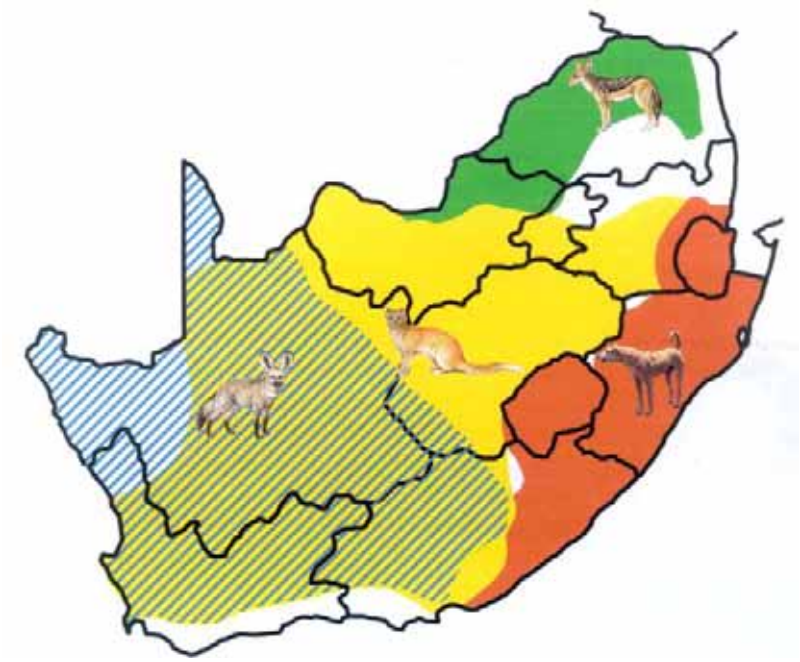


A. Liebenberg[1]

From the perspective of the Rabies laboratory at Onderstepoort Veterinary Institute

The epidemiology of rabies in South Africa is well understood due to decades of passive surveillance data. There are 4 maintenance hosts of the disease i.e. the stray dog in Kwazulu-Natal and surrounds, the yellow mongoose in the greater parts of the country, the bat-eared fox in the eastern half of the country and the black-backed jackal in the extreme North.

Figure 10: distribution and main vectors of rabies in South Africa.



The entire country is considered to be endemic to rabies.

ANIMAL RABIES SURVEILLANCE.

The rabies unit at the OVI (Onderstepoort Veterinary Institute) received 1009 samples in 2002 if which 326 were positive and 986 in 2001 of which 286 were positive. This follows the same trend as the previous 10 years.

[1] O.V.I. - P bag X5 - 110 Onderstepoort - SOUTH AFRICA

The breakdown of species affected follows the same pattern as always with domestic dogs contributing to 30% of positive cases followed by bovines and/or viverridae, wild canidae, wild felidae and other more rarely seen species.

Figure 11: species distribution of positive cases in 2001.

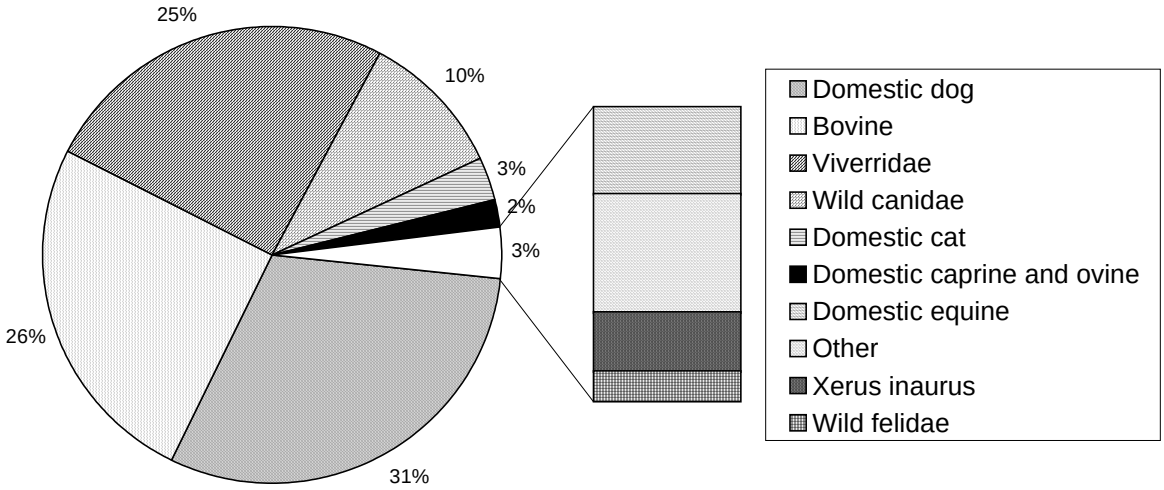
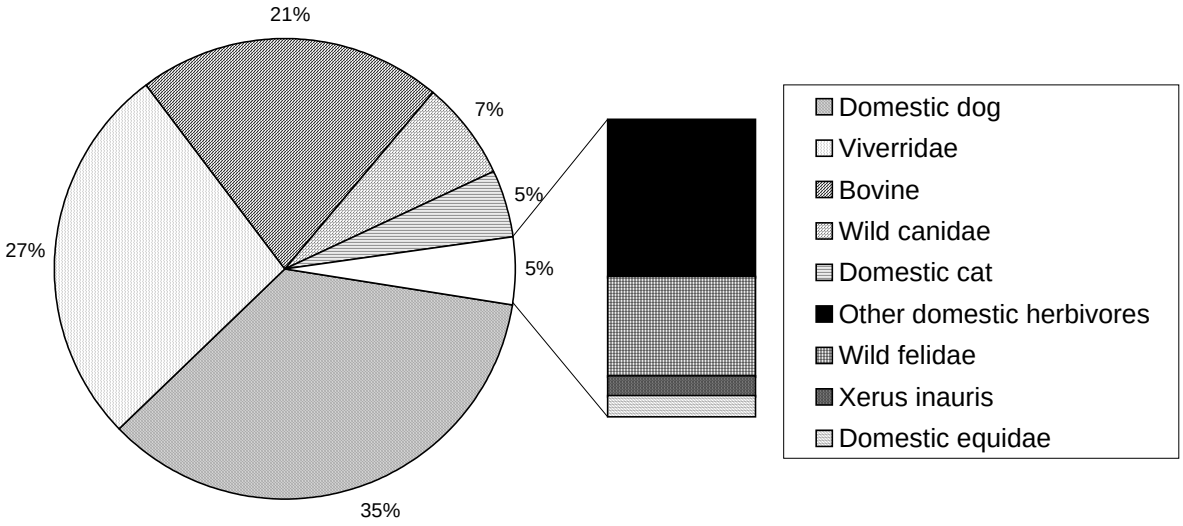


Figure 12: species distribution of positive cases in 2002.



The specific species seen were in sequence from most common to least common for 2001 and 2002 were as follows (this is similar to previous years). The *Canis adustus* cases are questionable as 3 out of the 4 specimens submitted were from an area 100 km west of the distribution region of the species.

There were no seasonal fluctuations in number of positive cases diagnosed for 2001. For 2002 there seemed to be an increase in August to December. Submissions are however affected by vacancies in state offices so it is therefore not possible to deduce anything from these distributions.

Table 19: wild species found rabies positive in 2001 and 2002.

Wild canidae		
	Bat-eared fox	*Otocyon megalotis*
	Black-backed jackal	*Canis mesomelas*
	Side-striped jackal	*Canis adustus*
	Cape fox	*Vulpes chama*
Wild felidae		
	African wild cat	*Felis lybica*
	Caracal	*Felis caracal*
	Small spotted cat	*Felis nigripes*
Viverridae (mongooses, surricates, genets, civets)		
	Yellow mongoose	*Cynictis penicillata*
	Suricate	*Suricata suricatta*
	Slender mongoose	*Galerella sanguinea*
	Small grey mongoose	*Galerella pulvurulenta*
	African civet	*Civettictis civetta*
Other		
	Aardwolf	*Proteles cristatus*
	Common duiker	*Sylvicapra grimmia*

BIOTYPE IDENTIFICATION.

Two biotypes are identified in South Africa with the use of monoclonal antibodies i.e. viverrid and canid biotypes. The specie distributions for the two biotypes are in sequence from most often affected to least affected. What is of interest is that bovines are affected 50:50 by both biotypes and wild canidae and viverrids therefore play an equal role in transferring the disease to bovines.

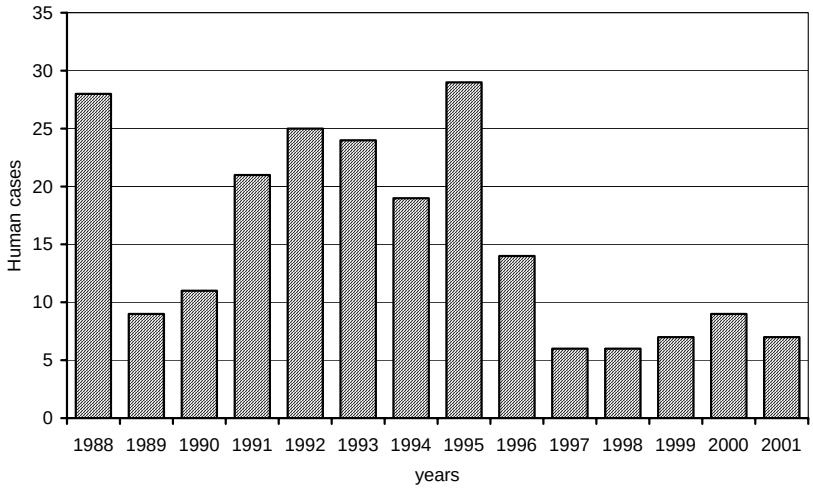
The distribution of the viverrid biotype for 2001 and 2002 fall within the known distribution region of mongoose rabies.

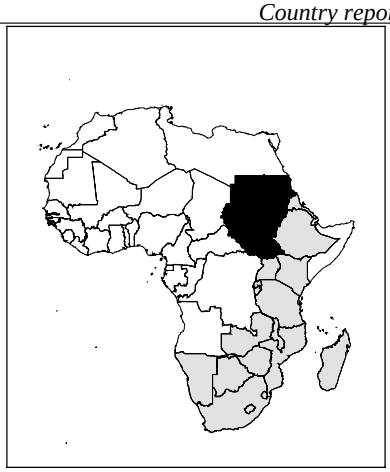
The distribution of the canid biotype varies from the known distribution region. A focus appeared in the Southeast Free State province in 2001. By 2002 the focus was still present and a second focus appeared to the west of it. These foci probably occur as a result of spillovers from Lesotho.

HUMAN RABIES.

The numbers of laboratory confirmed cases of human rabies have stayed constant in the last few years. The figures for 2002 have not yet been published.

Figure 13: laboratory confirmed human rabies between 1988 and 2001.





RABIES IN SUDAN

Yahia Hassan Ali[1]

1 INTRODUCTION.

Rabies exists in Sudan in humans and animals. As known from the literature the control measures plays an essential role in the incidence of the disease. The estimated animal population in Sudan in 2000 is 124844000 while, 91000 owned dogs and cats have been reported in Khartoum State, with an estimated 100000-150000 stray ones.

2 HUMAN RABIES.

Weekly, monthly, quarterly and annual reports for in and out patients visiting the hospitals and health centres in Sudan are regularly reported to the federal ministry of health in Khartoum. In the recent years the link between the state ministries of health and the federal ministry of health has been markedly weakened.

Rabies post-exposure treatment is now available in a large number of health centres, which is good, but it makes the data collection more difficult. Khartoum State is usually reporting the highest figures of human rabies post-exposure treatment, which is due to the shortage of the vaccine in most parts of the country, human rabies post-exposure treatment as well as deaths due to the disease during 2001-2002 is shown in Table 20.

Table 20: human rabies post-exposure treatment and deaths of the disease in Sudan 2001 – 2002.

		2001	2002
Khartoum	Post exposure treatment	10089	4812
	Deaths	*6*	*2*
Central states	Post exposure treatment	1809	1262
	Deaths	*0*	*8*
Northern States	Post exposure treatment	135	100
	Deaths	*2*	*1*
Eastern States	Post exposure treatment	622	353
	Deaths	*2*	*1*
Kordofan State	Post exposure treatment	664	578
	Deaths	*2*	*6*
Darfur States	Post exposure treatment	1121	1279
	Deaths	*0*	*6*
TOTAL	POST EXPOSURE TREATMENT	14440	8647*
	Deaths	*12*	*24*

- 263 exposures have been reported in the Southern States.

[1]Head of Rabies Unit - Virology Department - Central Veterinary Research Laboratories - P.O. Box 8067 - Khartoum - SUDAN.

Rabies vaccines.

Locally produced goat brain vaccine is still in use, it costs 6 USD per course (10 doses). Imported vaccines are Ducks embryo, which costs 100 USD (6 doses) and tissue culture vaccine (vero cells) which costs 110 USD (5 doses). The exposed person has to pay the cost, no role for NGOs in this respect. Human rabies diagnosis is based on case history and a clinical sign, laboratory confirmation is very rare.

3 ANIMAL RABIES.

The incidence of rabies in Sudan is variable according to the control measures adopted by the veterinary authorities. Almost all states of the Sudan report rabies annually. The main animal involved in rabies epidemiology in Sudan is dog. Goats followed by donkeys are usually the main victims to dog bites. Reporting system is not organized like that of the ministry of health. Monthly reports are collected from the different provinces to the director general of animal resources in each state then to the federal ministry of animal resources. There is usually a delay in sending the reports to the federal ministry of animal resources. During 2001-2002, a total of 1121 suspected rabid animals were reported in Sudan. Table 21 shows the species of rabies suspected animals reported.

Table 21: reported rabies suspected cases in Sudan 2001 – 2002.

	2001	2002
Dog	233	314
Cat	27	18
Monkey	5	18
Goat	58	31
Sheep	22	15
Equine	78	195
Cattle	73	29
Camel	19	16
TOTAL	485	636

4 DIAGNOSIS.

Laboratory diagnosis of rabies is mainly carried out in Rabies Unit, Central Veterinary Research Laboratory in Khartoum. Fresh samples as well as formalin preserved samples are sent to the laboratory. Very few samples were diagnosed at the National Heath Laboratory until February 2001. Fluorescent antibody test is the main technique applied for diagnosis, then histopathology. Table 22 shows the results of tested samples during 2001-2002.

Table 22: results of rabies laboratory diagnosis in Sudan, 2001 – 2002.

	2001		2002	
	positive	negative	positive	negative
Dog	7	8	2	2
Cat	9	4	4	1
Monkey	-	-	-	-
Goat	11	2	2	2
Sheep	2	-	-	-
Equine	6	3	2	-
Cattle	3	-	2	-
Camel	-	-	1	-
TOTAL	38	17	13	5

5 RABIES CONTROL STRATEGIES.

Rabies control programmes are carried out annually but with a variable efficiency in each State due to the independent budget in each state. This was the main reason for the frequent rabies outbreaks in

Sudan, especially at the western States (Darfur). Vaccination of susceptible animals and destruction of stray and unvaccinated animals are the main control strategy, adopted annually. Table 23 shows the figures of vaccinated and destroyed animals in Sudan during 2001-2002.

Table 23: Rabies control measures in Sudan 2001-2002.

	2001	2002
Vaccinated animals	7768	7423
Destroyed animals	14666	1343

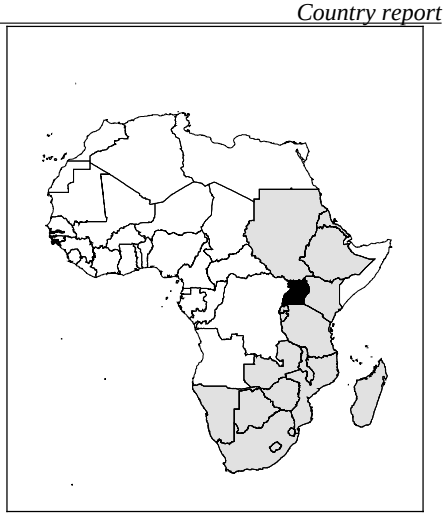
From the previous experience in control measures, it was noticed that the vaccination of dogs and other animals is usually restricted to a few numbers, due to the carelessness of the owners to vaccinate their animals.

During 2001 there was an outbreak of rabies in Darfur States and many other states. This was intensively followed by the national committee of rabies in Sudan, which resulted in establishment of national vaccination and destruction campaigns in all the states. Although there was a considerable number of vaccines distributed to all the state, the response to vaccination was poor. Table 24 shows the number of vaccines and bullets distributed to each state and the vaccination and destruction figures adopted.

Table 24: Distributed and used animal rabies vaccines and bullets in Sudan, 2001 – 2002

	Animal rabies vaccine		Bullets for destruction	
	Distributed	**Used**	**Distributed**	**Used**
Khartoum State	5410	825	4000	NA
Central states	6450	2283	8000	1742
Northern states	7900	5092	4000	11666
Eastern states	4500	NA	5000	NA
Kordofan states	6200	4429	5000	318
Darfur states	8190	NA	5000	2552
Southern states	3250	62	500	NA
TOTAL	41900	12691	31500	16278

The animal rabies vaccine used is an imported tissue culture vaccine which costs about 0.75 USD per dose, the production of tissue culture rabies vaccine locally is postponed due to lack of funds.



RABIES IN UGANDA

C. S. Rutebarika[1]

1 INTRODUCTION.

This report covers the period of mid 2001 and March 2003 and updates the previous reports of SEARG/WHO meetings.

Rabies is still a widespread problem in Uganda with 3904 suspected dog cases reported. Its significance is being overshadowed by the persistent epidemics of foot and mouth disease and contagious bovine peripneumonia in the country. The two epidemics, being fast spreading and of greater economic importance, competed more favourably for the meagre resources allocated to the department of livestock health and entomology. The disease remains endemic throughout the country.

The ministry of health has continued to spend progressively more money on antirabies vaccination because of insufficient funding for dog rabies control.

2 HUMAN RABIES

Table 25: human rabies data (1992 - March 2003).

Year	No. of post exposure treatments	ARV doses (imported)	No. of rabies cases	Estimated cost (USD)
1992	766	3976	50	35000
1993	1518	5720	23	52000
1994	2614	8298	15	74700
1995	3222	13623	14	122000
1996	1698	16000	9	144000
1997	2916	16000	10	144000
1998	3112	16000	10	144000
1999	4537	10000	5	90000
2000	5398	10000	9	90000
2001	5490	19570	16	119371
2002	4324	15133	12	98365
2003*	1543	8900		54290
TOTAL	31421	119630	173	955321

Source: Veterinary Public Health/ Ministry of Health.
* Information available up to March 2003.

A total of 5867 dog bite victims were reported to have received post exposure treatment with anti rabies vaccine in the various health units in the country for the period Jan 2002 to April 2003. This proves that control of dog rabies by the department of livestock health and entomology still leaves a lot to be desired.

[1] Department of Livestock Health and Entomology - Entebbe - UGANDA.

There is need for increased public health sensitisation programmes about the importance of rabies by members of the Technical Committee for Rabies control (TECOR) and to step up funding of the department of livestock health and entomology.

The dog contributed to over 95% of the rabies cases reported and there was no laboratory confirmation of any human rabies cases during this period. The collaboration between the district medical and veterinary staff has continued to grow and this has improved greatly on the reporting of bite victims and their management.

Figure 14: rabies cases, 1992 – 2002.

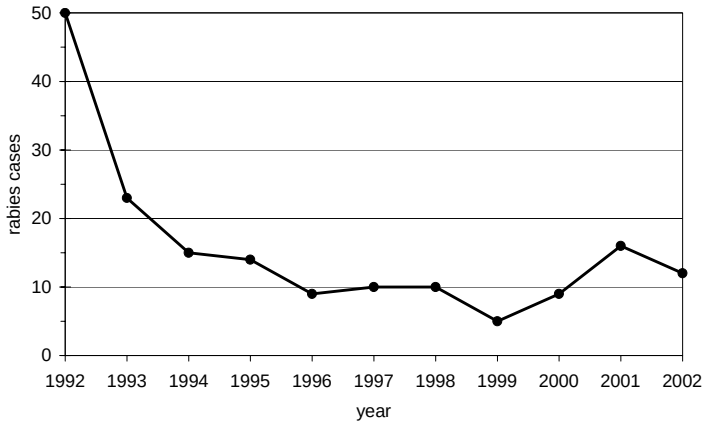


Figure 15: importation of antirabies vaccines, 1992 - 2002.

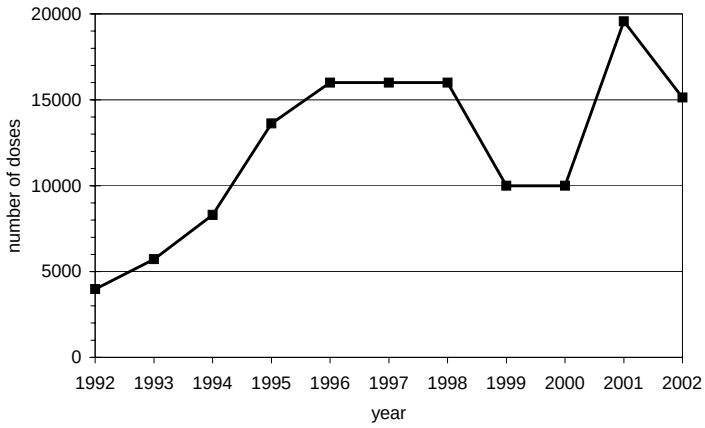


Figure 16: post-exposure treatments, 1992 - 2002.

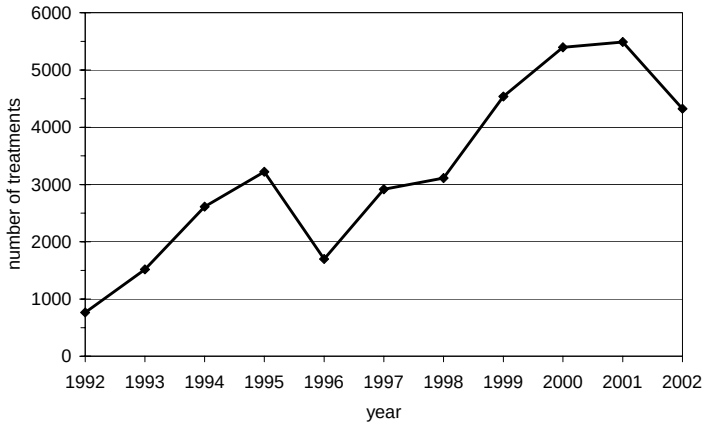
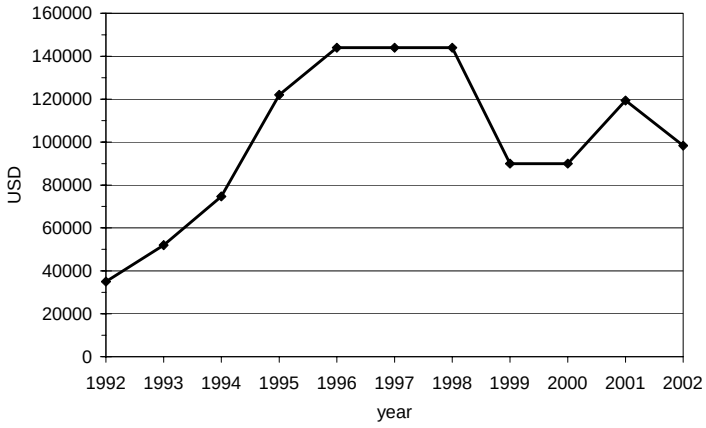


Figure 17: estimated cost of rabies (USD), 1992 - 2002.



3 ANIMAL RABIES CONTROL.

Rabies remains a very serious epidemic in the country. Outbreaks of other epidemics like foot and mouth disease, lumpy skin disease and malaria continue to overshadow its importance and took priority on resource allocation. Nevertheless, rabies was among the most predominantly reported diseases in the whole country. The surveillance and reporting has tremendously improved.

During the period of 2001-2003, 3409 suspected clinical dog cases were reported, 234479 dogs and cats were vaccinated, representing approximately 16% of the target population. 3323 stray dogs and cats were destroyed. Insufficient funding is a major factor contributing to the low vaccination figures. The policy for improved delivery of veterinary services is now in place and this allows both the public and private vets to participate in rabies control. The animal health rules are currently being reviewed and we expect that this will improve compliance during vaccination campaign. We also have a veterinary drug policy in place and this will ensure that only good quality vaccines are used in rabies control.

Public awareness, through a multisectoral approach (MSA), has continued to be a useful tool. The emergency of local FM radio stations/TV has contributed greatly to public sensitization about the disease.

4 FUNDING FOR RABIES CONTROL DIAGNOSIS AND REPORTING.

The procurement of rabies vaccine is the responsibility of central government (MAAIF) while implementation of vaccination campaigns is the mandate of local authorities whose funding priorities may not favour rabies control. MAAIF procured 240000 doses of rabies vaccine which was an improvement from the previous year but still insufficient for the whole country.

The contribution of private veterinarians, teaching institutions and the Uganda Society for Protection and Care of Animals (USPCA) in rabies control is significantly increasing especially around Kampala city.

The central diagnostic laboratory has reagents and uses FAT for diagnosis. However, very few samples are being submitted to the laboratories by district veterinary officers (DVOs) mainly because most times, the DVO receive information late after the offending animal has been killed and buried.

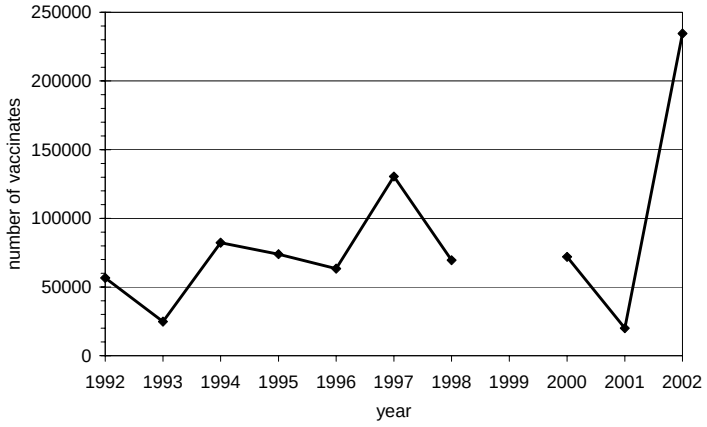
Public awareness programmes have been funded, though inadequately. Reporting from districts has improved a lot since PACE project started implementing its activities in August 2001. Regular surveillance formats are provided to DVOs for surveillance of major epidemics of which rabies is included. We have also installed a TADinfo system for data reporting and storage. The ministry of health and department of livestock health in MAAIF have produced and circulated guidelines for integrated surveillance of rabies for all districts in the country (Sept 2001). These guidelines are being used for both district veterinary and medical personnel when dealing with rabies.

Table 26: animal rabies data (dogs and cats vaccinated 1992-2003 April).

Year	Amount of vaccine procured	No. of vaccinates (cats and dogs)	Estimated cost of vaccine (USD)
1992	80000	56662	25600
1993	-	24875	-
1994	100000	82306	32000
1995	90000	73906	28800
1996	400000	63390	128000
1997	-	130480	-
1998	-	69555	-
1999	-	-	-
2000	74000	72000	29600
2001	-	20000	-
2002	240000	234560	96000
2003*	-	323	-
TOTAL	984000	828057	340000

Source: livestock health and entomology departmental annual reports, 1992-2002.
* Data available for up to March 2003.

Figure 18: Number of vaccinates, 1992 – 2002.



5 ROLE OF DONOR ORGANISATIONS / PROJECTS.

No major project funding rabies vaccination campaigns.

Although the government's "Plan for Modernisation of Agriculture" (PMA) has a component for control of epidemic disease and pest, rabies control has not yet benefited from this fund. PMA has extension services under the National Agricultural Advisory Services (NAADS) that will be based in the districts operating at parish level. PMA is a World Bank funded project. We shall include rabies sensitization in the extension messages for the NAADS programme implementers.

PACE project, that took off late 2001, has improved greatly the diagnosis and reporting of rabies.

6 USE OF DOG POPULATION REDUCTION.

The strategy of dog population is still being used in urban areas mainly. Such populations have been reduced by poisoning, shooting and hunting.

It is mainly the dogs of the elite which get reproductive surgical interventions and contraceptives. This is a class that usually keep their dogs vaccinated.

7 ANIMAL AND HUMAN VACCINATIONS.

7.1 Human vaccines.

Vaccines currently in use are the inactivated cell culture vaccines prepared on VERO Cells (Verorab). The human diploid cell culture (HDCV) and Duck embryo vaccines were last used in 1993.

The numbers of rabies suspect victims who received post exposure treatment and amounts of vaccine imported per year are shown in Table 25 for the period of 1992-2003. The vaccines were used in all the districts of the country and were free. But budgetary constraints limit the amount of vaccines procured.

7.2 Animal vaccines.

The vaccination returns for the year 1992 to 2003 are indicated in Table 26. There was an increase in the amount of vaccines procured in 2002.

The country has been importing inactivated tissue culture vaccines since 1992, which provides immunity for 1 year or 3 years. This 3 year immunity vaccine is ideal for our situation where vaccines are irregularly available and vaccination coverage is usually low. The numbers of doses of vaccines procured and used are also shown in Table 26. These vaccines have been used in all the districts of the country. We look forward to the era of thermostable vaccines or the use of oral baited vaccines that can be used at household level.

The liberalisation of vaccine procurement to the private sector could have eased the chronic shortage of vaccines and insufficient funding by government. Unfortunately no pharmaceutical company seems to be interested in rabies vaccine because it does not make money as would have been desired.

7.3 Constraints to rabies control.

Rabies control programmes are still constrained by:

a) Irregular and insufficient funding

b) Control of stray dog populations which are on the increase.

c) Public awareness on rabies is still insufficient.

d) Decentralisation policy and civil service reform.

e) Minimal role by the private sector

Table 27: Reported cases of Rabies in Uganda 1992 –2002

Species	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Dog	243	228	376	252	38	298	61	1214	442	1841	1568
Cat	-	-	1	4	1	5	3	7	13	11	19
Cattle	1	2	-	4	1	30	5	6	8	1	4
Goat/Sheep	2	-	7	5	8	4	-	-	-	2	1
Fox	-	2	-	5	8	-	-	3	3	-	9
Jackal	3	6	-	4	2	-	-		4	1	-
Monkey	-	-	1	-	3	3	-	2	-		
Rabbit	-	-	-	-	2	2	-	-	-	-	-
Mongoose	-	-	-	2	-	-	-	-	-	-	-
Pigs								1	1	-	-
Leopard								1	-	-	-
Bush back									1	-	-
TOTAL	249	238	385	276	73	268	69	1234	472	1856	1601

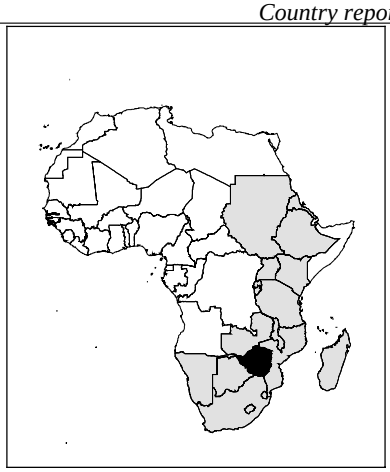
Source: livestock health and entomology departmental annual reports 1992-2002

ACKNOWLEDGEMENTS.

Dr. Winyi Kaboyo, Veterinary Public Health, Ministry of Health.

Dr K. Mugabi, Diagnostics and Epidemiological Unit.

The Uganda Veterinary Association small animal clinic.



RABIES IN ZIMBABWE 1999 - 2002

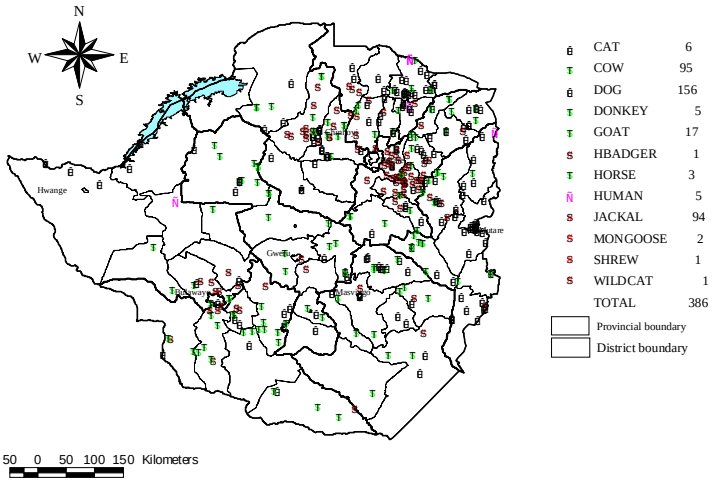
W. Shumba[1]

Figures of this paper are extracted from the presentation.

Animal and human rabies in Zimbabwe is a major zoonotic condition which requires regional and international support. The rabies cases are on the increase and this only needs a concerted effort from the stakeholders and the donor community to help alleviate major problems in the control of the fatal disease in both animals and humans.

Zimbabwe acts as the reference laboratory for rabies in the SADC region and has its fair share of problems in combating the disease. The Zimbabwe Rabies control unit has problems in improving diagnostics, publications for the region through SEARG and national communication with remote areas, quality control issues, short to medium term training courses to improve diagnostic capacity, attending regional and international workshops and the financial capacity to procure reagents and equipment.. Rabies vaccine for animals is not readily available due to foreign currency shortages. Need to establish records of rabies cases of vaccinated dogs thus establishing the efficacy of the vaccine as well as the management of the handling techniques of the vaccines in remote areas. The distribution pattern of the disease in the country has remained constant as shown by the annual rabies maps since 1999. The true situation is that rabies is on the increase in both animals and humans. From January 2003, 5 positive human cases have been confirmed by our laboratory and very remote areas do not have access to our courier service hence a similar distribution pattern every year.

Figure 19: rabies outbreaks in 1999, 386 reported cases.



[1] Central Veterinary Laboratory - PO box CY551 – Causeway – Harare - ZIMBABWE

Figure 20: rabies outbreaks in 2000, 330 reported cases.

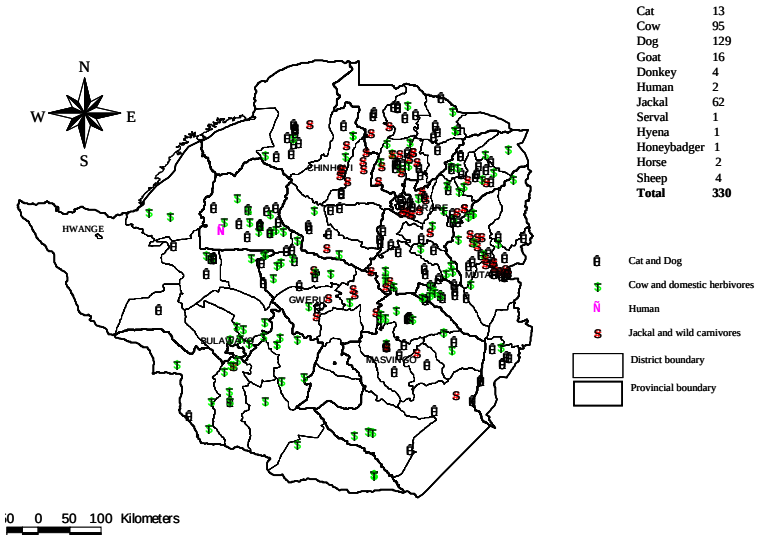


Figure 21: rabies outbreaks in 2001, 364 reported cases.

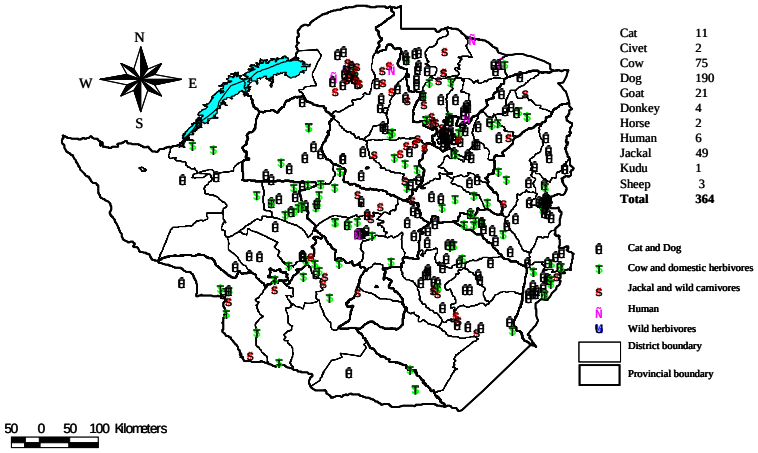


Figure 22: rabies outbreaks in 2002, 362 reported cases.

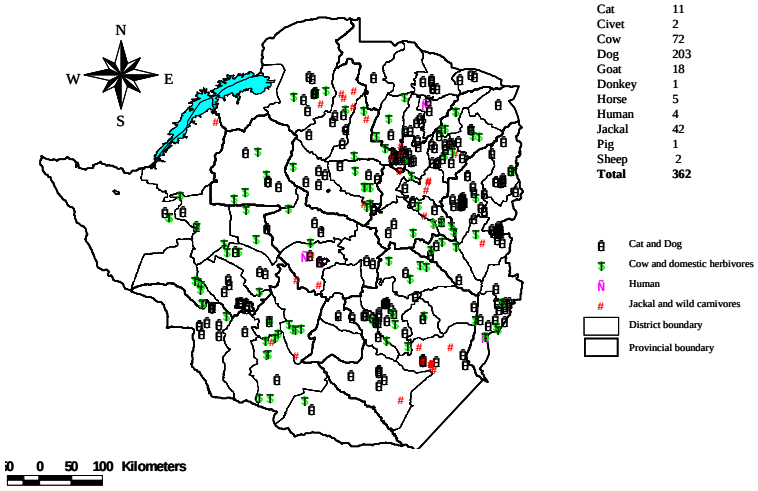


Figure 23: rabies outbreaks between 1999 and 2002.

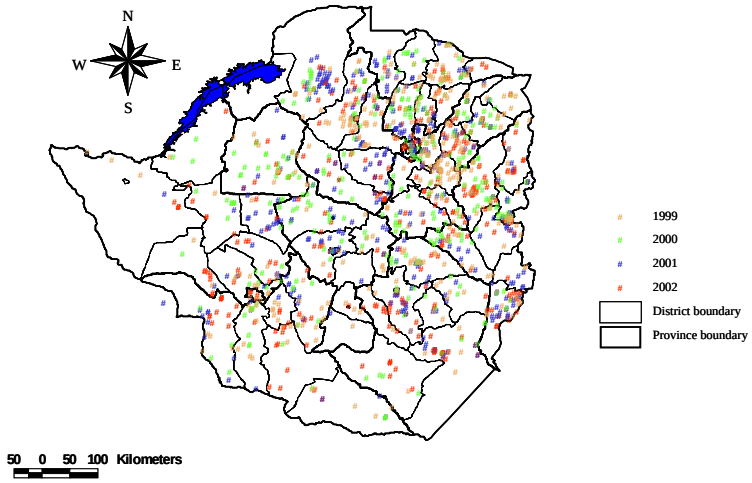


Figure 24: evolution of rabies incidence in Zimbabwe between 1999 and 2002.

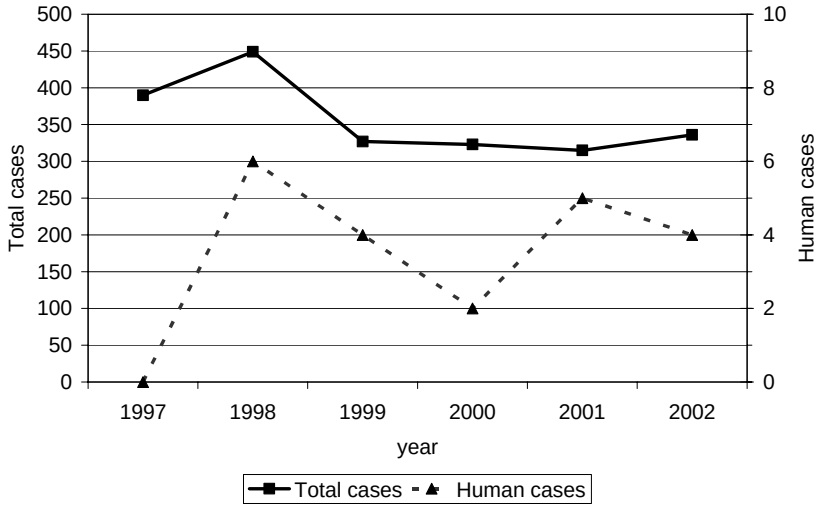


Figure 25: rabies cases per species in 2002.

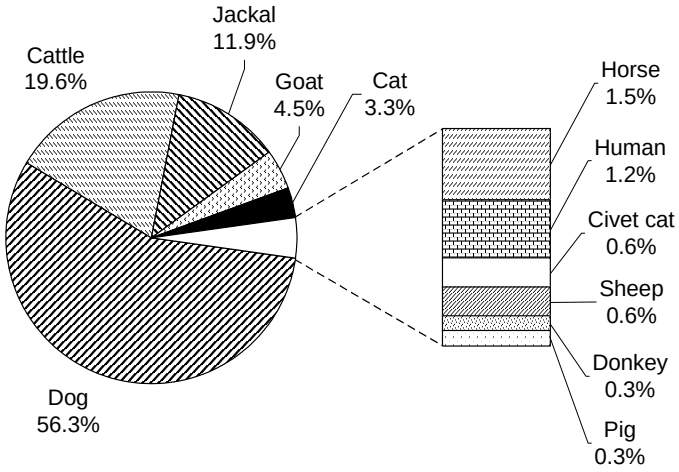


Figure 26: monthly incidence of rabies in 2002.

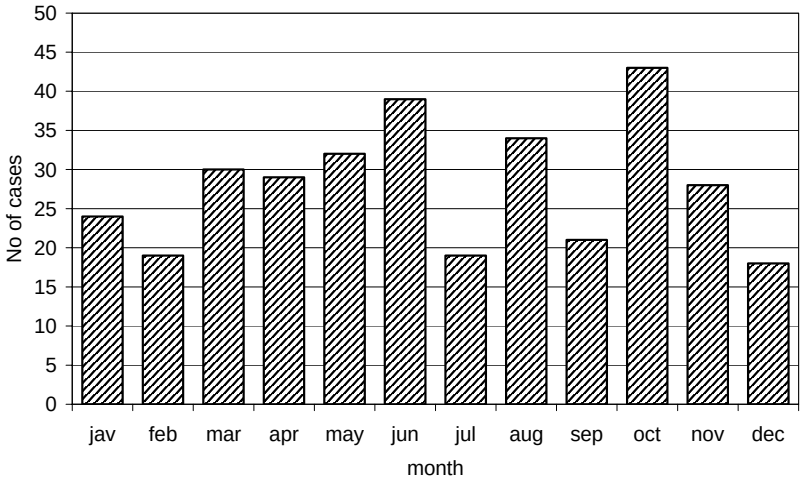


Table 28: rabies incidence and vaccination of dogs per province in 2002.

Province	Rabies cases	Number of vaccinated dogs
Manicaland	66	41588
Mashonaland Central	19	23194
Mashonaland East	63	40906
Mashonaland West	42	28406
Masvingo	68	107303
Matebeleland North	38	37290
Matebeleland South	21	25179
Midlands	19	10453
Grand Total	336	314319

SEARG
business meeting

QUO VADIS SESSION

SUGGESTIONS:

1) There is a need to have more human physicians involved in the meetings. Physicians and Veterinarians are both needed in the prevention of rabies. Although there is probably no need for 2 presentations (one regarding animal cases and one regarding human cases) from each country, it could be that physicians could present other data/problems that might be allocated to the second day of the meeting with country reports being on the first day.
2) The way participants are invited should be changed. Invitations should be mailed to the administrators/ministries with a copy to the invitee. This would help bring attention to the fact that a meeting on rabies is taking place and it is important for human health professionals (as well as veterinary professionals) to be involved.
3) Some meeting proceedings have not reached the person to whom they were sent. Since there is no copyright on the material, it is okay to copy all CD's, discs and paper copies and share them with whoever needs or wants a copy.
4) The SEARG members need to establish Universal rabies policy guidelines for countries in SE Africa.
5) The SEARG members should produce resolutions/recommendations as an outcome from the SEARG meetings. These resolutions/recommendations would have to be submitted to the administration of SEARG to be approved.
6) There should be an assessment of the general problems of member countries and potential recommendations as to how to help solve the problems as well as an assessment at the next SEARG to determine the progress.
7) There should be follow-up for PET in humans that do not receive the full treatment and also follow-up for animal vaccination. I.e. Surveillance and epidemiology data on human and animal vaccination: does it work? Are there vaccine failures? Etc.
8) WHO, (FX Meslin's request) would like epidemiological data from the SEARG countries to be entered in the new RABNET database. Please take time to fill out the forms from WHO and indicate who is the official (both from the animal and human health side) to be contacted regarding epidemiological information.
9) Is it time for SEARG to have legal status including a constitution?

Vaccinology and post-exposure prophylaxis

THE FRUSTRATIONS OF A RABIES TELEPHONIST

J. Godlonton[1]

1 INTRODUCTION AND BACKGROUND.

In December 2001 the South African Rabies Group published a handbook on Rabies, Guide for the Medical and Veterinary and Allied Professions. The handbook contained a section providing contact telephone numbers for expert advice and further information on rabies problems relating to humans and animals.

The phone numbers for human problems were Dr Godlonton's home and cell phone. The presentation centered on calls pertaining to human rabies from Jan 1st 2002 until April 30th 2003. Conclusions were drawn about public perception and knowledge of rabies, identifying certain problem areas in human rabies post exposure management.

2 SUMMARY AND STATISTICS OF CALLS.

221 calls received.

2.1 Rabies administration problems:

112 calls from:

- Rabies Advisory Group provincial, national
- Southern and East African Rabies Group
- Allerton Laboratory, Onderstepoort Veterinary Institute Rabies Unit
- Dept of Health. KwaZulu Natal. Communicable disease centre
- District Surgeons. State Veterinarians

2.2 Human rabies problems:

109 calls have been received.

Most calls were concerning post animal bite management and most of these were dog bites.

The majority of the calls where no or very low suspicion of rabies involved animals; besides dogs other animals included 4 cats, 3 wild monkey, 3 cows, 3 wild rats, 2 pet rats, 2 mice, 1 honey badger, (first recording and proved rabies positive), 1 hampster, 1 goat, 1 wild cat (positive human death), 1 bat and one human death in which the victim said he has been bitten by a leopard.

3 CONCLUSIONS AND FRUSTRATIONS ARISING OUT OF CALLS.

The single most important problem was the extent of ignorance of the callers about the correct management of post rabies exposure victims. The biggest frustrations about the human deaths from rabies was that the majority could have been prevented because the post exposure bite victims were taken to or presented at a treatment facility which could/should have implemented the correct post-exposure

[1]Edendale Hospital - 76 Desmond road – Scottsville - Pieternmaritzburg 3201 - SOUTH AFRICA

management and failed to do so. Giving consideration to the 4 major aspects of correct post-exposure management the major problems were:

3.1 Biting animal management.

This aspect was a major problem. Minimal effort was made to capture, observe, incarcerate, identify and where necessary euthanase and refer appropriate tissue to appropriate laboratories for rabies immunofluorescence test.

3.2 Wound management.

Details of how bite wounds were managed were scanty, use of appropriate virucidal agents and washing techniques were very inadequate. A number of bite wounds received primary suturing. Very few category 3 contact victims received rabies immunoglobulin into or around the wound.

3.3 Vaccine use.

Vaccine was under-used because of non-availability. Vaccine was incorrectly used in dae, timing following exposure, route of administration mainly due to administrator ignorance and fear of side effects.

3.4 Immunoglobulin (R.I.G).

R.I.G. was under used because of non-availability. Immunoglobulin stocks became severely depleted because of gross over use (miss-uses) having been used for incorrect indications. Following a period of poor availability it was subsequently significantly under used. Too little immunoglobulin was used into the wound site when indications existed.

Other problem areas frequently encountered concerned

3.5 Human rabies

Problems included poor knowledge about the fatality of rabies in appropriate management of rabid patient, very low incidence of attempts and ante-mortem diagnosis, “Cove” of nursing and family contacts, number of bite victims diagnosed as rabies had other problems and “survived” (chorea, acute depressive psychosis, viral encephalitis).

3.6 After rabies dermise.

Many problems here including

- non notification of post-rabies exposure
- non notification of rabid victims
- non notification of rabies death
- refusal to do a post-mortem by doctors
- refusal to allow a post mortem by family
- poor sample collection, preservation and transportation
- poor correlation and notification of positive rabies results

3.7 Pre-exposure prophylaxis

Poor knowledge of dates of immunisation

Poor knowledge of personal immunoglobulin levels

Rabies immunoglobulin still being given to victims who have had pre-exposure prophylaxis.

4 RECOMMENDATIONS ARISING FROM ABOVE MENTIONED PROBLEMS.

1) Education of health care workers at all levels of those likely to be involved in care of pest exposure victims.

   Re-enforcement and repeating education.

2) Making products available for correct post-exposure management, virucidal cleaning agents, vaccine and Immunoglobulin.

3) Improve the “follow-up” aspects of biting animal.

ALTERNATIVE RECOMBINANT POXVIRUS VACCINE FOR RABIES

J. Weyer[1], G.J. Viljoen[2] and Louis H. Nel[1].

1 INTRODUCTION.

1.1 The Rabies Situation in Africa.

Rabies has become primarily a problem of African and other developing countries. The African continent remains one of the most severely afflicted, with the majority of human cases reported from these countries (WHO, 1998b). It is estimated that up to 300 individuals are exposed to the rabies virus every 15 minutes (Rupprecht *et al.*, 2002). It is believed that this statistic is a great understatement considering the lack of proper surveillance in most African countries. A study on human rabies in Tanzania concluded that the underestimation of current rabies statistics is up to 100-fold (Cleaveland *et al.*, 2002). Although effective post exposure treatment (PET) for rabies is available, many problems plague the use thereof in the developing countries (Meslin *et al.*, 1994; Piper and Xenakis, 1998). It is often the case that rabies vaccine and, or anti-rabies virus immunoglobulin (RIG) are not available in these countries. The cost of proper cell culture vaccine and the immunoglobulin are also prohibitive. Furthermore, the success of PET hinges on the timely administration of the vaccine and RIG, a problem in Africa with its limited health care infrastructure. Furthermore, the failure of PET is most often associated with the incorrect administration of the biologicals or the failure to complete regimens. But rabies does not only lead to human mortality. Rabies is considered primarily an animal health threat. Rabies does not only account for the death of domestic pets and wildlife, but also of the loss of animals of agricultural importance. Especially, cattle rabies, leads to considerable economic losses. The implementation of continuous control and surveillance programs imposes an additional and sizeable economic burden on the limited resources of developing countries. Efforts to eradicate or to more economically control rabies are therefore justified.

In Africa, urban rabies is still the most prominent form of rabies. Concomitantly, human exposures to the rabies virus are most often associated with a rabid dog (WHO, 1998b). In African communities, many dogs remain free-roaming (either owned or stray) (Perry *et al.*, 1995; Kitala *et al.*, 2001). The parental vaccination of these animals is troublesome because these animals are often not approachable (Perry, 1993, Estrada *et al.*, 2001). The most sensible approach for the prevention of the disease in humans is the control of the disease in these dogs, a strategy of indirect protection for humans (Bőgel and Meslin, 1990). An oral vaccination program involving these dogs may provide a more feasible alternative and augment current vaccination strategies (Perry and Wandeler, 1993; Estrada *et al.*, 2001).

1.2 Oral vaccination of dogs.

An ideal rabies vaccine for the purpose of oral administration is defined by a few characteristics (Perry and Wandeler, 1993). The vaccine should infer adequate immunlogical protection against infection, so that a single administration of the vaccine would be sufficient to protect the animal. The oral vaccination of free-roaming dogs will bring the vaccine in closer proximity of humans, increasing the probability of human exposure to the vaccine. Exceptional safety is therefore critical, not only for the target but for non-targets as well. With the increasing number of people in African communities with acquired

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immunodeficiencies it is clear why the safety of such a vaccine should be undoubted. In addition, cost-effectiveness would be a deciding factor for the feasibility of such programs in poorer countries.

The rabies virus mutant, SAG-2, has been suggested as a probable oral vaccine for bait vaccination programs in South Africa (WHO, 1998a). The vaccine has undisputed efficacy and safety in target as well as a significant number of non-target recipients (Schumacher *et al.*, 1993; Fekadu *et al.*, 1996; Masson *et al.*, 1996; Bingham *et al.*, 1999; Lambot *et al.*, 2001; Knobel *et al.*, 2003). The vaccine is, however, less stable upon exposure to heat and other elements in the environment, posing problems if to be distributed in bait form into nature (Bingham *et al.*, 1999). Vaccines that are not only, safe and effective, but can be distributed in bait form and retain efficacy for an adequate period of time is required.

Lessons can be learnt from the successful oral vaccination programs in the United States of America and Europe (reviewed in Brochier *et al.*, 1996; and Mackowiak *et al.*, 1999). A recombinant Vaccinia virus (Poxviridae, Orthopoxvirus) expressing the rabies virus antigen, the glycoprotein, has been used extensively as an oral vaccine. The use of potent and replication competent viruses such as vaccinia virus as vaccine vehicles are, however, no longer desirable (Redfield *et al.*, 1987; Rupprecht *et al.*, 2001; Moussatché *et al.*, 2003). The dissemination of the vaccine virus from the vaccinees to non-target recipients is considered a significant risk (Rupprecht *et al.*, 2001). A more advantageous approach entails the development of safer vaccinia virus derivatives and the study of naturally host restricted poxviruses as vaccine vectors, in other words replication deficient vaccine carriers (reviewed in Moss *et al.*, 1996).

1.3 Replication-deficient Poxviruses as vaccine carriers.

Poxviruses prove to be exceptional as vaccine carriers, as proven with the application of the recombinant vaccinia virus expressing the rabies virus glycoprotein (Reviewed in Pastoret and Vanderplasschen, 2003). The advantages of using poxviruses as vaccine vehicles include the tolerance of large insertions of foreign DNA (up to 30 Kilobasepairs), which provides the opportunity of developing multivalent vaccines. Poxviruses serve as live vaccine vectors and because of the natural virus infection both humoral and cellular responses are elicited. Poxvirus DNA is not infectious and gene expression occurs in the host cytoplasm. Poxvirus virions are also relatively temperature stable. A poxvirus that is unable to replicate in mammalian cells or is naturally host range restricted, produces diminished cytopathic effects in target and non-target organisms, retains the capacity for high-level gene expression and immunogenicity while promising exceptional safety for laboratory workers and potential vaccine recipients, would provide an ideal vaccine carrier candidate for the development of an oral rabies vaccine.

One such virus is the Lumpy Skin Disease virus (*Capripoxvirus*, Poxviridae) (LSDV), a naturally host restricted virus indigenous to Africa (Barnard *et al.*, 1994). The attenuated Neethling vaccine strain, LSDV-SA promises great potential as a vaccine vector for veterinary application in Africa. The use of this virus as a vaccine vector has been investigated in limited number of studies (Kitching *et al.*, 1987; Romero *et al.*, 1993; Romero *et al.*, 1994a ,b and c; Wade-Evans *et al.*, 1996; Ngichabe *et al.*, 1997; Aspden *et al.*, 2002; Ngichabe *et al.*, 2002; Aspden *et al.*, 2003; Berhe *et al.*, 2003). LSDV-SA as a replication deficient vaccine vehicle has been explored with encouraging results (Aspden *et al.*, 2003). Additionally, LSDV-SA as a vaccine vector also provides the possibility of developing dual vaccines against capripoxvirus infections and the heterologous veterinary disease of interest (Kitching *et al.*, 1987; Romero *et al.*, 1993; Romero *et al.*, 1994a and c; Aspden *et al.*, 2002; Berhe *et al.*, 2003).

2 THE AIM OF THE STUDY.

It is suggested that poxviruses, such as Lumpy Skin Disease virus (LSDV), expressing the full-length rabies virus glycoprotein will provide an effective (single dose/long-term protection/non-invasive administration/more economical than cell culture vaccines) and safe (severely host restricted or attenuated viruses used) rabies vaccine for application in Africa.

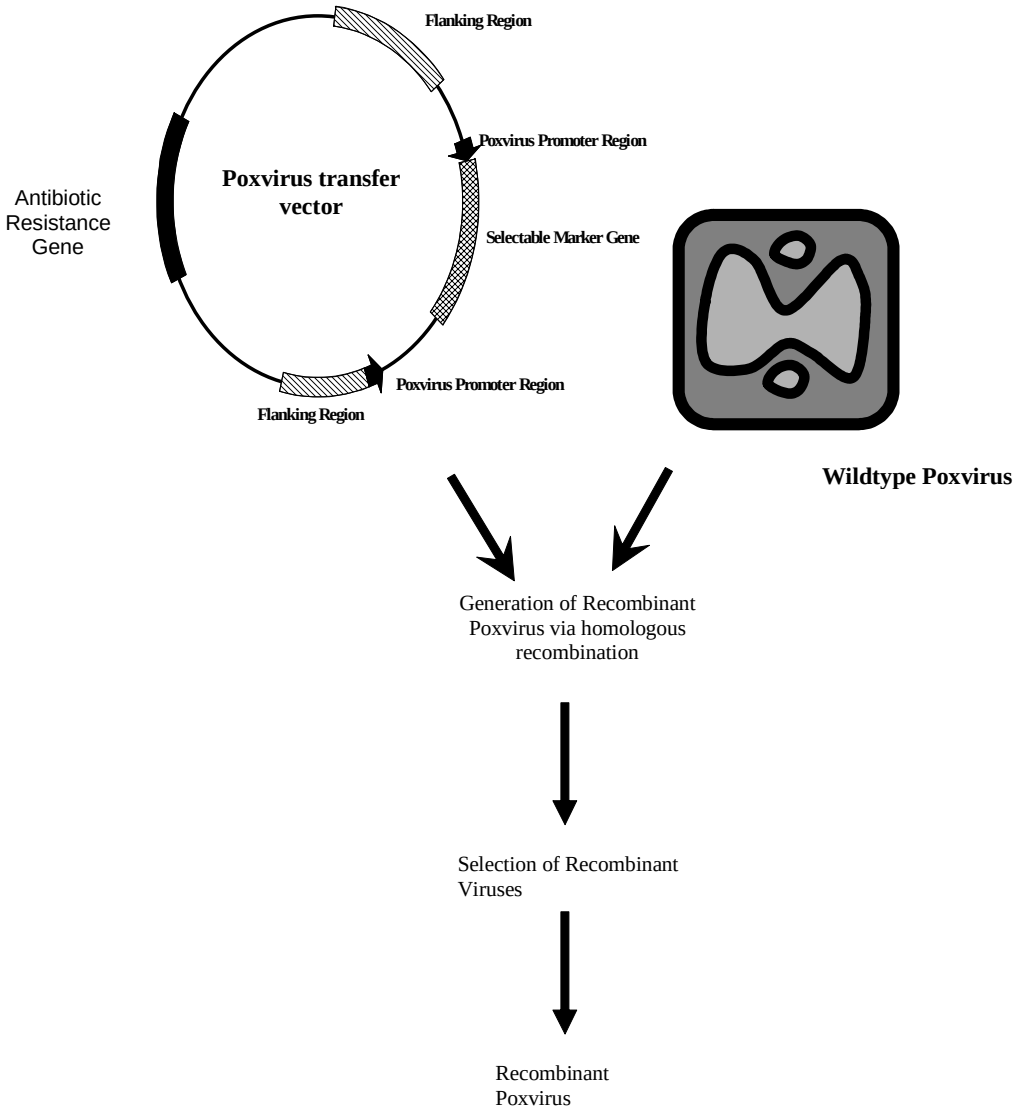
The first, and current, phase of this study entails the generation of homogenous recombinant LSDV-SA expressing the rabies virus glycoprotein gene. The recombinant viruses will be used in a future study to compare the efficacy of the different recombinant vaccines in laboratory animals. Further-

more, different protocols for the selection of recombinant LSDV were compared in an effort to standardize the procedure (Romero *et al.*, 1993, Wallace, Personal communication).

3 BRIEF OVERVIEW OF MATERIALS AND METHODS.

3.1 Generation and selection of recombinant LSDV-SA.

Figure 27: A diagrammatic representation of the generation of recombinant poxviruses (such as LSDV-SA). A recombinant transfer vector (containing the heterologous gene of interest) is transfected in wild type poxvirus-infected cells. Homologous recombination occurs and the gene of interest is inserted unto the virus genome. The recombinant virus is then subjected to selection to eliminate wild type virus background. The generation of recombinant poxviruses is summarized in Smith and Mackett, 1992.



Recombinant lumpy skin disease viruses (Neethling vaccine strain) (LSDV-SA) were generated as previously described (Romero *et al.*, 1993; Fick 1998). The integration of the expression cassette in the wild type virus genome was verified by polymerase chain reaction (PCR) analysis using an internal primer (binding within the rabies virus glycoprotein coding sequence) and an external primer (binding within the LSDV-SA genome). The purification of recombinant LSDV-SA from wild type virus background proves to be more troublesome than for other recombinant poxviruses (Berhe *et al.*, 2003; Personal communication Professor G.J. Viljoen and Mr. D.B. Wallace, Agricultural Research Council –

Onderstepoort Veterinary Institute). Two methods for the selection of recombinant LSDV-SA were evaluated (Romero *et al.*, 1993; Mr. D.B. Wallace, Agricultural Research Council – Onderstepoort Veterinary Institute). The homogeneity of the recombinant virus samples was routinely assessed (between subsequent passages) by PCR analysis using primers binding within the thymidine kinase (TK) flanking regions of the recombinant viruses. Although recombinant LSDV-SA could be purified following either protocol, it was found that the protocol suggested by Romero *et al.* (1993) was less time consuming. In addition the value of detergent treatment in the purification of recombinant LSDV-SA was investigated. Although slight enrichment for recombinant viruses could be achieved when the samples were treated with CHAPS or SDS, no homogenous samples were isolated upon such treatment. Stable expression of the rabies virus glycoprotein from the recombinant LSDV-SA was indicated by indirect immunofluorescence assay. A set of four monoclonal antibodies was used as primary antibody, respectively (The monoclonals were a generous gift form Dr. A.I. Wandeler, Canadian Food Inspection, Agency). The monoclonals are each directed towards a different major antigenic site on the rabies virus glycoprotein external domain. The positive results with each of the monoclonals indicated that the protein is expressed in an immunogenic manner.

4 SUMMARY OF RESULTS.

A recombinant LSDV-SA expressing the rabies virus antigen, the glycoprotein gene, was generated (results not shown). In addition, different strategies for the purification of recombinant LSDV-SA have been evaluated (results not shown). This represents the first comprehensive and parallel comparison of selection strategies for recombinant LSDV. The information generated during this study will facilitate the process of purification of recombinant LSDV and is expected to be most useful to future workers in this field.

5 CONCLUDING REMARKS.

Oral vaccination of free-roaming dogs will be advantageous in the struggle to control rabies in Africa. A vaccine similar to the recombinant vaccinia virus vaccine, used in oral vaccination programs in the United States and Europe, but which offers improved safety will present an ideal vaccine for such programs in African countries. In this study it was put forward that a recombinant LSDV-SA expressing the rabies virus antigen could be the answer. A recombinant LSDV-SA could also be applied as a dual vaccine, against rabies virus and capripoxvirus infections in cattle (such as lumpy skin disease). Such a virus was generated and purifed, and shown to express the rabies virus antigen. It is envisioned to use the recombinant LSDV-SA in a study to evaluate the comparative efficacy of different poxviruses in laboratory animals.

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CHALLENGES OF INTRODUCING RABIES INTRA-DERMAL POST-EXPOSURE TREATMENT IN UGANDA

R. Winyi Kaboyo[1]

1 INTRODUCTION.

Dog rabies is endemic in Uganda; from 1990 to1994 dog bites were responsible for 96% of all suspected human rabies cases who received rabies post-exposure treatment (Winyi-Kaboyo *et al.*, 2001). The need for rabies post-exposure treatments has been on the increase although with varying degrees from one district to another. Consequently, there has been a corresponding increase in the number of doses and costs of the rabies vaccine imported into the country (Table29).

Table29: number of post-exposure treatments, rabies cases and vaccine costs, 1998-2001.

Year	No. people treated (rabies PET)	Rabies cases	Vaccine imported doses	Cost in USD
1998	3112	10	16000	144000
1999	4537	5	10000	90000
2000	5398	9	10000	90000
2001	5490	16	19570	119371
TOTAL	18537	40	55570	443371

Since the early 1990's, the ministry of health stopped using rabies vaccine of nervous tissue origin and changed to tissue culture vaccine in line with World Health Organization recommendations for rabies post-exposure treatment (WHO, 1992).

The ministry of health routinely procures purified verocell rabies vaccine manufactured by Aventis Pasteur (France) and marketed as VERORAB®. The vaccine is very expensive in light of other competing health priorities like HIV/AIDS, malaria and tuberculosis. The unit cost of vaccine has also been increasing, for example within the year 2000, the cost had gone up by 17% from 9410 to 11000 Uganda shillings (i.e. 4.74 to 5.54 USD) per dose (Winyi-Kaboyo, 2001).

In 1996, the ministry of health changed from the standard 5 doses intra-muscular regimen to the 4 doses (2-1-1) intra-muscular regimen to save costs, reduce the number of hospital visits to three and thus improve on patient compliance. Despite the change, vaccine costs remain high. This is evidenced by frequent shortages of vaccine at designated rabies treatment centres leading to anxiety and death of rabies exposed individuals who could not be treated because of vaccine stock out.

In 2002, the ministry of health decided to introduce the 2-2-2-0-1-1 intra-dermal regimen as per WHO recommendations of 1996 (WHO, 1997).

The primary objective of this change is to increase accessibility of rabies post-exposure treatment without necessarily reducing the ministry of health budget towards the purchase of rabies vaccine.

2 MATERIALS AND METHODS.

The intra-dermal regimen is recommended for centralised animal bite clinics that see more than one PET patient daily (Komaltham *et al.*,). To identify the busy clinics/treatment centres the following was done:

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- post-exposure treatment data reviews for the years 2000, 2001 and 2002 were conducted, using EPIINFO 6 software.
- A questionnaire was designed and administered to health workers in 21 randomly selected rabies treatment centres.

In this study, a rabies treatment centre was defined as any health facility treating animal bite cases suspected of exposure to rabies using rabies vaccine, immunoglobulin or both.

2.1 Post-exposure treatment data review.

Rabies post-exposure treatment data is routinely collected from all rabies treatment centres on specifically designed forms as part of rabies surveillance and monitoring of vaccine usage.

Information on a number of variables is collected (e.g. District, name of treatment centre, name, age, sex of patients, species of biting animal, treatment schedule, doses given, patient outcome/prognosis and date of hospital visit to the treatment centre). For purposes of determining how busy a rabies treatment centre was, the dates of hospital visits were analysed to determine which treatment centre see more than one post-exposure treatment patient on any given day.

A busy treatment centre was defined as a treatment centre that was seeing at least two rabies post-exposure treatment patients on a given day.

2.2 Questionnaire survey.

The questionnaire was to assess the need and ability of rabies treatment centres to change from intra-muscular to intra-dermal regimen. The key questions asked were:

- Catchment area of the treatment centre for animal bite patients requiring rabies post-exposure treatment.
- Average number of post-exposure treatments given daily, weekly and monthly.
- Availability of 0.1ml needles and syringes for intra-dermal administration.
- Experience of clinical staff in intra-dermal administration of drugs and/or vaccines.
- Staff training needs for the new 2-2-2-0-1-1 intra-dermal regimen.
- Commonest epidemiological pattern of rabies exposure to humans; whether “Single Source – Single Exposure” (SSSE) or “Single Source – Multiple Exposure” (SSME).
- Whether the need to change from intra-muscular to intra-dermal was appreciated or not.

3 RESULTS.

Rabies post-exposure treatment data review showed that only 9 out of 60 treatment centres (15%) could be classified as "busy treatment centres".

A recommendation was made for these centres to change from the 2-1-1 intra-muscular to the 2-2-2-0-1-1 intra-dermal regimen. The centres are located in the districts of Arua, Kabarole, Kotido, Wakiso (Entebbe Hospital) Masaka, Mbale, Mbarara, Soroti. and Kampala (Mulago National Referral Hospital). However only the first four mentioned above had by May 2003 started using the intra-dermal regimen. (Questionnaire assessment in 21 treatment centres indicated that of these 20 treatment centres (95%) had staff with experience in intra-dermal drug or vaccine administration.

The need for training and orientation of clinical staff for the 2-2-2-0-1-1 intra-dermal regimen was expressed by 13 (62%) of the 21 treatment centres that were surveyed.

Only 4 treatment centres (19%) had a regular supply of 0.1 ml syringes and needles suitable for intra-dermal drug administration. Seventeen (81%) treatment centres did not have these syringes and needles. However only 8 (38%) treatment centres were willing to purchase them from their own budget if the intra-dermal regimen were to be introduced. The other 9 treatment centres requested that the central ministry of health should provide them free of charge alongside the vaccine.

Figure 28).

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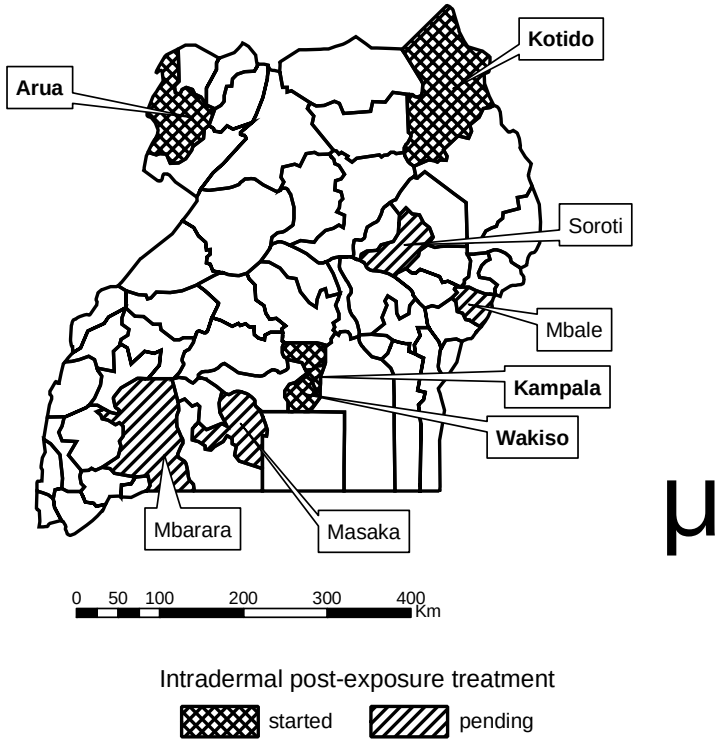
Figure 28: Districts recommended for rabies id PET in Uganda.



Out of the 18 treatment centres that responded to the type of exposure, 11 (61%) reported SSSE and 7 (39%) reported SSME as the commonest rabies exposure pattern to their clients in their respective catchment areas.

The busy treatment centres were more agreeable to change to intra-dermal regimen as soon as possible citing the need to avoid vaccine stock-outs. The less busy treatment centres preferred to continue with the intra-muscular regimen.

4 DISCUSSION/CHALLENGES.

In this article the main challenges in the introduction of the intra-dermal regimen will be discussed.

Cost Comparison: in terms of vaccine costs, the intra-dermal is twice as economical compared to the intra-muscular regimen as shown: (using VERORAB®, 0.5ml vial at USD 6.00)

In spite of the intra-dermal being more economical , there are number of challenges associated with its introduction. This is more so when changing from a relatively “easy” 2-1-1 intra-muscular to a “complicated” 2-2-2-0-1-1 intra-dermal regimen.

Table 30: Compared costs of the IM and ID regimens.

im:	**Day**		**0**		**7**		**21**	
	Doses (vials)		2	1		1		
	Total vials: 4							
	Total cost: 6.00 x 4 vials = 24 USD							
id:	**Day**		**0**	**3**	**7**	**14**	**30**	**90**
	Doses (ml)		0.2	0.2	0.2	0	0.1	0.1
	Total volume: 0.8mls (i.e. ≈ 2 vials)							
	Total cost: 6.00 x 2 vials = 12 USD							

Policy change: Approval is needed from the director general health services at the national level. The regimen change has also to be accepted at the implementation level (i.e at treatment centres in the districts). This requires time to coordinate the change at both levels. The need for cost saving is not easily appreciated by the treatment centres since the vaccine is purchased centrally by the ministry of health and given to the treatment centres at no cost.

Identification of busy treatment centres: This is very important in order to have a critical number of post-exposure treatment patients on a given day to avoid unacceptable vaccine wastage. The challenge is to have well kept rabies post-exposure treatment data from as many treatment centres as possible and collected over a number of years. These data are then reviewed to identify the busy treatment centres for which intra-dermal post-exposure treatment would be appropriate.

Merging treatment centres: Rabies post-exposure treatment should be merged into one treatment centre where there are many treatment centres in close proximity to each other but each one seeing only a few post-exposure treatment patients. The chosen treatment centre would now fit the criteria of a busy treatment centre. The other less busy treatment centres, however may not be agreeable to this strategy and may feel downgraded or discriminated against. Similar sentiments were expressed when intra-dermal regimen was recommended for introduction at Mulago National Referral Hospital in Kampala city and all other hospitals within the city were required to refer rabies patients to Mulago.

Staff training: This is necessary to orient health staff to the new id regimen. A number of meetings and seminars were organised with clinical staff to explain the intra-dermal regimen schedules, discuss the advantages and disadvantages and address the concerns of the staff regarding vaccine reconstitution, storage and technique for the intra-dermal route. This enables staff to gain confidence and encourages them to willingly change from the intra-muscular to intra-dermal regimen.

It was also explained that the advantage of SSME rabies transmission pattern lies in having a large group of exposed individuals seeking rabies post-exposure treatment at the same time. This minimises losses of reconstituted vaccine since a large number of post-exposure treatment patients would be available on day 0 and other scheduled days of treatment.

Provision of logistics: The intra-dermal regimen requires 0.1ml syringes and needles these have to be purchased separately. (Unlike for the intra-muscular regimen syringes and needles are not supplied by the vaccine manufacturer). This requires extra funds. The ministry of health has also to provide other post-exposure treatment report forms reflecting the new intra-dermal schedule. These are additional costs that add to the challenges of implementing the intra-dermal regimen.

Other challenges were in form of **specific questions and concerns:**

- What is the effect of interchangeably using the intra-muscular and intra-dermal regimens on the same patient?
- Is it safe to give the last 2 doses together at day 30 instead of extending the treatment to day 90?
- Could manufacturers consider repackaging the vaccine into 0.2ml vials instead of 0.5ml vials and provide 0.1ml syringes?

5 CONCLUSION.

The intra-dermal is still new and being evaluated as to achieving its intended objective; that is increasing accessibility to rabies post-exposure treatment.

More treatment centres will be evaluated for a change to intra-dermal regimen while the less busy treatment centres will continue using the 2-1-1 intra-muscular regimen.

ACKNOWLEDGEMENT.

I am very grateful to the District Directors of Health Services for the routine rabies post-exposure treatment data submitted to the ministry of health.

The health staff in the surveyed treatment centres are sincerely thanked for their active participation in the questionnaire survey, training sessions and giving their views on the intra-dermal regimen.

The director general health services, ministry of health is acknowledged for giving permission to publish this article.

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EXPERIENCE WITH TISSUE CULTURE RABIES VACCINE USED INTRA-DERMALLY IN THAILAND

T. Kamoltham[1] and W. Thinyounyong,

ABSTRACT.

A five-year project (1996 – 2001) was initiated in Phetchabun Province, Northern Thailand to prevent human rabies death. It involved:

- a better accessibility to educational awareness,
- intensifying documentation of post-exposure treatment,
- reducing the dog population by monitoring and implementing vaccination and sterilization programmes,
- increasing the cooperation between the ministries of public health, education on a provincial level,
- intensifying follow-up of patients exposed to suspected and laboratory-confirmed rabid animals

In a retrospective study, post-exposure treatment was documented through the use of a reporting form that the medical staff completed for every patient who presented at clinics and hospital emergency rooms.

The canine population was monitored through the Phetchabun livestock department of the ministry of agriculture.

All patients were followed up by health volunteers in the villages, public health personnel in sub district health station. 10350 patients received rabies post exposure treatment 7227 patients were administered intra-dermal regimens known as the Thai Red Cross intra-dermal regimen (TRC-ID ie.2-2-2-0-1-1 schedule). 40% of patients were children below the age of 9. Of the 1114 animals that were actually submitted for laboratory testing after exposure of humans, 26% (108 animals) were confirmed rabid. All animals (dogs, cats and rats) confirmed positive for rabies were tested by FA and/or mouse inoculation tests.

There were 189 human exposures to the laboratory confirmed rabid animals. One hundred and sixty-eight of these 189 patients (89%) received 0.1ml of tissue culture vaccine intra-dermally except when they received PDEV. When PDEV was administered, patients received 0.2 ml intra-dermally. The breakdown of vaccine administered to the 168 patients was as follows: 148 patients received PCEC, 10 patients received PVRV and 10 patients received PDEV. Local side effects such as itching, redness and pain were rare. No systemic or allergic reactions were reported in our series.

40% of the wounds were category III, 30% were category II and 30% were category I. The most common wound was inflicted in the hand and leg region respectively.

All patients were followed up for one year post-treatment through the use of an effective primary heath care system which included: health volunteers in the villages, health workers in sub district health stations and the provincial health organization of Phetchabun. All patients were alive one year after exposure. Two human deaths occurred in the first two years of the programme–neither patient had received vaccine or rabies immunoglobulin after exposure. During the last three years of the programme no human death was recorded, which indicated that the programme was successful.

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1 INTRODUCTION.

Canine rabies is endemic in Thailand and is an especially serious health threat to people living in more rural areas of the country. The number of human deaths attributed to rabies has dramatically decreased in the past decade in Thailand. This has been accomplished through the increased use of highly purified tissue culture rabies vaccines (TCV) supplied free of charge by the Thai ministry of health and also by the Thai Red Cross organization to patients unable to pay for post-exposure treatment. However, the projected cost of vaccine for administration to the general population using the original Essen post-exposure treatment regimen (1.0 ml administered intramuscularly on days 0,3,7, 14 and 30) was a too high financial burden for the Thai government to undertake and maintain it (WHO, 1992; Chutivongse *et al.*, 1990; Ministry of health, 1992). Therefore, a reduced dosage intradermal post-exposure treatment was adopted, based on numerous intra-dermal rabies vaccination studies conducted in the 1980s and 1990s (Chutivongse *et al*, 1990; Wasi *et al.*, 1994; Phanupak *et al.*, 1987). Due to the success of these and other more recent studies, WHO has acknowledged and promoted the use of 0.1 ml per dose of purified vero cell rabies vaccine (PVRV) and purified chick embryo cell rabies vaccine (PCECV) in what is now known as the traditional Thai Red Cross intradermal (TRC) or "2-2-2-0-1-1" regimen (WHO, 1997; WHO, 2000). In this regimen, 0.1 ml of PVRV or PCECV is administered intra-dermally at two sites on days 0,3 and 7, no vaccine on day 14 and at one site on days 30 and 90. Intra-dermal regimens have considerably lowered the cost of post-exposure treatment due to the lower amount of vaccine required per treatment (Wasi *et al.*, 1994; Phanupak *et al.*, 1987)

Although the number of human rabies deaths has declined dramatically throughout Thailand, unnecessary human deaths still occur. In fact, between 50 and 70 human rabies deaths continue to be reported each year in Thailand. Twenty-five deaths were reported in the northern province of Phetchabun between 1989 and 1996. In order to prevent human rabies deaths in Phetchabun province, a rabies control program was initiated in March 1993 with the specific aim to eliminate human rabies throughout the province by 2000. The program approached the target of elimination of human rabies through several strategies including: increasing the accessibility of post-exposure treatment for humans exposed to potentially and confirmed rabid animals; intensifying the documentation of post-exposure treatment in humans; increasing the educational awareness through advocacy in provincial schools, television programs and newspaper coverage; reducing canine rabies by monitoring the dog population and by implementing vaccination and sterilization programs; increasing the cooperation between the ministries of public health, agriculture and education on a provincial level and finally by assessing the impact of the program through intensified follow-up of patients exposed to both laboratory confirmed and suspected rabid animals. Increased post-exposure treatment for humans was achieved by expanding the usage of the 0.1 ml dose per site of the Thai Red Cross regimen using both PCECV and PVRV and implementing post-exposure treatment immediately for all human contact cases in lieu of observing a dog after the exposure for 10 days prior to initiating treatment. Documentation of post-exposure treatment was achieved through the development of a reporting form that was completed by the attending medical staff for every patient presenting at clinics and hospital emergency rooms. The canine population was monitored through the Phetchabun livestock department belonging to the ministry of agriculture. In addition, a canine vaccination project was initiated in 1996. The enclosed study summarizes the results of the strategic rabies prevention program implemented in Phetchabun province between 1997 and 2001.

2 MATERIALS AND METHODS.

The public health system of Phetchabun province is composed of 15 hospitals including one 400-bed provincial hospital; two 90-beds, seven 60-beds, two 30-beds and one 10-beds district hospitals; two private hospitals; 44 clinics and 150 health centres. All of these facilities receive vaccines supplied by the Phetchabun provincial health office and report disease incidence to this office.

Rabies is a reportable disease in Thailand. In order to document the number of post-exposure treatments throughout Phetchabun province, a specific animal bite reporting form was devised and distributed to all hospitals, clinics and health centres treating patients after exposure to suspect rabid animals. Prior to beginning the program, the medical staff of each clinic was required to participate in an intense rabies education program and was instructed as to the importance of completing the form in an accurate and precise manner. The information from these animal bite report forms was compiled

and evaluated to determine the number of post-exposure treatments administered and the ultimate success or failure of the rabies prevention program. In order to complete the animal bite form, each patient was interviewed by the attending physician or medical staff who then completed the report form, which included: name, address, age, sex, occupation, date of exposure, category of wound, type of animal, name of vaccine administered, regimen of vaccination, whether equine rabies immune globulin (ERIG) was administered, date of all subsequent vaccinations and any side effects to the vaccine.

Patients were initially examined by a physician when they presented at one of the emergency treatment centres. Wounds were cleaned and debrided as required. Suturing was avoided if possible. Even in category III exposures, ERIG was rarely administered because it was only sporadically available. Patients received either the traditional five dose intramuscular Essen regimen or the intra-dermal Thai Red Cross regimen. The vaccines used intra-dermally were PCECV and PVRV administered as a 0.1 ml dose per site, or purified duck embryo cell rabies vaccine (PDEV) administered as a 0.2 ml dose per site. All vaccines had a potency of at least 2.5 IU/intramuscular dose.

A parenteral canine vaccination program was initiated in 1996 with the cooperation of the Phetchabun livestock department and the public health office of Phetchabun. In this program health volunteers and staff traveled to various sites administering free animal vaccinations and conducting educational programs in a strategy to "educate the educators". In addition, the dog population was controlled by contraception and sterilization, especially targeting stray dogs and community dogs living around temples and schools. The livestock department of Phetchabun conducted a census of the dog population through the support of its officers and additional volunteers.

Whenever possible, the biting animal was captured, euthanased and tested for the presence of rabies virus. These animals were examined by the central laboratory of the livestock department of Phetchabun. Submitted specimens were analyzed using the direct fluorescent antibody test (FAT) as previously described (Trimarchi and Debbie, 1991). As a confirmatory test, tissues from animals that initially tested negative by the FAT were further analyzed using the mouse inoculation test (MIT), as previously described (Sureau *et al.*, 1991).
In the event that an animal was confirmed positive for the presence of rabies, the public health office was contacted and further investigation was conducted including the contact of all potential exposed persons. All confirmed exposed patients were immediately immunized and followed for a minimum of one year. In addition, 'Ring Vaccination' was conducted. The five human rabies deaths that occurred within Phetchabun province between 1996 and 1998 were thoroughly investigated to determine the circumstances surrounding their death.

Pre-exposure and post-exposure immunization in animals and humans were administered liberally to the extended family and neighbourhood where laboratory confirmed rabid animals were located.

3 RESULTS.

Table 31: distribution of post-exposure treatment regimens and WHO category of exposure in patients presenting at rabies treatment centres in Phetchabun province Thailand between 1997 and 2001.

YEAR	Number of PET	TRC PET (%)	PET with RIG (%)	TRC with RIG (%)	Category I (%)	Category II (%)	Category III (%)
1997	1939	923(47%)	20(1.0%)	8(0.4%)	91(5%)	566(29%)	1282(66%)
1998	1690	1125(66%)	80(4.7%)	22(1.3%)	47(3%)	438(26%)	1205(71%)
1999	2212	1412(64%)	127(5.7%)	27(1.2%)	212(10%)	515(23%)	1485(67%)
2000	2816	2282(81%)	34(1.2%)	34(1.2%)	96(3%)	259(10%)	2461(87%)
2001	1693	1485(88%)	48(1.6%)	30(1.7%)	139(8%)	413(24%)	1141(68%)
TOTAL	10350	7227(70%)	309(3.0%)	121(1.2%)	585(6%)	2191(21%)	7574(73%)

Data were compiled by Phetchabun provincial public health (Ministry of health, 2001).

10350 post-exposure treatmentswere administered in Phetchabun province of which 73% had received WHO Category III wounds (Table 31). The number of post-exposure treatments tended to increase annually from 1997 to 2001, mainly due to an increase in the use of the Thai Red Cross regimen and then decreased in 2001. During the years 1997 to 1998, there were two human deaths reported .During the last three years of the study period (1999 to 2001) no human deaths were reported (Table 32). Investigation of the two deaths that occurred from March 1996 through December 1998

revealed that these deaths occurred because neither patient had received vaccine or rabies immunoglobulin after exposure.

Table 32: number of exposures to suspected and proven rabid animals and human rabies deaths reported in Phetchabun province from 1992 to 2001.

Year	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	Total
Exposures	1698	1256	1340	1493	1692	1939	1690	2212	2816	1693	17829
Deaths	3	3	1	2	3	1	1	0	0	0	14

Data were compiled by Phetchabun Provincial Public Health (Ministry of health, 2001).

Compilation of information collected from the animal bite reporting forms indicated that 23% of all persons exposed to potentially rabid animals between 1997-2001 were involved in the agricultural industry (Ministry of health, 2001). Seventy-three percent of all recorded animal bites were categorized as WHO category III wounds (penetration of the intact skin with bleeding) with 93% of all potential exposures being caused by dog bites. Potential rabies exposures were reported most frequently in children under 10 years of age (43% of all exposures), followed by adolescents between the age of 10 to 19 (13.8%) and adults between the age of 30 to 39 (10.8%). Fewer than 3.0% of the exposed population in Phetchabun received ERIG between 1997 to 2001 due to the lack of production and availability (Table 31).

While the number of patients that received post-exposure treatment increased from 1997 to 2000, the number of vials used decreased due to the more frequent use of the Thai Red Cross regimen. At the end of 2001, 88% of all patients were receiving post-exposure treatment via the intra-dermal route. It was estimated that the Thai Red Cross regimen would save 60 to 80% of the costs associated with the intramuscular Essen regimen. However, due to various reasons, the real cost savings was to be calculated closer to 40%. The difference in real cost savings was attributed to the fact that some physicians were reluctant to use intra-dermal regimens or had not mastered the intra-dermal technique. In other cases, private patients preferred to purchase sufficient vaccine to receive the five doses intramuscular Essen regimen. In addition, some clinics were too small to use the intra-dermal regimen effectively since once opened, according to the manufacturers' recommendation, a vial can only be stored for 8 hours. Recently, studies conducted in Thailand have demonstrated that opened vials of tissue culture vaccines may be stored at 4 to 8°C up to seven days if sterility is maintained (Kamolthan *et al.*, in press; Khawplod *et al.*, in press).

According to the dog population census, the canine population increased by 10% between 1996 and 2001, expanding from approximately 91000 in 1996 to more than 105000 in 2001 (human:dog ratio equals 10:1). Based on census data, 89% of the dogs had identifiable owners and 64% of the canine population had received at least one vaccination against rabies (Table 33). During the five years period, there were 1114 animals submitted for laboratory testing after exposure of humans. One hundred and eighty animals (16%; range = 8-32%) were confirmed to be rabid by FAT or MIT. This variation of percentages was due to continuous dog vaccination campaign and population control projects in Phetchabun. Twenty patients exposed to laboratory confirmed rabid animals received intramuscular post-exposure treatment, another 168 patients exposed to laboratory confirmed rabid animals received post-exposure treatment using the Thai Red Cross regimen. These patients received the following vaccines: 148 patients received 0.1ml of PCECV per site, 10 received 0.1ml of PVRV per site and 10 received 0.2ml of PDEV per site. Of the 188 patients exposed to laboratory confirmed rabid animals, 57 (30%) had WHO Category I wounds, 56 (30%) had WHO Category II wounds and 75 patients (40%) experienced WHO Category III wounds. Of these patients with Category III wounds, 6 were treated with the intramuscular regimen; 69 patients received the TRC regimen (Figure 29). All patients were followed up for at least one year post-treatment through the use of an effective primary health care system which included: health volunteers in the villages, health workers in sub-district health stations and the provincial public health organization of Phetchabun province. All patients were confirmed to be alive.

Table 33: the estimated dog population and vaccine coverage in Phetchabun province between 1996 and 2001.

Dog population	1996	1997	1998	1999	2000	2001
Estimated total	91190	93792	95144	99838	102292	105272
Vaccinated (Percent)	66568 (73%)	66654 (70%)	74212 (78%)	76875 (77%)	65466 (64%)	67372 (64%)

Data were compiled by the livestock department of Phetchabun.

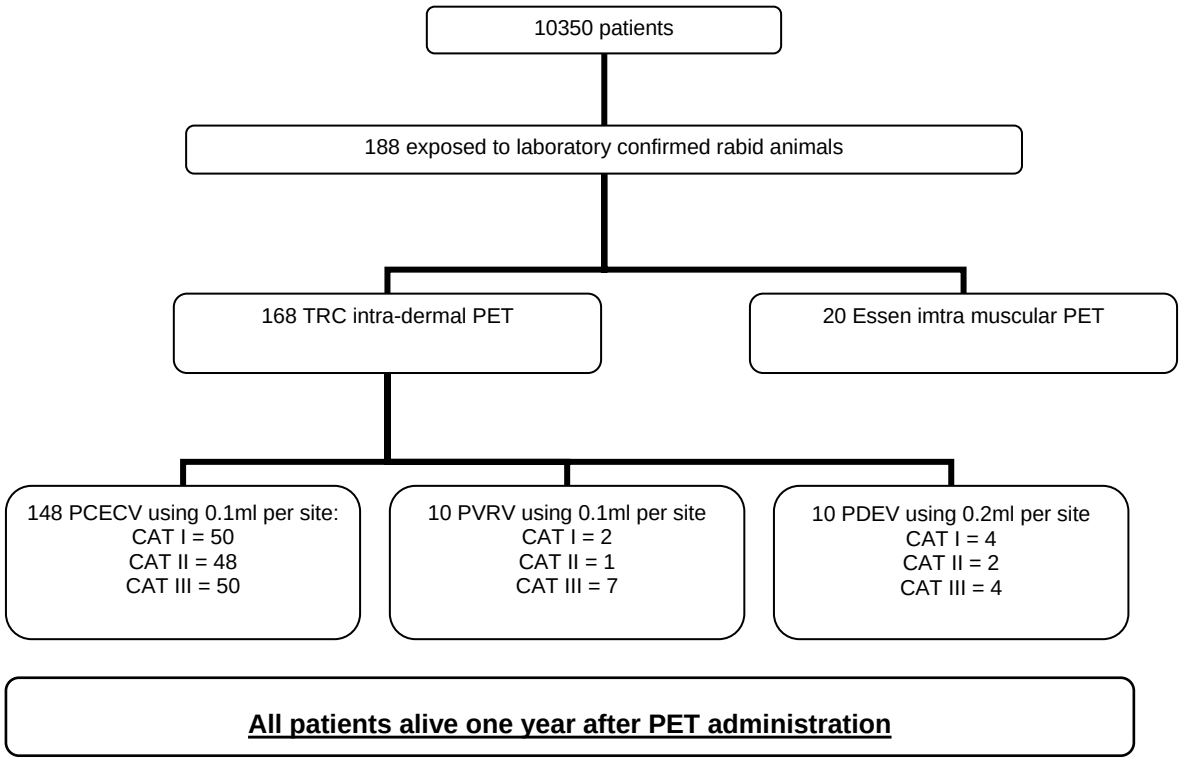
Side effects to the vaccines, including local itching, redness and pain, were rarely reported. No systemic or allergic reactions or serum sickness were reported.

4 CONCLUSIONS.

The data presented in this report represent the results of the first large scale program in Southeast Asia, through joint cooperation of human and animal health authorities as well as government and non-governmental organizations. The cornerstone to the implementation of a successful rabies prevention program is the education of medical officials and local residents. The fact that no human death occurred during the last three years of the five years program indicate that the program has been successful.

Educational programs initiated at the beginning of the program in Phetchabun province helped to increase the awareness of the correct post-exposure treatment in the medical community. Additional media coverage accompanied by the assistance of community volunteers augmented the existing rabies awareness programs, thereby spreading rabies prevention information throughout the general population. The enhanced educational focus certainly accounts for the continual increase in the number of patients that received post-exposure treatment from 1997 to 2000. The significant decrease in the number of post-exposure treatments administered in 2001 is attributed to changes in the reporting system when a new public health system was implemented. It is of concern that 73% of the patients experienced Category III exposures between 1997 and 2001 but only 3% received ERIG. This unfortunate situation highlights the current state of affairs in Asian countries facing critical shortages of RIG. For the past decade supplies of human as well as equine RIG have continually declined throughout the world, and from all reports this trend will continue (Briggs, 2002). Increasing current supplies and/or funding of new technologies to replace RIG is urgently needed and should be a top priority for WHO and other global health foundations.

Figure 29: number of patients and treatment regimens administered to patients presenting at emergency clinics after exposure to suspect and confirmed rabid animals in Phetchabun province between 1997 to 2001. All patients treated received post-exposure treatment by the Thai Red Cross intra-dermal regimen or by the intramuscular Essen regimen.



As has been reported previously, the incidence of exposures in children was similarly high in Phetchabun province (WHO, 2001). Protecting children from rabies exposure is a difficult if not impossible task in Asian countries where canine rabies is endemic and stray dog populations continue to increase as is evidenced in this report. Parents that are educated as to the lethality of rabies and proper post-exposure treatment protocol will certainly make every effort to insure that their children receive treatment after exposure to a potentially rabid animal. Thus it is critical for the general public to receive education as to the consequences of a rabies exposure, the location of local treatment centres within their area, and the necessity for prompt action after an exposure occurs.

Decentralization of the Public Health system in Thailand which occurred in 2001 could potentially hamper the availability of vaccines in local communities. One effect already evident is the lack of national reporting information as seen in 2001. Procurement of vaccines and other pharmaceuticals is now under the control of the local government. Thus, some local governments may choose to spend their allocated budget on pharmaceuticals other than rabies vaccines. This must be carefully monitored in the future.

The efficacy for using reduced dosages of TCV in the Thai Red Cross regimen was confirmed in this five year study where 7227 patients were administered this specific regimen. Regarding the dosage per site: 0.1ml of PCECV or PVRV was used whereas the dosage for PDEV was 0.2ml per site. Thus, this is the largest single database available to date, with continued follow-up, to validate and endorse the use of 0.1 ml per site of both PCECV and PVRV for treatment in patients that present with Category III wounds. The use of these vaccines in reduced intra-dermal regimens is currently the only means by which most developing countries will be able to financially afford the replacement of nerve tissue vaccines.

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EUROPEAN INTERLABORATORY F.A.T. COMPARISON TEST 2001

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During previous meetings organised on rabies control in central and eastern Europe, it was decided to establish a regular interlaboratory testing for rabies diagnosis. This paper presents how the test has been prepared and the first results obtained.

1 RECOMMENDED TECHNIQUES USED FOR LABORATORY DIAGNOSIS OF RABIES.

The 4th edition of WHO "Laboratory techniques in rabies" and the "Manual of standards for diagnostic tests and vaccines" edited by OIE recommend different tests for routine rabies diagnosis.

Rabies diagnosis cannot be based on a simple clinical examination of suspected animals. Some symptoms are quite characteristic of rabies (like the bi-tonal barking of a rabid dog) but most often they are signs of a non specific nervous disease (wild animals without fear of man, difficulties in swallowing …). Another point is that these signs may not be all observed on a rabid animal.

The only way to be sure of a diagnosis of rabies is then to use laboratory techniques. These are used to detect the virus itself, some of its components or tracks of its replication in infected tissues.

Historically, histological methods have been used to detect the Negri bodies. These intra-cytoplasmic acidophilic inclusions are specific of rabies. They correspond to a matrix containing a big quantity of N protein. So, these techniques (as Mann's or Seller's ones) are based on a non-specific tinctorial affinity to detect specific organites. Like for every histological method, the main withdraw of these techniques is that it needs fresh material to produce a reliable result.

1.1 Detection of rabies antigen.

Different immuno-chemical methods have been developed to detect the virus or its antigens. The most used test is the fluorescent antibody test (FAT). The general principle is to apply on a fixed specimen a specific conjugate. This antibody is generally coupled to FITC, it may be a polyclonal one or a mix of monoclonal ones.

This test is the central technique of laboratory diagnosis of rabies because it gives alone a diagnosis and it is also used to confirm rabies in the inoculation tests.

1.2 Detection of rabies virus replication: inoculation tests.

The other group of available techniques aim at detecting the replication of the virus on living substrates, i.e. cells or mice.

In mice rabies induces clinical signs that are relatively typical but it is better to confirm with a FAT control on the first mouse.

In cells, rabies virus grows generally without cytopathic effect; once again it is necessary to use FAT to confirm the presence of rabies virus in cells.

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2 WHAT IS THE INTERREST OF INTERLABORATORY TESTS IN RABIES DIAGNOSIS?

The general principle that was decided during Zagreb meeting is the following:

1) Every participating country nominates a national reference laboratory for rabies diagnosis.
2) This laboratory will participate to the test
3) And then if there are some regional laboratories in the country, this national laboratory will organise tests for them.

3 PREPARATION OF THE TEST.

3.1 The prerequisites.

- This test is the first step of rabies diagnosis interlaboratory testing; the next one will be the control of inoculation tests. It is then necessary that the panel of test specimens may be used indifferently for FAT and inoculation tests. *Consequence: the panel should be prepared with non inactivated material.*
- To be close to real conditions different "titres" must exist in the panel. *Consequence: it is necessary to prepare homogenates of infected material in non-infected brain material used as diluant.*
- To be close to real conditions different "strains" and genotypes must exist in the panel. *Consequence: the panel should include different strains that are collected in Europe.*
- All laboratories must receive identical parts of the same test preparation. *Consequence: central preparation and testing of the panels.*
- The panel should be easy to send and it should be prepared in a way that makes it as independent from cold and speed of transport as possible. *Consequence: the test preparation should be freeze-dried.*

3.2 Choice of strains.

The following rabies virus strains have been selected for the interlaboratory F.A.T. comparison test 2001:

- A fox strain isolated in the field in France in 1986 and maintained by serial passages on fox since then. This strain belongs to genotype 1.
- A polar fox strain isolated in Estonia in 1996. This strain belongs to genotype 1.
- A bat strain isolated in France and characterised as EBL1b.
- An atypical fox strain isolated in Eastern Germany, genotype 1.

3.3 Choice of the dilution medium and dilution method.

The fox strain was obtained from the brain of control foxes inoculated with a fox street strain maintained by serial passages on foxes during a challenge. After collection, brains have been kept in deep freezer. The other strains have been produced by intra-cranial inoculation to mice; this corresponds to the first passage from the original specimen.

In this FAT test, we decided to dilute positive brain material to make different "titres" (i.e. to prepare strong and weak specimens) because it is the best way to be sure that all participants to the test will receive identically infected tissues. It is also a way to include in the test the making of the smear and the fixation step, which should not have been possible if the panel was constituted of already fixed slides ready to stain. Moreover, homogenizing the material is a better way to obtain identical aliquots than to prepare prints or smears on glass slides from an infected piece of brain.

The choice of the dilution substrate was made according to the following points:

- rabies diagnosis is carried out on brain tissue
- rabies is maintained by red fox in Europe hence, fox is the most often tested species.

Consequently, the dilution substrate is made of negative fox brain tissue that is homogenised manually with mortar and pestle with the different positive brain tissues. This way, it is easy to prepare strong and weak specimens.

3.4 Constitution of the test panels.

Once a batch of a test preparation is ready, the whole homogenate is aliquoted and freeze-dried. After control of the final product, this batch may be sent for testing.

Each panel comprises:

- Three control tubes: a negative, a strong positive and a weak positive.
- Eight test tubes: three negative ones, a strong positive and a weak positive fox strain, "medium positive" with atypical fox strain, polar fox strain and bat strain (EBL1).

Each test tube of the panel was coded and the codes are changed between panels.

4 RESULTS OF THE TEST.

As it was stated above, national reference laboratories for rabies diagnosis have been identified in 16 countries. All of them have been contacted to send us import licence that must be included in the inter-laboratory test panel.

Fifteen countries sent an import licence and received a test panel.

It was asked to the participants to test twice the panel during two different diagnosis sessions and to provide us a copy of the technical procedure routinely followed in the laboratory. Three countries tested the panel according two procedures (with two different conjugates or two concentrations of the conjugate).

The global analysis of results is summarised in Table 34

Table 34: global results of the interlaboratory tests.

Country code	Number of tests	Correlation between the two tests	Errors in controls	Errors in tests
A	1	-na-	0	negative
B	2	good	0	0
C1	2	good	0	fox weak positive atypical fox strain
C2	2	1 discrepancy in controls	weak positive	EBL1 fox weak positive
D	1	-na-	0	0
E	2	good	0	0
F1	2	good	0	0
F2	2	good	0	0
G	2	1 discrepancy in test	0	negative
H	1	-na-	0	2 negative fox weak positive
I	2	good	0	0
J1	2	good	0	0
J2	2	good	0	0
K	2	good	0	0
L	2	good	0	0
M	2	good	0	0
N	2	good	0	0
O	2	good	0	0

The summary of this shows that 5 out of the 15 laboratories (or out of the 18 procedures tested) made errors.

Four false positive diagnoses were made in 3 different laboratories.

The errors in positive samples correspond to fox strain weak positive (3 test slides and one control slide), EBL1 or atypical fox strain. These samples may be more difficult to stain with some conjugates that have been diluted or that have not a large enough range of detection.

These data could have been correlated with the examination of detailed technical procedures. Unfortunately, only 6 of them have been received so far.

5 SUMMARY OF THE LAST TECHNICAL QUESTIONNAIRE (PRESENTED IN ZAGREB, 1998)

5.1 Analysis of answers.

The reliability of experimental diagnosis of rabies depends mainly on the regular practice of the techniques. Which means that if training technicians is important, it is as important to maintain this trained status by the regular practice and by regular quality control of the work performed.

I.e. a more or less centralised structure of experimental diagnosis in the country is necessary because it is the only way to allow a daily practice of the tests.

5.1.1 Technicians performing the diagnosis.

31 laboratories answered to this part of the questionnaire. In 21 laboratories less that 5 technicians are involved in rabies diagnosis, which should allow a regular training and so the reliability of diagnosis.

Only 2 laboratories have „full time“ technicians. Most often (16/31) rabies diagnosis is a part of the activity of the technicians (generally more than 75%). The other activities of these technicians consist principally in other diagnosis work.

5.1.2 Fluorescent antibody test.

The **number of examinations** performed in a laboratory ranges between 150 and 3500 in the rabies infected countries (i.e. .between one diagnosis every other day and 9 every day). The "low" daily activity is also a financial problem regarding the conservation of diagnosis conjugate because the use-by date will be reached before the end of the vial.

Most often, the fluorescent antibody test is performed on an impression of brain material (23/27), smears are prepared in 13 laboratories. Nine laboratories use both impression and smears. Both techniques suppose that the specimen is in a good state to make a thin smear/impression that will be easy to examine and so FAT will be more reliable. The study of discrepancies between techniques when FAT and MIT or FAT and CCT are used shows that the percentage of discrepancies is significantly higher when specimens are autolysed than when they are in a good state.

Different sections are routinely examined. Ammon’s horn is tested in 26 laboratories, cerebellum in 19, medulla oblongata in 19 and cortex in 15. Twenty-three laboratories examine at least Ammon’s horn with other parts of brain. Four out of 23 laboratories examine routinely salivary glands.

Once the slide is prepared, fixation is performed. **Fixation** is easier for thin specimens that have been allowed to dry correctly on the slide; once again the importance of the state of conservation appears. To obtain this it is very important to avoid any autolysis between the discovery of the animal and the diagnosis, i.e. rapid transport with cold chain to the laboratory. Twenty-four laboratories fix the slides in acetone, 7 heat the slide to fix the specimen. Two laboratories use both heat and acetone fixation. When acetone is used, the fixation time ranges between 5 minutes and 4 hours, 10 out of 21 laboratories fix for 30 minutes. The fixation is generally made around –20°C (14 laboratories out of 21), 4 laboratories fix at room temperature. Acetone bath is changed daily (8/21), twice a week (3/21), weekly

(6/21), twice a month (3/21) or monthly (1/21), generally the higher is the number of diagnosis performed, the more frequent is the changing of acetone.

Twenty six laboratories use a commercial **conjugate**. Twenty three of them use it at the „normal“ dilution, 3 dilute the reconstituted conjugate more then it is recommended by the producer. It is a classical way to spare money but some "over-diluted" conjugates are not stable and must be used on the first day.

Eleven out of 15 lab use glycerol associated with a buffer as **mounting medium** for the slides. The pH of this medium is generally set around 7.0, which is not the optimal pH for fluorescence of FITC (>8.5); only 3 laboratories use such an alkaline mounting medium.

The **microscopes** used to examine these slides are generally equipped with a mercury bulb (23/27). Halogen bulbs are used only in 7 laboratories. Both bulbs are suitable for FITC which is excited by blue light (490nm). The objective magnification ranges between 10 and 100. Fifteen laboratories use magnification less than 50 which will give a clear image even with thick specimens that cannot be clearly read clearly with a 90X or 100X magnification objective. The eyepiece magnification ranges between 4 and 13, which gives a global magnification ranging between 100 and 1000. The „mean“ combination is 10X eyepiece associated with a10 to 40X objective.

The slides are generally examined by 2 technicians (13/22). This **double reading** is a good way to have a reliable diagnosis. The stained and examined slides are kept for at least 1 month (up to 24).

A **"safety“ sample** is collected by 23/25 laboratories. This sample is generally kept frozen (-20°C) for at least 1 month (up to 24).

Seven countries that perform or have performed oral vaccination campaigns look for the possibility of vaccine induced rabies. This test is made in the laboratory that performs the diagnosis.

Different **controls** may be used to validate the fluorescent antibody test :

- Positive CVS controls (mouse brain) are used by 18/24 laboratories.
- A positive specimen from the day before is used as a control by 14/23 laboratories.
- A negative control (mouse brain) is used by 11/22 laboratories.
- A negative specimen from the day before is used as a control by 11/22 laboratories

These controls are used in every staining process by 16/21 laboratories. Others use controls once or twice a week or once or twice a month.

5.2 Minimum requirements.

To compare results of rabies surveillance, correlation of results is the first thing to assess. The standardisation of the techniques is the second step.

With respect to diagnostic procedures, there exist three main international references dealing with laboratory techniques in rabies which can be regarded as the current standard works of rabies diagnostics. Based on ongoing developments in the field of diagnostic research the recommendations given there are permanently brought up to date:

· Meslin, F.-X., M.M. Kaplan, and H. Koprowski (1996),WHO, Laboratory techniques in rabies, 4th edition, Geneva,

· O.I.E. Manual of standards for diagnostic tests and vaccines (2000),

The widest spectrum of diagnostic standard tests is presented by the WHO with detailed information concerning preconditions to be needed, materials and methods and comprehensive discussions on the advantages and disadvantages of the tests available.

Fluorescence antibody test minimum requirements:

- The suitability of an anti-rabies conjugate to be used for FAT in rabies routine diagnosis has to be proven with respect to its sensitivity and specificity.

- Commercially available anti-rabies conjgates should be diluted according to the producers instruction, especially when it contains of a mix of monoclonal antibodies.
- Positive and negative controls should be used in every staining process.
- Rabies-positive samples have to be proven for the presence of vaccinal or field induced rabies.

The regular daily practice of FAT is the only way to maintain the quality of rabies diagnosis. That is why a centralised structure is necessary. It is much better to have one national lab that performs 5 diagnosis a day than 10 regional labs that receive 1 specimen every other day. This structure is also a good way to use expensive reagents like conjugate completely before the expiry date.

6 CONCLUSION.

This first interlaboratory test for rabies diagnosis was long to begin but it may now be regularly maintained on an annual basis for instance according to the following agenda:

1. first quarter: reception of the import licences
2. second quarter: sending panels and reception of results with a second import licence (for a possible secont panel)
3. third quarter: analysis of results and possible second test if needed.
4. end of the year: global analysis.

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MOLECULAR DIAGNOSTIC TOOLS FOR THE RAPID STRAIN IDENTIFICATION OF RABIES IN HUMANS

Anthony R Fooks[1]

1 INTRODUCTION.

Rabies is routinely diagnosed on clinical and epidemiological grounds, especially when exposure in a rabies-endemic country has been reported. Negative diagnostic tests using conventional assays, even late in the disease, do not exclude the clinical diagnosis as these tests are never optimal and are entirely dependent on the nature and quality of the sample supplied. For this reason, molecular tools and virus typing are becoming more widely used for the rapid detection and strain identification from clinical specimens. An early laboratory diagnosis is critical and may decrease the number of unnecessary contacts with the patient, reduce the requirement for invasive and costly interventions and enable the appropriate medical treatment regimen for the patient to be administered. Three human cases of rabies in the UK (2001 – 02) are described to highlight the capability of molecular diagnostic tests for the rapid identification of rabies virus and the potential to type strains to both host and geographical location.

2 HUMAN RABIES CASES IN THE UNITED KINGDOM.

Between 1977 and 2002, eleven human rabies deaths occurred in the UK as a result of infection in India [4], Pakistan [2], Nigeria [2], Zambia, Bangladesh and The Philippines. None of these documented cases of human rabies received post-exposure treatment for rabies before the appearance of symptoms. In April 2001, the first case of human rabies that year, imported from the Philippines to the UK, was recorded (Smith *et al.*, 2003). In May 2001, a second case of human rabies was suspected in a patient who had travelled to the UK from Nigeria (Johnson *et al.*, 2002). Rabies remains widely distributed in almost all of the inhabited islands throughout the Philippine archipelago and is widespread throughout West Africa. In November 2002, an indigenous case of human rabies-encephalitis following exposure to bats was reported in the UK (Fooks *et al.*, 2003).

2.1 Case 1 (UK ex. Philippines).

A 55-year-old man, presented to hospital after having been bitten on the palm of the hand by a dog while visiting the Philippines 6-weeks earlier. The patient was managed supportively but developed progressive cardiovascular instability and died 12 days after the onset of symptoms.

2.2 Case 2 (UK ex. Nigeria).

A 52-year-old female was admitted to hospital following a short illness and after having been bitten on the leg by a dog in Nigeria some five months previously. Within twenty-four hours of admission she showed a marked deterioration and died shortly afterwards.

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2.3 Case 3 (Scotland – UK).

A 55-year-old bat conservationist was admitted to hospital with an unexplained neurological illness. He presented with a 5-day history of pain and paraesthesia in the left arm followed by increasing weakness of his limbs with evidence of evolving encephalitis. Nineteen days after the onset of illness the patient died.

3 MATERIALS AND METHODS.

3.1 Polymerase chain reaction.

Total RNA was extracted from clinical samples using the TRIzol method following the manufacturer's instructions (Invitrogen Life Technologies). In samples of low cellular content including saliva, 15 µg yeast tRNA (Invitrogen Life Technologies) was added as a carrier to each extraction mix. Extracted RNA was quantitated using a Genequant II spectrophotometer (Pharmacia Biotech). The RNA concentration was adjusted by dilution with HPLC grade H20 to 1µg/µl. The precautions for PCR were strictly followed to avoid false positive PCR results, as described by others (Kwok and Higuchi, 1989).

3.1.1 Reverse transcription and 1st-round PCR.

Reverse transcription was performed using a pan-Lyssavirus primer JW12. The cDNAs were amplified using the specific primer set JW12 and a cocktail of JW6 (DPL, M and E).

3.1.2 2nd-round PCR.

A hemi-nested second-round PCR reaction was carried out by employing a set of three internal primers JW10 (DLE2, ME1 and P).

Table 35: oligonucleotide primers used for RT-PCR.

Primer	Sequence (5' to 3')	Messenger / genomic sense	Position in genome	Use of primer
JW12	ATGTAACACCYCTACAATG	M	55-73	RT
JW6 (DPL)	CAATTCGCACACATTTTGTG	G	660-641	1st-round PCR
JW6 (M)	CAGTTAGCGCACATCTTATG	G	660-641	1st-round PCR
JW6 (E)	CAGTTGGCACACATCTTGTG	G	660-641	1st-round PCR
JW10 (DLE2)	GTCATCAAAGTGTGRTGCTC	G	636-617	2nd-round PCR
JW10 (ME1)	GTCATCAATGTGTGRTGTTC	G	636-617	2nd-round PCR
JW10 (P)	GTCATTAGAGTATGGTGTTC	G	636-617	2nd-round PCR

4 RESULTS.

4.1 Case 1 (UK ex. Philippines): ante-mortem detection of rabies nucleic acid within the skin and saliva using PCR.

The clinical diagnosis of rabies was confirmed on ante-mortem specimens using molecular techniques only. Only the PCR based assays provided unambiguous results, possibly reflecting the low levels of virus present in these peripheral sites at the time of sampling (Table 36). The PCR products were sequenced within 36 hours of sample receipt and in the absence of post-mortem samples provided the only confirmation that this was a case of rabies.

A 309-nucleotide sequence from the wound sample (day 4) was obtained and sequence analysis demonstrated that this isolate was closely related to other Asian isolates. The acquisition of the Philippine isolates and sequence data enabled us to confirm that the UK isolate was of Philippine origin and most closely resembled that of a previously submitted rabid dog sample from the Philippines.

Table 36: case 1 (UK ex. Philippines): ante-mortem PCR tests.

Specimen	Date sampled	1st round PCR (N-gene)	2nd round PCR (N-gene)
Saliva	Day 2	-	-
Skin (Nape)	Day 2	-	-
CSF	Day 4	-	-
Skin (Wound)	Day 4	+	+++
Skin (Nape)	Day 4	+	+++
Saliva	Day 4	+	+++

Key: - negative; + weak positive; ++ positive; +++ strong positive

4.2 Case 2 (UK ex. Nigeria): post-mortem detection of rabies nucleic acid within the brain using PCR.

Clinical diagnosis in this case was confirmed only on post-mortem brain material (Table 37). RT-PCR was performed on samples from a number of compartments of the brain, the skin, salivary gland and heart tissue. Positive results were found for every tissue compartment apart from the heart. Stronger PCR bands were consistently found within the brain samples and weaker bands found for the salivary gland and skin tissue.

Table 37: case 2 (UK ex. Nigeria): post-mortem PCR tests.

Tissue sample	PCR (N-gene)*
Hippocampus	+++
Cortex	+++
Cerebellum	+++
Pons	+++
Salivary gland	+
Skin (Nape)	+
Heart	-

*Results are represented as a strong band (+++), a weak band (+) or negative (-).

All of the brain samples tested were FAT positive (+++). In contrast, the salivary glands, neck and heart tissue samples were not tested by FAT (Johnson et al, 2002).

4.3 Case 3 (Scotland – UK): ante- and post-mortem detection of rabies nucleic acid using PCR.

A saliva specimen identified the presence of low levels of RNA in a first round PCR reaction (Table 4). Using a hemi-nested 2nd-round RT-PCR, saliva samples collected on different days proved positive. Within 36 hours of a positive PCR result, a 400-bp region of the nucleoprotein gene was sequenced and identified a strain of European bat lyssavirus (EBLV) type-2a. One saliva sample (sample 3), however, was negative by both first and second-round PCR reactions indicating transient excretion of virus in saliva. Interestingly, both the submandibular and parotid salivary glands were negative by first-round PCR reactions but positive by second-round (nested) PCR reactions confirming the low levels of virus present in these tissues. Positive results from autopsy samples were however, found for each of the three brain regions using RT-PCR.

Table 38: case 3 (Scotland – UK): detection of rabies virus by PCR.

Ante-mortem samples		
	1st Round PCR	**2nd Round PCR**
Human saliva – sample 1	-	+
Human saliva – sample 2	+ (weak)	+
Human saliva – sample 3	-	-
Human saliva – sample 4	-	+
Human saliva – sample 5	-	+
Positive Control	+	+
Negative Control	-	-
Post-mortem samples		
	1st Round PCR	**2nd Round PCR**
Cerebellum	+	ND
Medulla	+	ND
Hippocampus	+	ND
Submandibular salivary gland	-	+
Parotid salivary gland	-	+

ND: Not done

5 CONCLUDING REMARKS.

Diagnostic tests for the confirmation of human rabies should include a combination of both conventional and molecular tools. In our laboratory, ante-mortem diagnosis mainly from saliva samples is made using virus isolation, PCR and sequencing. In contrast, post-mortem diagnosis from brain samples is most rapidly made using an FAT and confirmed by PCR and sequencing. In many circumstances, an inconclusive laboratory-based ante-mortem diagnosis for rabies is only plausible from autopsy brain samples.

In a study of 10 rabies patients in the USA for whom RT-PCR was used for diagnosis, viral RNA was detected in all of the patients tested including three patients in whom saliva was negative for virus isolation (Noah *et al.*, 1998). Using molecular tools for diagnosis however, involves extreme care and diligence to avoid false-positive results by PCR. Confirmation of rabies in a patient should only be made if two or more tests generate a positive result, preferably from samples taken on different days.

These three human rabies cases in the UK demonstrate the potential of PCR-based assays to rapidly diagnose rabies in a patient where other conventional diagnostic methods have been unsuccessful.

Although countries in Africa with a poor healthcare infrastructure recognise that molecular-based diagnostic assays will be unaffordable for routine use, the cost / benefit ratio should still be measured. Importantly for laboratories throughout Africa, is the benefit that molecular tools offer the capability for a differential diagnosis of human encephalitic diseases that present with similar clinical symptoms.

The principal goal for diagnostic laboratories in Africa will involve a co-operative approach between neighbouring countries in Africa and between laboratories in Africa and other recognised collaborating laboratories around the world. This should include the sharing of diagnostic material for genotyping rabies virus isolates to obtain a better understanding of the epidemiology of rabies in different hosts and geographical regions within Africa.

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DEVELOPMENT OF A HEMI-NESTED RT-PCR METHOD FOR THE SPECIFIC DETERMINATION OF EUROPEAN BAT LYSSAVIRUS 1. - COMPARISON WITH OTHER RABIES DIAGNOSTIC REFERENCE METHODS

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ABSTRACT.

A simplified Hemi-Nested Reverse Transcriptase Polymerase Chain Reaction (hnRT-PCR) has been developed to determine specifically the European Bat Lyssavirus 1 (EBL1) nucleoprotein gene. The specificity of this method was determined by using the seven genotypes of lyssavirus by RT-PCR, Southern Blot and sequence analysis. Compared to the reference rabies diagnostic methods, the technique showed a higher sensitivity. In view of these results, we suggest this new hnRT-PCR should be performed for the epidemiological survey of bat colonies, also providing rapid detection and genotyping of EBL1 until now encountered in all naturally infected bats in France.

1 INTRODUCTION.

Rabies Virus (RV) and Rabies-Related Viruses (RRV) belong to the Rhabdoviridae family, *Lyssavirus* genus, which is subdivided into seven genotypes based on RNA sequencing [1,2,3,4,5]: genotype 1 (classical rabies virus), genotype 2 (Lagos Bat virus), genotype 3 (Mokola virus), genotype 4 (Duvenhage virus), genotype 5 (European Bat Lyssavirus 1, EBL1), genotype 6 (EBL2) and genotype 7 (Australian Bat Lyssavirus, ABL).

In Europe, bat rabies is caused by two specific virus genotypes, EBL1 and EBL2 [4]. Infected bats have been reported in several European countries, from Russia to Spain, particularly in coastal regions [6]. EBL1 isolates are mainly obtained from *Eptesicus serotinus* and sometimes from *Vespertilio murinus*, *Myotis myotis*, *Myotis nattereri*, *Miniopterus schreibersii*, *Rhinolophus ferrumequinum*, while EBL2 isolates were mainly identified in *Myotis dasycneme* and *Myotis daubentonii* [7]. The European bat lyssaviruses 1 and 2 have recently been divided into the phylogenetic lineages EBL1a, EBL1b, EBL2a and EBL2b [1]. EBL1 is mainly distributed in Western European countries such as France, Germany, Spain [6,7,8,9] whereas EBL2 seems to be recorded in North-western European countries such as United Kingdom, Finland, Holland [6,10]. In France, 14 cases of bat rabies due to EBL1 virus have been diagnosed from 1989 to the end of 2002, all occurring on *Eptesicus serotinus*. Until now and despite an improvement of the surveillance system in the last few years, no EBL2 isolate has been detected in France. An additional case was recorded in May 2000 on an imported tropical fruit bat *Roussetus ageapticus* [11,12], infected with a Lagos Bat virus (genotype 2).

Three major methods of laboratory diagnosis of rabies have been standardised and recommended by the World Health Organisation [13]: the fluorescent antibody test (FAT), the rabies tissue-culture infection test (RTCIT) and the mouse inoculation test (MIT). These methods vary in efficiency, specificity and reliability. FAT is the most commonly used diagnostic test, it detects virus antigen in the brain using FITC-labelled anti-rabies antibodies [14]. FAT is the reference technique for post-mortem examination, however the sensitivity of this method depends on the biological quality of the specimen submitted for diagnosis. The two other techniques, RTCIT and MIT, detect rabies virus replication respectively in cells and in mice. Results of both tests have to be confirmed by FAT [15]. The MIT

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presents the advantage of allowing further identification and characterisation of the isolate from the brain of inoculated animals [15].

For about 10 years, developments in molecular biology have been improving in rabies research field. The Polymerase Chain Reaction (PCR) provides an interesting tool for genetic characterisation of virus strain by sequencing the amplified products. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) in rabies diagnosis may be a rapid and sensitive alternative technique to the previous rabies diagnosis methods [16-23].

This study describes the development of a simplified hemi-nested RT-PCR (hnRT-PCR) which is sensitive as well as specific and allows a rapid identification of EBL1 a and b viral isolates without sequencing PCR products. In addition, this paper compares the sensitivity of this hnRT-PCR to the rabies diagnosis reference techniques, namely MIT and RTCIT.

2 MATERIAL AND METHODS.

2.1 Material.

2.1.1 Rabies virus isolates.

Table 39: characteristics of rabies and rabies-related viruses.

RabiesVirus	Identification number	Animal species	Origin and year of death	Biological origin of the isolate	GenBank accession number
Laboratory fixed strains					
CVS-11	CVS-11			BHK-21 passaged	
CVS-27	CVS-27			Mice passaged	
Genotype 1 (Rabies virus)					
Genotype1	GS9	*Vulpes vulpes*	France, 1986	P4 fox brain	
Pasteur Virus				M13215	
Genotype 2 (Lagos bat virus)					
Lagos bat virus	119645	*Roussetus aegyptiacus*	*France, 1999	P2 brain	
Lagos bat virus	Lagos_af_bat	*Eptesicus helvum*	1958	ND	U22842
Genotype 3 (Mokola virus)					
Mokola virus	ND	ND	ND	ND	
Mokola virus	Mokola_af	Cat	Africa, 1991	ND	U22843
Genotype 4 (Duvenhage virus)					
Duvenhage virus	ND	ND	ND	ND	
Duvenhage virus	86132_af	Human	Africa, 1986	ND	U22848
Genotype 5 (European Bat Lyssavirus type 1: EBL1)					
EBL1a	RV631	ND	ND	BHK passaged	
EBL1a	9367_hol	*Eptesicus serotinus*	Poland, 1985	ND	U22844
EBL1b	122319	*Eptesicus serotinus*	France, 2001	P1 brain	
EBL1b	122276	*Eptesicus serotinus*	France, 2001	P1 brain	
EBL1b	122154	*Eptesicus serotinus*	France, 2001	P1 brain	
EBL1b	121411	*Eptesicus serotinus*	France, 2000	P3 brain	
EBL1b	RV266	*Eptesicus serotinus*	France, ND	BHK passaged	
EBL1b	8918_fra	*Eptesicus serotinus*	France, 1989	ND	U22845
Genotype 6 (European Bat Lyssavirus type 2: EBL2)					
EBL2a	RV228	*Myotis daubentoni*	England, 1996	BHK passaged	
EBL2a	9018_hol	*Myotis dasycneme*	Holland, 1987	ND	U22847
EBL2b	RV8	Human	Finland, 1985	BHK passaged	
EBL2b	9007_fin	Human	Finland, 1986	ND	U22846
Genotype 7 (Australian Bat Lyssavirus: ABL)					
ABL	ABL	ND	Australia, 1998	P5 brain	
ABL	ND	Bat	Australia, ND	ND	AF081020

P1: first mouse passage material brain, N.D.: not determined.
*: Imported animal from Africa

Four isolates of EBL1 virus from France (genotype 5), two isolates of rabies-related viruses (genotype 2 and 7), one fox strain (identified GS9: genotype 1) and two laboratory fixed strains (CVS-27 and CVS-11) were chosen to validate the technique (Table 39). Nine viral sequences (genotype 1 to 7) published and extracted from GenBank were used in the phylogenetic analysis. We received cDNAs of

genotypes 3 and 4 from the university of Pretoria (Africa) and genotypes 5 and 6 from VLA (UK) (Table 39 and Table 41). Table 39 summarises all the characteristics of these viruses (species from which the viruses were isolated, geographical origin sources, year of animal deaths, GenBank accession number). All strains from original brains were first passaged on mouse then mouse brains were collected under sterile conditions. Ten % mouse brain suspension (weight/volume) was prepared in Dulbecco's Minimum Essential medium (DMEM) supplemented with 10% heat inactivated fœtal calf serum (Invitrogen, France) and antibiotics and immediately kept at –70°C until use.

2.1.2 Preparation of the brain homogenate dilutions.

To estimate the sensitivity of hnRT-PCR and rabies diagnostic reference techniques, twelve 1:5 (weight:volume) serial dilutions of an EBL1b suspension (N°121411, France, 2000, see Table 39) ranging from $10^{-1,4}$ to $10^{-9,1}$ were prepared in a DMEM medium supplemented with 10% heat inactivated fœtal calf serum. The titre of the virus stock was established in mice at $10^{5.7}$ $MICLD_{50}$ /ml.

2.2 Rabies reference diagnostic methods.

Fluorescent antibody test, cell inoculations on BHK21 (ATCC: CCL10) and Neuro2a (ATCC: CCL131) and mouse inoculation tests were performed as previously described [14, 15, 24].

2.3 Development of the hnRT-PCR.

2.3.1 Primers.

Table 40 details all primers used for RT-PCR and Southern Blot hybridisation. Based on the alignment of 7 nucleotide sequences published in the GenBank covering the nucleoprotein region of different RRV isolates including the genotypes 1 to 7, both consensus regions and regions with less homology were identified. From these, a pair of universal primers JW12-JW6 which had previously been described by Heaton *et al.* [17,18] was designed from nucleotide position 55 to 661 as compared with the PV strain [25] with minor modifications including modified nucleotides (R, V, Y) with an amplification product of 606-bp as previously described [17] for the seven genotypes of RVs. A primer pair JW12-Jebl1 was designed with an expected amplification product of 410-bp for the specific amplification of EBL1 (Table 40). Jebl1 was selected from a published sequence of EBL1 (GenBank accession number No. U22845) to selectively amplify a portion of the nucleoprotein gene of EBL1.

Table 40: characteristics of the oligonucleotides used for RT-PCR. Nucleotide localisations are based on the PV strain of rabies. N designates rabies nucleoprotein gene. Pair of primers JW12 and JW6 which has been described previously by Heaton *et al.* [17,18] was designed with minor modifications: R (A or G); V (A or C or G); Y (C or T).

Primer	Sense	Sequence (5' to 3')	Nucleotide position	Target gene	Genotypes amplified	Function
JW12	Forward	ATG TAA CAC CYC TAC AAT G	55-74	N	-	RT-PCR
JW6	Reverse	CAR TTV GCR CAC ATY TTR TG	660-641	N	1, 2, 3, 4, 5, 6, 7	First PCR
Jebl1	Reverse	GTC CCG AGT GAG ATC TTG A	465-447	N	EBL1	Second PCR

2.3.2 Assessment of the hnRT-PCR performance.

The specificity and the sensitivity of the hnRT-PCR were assessed as summarised in Table 41. The cDNAs obtained from the University of Pretoria and the Veterinary Laboratories Agency have been obtained after incubation at 42°C for 60 minutes of 2 micrograms of total RNA (extracted with the Trizol methodTM), 20 pmol of primer JW12 and 200 U of the Moloney Murine Leukemia virus reverse transcriptase (M-MuLV) (Applied Biosystems, France) [15]. The primary amplification was then performed with 5 pmol of primer JW12, 5 pmol of primer JW6 and 1.25 U of Taq polymerase (Invitrogen,

France) [17]. A second amplification of the 10-fold diluted primary PCR products was conducted by using 5 pmol of primer JW12 and 5 pmol of primer Jebl1. The first amplification was conducted with 30 cycles and the second PCR with 25.

To evaluate the specificity and the sensitivity, a one step hemi-nested RT-PCR was developed according to Qiagen instructions for all the AFSSA laboratory EBL1 isolates (see Table 41) using the same pair of primers as described above.

Table 41: characteristics of the techniques used in the different laboratories for the amplification of rabies viruses.

RabiesVirus	Identification number	RNA extraction method	RT-PCR method	Laboratory
Genotype1	GS9	Qiagen Viral RNA TM	One step RT-PCR, PCR2	AFSSA, France
Lagos bat	119645	Qiagen Viral RNA TM	One step RT-PCR, PCR2	AFSSA, France
Mokola	ND	Trizol TM	RT, PCR1, PCR2	Pretoria, Africa
Duvenhage	ND	Trizol TM	RT, PCR1, PCR2	Pretoria, Africa
EBL1a	RV631	Trizol TM	RT, PCR1, PCR2	VLA, UK
EBL1b	RV266	Trizol TM	RT, PCR1, PCR2	VLA, UK
EBL1b	122319	Qiagen Viral RNA TM	One step RT-PCR, PCR2	AFSSA, France
EBL1b	122276	Qiagen Viral RNA TM	One step RT-PCR, PCR2	AFSSA, France
EBL1b	122154	Qiagen Viral RNA TM	One step RT-PCR, PCR2	AFSSA, France
EBL1b	121411	Qiagen Viral RNA TM	One step RT-PCR, PCR2	AFSSA, France
EBL2a	RV228	Trizol TM	RT, PCR1, PCR2	VLA, UK
EBL2b	RV8	Trizol TM	RT, PCR1, PCR2	VLA, UK
ABL	ABL	Qiagen Viral RNA TM	One step RT-PCR, PCR2	AFSSA, France

2.3.2.1 Assessment of the sensitivity of the hnRT-PCR.

Viral RNA was extracted from 100 µl of the twelve 1:5 EBL1b (weight:volume) serial dilutions as described above according to the manufacturer's instructions (Qiagen, France) by using the Qiagen Viral RNA minikit. To provide a carrier function to maximise RNA recovery from highly diluted samples, twenty µl of 10% mouse brain suspension obtained from healthy mice, i.e. not inoculated with rabies, were included in each 100µl dilution before the viral RNA extraction.

Five µl (250 ng) of viral RNA sample was mixed with 10 pmol each of forward (JW12) and reverse (JW6) primers in 15 µl of RT-PCR solution containing 1x Qiagen One step RT-PCR buffer, 1 mM each dNTP, 0.04% (v/v) one step RT-PCR enzyme mix (Qiagen) containing the reverse transcriptase and the Taq polymerase. A second amplification of 1 µl of the primary products PCR using 5 pmol of primer JW12 and 5 pmol of primer Jebl1 was then conducted.

Amplifications were performed in a DNA thermocycler (Eppendorf, France) using the following programme: one cycle of RT at 50°C for 30 min, followed by denaturation of 15 min at 95°C, 35 amplification cycles with denaturation at 94°c for 30 sec, annealing for 30 sec at 55°C and extension for 1 min at 72°C, and a final extension at 72°C for 10 minutes. The first amplification was achieved with 35 cycles whereas the second PCR was conducted with 25 cycles. The amplified products were analysed by electrophoresis on a 2% agarose gel stained with ethidium bromide and then photographed.

2.3.2.2 Assessment of the hnRT-PCR product specificity.

2.3.2.2.1 Nucleotide sequencing.

The isolated bands of the 410bp- PCR products obtained from all isolates were excised from the gel, purified using a commercial kit (QIAquick PCR purification kit, Qiagen, France) and cloned into a pDrive vector (Qiagen PCR Cloning, Qiagen, France). The recombinant plasmid was purified using an Eppenforf kit according to the manufacturer's instructions. Sequencing performed with the sense primer T7 (5'GTAATACGACTCACTATAG3') and the antisense primer SP6 (5'CATTTAGGTGACACTATAG3') was undertaken by the Genome Express company (Meylan, France) on an Applied Biosystems 3730 sequencer.

2.3.2.2.2 Nucleotide analysis.

Multiple sequence alignment of four EBL1 sequences (410-bp PCR products), one fox isolate and two laboratory fixed strains (606-bp PCR products) with nine sequences available in the Genbank database (Table 39) was generated by using Genedoc [26] and Clustal X programs [27] based on the 400 bp region of the nucleoprotein gene. Sequence identity matrix calculated from the multiple alignment was constructed using the BioEdit program [28].

2.3.2.2.3 Southern Blot hybridisation.

Southern analyses of PCR products were performed with an EBL1 probe labelled with digoxygenin 11-dUTP in a DNA random primed labelling reaction according to the manufacturer's instructions (Roche, France). EBL1 probe was obtained by cloning the 410-bp fragment of PCR products (N°122276, France 2001, Table 39) into the pDrive Cloning vectorTM (Qiagen, Courtaboeuf, France). The reaction consisted in a mixture of 400 ng EBL1 DNA probe, 4µl of 5x concentrated labelling mixture of random hexamers, 1mM each dNTPs containing 0.35 mM DIG 11-dUTP (Roche, Meylan, France), and labelling grade Klenow enzyme in an appropriate reaction buffer adjusted to 20 µl with RNase free water. The reaction mixture was incubated overnight at 37°C before the addition of 2µL of 0.2M EDTA pH 8.0.

DNA fragments were transferred to Hybond N Nylon membranes (Amersham Pharmacia, France) and immobilised on the blots by UV-cross linking. After blocking for 30 minutes at 42°C in 20 ml hybridisation buffer containing 6x SSC, 50% formamide and 0.5% SDS, the filters were probed with 10 ml of hybridisation buffer containing 1 µl DIG labelled EBL1 probe previously denatured for 5 min at 95°C. Hybridisation was performed overnight at 42°C. Membranes were then washed twice for 10 min at room temperature with 50 ml 2X SSC, 1% SDS, 15 min at 65°C with 50 ml 0.5X SSC, 1% SDS and rinsed 5 min with 50 ml of washing buffer (0.1M Maleic Acid, 0.15M NaCl, 0.3% Tween 20). After blocking for 30 minutes at room temperature in 50-ml blocking solution, the filters were incubated for 30 min with anti-digoxygenin conjugated phosphatase alkaline diluted 1/10000. Specific complexes were revealed by chemiluminescence detection according to the manufacturer's instructions (Roche Molecular, France).

2.3.2.2.4 Controls.

To assess the reliability of PCR results in consideration of both specificity and sensitivity, negative and positive controls were added to each reaction. The PCR negative control was obtained by substituting the reverse transcribed cDNA with ultra pure water in the PCR reaction mixture. The RT negative control was obtained by omitting the RNA in the reverse transcription step. The viral RNA extraction negative control was obtained by substituting the positive brain homogenate with a mouse brain suspension obtained from healthy mice, which was incorporated in each serial EBL1b dilution performed to test the hnRT-PCR sensitivity.

To control the absence of laboratory contamination, every single RNA extraction as well as RT-PCR were performed with an additional negative control consisting of brain of healthy mice. To prevent any cross contamination, the viral RNA isolation and the master mix for the RT preparation were prepared in different rooms dedicated for molecular biology. Finally, a positive control derived from FAT positive brains of EBL1 inoculated mouse (N°121411P4) was included in each single reaction.

3 RESULTS.

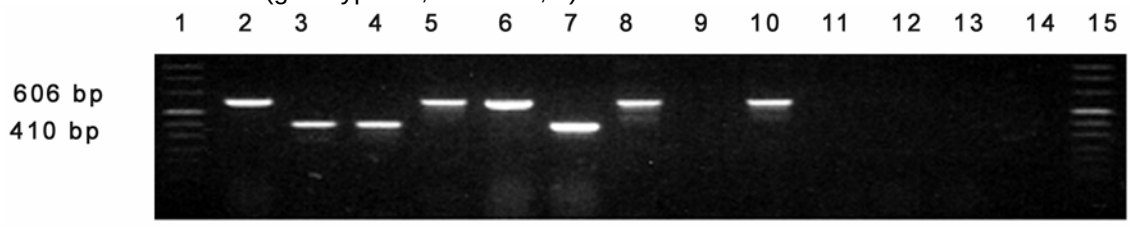
3.1 Design of primers and specificity.

A panel of eight isolates belonging to the Lyssavirus genus was used to test the hnRT-PCR specificity. As previously described by Heaton et al. [17], amplification of cDNAs with primers JW12-JW6 gave a positive signal for each tested isolate, producing a specific band of 606-bp. Amplification of the primary PCR products with the primer pair JW12-Jebl1 produced a single band of 410-bp with both EBL1 isoforms EBL1a and EBL1b (Figure 30) while no specific band was detected in any of the other genotypes (Figure 30). The specificity of the 410-bp products was confirmed by Southern Blot hybridisation

by using the ebl1 probe (Figure 30) and was definitely confirmed by direct sequencing of the PCR products.

Figure 30: assessment of the specificity of EBL1 amplification by RT-PCR and Southern Blot.

1A: RT-PCR amplification of rabies nucleoprotein gene from two European bat rabies virus isoforms (genotype 5a, b and 6a, b).



Lanes 1 and 15: Southern blot marker VIII (Roche, France), lanes 2-3: PCR products of genotype 5a (Denmark), lanes 4-5: PCR products of genotype 5b (France 1989), lanes 6-7: PCR products of genotype 5b (France 1989), lanes 8-9: genotype 6a (England 1996), lanes 10-11: PCR products of genotype 6b (Finland 1985), lanes 12-13: PCR products of FAT negative mouse brain, lanes 14: PCR negative control, lanes 2, 5, 6, 8, 10, 12, 14: amplification of cDNA with the pair of primers JW12-JW6, lanes 3, 4, 7, 9, 11, 13: amplification of cDNA with the pair of primers JW12-Jebl1. PCR products were electrophoresed through 2% agarose together with 100-bp DNA ladder marker (Promega, France).

1B: Southern Blot hybridisation of RT-PCR products of the same gel described above.

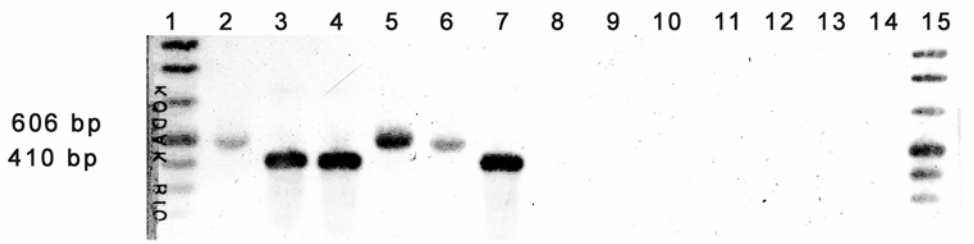
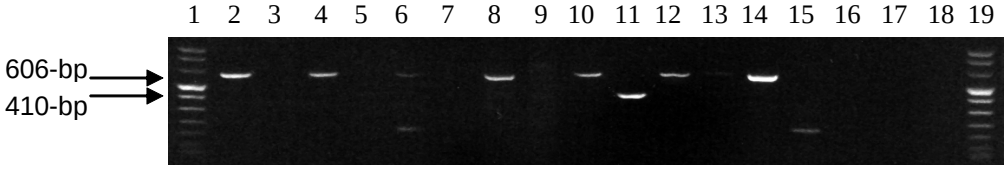


Figure 31: assessment of the specificity of EBL1 amplification by hnRT-PCR with the different genotypes of rabies virus.

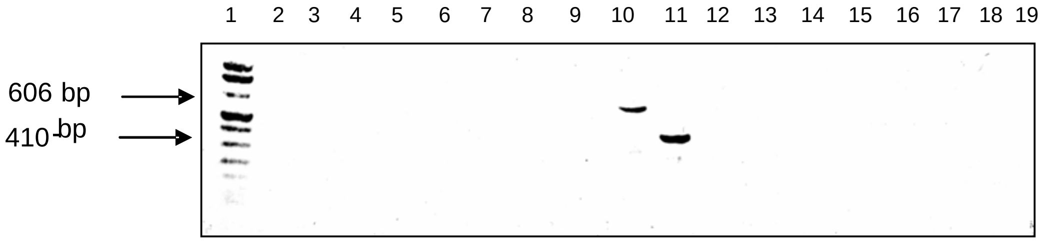
2A: RT-PCR amplification of rabies nucleoprotein gene from the seven genotypes of rabies virus.



Lanes 1 and 19: 100-bp DNA ladder, lanes 2-3: genotype 1 (France 1986), lanes 4-5: genotype 2 (France 1999), lanes 6-7: genotype 3, lanes 8-9: genotype 4, lanes 10-11: genotype 5 (France 2000), lanes 12-13: genotype 6 (England 1996), lanes 14-15: genotype 7 (Australia 1998), lane 16: total RNA extraction negative control, lane 17: RT negative control, lane 18: PCR negative control.

Even-numbered lanes correspond to amplification with JW12-JW6 primers, odd-numbered lanes correspond to amplification with JW12-Jebl1 primers. PCR products were electrophoresed through 2% agarose together with 100-bp DNA ladder marker (Promega, France).

2B: Southern Blot hybridisation of PCR products of the same gel described above.



The comparison of the 393-nt long sequences of the EBL1 virus sequences by using the Bio-Edit software showed more than 96% identity for the six EBL1 sequences (data not shown) (Figure 32). To confirm the specificity, the one step RT-PCR followed by the second PCR has been performed with additional genotype 1 isolates (GS9, CVS-11, CVS-27) used in the laboratory. The neighbour-joining tree (Figure 33) showed that rabies viruses were divided into seven groups representing the seven genotypes and that the four French isolates belong to the cluster formed by the European bat Lyssavirus 1b (genotype 5b) with a bootstrap probability of 842 out of 1000 (84,2%).

Figure 32: schematic alignment of four EBL1 isolates, two laboratory fixed strains, one genotype 1 and nine viral sequences retrieved from GenBank databases. Alignment was performed with GenDoc software [26], aligned with Clustal X and visualised with the BioEdit software [27,28].

```
                         80         90        100        110        120        130        140
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
PV              ATGGATGCCG ACAAGATTGT ATTCAAAGTC AACAATCAGG TGATTTCTTT GAAGCCTGAG ATCATCGTGG
GS9             ..T....... .......... .......... ..T....... ..G.C..... ...A..A... ..T.......
CVS-11          ..T....... .......... G......... ..T....... ..G.C..... .......... ..T.......
CVS-27          ..T....... .......... G......... ..T....... ..G.C..... .......... ..T.......
Lagos_bat       ......T.A. .A.G...... T....G...T C.T....... .AG.G..AC. C..A..A... ..T..ATCA.
Mokola          .....GT.T. .......... G.....G..G ..T..C..A. .TG....... .......... G....ATCA.
Duvenhage_virus ........T. .A.GA..... C..T..G... CGT......C .AG.C...G. A..A..A... G.....TCT.
EBL1a_9367hol   .......TTA .....G.... T..T..G... C.T......C ..G.C..GG. A.G....... G.G..TTCT.
Ebl1b_121411    ..T....TTA ...G.G.... T..T..G... C.T......T ..G.C..GG. A..A...... G.G..TTCT.
EBL1b_122319    ..T....TTA ...G.G.... T..T..G... C.T......T ..G....GG. A..A...... G.G..TTCC.
EBL1b_122154    .......TTA ...G.G.... T..T..G... C.T......T ..G.C..GG. A..A...... G.G..TTCT.
EBL1b_122276    ..T....TTA ...G.G.... T..T..G... C.T......T ..G.C..GG. A..A...... G.G..TTCT.
EBL1b_8918FRA   .......TTA ...G.G.... T..T..G... C.T......T ..G.C..GG. A..A...... G.G..TTCT.
EBL2a_9018hol   ........T. ...GA..... C......... C.T......T ..G.G...G. A.....A..A G.A.....A.
EBL2b_9007fin   .....C..T. ....A..... C......... C.T......T ..G.G...G. A..A..A..A G.A..T..A.
ABL             ......T.T. .T........ C..T..G... .........T ..G.G...G. T.....G... G.G..A..A.

                        150        160        170        180        190        200        210
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
PV              ATCAATATGA GTACAAGTAC CCTGCTATCA AAGACTTGAA AAAGCCCTGT ATAACCCTAG GGAAAGCCCC
GS9             .......... .......... .......... ....T..... .......... .......... ..........
CVS-11          .......... .......... .....C.... .G..T..... ......T... ..C....... ..........
CVS-27          .......... .......... .....C.... .G..T..... ......T... ..C....... ..........
Lagos_bat       .......... A..T..A..T ..A.....T. C...TGG... ...A..AG.C ..C..TT... .A.GG..T..
Mokola          .......... ...T..A..T ..C..C..TC T...TGG... G..A..AG.G ..C...T.G. ....G..A..
Duvenhage_virus ....G..... ......A..T ........T. CT..TAA... G.....TA.. ..C..A..T. .A.GG..T..
EBL1a_9367hol   ....GG.... ...T...... .......... .....AA... G..A..AA.C ..C..T..C. .A....AT..
Ebl1b_121411    ....G..... ......A... .....C..T. .....AA... G..A..GA.C ..C..T..C. .A....AT..
EBL1b_122319    ....G..... ......A... .....C..T. .....AA... G..A..GA.C ..C..T..C. .A....AT..
EBL1b_122154    ....G..... ......A... .....C.... .....AA... G..A..GA.C ..C..T..C. .A....AT..
EBL1b_122276    ....GA.... ......A... .....C..T. .....AA... G..A..GA.C ..C..T..C. .A....AT..
EBL1b_8918FRA   ....G..... ......A... .....C.... .....AA... G..A..GA.C ..C..T..C. .A....AT..
EBL2a_9018hol   .......... ......A... .....C..T. ....TCG... ...A...A.C ........T. AA.....T..
EBL2b_9007fin   ....G..... ......A... .....C.... ....TCG... ...A...A.C ........C. .A.....T..
ABL             .......... ......A..T ..C....... .....CAA.. G.....TA.. .....T..T. ....G..T..

                        220        230        240        250        260        270        280
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
PV              CGACTTAAAC AAAGCATACA AGTCAGTCTT ATCAGGCATG AATGCAGCCA AACTTGATCC TGATGATGTA
GS9             .......... .......... .......... ...G...... .......... .......... ..........
CVS-11          ......G... .......... .A.....T.. .......... .....C.... .......... G.........
CVS-27          ......G... .......... .A.....T.. .......... .....C.... .......... G.........
Lagos_bat       T..T..G.G. .C........ ....TA.A.. G..T..T... .....C.... .GT.A...T. A.....C..T
Mokola          T..TC..... .CT....... .A..CA..C. ......T... ..G..T..A. .G.....C.. A..C.....T
Duvenhage_virus G.....G..G .C........ ....CA.... G..G..G... .....C.... .G..G..... A.....C...
EBL1a_9367hol   ...T..G..A .C...C.... ....TA.... G.....G... .....T..T. ..T.A..C.. A.....C..C
Ebl1b_121411    ...T..G..A .CC..C.... ....TA.... G.....G... .....T..T. ..T.G..C.. A.....C..C
EBL1b_122319    ...T..G..A .C...C.... ....TA.... G.....G... .....T..T. ..T.G..C.. A.....C..T
EBL1b_122154    ...T..G..A .C...C.... ....TA.... G.....G... ..C..T..T. ..T.G..... A.....C..C
EBL1b_122276    ...T..G..A .CC..C.... ....TA.... ......G... .....T..T. ..T.G..C.. A.....C..C
EBL1b_8918FRA   ......G..A .C...C.... ....TA.... G.....G... .....T..T. ..T.G..... A.....C..C
EBL2a_9018hol   G.....G... .G...T..T. ....CA.TC. G..C..A..A .....C..T. GGT.G..... .........G
EBL2b_9007fin   G.....G..T .G...T..T. ....CA.TC. G..C..A..A .....T..T. GGT.G..... .........G
ABL             A..T.....T ..G.....T. ....CA.A.. ...G..A... .....T..A. .G..G..C.. C.........

                        290        300        310        320        330        340        350
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
PV              TGTTCCTACT TGGCAGCAGC AATGCAGTTC TTTGAGGGGA CGTGTCCGGA AGATTGGACC AGCTATGGAA
GS9             .......... .......... .......... .......... .......A.. ...C...... ..........
CVS-11          ..C....... .......... .......... .......... .A........ ...C...... ..........
CVS-27          ..C....... .......... .......... .......... .A........ ...C...... ..........
Lagos_bat       ..C.....TC ....T..... T.....AC.. .....A...G TC.....A.. G.......TA ..T..C....
Mokola          ..C..T.... .A.....T.. T.....TC.A ..C......G TC.....C.. G..C...GTT ..T.....G.
Duvenhage_virus .....A.... .A....GT.. T...ATT..G ........TG T......C.. .......GTT ..T.......
EBL1a_9367hol   ..C..T..T. .A..T.G... C...GTC..G ........C. TC..C..... .........T ..T..C....
Ebl1b_121411    ..C.....T. .A..T.G... C...GTC..G ........C. TA..C..A.. .........T ..T..C....
EBL1b_122319    ..C..T..T. .A..T.G... C...GTC..G ........C. TC..C..... .........T ..T..C....
EBL1b_122154    ..C..T..T. .A..T.G... C...GTC..G ........C. TA..C..... ...C.....T ..T..C....
EBL1b_122276    ..C.....T. .A..T.G... C...GTC..G ........C. TA..C..A.. .........T ..T..C....
EBL1b_8918FRA   ..C..T..T. .A..T.G... C...GTC..G ........T. TA..C..... ...C.....T ..T..C....
EBL2a_9018hol   ........TC ....T..... T...GCTC.A .......... TT.....T.. C......GAG .....C..G.
EBL2b_9007fin   .....T..TC ....T..... T...GCTC.G .......... TT.....T.. C......GAG .....C..G.
ABL             .....T.... .A..T..... T...G....A .........G TC..C..A.. .........G ..T.....G.
```

Figure 33: phylogenetic relationships based on 400-nt of the N gene between European bat lyssavirus 1 isolates and the others genotypes isolates extracted from GenBank databases. The relationships are presented as an neighbour-joining tree (A) and unrooted tree (B) performed with Clustal X software. The bold numbers refer to the genotype. Bootstrap values are shown within the figure A, with values over 70% considered significant.

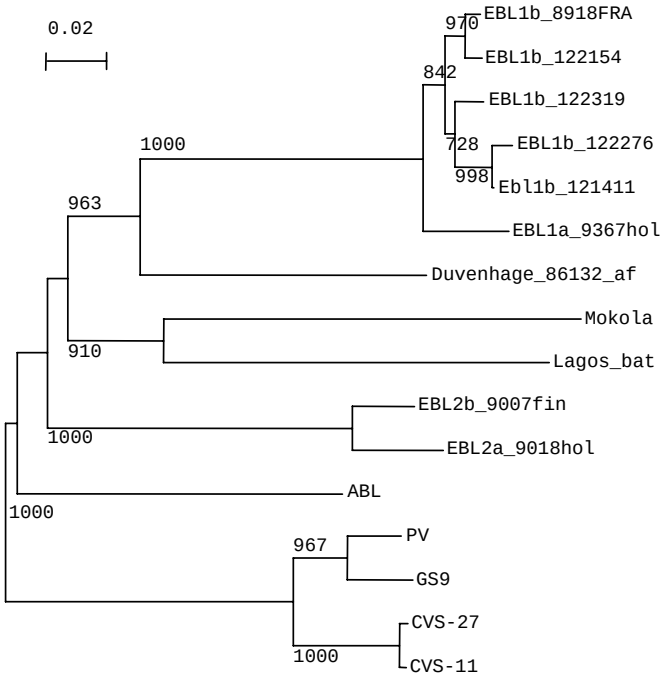


Figure 4 A:

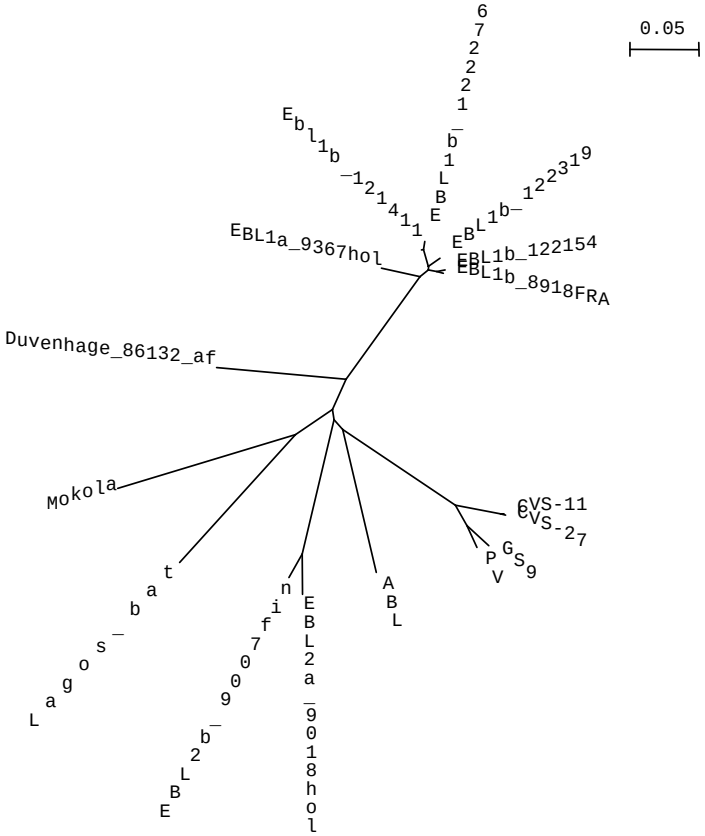


Figure 4B:

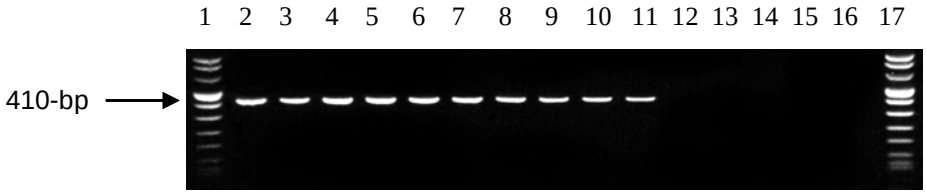
3.2 Sensitivity of the hnRT-PCR and of other classical diagnostic methods.

From the twelve groups of five mice each intracerebrally inoculated, no mouse died within the 2 first days post-inoculation. Mice inoculated with the dilutions ranging between $10^{-1.4}$ and $10^{-2.1}$ died between 7 and 12 days post inoculation, whereas mice inoculated with the dilutions $10^{-2.8}$ to $10^{-4.2}$ died between 12 and 20 days post inoculation. A FAT positive result was found among 23 out of 60 (38 %) of the mice intracerebrally inoculated. All surviving mice inoculated with the dilutions $10^{-4.9}$ to $10^{-9.1}$ did not develop any rabies clinical signs and those sacrificed 4 weeks after inoculation remained FAT negative.

Virus isolation on Neuro2a cells gave positive results for virus dilutions ranging from $10^{-1.4}$ to $10^{-4.2}$ and negative results for the last dilutions ($10^{-4.9}$ to $10^{-9.1}$) and for the negative control. Similar results were observed with BHK_{21} cells. However, at higher concentrations of brain suspension, the 5 samples that were found positive by both RTCIT procedures showed more positive cells with Neuro2a than with BHK_{21} cells.

Results of the hnRT-PCR obtained with the same virus dilutions are presented in Figure 34. Positive results with the EBL1 amplification product of 410-bp were observed for the serially 1:5 virus dilutions ($10^{-1.4}$ to $10^{-7.7}$) and negative results were observed for the dilutions $10^{-8.4}$ and $10^{-9.1}$ and for the negative controls (total RNA extracted from a naive mouse brain suspension, RT and PCR control negative reaction).

Figure 34: determination of the sensitivity of amplification of EBL1-N by hnRT-PCR.



Serial 5-fold dilutions of the EBL1b virus stock containing 105.3 MICLD50/ml/IC were amplified by the one step RT-PCR followed by an hnPCR using the primers JW12-Jebl1.
Lanes 1 and 17: 100-bp DNA ladder, lanes 2-13: serial dilutions of EBL1-b (France 2000) stock virus, lane 14: total RNA extraction negative control, lane 15: RT negative control, lane 16: PCR negative control. PCR products were electrophoresed through 2% agarose together with 100-bp DNA ladder marker (Promega, France).

In order to determine the corresponding amount of virus detected by the different tests, the actual number of MIC LD_{50} was calculated according to the volume of the dilution of the virus stock entering the test (50µl for RTCIT, 30µl for MIT and 0.03µl for hnRT-PCR). According to this calculation method, the end-point detection for MIT, RTCIT and hnRT-PCR was respectively $10^{-0.42}$, $10^{-0.2}$ and $10^{-5.93}$. In our experimental conditions, hnRT-PCR is therefore theoretically 5 10^5 ($10^{5.7}$) more sensitive than RTCIT and 3 10^5 ($10^{5.5}$) more sensitive than MIT.

4 DISCUSSION.

In France, from 1989, year of the first bat rabies diagnosis until the end of 2002, 14 bat rabies cases due to EBL1 virus have been recorded in our country [8, 29, 30] all recorded on *Eptesicus serotinus*.

In this study, a hemi-nested RT-PCR based on the amplification of specific EBL1 nucleoprotein with an optimised pair of primers JW12-Jebl1 was developed to allow the rapid identification of EBL1 a and b viruses in all infected bat specimens received for diagnosis. No specific band was obtained with the six other lyssavirus genotypes tested to validate the technique. The specificity of the PCR products was demonstrated by Southern Blot with an EBL1 probe and by sequencing. Phylogenetic analysis allowed the validation of the specificity of the hnRT-PCR and the differentiation of the two isoforms a and b of the virus, as showed in the neighbour-joining tree.

The hnRT-PCR showed sensitivity higher than both inoculation tests (MIT or RTCIT on Neuro2a or BHK-21 cells). The smallest doses of virus detected by both cell systems were identical ($10^{-0.2}$ $MICLD_{50}$) whereas the limit reached by hnRT-PCR was $10^{-5,93}$ $MICDL_{50}$. Additionally, the data obtained

during this study suggest that for concentrated brain suspension, Neuro2a cells exhibit more fluorescent signal than BHK-21 cells. High concentrations of brain may induce an inhibitory effect on growing BHK-21 cells [31, 32].

The MIT and RTCIT tests detect only complete and fully infective virus particles while hnRT-PCR detects RNA molecule that may come from complete infective particles as well as from inactivated or defective particles. This may partially explain the great difference between molecular methods and classical inoculation tests.

FAT and MIT methods are classical routine rabies diagnosis techniques. Although the sensitivity of these diagnostic tests is satisfactory, fresh brain material is required to obtain reliable results [19, 33]. In addition, FAT is generally performed with selected portions of the brain because the rabies virus antigens are not uniformly distributed in the brain of infected animals [33]. When the material is decomposed or putrefied, FAT is more difficult to read and MIT or RTCIT may be unusable because of the presence of bacteria and/or toxins. The laboratory diagnosis is therefore less reliable or even impossible to perform on such putrefied tissues. The specimens of dead European bats received in laboratory may be in a high decomposition state due to the size of animals, delays in recovery, storage or dispatch. In 2002, classical rabies methods were not performed on 39 out of 195 (20%) dead French bats received in our laboratory due to their high level of decomposition.

Additionally, rabies diagnosis on live bats cannot be performed by using classical rabies diagnostic techniques that need brain tissue; alternative methods are therefore also necessary because all European bat species are protected by law [34]. According to this regulation, new sensitive and reliable techniques should be developed for micro-samples collected on live bats with minimum disturbance such as blood, saliva or skin. These techniques should also be validated and applied to decomposed bat tissues. A study from Heaton *et al.* [18] demonstrated that the RT-PCR is able to detect the nucleoprotein gene of rabies from brain tissue material that was left at room temperature for 72 hours or at 37°C for 15 days, while FAT was negative after 2 days [19]. More recently, Echevarria *et al.* [16] have detected by RT-PCR the expression of Lyssavirus in 15 out of 71 oropharyngeal swabs in a serotine bat colony involved in a case of human exposure.

In conclusion, the MIT allows in vivo propagation of the virus and the subsequent typing of the viral strain (by Mabs and/or RT-PCR) and the sequencing, while the FAT is a cheap, rapid and sensitive technique to detect rabies virus antigens in a suspected tissue. hnRT-PCR is at least as sensitive as virus inoculation tests in neuroblastoma cells and mouse inoculation for demonstrating small amounts of EBL1 virus. This technique reduces the time required for diagnosis from 2-3 or 28 days when result is negative (cell culture test or MIT respectively) to 2 days with the advantage of typing of the strain. In complement to FAT, the sensitive RT-PCR described in this paper would potentially constitute a suitable technique for primary diagnosis on bat when brain is autolysed. Moreover, this technique should be used for epidemiological surveys of bat colonies, by collecting samples on alive animals (saliva, blood samples) providing rapid detection and identification of the viral strain by sequencing the PCR products.

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Epidemiology, dog ecology and oral vaccination

ORAL VACCINATION CAMPAIGNS OF DOGS AGAINST RABIES

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1 INTRODUCTION.

Effective control of terrestrial wildlife rabies seemed almost impossible in Europe until the development of oral vaccination. Nowadays, oral vaccination of foxes (*Vulpes vulpes*) by distributing vaccine baits is the method of choice in rabies control in most European countries. Consequently, large areas are now rabies-free and here baits are no longer distributed or the distribution of baits is limited to border regions in order to prevent re-infection. Hence, this successful method has also been suggested for dog rabies control. Especially, in countries where a large segment of the dog population is not accessible for vaccination by the traditional parenteral route. Next to a safe and efficacious vaccine virus and attractive bait, a bait distribution system is required that assures bait availability to the target species, the domestic dog (*Canis familiaris*), meanwhile minimizing bait accessibility to non-target species, including humans. The latter constraint is essential, because of the safety concerns associated with the presently available oral rabies vaccine viruses. All licensed products are live viruses, live-modified or recombinants, therefore certain safety risks are associated with their use. Dogs live in close proximity of humans, thus unintentional direct or indirect human contact with the vaccine virus cannot be completely ruled out.

The following three bait distribution systems have been suggested:

- distribution of baits at selected sites,
- distribution of baits at a central location,
- and distribution of baits during a house-to-house campaign (WHO, 1993).

These different strategies are aimed at different segments of the dog population. In this paper, all three baiting systems will be reviewed and the major advantages and disadvantages discussed.

2 DISTRIBUTION OF BAITS AT SELECTED SITES.

Baits are distributed at selected sites and the dogs have to locate the baits themselves. The target population are therefore free-roaming dogs, irrespective of their vaccination and ownership status. This system is similar to oral vaccination of wildlife. A major disadvantage is the fact that not only the target population can locate and consume the baits, but other animals as well. To estimate bait depredation by non-target species, baits were distributed at selected sites in different urban areas of Istanbul, in Turkey. A total of 400 Köfte-baits (minced meat mixed with bread crumbs) were distributed and possible bait-uptake was investigated by direct observation. The baits were placed during day and night and observed, on average, for two hours. 53% of the baits were consumed by dogs. Birds, especially hooded crows (*Corvus frugilegus*), were the most important bait competitors during daytime; 26% of the baits were removed by birds. Cats (*Felis catus*) consumed 13% of the baits distributed. The fate of 8% of the baits could not be assessed; especially during the evening and night it was sometimes impossible to determine which animal species located the bait. Astonishingly, only once a man was seen to kick a bait aside with his shoes. It was never observed that a human picked up a bait or a discarded vaccine container. However, it is clear that this distribution system is associated with a potential high number of human contacts, especially children playing (Vos and Sanli, 1998).

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Although more than 50% of the baits were consumed by dogs, it doesn't mean that also more than 50% of the target population of free-roaming dogs has been vaccinated. To assess the vaccination coverage by distributing baits at selected sites, free-roaming dogs were caught, bled and tagged in two areas of Istanbul; Sarigazi and Ferhatpasa. Most of these dogs were ownerless. Subsequently, baits were distributed at selected sites and one month later, tagged dogs were recaptured and a second blood sample was collected (Gleixner *et al.*, 1998; Vos, unpublished data). The results are summarized in Table 42. In this study the seroconversion rate (>0.5 IU/ml) was selected as an indicator of the vaccination coverage. Vaccination coverage is sometimes also determined by detection of a biomarker incorporated in the bait, like tetracycline or rhodamine-B. However, this will only determine bait-uptake and not the actual vaccination coverage. The latter is often lower than the observed bait-uptake. For example, dogs remove the vaccine container from the bait or swallow the vaccine container without puncturing it with their teeth. Hence, the vaccine virus is not released in the oral cavity. Also, dogs may puncture the vaccine container but most of the vaccine is spilled on the ground.

Table 42: vaccination coverage of the free-roaming dog population after distribution of baits at selected sites in two municipalities, Sarigazi and Ferhatpasa (Istanbul, Turkey).

	Sarigazi	**Ferhatpasa**
Number of dogs tagged	22	31
Bait density (bait/km²)	43	150
Bait disappearance rate (%)	64.7	92.7
Seroconversion rate (%)	4.5	23.3

The results were disappointing, only a very small percentage of the target population was vaccinated. A similar study conducted in Tunisia confirmed these results (Matter *et al.*, 1998).

To reach high vaccination coverage at least hundreds, if not thousands, of baits per km² would have to be distributed. The associated costs would be unacceptably high and of course the high number of baits would also increase the potential risks associated with unintended human contacts with the vaccine virus considerably. Thus, this method is not very suitable in terms of cost-effectiveness and safety. Only in certain situations this method can be useful, e.g. to reach true feral dogs. For this purpose, baits could be distributed at areas like dump sites where these animals gather regularly.

3 DISTRIBUTION OF BAITS AT A CENTRAL LOCATION.

Baits can also be distributed to dogs at a central vaccination location. The baits can be offered directly to the dogs, or they can be handed over to the dog owners who will take the baits back home and give it to their dog(s). Oral vaccination of dogs at a central location is aimed at owned dogs that can be handled by their owners. Therefore, it seems more appropriate to vaccinate these dogs by the traditional parenteral route. Mass parenteral vaccination campaigns at a central location have shown to be very effective. In Turkey, this approach was especially effective in rural villages. Here, there are no or few true ownerless dogs and most dogs can be restrained without problems by their owners. A well organized campaign can result in very high vaccination coverage. However, in urban areas the situation is likely to be completely different. Many dogs can not be handled by their owners, so these dogs will not show up at the vaccination centre. Simultaneously with the trial to determine the vaccination coverage of free-roaming dogs by distributing baits at selected sites in Sarigazi, Istanbul, a vaccination campaign at a central located veterinary clinic was organized. The dogs were vaccinated free of charge during a three-day period. To promote this campaign different methods were used, e.g. banners, posters, radio announcements, driving around with a megaphone. A survey conducted directly afterwards revealed that 206 owned dogs were present in this area. Only 45 dogs (21.8%) showed up for vaccination at the clinic during the campaign. 18% of the owned dog population had been vaccinated against rabies during the previous 12 months. So, after this campaign only 39.8% of the owned dogs were vaccinated against rabies. This figure is most likely not sufficient to control dog rabies, Coleman and Dye (1996) mentioned a vaccination coverage of 70% to prevent a dog rabies outbreak. The distribution of baits to dog owners at a central location must be discouraged, although it has been suggested that this distribution system could achieve high vaccination coverage (Matter *et al.*, 1995). A study in Tunisia showed that by using this method 1.7% of the human population had unintended contact with the vaccine virus (Ben Youssuf *et al.*, 1998). In view of the previously mentioned risks associated with the use of live virus vaccines, this is unacceptable.

4 DISTRIBUTION OF BAITS DURING A HOUSE-TO-HOUSE CAMPAIGN.

The last suggested method is by going house-to-house and vaccinate all dogs encountered.

The selected area is systematically covered and every dog encountered is approached. To achieve better results, it is suggested that the vaccination team is accompanied by locals. This to ensure community acceptance. People are often reluctant to cooperate and distrust vaccination teams as a result of, for example, previous dog removal campaigns. Local children are often more than willing to help. They often know all dogs, if they are owned or not, where the owners live and where the ownerless dogs can be found. It was even observed that children were able to handle many ownerless dogs without difficulties. These animals could be handed over to the vaccinators, and these animals could subsequently be vaccinated by the parenteral route. In case of a dog with an owner who could be located within a reasonable period of time and could restrain the animal, the dog was vaccinated by the parenteral route and the owner became a vaccination certificate. If the dog did not have an owner, or the owner was afraid to handle the animal, a bait was offered. The bait was thrown to the animal by one of the vaccinators and later on the empty discarded capsule was picked up by a member of the vaccination team. An enormous advantage of this method is that the risk of unintentional human exposure to the vaccine virus is almost eliminated.

This above described system was also tested in Sarigazi (after the campaign at the central located clinic) and Ferhatpasa, Istanbul - Turkey. The house-to-house campaign was combined with a survey to estimate the number of owned dogs in these areas. The results of these campaigns are summarized in Table 43.

Table 43: vaccination coverage of the owned dog population in Sarigazi and Ferhatpasa, Istanbul – Turkey.

Vaccination coverage (%)*	Sarigazi	Ferhatpasa
Prior to campaign	18.0	15.5
Campaign at clinic	21.8	-
House-to-house campaign		
Parenteral	22.8	40.5
Oral	21.2	18.1
Total	83.8	74.1

* - percentage of dogs vaccinated by the oral or parenteral route

During these house-to-house campaigns, no blood samples were collected to examine seroconversion. So, the vaccination coverage achieved by oral vaccination is based on the number of dogs that accepted bait, and subsequently punctured the vaccine container. Dogs that swallowed or discarded the vaccine container without being punctured were not included. In Sarigazi, an additional 44% of the owned dog population could be vaccinated, of which 21.2% were inaccessible for parenteral vaccination but could be offered bait successfully. From the results obtained, it becomes clear that the dogs vaccinated at the clinic in Sarigazi could easily have been vaccinated during the house-to-house campaign. In both areas, vaccination coverage of more than 70% was achieved. So, by going house-to-house and include oral vaccination for all dogs not accessible for vaccination by the traditional parenteral route, the overall vaccination coverage was raised to levels that are assumed to be sufficient to stop the spread of rabies. The remaining dogs not vaccinated were too young (less than 3 months old), or could not be located. As a result of the very high population turn-over, it may be necessary to organize a vaccination campaign twice a year, in order to maintain high vaccination coverage. However, oral vaccination does not only have a quantitative effect by increasing the overall number of dogs vaccinated, it also has a qualitative effect by immunizing dogs that play an important role in the maintenance and spread of rabies; the free-roaming owned and ownerless dogs. During a mass vaccination campaign in Kusadasi, a coastal town in Turkey, 8.2% and 43.3% of the restricted (owned) and free-roaming (owned and ownerless) dogs, respectively, could not be vaccinated by the parenteral route and were subsequently offered bait. The advantages of oral vaccination become even more pronounced when looking at the ownership status. 12.3% of the vaccinated owned dogs could not be vaccinated by the parenteral route and were given bait. However, 74.1% of the vaccinated ownerless dogs could only be reached by offering them vaccine bait (Güzel *et al.*, 1998). An even more extreme situation was observed in the coastal village of Mindoro, the Philippines. Here, none of the 216 owned dogs had previously been vaccinated against rabies. All, except one dog, were free-roaming and no ownerless dogs were present in Mindoro. During the vaccination campaign, only 17 dogs (8%) could be restrained by the vaccinators and 19% were considered not eligible for vaccination (less than 3

months old). 56% of the dogs was offered a bait successfully. During a survey conducted afterwards to assess possible adverse events, it was determined that some dogs were offered bait twice. Thus the overall vaccination coverage of the dog population was corrected from 64% to 62%. Looking at the juvenile (>3 months old) and adult dog population only, a vaccination coverage of 76% was attained (Estrada *et al.*, 2001b).

5 DISCUSSION.

The data presented here clearly show the feasibility of oral vaccination of dogs as a promising additional tool in dog rabies control. Oral vaccination does not only increase the total number of dogs vaccinated, but can be used to immunize especially those animals that play an important role in the maintenance and transmission of this virus disease; the free-roaming dogs. However, in order to optimise vaccination campaigns, including oral vaccination, the selected system must be adapted to the local situation. For example, in certain areas a well organized mass parenteral vaccination campaign at a central location can reach enough dogs to halt or prevent a rabies outbreak. Hence, oral vaccination of dogs is not required. Also parenteral vaccination campaigns by going house-to-house have shown to be effective. Unfortunately, in areas with a high number of free-roaming dogs, owned or ownerless, that are not accessible for vaccination by the parenteral route, the obtained vaccination coverage, by using the latter method only, is often not sufficient for effective rabies control. In Turkey, this was often the case in urban and semi-urban areas (Vos and Aylan, 1999). In other countries, like the Philippines, also in rural areas only few dogs were accessible for parenteral vaccination. In these areas, oral vaccination of dogs could increase the vaccination coverage considerably. The potential advantages of oral vaccination of dogs has been studied in many different countries; Tunisia, Turkey, Sri Lanka, the Philippines, Zimbabwe and the Republic of South Africa (Perry *et al.*, 1988; Matter *et al.*, 1995, Güzel *et al.*, 1998; Bishop *et al.*, 1999; Perera *et al.*, 2000; Estrada *et al.*, 2001b).

However, it seems that all these and other studies were not able to convince authorities to incorporate oral vaccination of dogs in their (national) rabies control programme. Two major reasons for the reluctance to use this promising new tool can be identified; financial constraints and safety concerns. In most countries with dog-mediated rabies, the financial resources for rabies control are limited and the available funds are predominantly used for human treatment. This is by all means understandable; however one should not forget that it does not solve the rabies problem. To eradicate rabies, including human rabies, the most cost-effective method is by controlling the disease in the main vector species; the domestic dog. To lower the acceptance threshold for oral vaccination, the price of vaccine bait should be as low as possible and not surpass that of a single dose for parenteral vaccination. Presently, the price of manufactured vaccine baits is much higher. To circumvent this problem, for example, baits prepared from local available cheap material can be used (Estrada *et al.*, 2001a). Thus, only the capsule filled with vaccine virus must be imported, reducing costs considerably.

Also, the safety issue associated with the present available oral rabies vaccines seems to be an almost insurmountable barrier. It is clear that for oral vaccination of dogs more stringent safety precautions are required than for oral vaccination of wildlife. However, the safety issue should not be overrated. In Europe, more than 150 million vaccine baits have been distributed since 1978. So far, not a single serious incident of human exposure to any of the vaccine viruses used has been reported. Furthermore, the suggested bait delivery system by going house-to-house and re-collection of all discarded vaccine containers will greatly reduce any possible unintended human exposure to the vaccine virus. Many studies with potential safer vaccine candidates have been investigated, including more attenuated live rabies viruses (Visser *et al.*, 2001; Dietschold *et al.*, 2001), replication-defective recombinants (Wandeler *et al.*, 2001. Vos *et al.*, 2001), DNA-vaccines (Tordo *et al.*, 2000), transgenic plants (Loza-Rubio *et al.*, 2000). However, most of these candidates are not capable of inducing a protective immune response after a single oral administration. Hence, it will most likely take many years before a suitable candidate will be available, meanwhile every year thousands of people are dying of rabies after being bitten by a rabid dog. On the one hand we have the live-savings potential of oral vaccination of dogs and on the other hand we have possible adverse effects; reduced human deaths and number of PET versus human exposure to the vaccine virus. Evaluating this moral dilemma we must be aware that the perception of risks and benefits are tremendously different in developing and developed countries.

In Europe, with only a few (imported) human rabies cases annually, oral vaccination of wildlife is widely accepted and developed into the preferred method to control and eradicate rabies. The ratio to

withhold this method for countries where hundreds, if not thousands, of people die from rabies every year seems, at least, puzzling. Especially, considering that the suggested vaccine virus candidates for oral vaccination of dogs have been used extensively in Europe without any reported human incident. Of course, the safety risks associated with oral vaccination of dogs should not be ignored, but by applying the suggested baiting strategy unintended human contacts with the vaccine virus can almost be eliminated. Oral vaccination of dogs against rabies therefore deserves the (increased) active support and advocacy of (international) organizations involved in rabies control.

6 CONCLUSIONS.

Parenteral vaccination of dogs remains the cornerstone of dog rabies control. However, when a large segment of the dog population is not accessible for parenteral vaccination, oral vaccination can increase the vaccination coverage of the dog population considerably.

A house-to-house campaign is the most efficient and safest method to distribute oral rabies vaccine baits. The risk of unintentional direct human contact with the vaccine virus are minimized and almost non-existent. Therefore, oral vaccination of dogs as a supplementary tool to parenteral vaccination should be encouraged, especially in view of the present impasse in dog rabies control as observed in many countries.

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RE-EVALUATING THE BURDEN OF RABIES IN DEVELOPING COUNTRIES: CANINE RABIES IN AFRICA

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1 INTRODUCTION.

A method has been developed which allows the number of human rabies deaths to be predicted from reported cases of suspect rabid dog bites (Cleaveland *et al.*, 2002). The model uses the distribution of bite injuries on the body, together with the likelihood of the patient receiving successful post-exposure treatment, to predict the outcomes of rabid dog bites. The model thus allows the incidence of suspect rabid dog bites among the at-risk human population to be used as a determinant of the number of human rabies deaths. This report presents the results of a first attempt to apply the predictive model on a regional level, to estimate the burden of canine rabies on human health in Africa.

2 METHODS.

The human population at risk from canine rabies was taken as the number of people living in rabies-infected areas where the dog population density exceeds the threshold density at which canine rabies is capable of being maintained endemically (4.5 dogs/km^2 – Kitala *et al.*, 2002). Model parameters were determined by examination of literature sources and consultation with experts in the fields of rabies epidemiology and control. All parameter estimates used in the model are published elsewhere. Uncertainty in parameter estimates, and inherent parameter variability due to between-country differences, was incorporated into the model by assigning confidence distributions to input parameters. Parameter distributions were sampled iteratively (until convergence at <1.5%) using a Monte Carlo simulation procedure (@Risk Pro 4.5, Palisade Corp., Newfield, New York). Model predictions are reported using the means of the resulting probability distributions, with the 5th and 95th percentiles used as the lower and upper bounds, respectively. The annual number of human rabies deaths estimated in this way was then used to determine a disability-adjusted life year (DALY) score for rabies in Africa, using previously-described methodologies (Mathers *et al.*, 2001). The DALY score is a standardised, comparative measure of the burden of disease (Murray, 1994). For the calculation of a DALY score for rabies the following components were considered:

1) mortality due to the disease,
2) mortality due to post-exposure treatment reactions to nerve-tissue vaccines,
3) morbidity following side-effects of nerve-tissue vaccines, and
4) psychological impact of fear and trauma on a person bitten by a suspect rabid dog.

Previously-reported DALY estimates for rabies have focused only on disease-induced mortality (Fèvre *et al.*, 1999), but evidence suggests that the latter three above-mentioned components may constitute a sizeable proportion of the rabies burden in developing countries.

3 RESULTS.

Using dog population density data as a predictive indicator, approximately 634 million people are estimated to be at risk from endemic canine rabies in Africa (80% of the total population). Over 800,000

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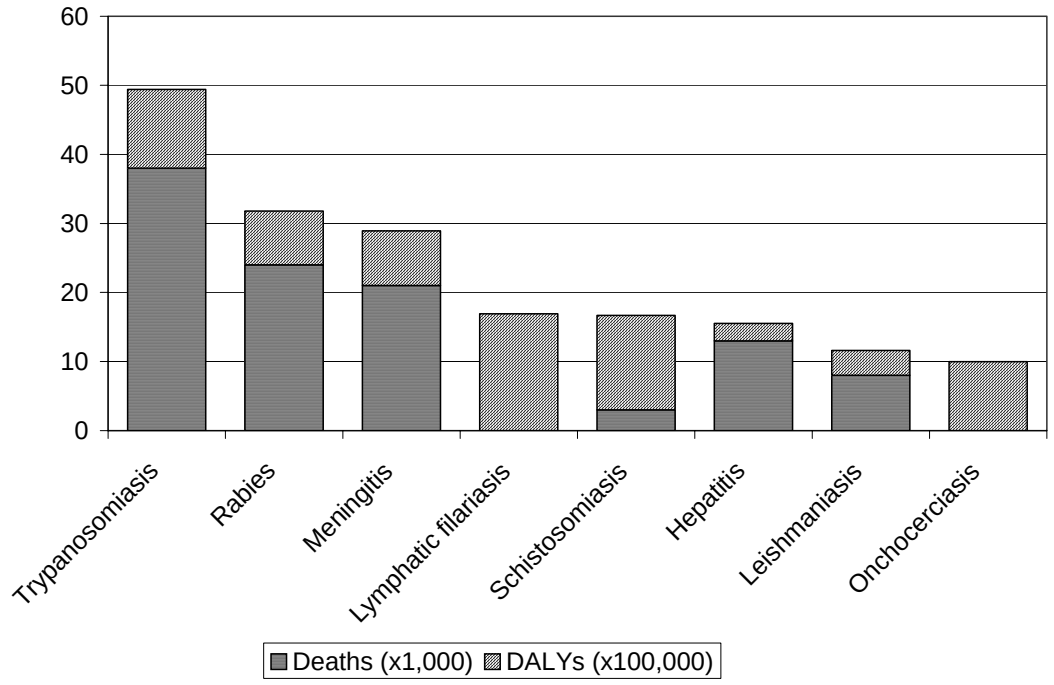
people are bitten by suspect rabid dogs each year, resulting in a predicted total of 24000 human deaths annually. The estimated incidence rate per 100000 inhabitants varied from 2.0 in urban areas to 3.6 in rural locations. More than 780000 DALYs are lost to the disease, 4% (30000) of which are as a result of the fear and anxiety suffered by people following the bite of a suspect rabid dog. Figure 1 presents a comparison of the rabies burden with that of other infectious and parasitic diseases in Africa.

4 DISCUSSION.

From this report rabies emerges as a significant public health problem on the continent of Africa. The burden of the disease is not evenly distributed across all sectors of society, but is rather influenced by age-related and socio-economic factors. Disparities in the affordability and accessibility of post-exposure treatment, levels of rabies awareness and risks of exposure to rabid dogs result in a skewed distribution of the disease burden across society, with the major impact falling on those members, particularly children, of poor rural communities. Although effective and economical control measures are available, rabies remains a neglected disease throughout Africa

Figure 35:



Source: World Health Report 1999

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CANID RABIES IN ZIMBABWE AND SOUTH AFRICA A REVIEW

C.T. Sabeta[123] and L.H. Nel[1]

1 BRIEF HISTORICAL REVIEW.

Despite the advances and progress towards the control of infectious diseases made in the past century, rabies is still a disease of serious public health concern. As many as 50000 preventable deaths occur annually in the developing nations of Asia, Latin America and Africa (Meslin *et al.*, 1994; Blancou, 1988).

In comparison to the other continents where rabies is endemic, the history of rabies in Africa before the 19th century, is rather fragmentary and largely anecdotal. There are no definite reports to indicate that rabies was present in Zimbabwe before 1902, although Edmonds (as cited by Foggin, 1988 and Swanepoel *et al.*, 1993) reports anecdotal evidence from older members of the population who claimed to remember cases from before the European occupation. The first definite cases of rabies in Zimbabwe were reported in 1902 in dogs and thought to have originated from Zambia. The disease spread throughout most parts of Zimbabwe and was subsequently controlled through destruction of stray dogs, compulsory vaccination of all dogs and introduction of a dog tax. Zimbabwe remained rabies-free between 1913 and 1950, with the exception of two other dog rabies cases diagnosed in dogs that were imported from Zambia in 1938.

In South Africa, an epizootic involving domestic animals in 1893 could be traced back to an Airedale Terrier that had recently been imported from England (Hutcheon, 1894 as cited by King *et al.*, 1994). Furthermore, traditional folklore of the Baralong tribe from the northern Cape, writings of early travellers and explorers contain accounts of rabies, are strongly suggestive of the existence of rabies before the 1900s (Snyman, 1940). From 1916 onwards, the disease occurred with more frequently in the yellow mongoose *Cynictis penicillata*. There was doubt as to whether this was indeed rabies (Cluver, 1927) and only in 1928, this doubt was removed when rabies was confirmed in two schoolboys who had died after being bitten by a tame yellow mongoose.

Canid rabies was recently introduced into southern Africa from Angola by dogs (Swanepoel *et al.*, 1993). It then spread to Zimbabwe and South Africa in the dog population in the 1950s and in the 1960s spread into KwaZulu-Natal and has persisted ever since.

In Zimbabwe, the domestic dog *Canis familiaris*, side stripped jackal *Canis adustus* and black backed jackal *Canis mesomelas* are the principal vectors of rabies (Foggin, 1988; Bingham 1999a, 1999b, 1999d). The majority of domestic dogs live in communal lands (Brooks, 1990; Butler, 1998; Butler and Bingham, 2000) and account for approximately 50% of all confirmed rabies cases. Jackal rabies accounts for 25% of all confirmed rabies cases. In South Africa, rabies cycles in several reservoir species with specific geographical associations. These include the domestic dog in KwaZulu-Natal, black-backed jackals in the northern border areas with Zimbabwe and bat-eared foxes *Otocyon megalotis* in the western Cape region (Swanepoel *et al.*, 1993). The geographic distribution of the principal vector species in Zimbabwe and South Africa is shown in Figure 36.

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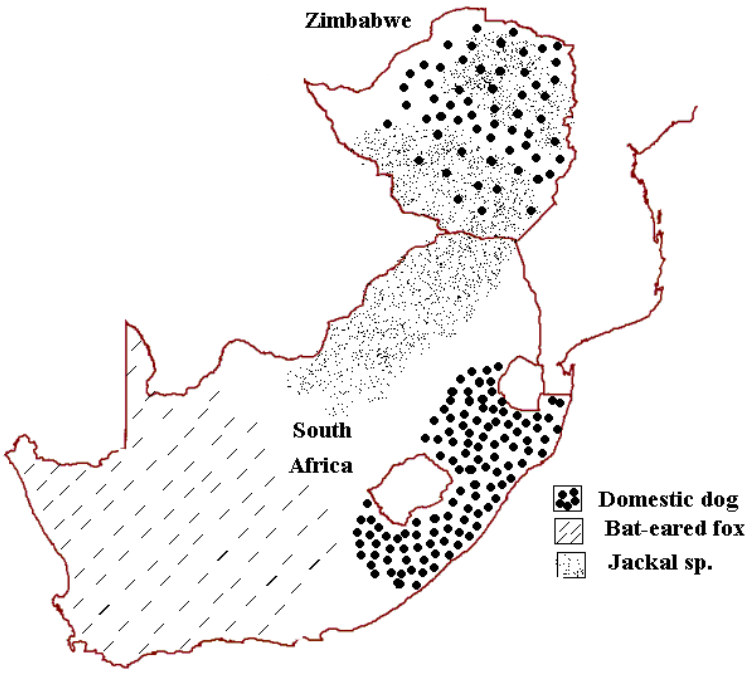
Figure 36: Approximate enzootic regions for canid rabies in Zimbabwe and South Africa.



2 ANTIGENIC DATA.

Monoclonal antibodies (Mabs) can be used to detect specific epitopes on the viral proteins and presence or absence of these epitopes can define a virus taxonomically. Mab studies have demonstrated the existence of two rabies biotypes in southern Africa (King *et al.*, 1993, 1994). The two biotypes are referred to as canid (infecting carnivores of the family Canidae) and viverrid (infecting carnivores of the subfamily Viverrinae). The demonstration of the two distinctive reaction patterns in the nucleoprotein (N) of rabies viruses isolated from species of South Africa supported Foggin's postulation (1988) that isolates of mongoose origin differed from those that commonly circulated in domestic dogs and jackal species.

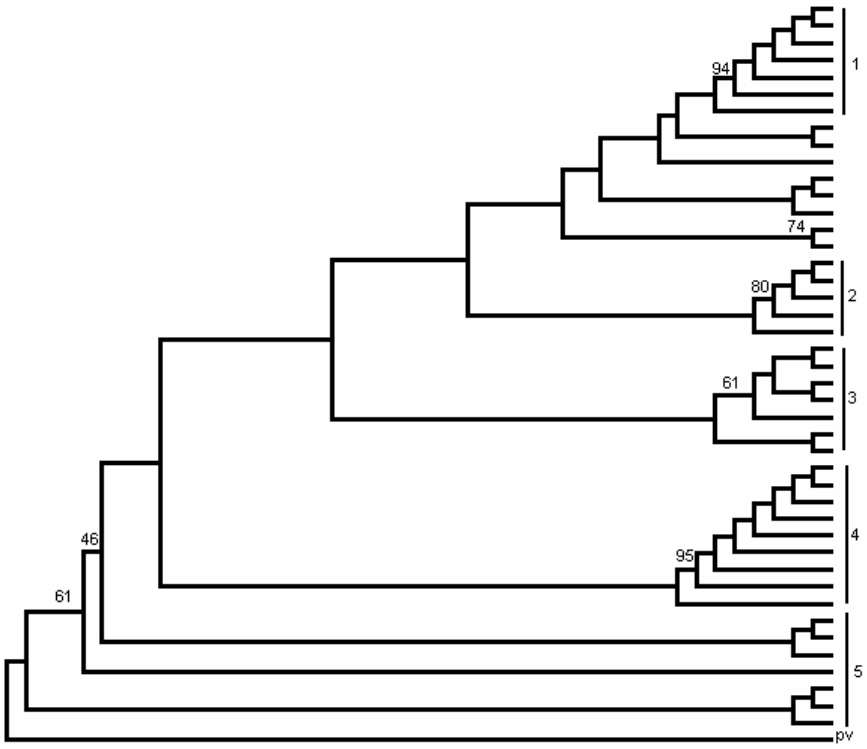
Today, nucleoprotein directed Mabs are still an important diagnostic tool for differentiating lyssavirus genotypes. or instance, the Onderstepoort Veterinary Institute (OVI) is presently involved in routine typing of lyssaviruses. A case in point is a rabies virus from an African wild civet *Civettictis civetta* (from Zimbabwe), which gave an inconclusive result when typed in Harare but was later classified as viverrid using a polyclonal conjugate at OVI (Meredith, 1995). Rabies samples are part of a few repositories in Africa and retrospective antigenic screening can be useful in lyssavirus surveillance on the continent. In this way unusual lyssaviruses have been identified from a collection of rabies isolates (Bingham *et al.*, 2001).

3 MOLECULAR ERA.

Although antigenic analysis of viruses has been very useful in lyssavirus epidemiology by discriminating between variants, tools of molecular biology have added dimensions of speed and precision. Sequences of genes of the nucleoprotein, phosphoprotein and the glycoprotein and G-L intergenic region have been particularly useful in determining the molecular epidemiology of rabies throughout the world (Tordo *et al.*, 1986; Nadin-Davis *et al.*, 1993, 2001; Sacramento *et al.*, 1992). In southern Africa, a few molecular studies on a limited number of virus isolates were conducted (Nel *et al.*, 1993; Von Teichman *et al.*, 1995; Nel *et al.*, 1997) although they were focused on the genetic distinction of the two rabies biotypes (Nel *et al.*, 1993, 1997, 1998; Von Teichman *et al.*, 1995).

Recently, we embarked on a comprehensive and regional comparative genetic study of viruses of the canid biotype from different host species throughout Zimbabwe and South Africa. We targeted the cytoplasmic domain of the glycoprotein and the G-L intergenic region, shown to be most divergent region of the rabies genome (Tordo *et al.*, 1986), and thus useful for short-term evolutionary studies. All the southern African virus isolates were found to lack one of the two polyadenylation sites postulated for the G genes of ERA and Pasteur Virus PV (Tordo *et al.*, 1986). The absence of this signal has been shown in street viruses from Europe, and was suggested to be of transcriptional rather than evolutionary significance (Sacramento *et al.*, 1992). Phylogenetic analysis delineated the viruses into 5 groups that had significant bootstrap support (Figure 37). The five groups corresponded to viruses from dog and jackal species from Zimbabwe and South Africa (group 1), from dogs only from north-eastern Zimbabwe (group 2), a group from bat-eared fox isolates (group 3), jackal and dog viruses from central Zimbabwe (group 4) and the last one from domestic dogs and jackal species from southern Zimbabwe and northern bordering areas of South Africa (group 5).

Figure 37: Phylogenetic tree of nucleotide sequences of the cytoplasmic domain of the glycoprotein and the G-L intergenic region of selected canid viruses from Zimbabwe and South Africa. Principal bootstrap values are indicated at the nodes. The sequence PV was included as outgroup.



The most important finding of this investigation is that all the southern African canid viruses were closely related with an average sequence homology of 96.6% (Sabeta *et al.*, 2003) and could be distinguished from PV used as the outgroup irrespective of geographical origin. This result is suggestive of a common and recent origin, consistent with the historical emergence of canid cycles (Swanepoel *et al.*, 1993). The results from this investigation further support findings of other studies in which rabies virus isolates from both domestic dogs and jackal sp. were shown to have no antigenic distinctions, implying that they were closely related (King *et al.*, 1994; Bingham *et al.*, 1999b).

4 FUTURE PERSPECTIVES.

It is evident from this investigation that the canid lineage is now well established in the southern African sub-continent and is most likely to initiate future cycles. Two aspects of epidemiology, viz surveillance and knowledge of the distribution of the antigenic and genetic virus variants, are essential components of an efficient and economical rabies control program. In this regard, results from this study underscore the need for continual surveillance of lyssaviruses in the greater part of Africa, in man, domestic and wild carnivore species.

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THE USE OF MONOCLONAL ANTIBODIES FOR RABIES VARIANT TYPING WITH SOME APPLICATIONS TO RABIES IN THE SEARG REGION

A.I. Wandeler[1], J.M. Armstrong and S. Nadin-Davis

1 MONOCLONAL ANTIBODIES.

In 1975 Köhler and Milstein published their groundbreaking paper on fusing spleen cells of immunised animals with myeloma cells in order to obtain continuous hybridoma cultures that produce antibodies of predefined specificity. The antibodies secreted by cloned hybridoma cell lines are commonly called monoclonal antibodies (Mabs). Mabs have unique attributes, they can be produced in vitro in unlimited quantities, they have consistent binding specificity to particular sites (epitopes) on antigenic molecules, and they display other homogenous properties such as isotype, avidity, and labelling properties (Harlow and Lane, 1988).

The development of Mab technologies had enormous consequences for diagnostics and research. The vast majority of current Mabs is of rodent origin (=murine Mabs), but it is possible to generate hybridomas with cells originating from a variety of species, incl. humans. Anti rabies Mabs are used today for routine diagnosis (as FITC-labelled cocktails), for serology in competitive ELISAs, and for Lyssavirus species and variant identification. Cocktails of neutralising anti G-protein Mabs are currently investigated as possible alternatives to replace, in post-exposure prophylaxis, the scarce H-RIG and E-RIG preparations.

2 THE PRODUCTION OF MONOCLONAL ANTIBODIES TO LYSSAVIRUSES ANTIGENS.

The first step in the production of murine Mabs is to immunise mice with a preparation that contains the proper antigens. Peptide or protein preparations, whole inactivated virus, live attenuated or recombinant viruses can be injected for immunisation. We like to use viral capsids (RNP) purified from infected cells, if the intended use of the Mabs is virus variant identification by indirect immunofluorescence. Some weeks after the initial immunisation a lymphoblast response is stimulated by a booster injection. After a few days spleen cells are harvested and fused to myeloma cells, usually by adding polyethylene glycol to the suspension. Unfused myeloma cells are then eliminated and the surviving hybridoma cells are cloned. Culture supernatant from each clone is then screened for the desired antibody activity. We usually do this either on infected cell cultures by indirect immunofluorescence or with ELISA on antigen coated polystyrene plates. Other criteria for selecting particular hybridoma clones are their growth property, Mab avidity, and Mab labelling properties. The isotype of the secreted immunoglobulins are determined. We generally reject IgM's as reagents for indirect immunofluorescence and ELISA.

3 LYSSAVIRUSES, LYSSAVIRUS VARIANTS AND MONOCLONAL ANTIBODIES.

The original classification of Lyssaviruses into serotypes has long been superseded by a grouping based on genome sequences (Bourhy *et al.*, 1993). At present we recognise seven Lyssavirus species (genotypes): Rabies, Lagos Bat, Mokola, Duvenhage, European Bat Lyssaviruses Type I and II and Australian Bat Lyssavirus. All species for which we have a sufficient number of isolates can be further

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subdivided into genetic and antigenic variants. We call a variant a "strain" if it has well described biological properties and a passage history in laboratory animals and/or cell culture.

The Lyssavirus genome codes for 5 structural proteins: N, P, G, M, and L. Virions contain host-derived molecules in addition to these structural proteins. Monoclonal antibodies to N- and P-proteins applied in indirect immunofluorescence usually bind to inclusion bodies, less frequently they also light up diffusely the cytoplasm of acetone-fixed infected cells. Indirect immunofluorescence with anti G-protein Mabs usually produces a more diffuse staining of acetone- or methanol-fixed infected cells. Most anti-N and anti-P Mabs work very well on acetone fixed smears of infected brains, while most anti-G Mabs do not, though all of them can be applied in indirect immunofluorescence on infected cell culture.

The WHO Collaborating Centre at CFIA's rabies laboratory in Ottawa has a collection of about 500 distinct anti-Lyssavirus Mabs. These Mabs have been extensively used to characterise Lyssavirus isolates from Africa, Europe and North America. Analysing the ability of our Mabs to discriminate between different Lyssavirus species, related groups within species, and variants, we have to take into account that we looked at several thousand rabies isolates, but that we had only a few isolates from each of the other Lyssavirus species at our disposal. In this context, Mabs that recognise epitopes that occur on different viruses of only one Lyssavirus species are fairly common. However, with our Mabs we have not succeeded to detect any epitopes that would identify a Lyssavirus as belonging to the species rabies. In addition, we have not been able to generate a Mab that is binding exclusively to a single rabies virus variant.

The reaction of a Mab with more than one variant does not always reflect a close phylogenetic relationship between these viruses. For example, 11DD1, raised from hybridised spleen cells of a mouse immunised with ERA, recognises ERA virus and does not bind to any other tested Lyssaviruses, except to isolates from *Lasiurus* species. ERA strain and *Lasiurus* (an American bat genus) isolates are genetically not closely related. M1806, with the spleen cell donor immunised with raccoon rabies virus RNP, recognises raccoon rabies virus, but also a few distinct variants circulating in big brown bat (*Eptesicus fuscus*) populations. There may be a closer phylogenetic relationship between these viruses since raccoon rabies and North American bat rabies viruses have a common root, though the majority of the latter do not bind M1806.

4 LYSSAVIRUS VIRUS VARIANT IDENTIFICATION.

Molecular analysis has largely replaced serological methods for the classification of viruses. The characterisation of selected parts of viral genomes permits reconstructing phylogenetic relationships. These can be displayed as evolutionary trees. Likelihood values can be calculated for different conceivable branching topologies. Molecular epidemiology is the reconstruction of the history of an epidemic using these powerful tools. Monoclonal antibodies recognise phenotypic aspects of viral proteins and can therefore not provide phylogenetic information with the same accuracy. However, they bind to molecular surface structures recognised by a host's immune system, structures that are likely to have functional properties on which natural selection may act.

While molecular studies remain fairly laborious and costly processes, the antigenic characterisation with monoclonal antibodies is an inexpensive "workhorse" approach to identify large numbers of virus isolates in a diagnostic laboratory setting. Though the binding of a larger number of Mabs can be studied on infected cell cultures, it is important that the tests can be conducted on the original brains in order to remain low in cost and efforts. This is usually accomplished on acetone-fixed brain smears that are first incubated with the selected Mabs, then with anti mouse-Ig FITC conjugate.

Probably not less than six and certainly not more than twenty different Mabs should be included in a panel for routine variant identification. The following criteria might be used to select a panel from a larger collection of Mabs:

a) The panel must discriminate between the different species and variants occurring in an area. Variants should be defined as members of a clade (branch) in a phylogenetic tree. There is a possibility that a selected Mab panel shows similar reaction patterns for phylogenetically distant variants if this molecular analysis is not accomplished first. This risk is higher if the geographical area investigated is large.

b) The Mabs must give clear positive or negative results when applied in indirect immunofluorescence on fixed brain smears of Lyssavirus infected animals or humans.

c) The panel should include a control Mab, directed against an unrelated antigen that is unlikely to occur in brain tissue. Antibodies can bind nonspecifically, e.g., to FC receptors. The use of a non-lyssa Mab helps to recognise structures that could be misinterpreted as Lyssavirus antigen accumulations. Staining of Lyssavirus inclusions might occur due to a cross-reaction of the anti-mouse conjugate with autochthonous antibodies when the animal under examination had made a strong immune response to the infection and immunoglobulins were leaking post-mortem into the brain tissue. The test results for a specimen should be discarded when the brain smear incubated with the non-lyssa Mab shows suspicious fluorescence. In such a case another technique (genome analysis, Mabs on infected tissue culture) needs to be applied for species or variant identification.

5 MABS AND THE VIRUSES OF THE SEARG REGION

Panels of monoclonal antibodies have been used in the past to discriminate between different virus variants (Wiktor *et al.*, 1980; Schneider *et al.*, 1985; Baer and Smith, 1991; Rupprecht *et al.*, 1991; King, 1991). It is Arthur King's merit to propagate the technique in Southern Africa (King *et al.*, 1994).

Bingham *et al.* (2001) have published a good example of using molecular data for selecting a Mab panel. A phylogenetic tree derived from N-gene sequences of Mokola virus isolates from Zimbabwe and South Africa can be interpreted as composed of four major branches or clades. Eleven anti-N and two anti-P Mabs of the Ottawa collection bind to epitopes of some, but not all of these isolates. These Mabs provide four reaction patterns that concur with the branches of the gene tree. A panel of four Mabs could be selected for discriminating between members of the different recognised Southern African Mokola clades (Table 44).

Table 44: Mab reaction patterns in indirect immunofluorescence with Mokola isolates from South Africa and Zimbabwe.

	Mab ID			
Isolate ID	**20HF8 (N) M879 (N)**	**11DG10 (N) M1335 (N) M1336 (N)**	**M878 (N) M1700 (P) M1718 (P)**	**M862 (N) M882 (N) M1015 (N) M1025 (N) M1027 (N)**
21846 (Zim)	-	-	-	+++
97/229 (SA)	+++	-	+++	-
97/252 (SA)	+++	-	+++	-
98/071 (SA)	+++	-	+++	-
700/70 (SA)	-	-	+++	+++
543/95 (SA)	-	+++	+++	+++
112/96 (SA)	-	+++	+++	+++
322/96 (SA)	-	+++	+++	+++

The protein specificity of each Mab is indicated in brackets behind their identification number. For details see Bingham *et al.*, 2001.

The same paper (Bingham *et al.*, 2001) describes a Mab panel for discriminating between canid rabies isolates and viruses of mongoose (Herpestidae) origin. This panel can be expanded to identify also the different Lyssavirus species occurring in the SEARG region (**Erreur ! Référence non valide pour un signet.**). The adding of additional Mabs to the panel will permit to differentiate between canid viruses originating from different areas of the SEARG region (Table 46).

Table 45: a Mab panel for discriminating between different Lyssavirus species occurring in the SEARG region.

	38HF2 control	**M853**	**M1386**	**M612**	**M1001**	**M837**
Rabies: Canid	+++	+++	-	-	-	-
Rabies: mongoose	+++	-	+++	-	-	-
Lagos bat	+++	-	-	+++	-	-
Mokola	+++	-	-	-	+++	-
Duvenhage	+++	+++	-	-	-	+++

Table 46: a Mab panel for differentiating between canid rabies viruses from different areas of the SEARG region.

	20HF8 M1336	**7AG8 M1335**	**M1386 M1400**
Ethiopia	+++	+++	+++
Tanzania, Zimbabwe	+++	+++	-
Zimbabwe, South Africa, Namibia	+++	-	-

6 CONCLUSIONS.

Mabs are valuable tools for epidemiological research. However, it is strongly recommended that Mab panels for virus classification be validated by molecular studies that permit to establish phylogenetic relationships.

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Chiron award presentation

CHIRON AWARD 2001 FEED-BACK

TITLE OF PROJECT : "SOCIO-ECONOMIC IMPACT ANALYSIS OF RABIES IN SOUTH AFRICA."

This project, which is basically descriptive epidemiology, will hopefully provide some tool that will assist in emphasizing the much-ignored problem of rabies as a disease effecting humans.

HYPOTHESIS/PROBLEM:

The importance of rabies has never been quantified or qualified in this country in real economic terms and the effect that rabies has on people's lives has never been measured. The current situation is that it is quantified and qualified by the number of laboratory confirmed human deaths and to a lesser degree by the number of animal cases. The role that the public and private sectors play respectively in the control of the disease has never been quantified (public good vs. private good).

As a result no cost-benefit analyses of control programs are possible.

Significant levels of ignorance probably still exist among the public and where people do come into contact with rabies there is probably a lot of confusion and anxiety surrounding such an incident.

The first objective of this project was to determine the amount that rabies costs a province in terms of deaths, vaccines (human and animal) and antiglobulin usage, loss of livestock and time of productive activity

The second objective is to determine the importance of rabies for various socio-economic and ethnic groups.

Once the above objective have been met data will be available that will assist decision makers in diseases priority assessment for better resource allocation to both field services and research (e.g. the need for effective oral vaccines for wildlife in South Africa). Information about affected communities is also needed for planning and policy making and for judging the effectiveness with which projects eventually met their goals. It is probably necessary to precede a vaccination campaign with an educational campaign.

Cost-benefit analysis of control strategies will also be possible.

Due to the magnitude of this study it will be piloted in one province. Compiling of epidemiological data and plotting of spatial and temporal distributions of cases will be done to determine if there is a pattern.

Division of the province into sociological zones based on demographic data and selecting a statistically meaningful number of households from each zone to be interviewed using an unbiased method.

At the end of each year the provincial director of veterinary services of each province releases a report on the budget expenditure per disease. This information can be used as an indication of the public good service rendered in combating the disease.

The private good aspect is reflected mainly in the number of vaccines administered by private veterinarians and welfare companies. South Africa has over 2000 registered private veterinarians. A mailing list can be obtained from the SAVC and veterinarians can then be asked to give an indication of the number of vaccines administered in one year. Alternatively the manufacturers of the vaccines could be approached for similar records.

Suggestions made by the selection committee were the following:

1) Biggest concern was the use of mailed questionnaires as they tend to be unsuccessful or result in biased feedback.

2) Interview questions should be asked by trained interviewers in order to guarantee uniformity.
3) The follow-up of human contacts from cases diagnosed in the laboratory might not provide the complete picture and it will also be important to get information from hospitals on dog bites.
4) Telephone interviews do not seem appropriate

The study will be piloted in Kwazulu-Natal province as dog rabies probably has the biggest socio-economic impact. The data for that province is updated and corrected and ready to use. The next remaining step is to design the questionnaires and select the communities to be interviewed.

The number of unreported bovine cases, human contacts and PEP (post-exposure prophylaxis) treatments for the preceding year also has to be determined.

There is no official recording system for PEP's administered in this country so this data is not available.

Hospital/clinic records will be the only records available. Therefore a study to determine the number of PEP's administered will have to go through the records of several clinics or hospitals from different socio-economic areas of the country to arrive at a representative samples. The one advantage if this approach is that data can also be gathered for the number of suspected neurological cases and the number of bite wounds seen.

This however is based on the assumption that the clinics keep good records.

Another method of obtaining this information is to look at vaccine/RIG (rabies immunoglobulin) administration at hospital/pharmacy level.

The only other remaining way of getting a reasonable estimate is to look at vaccine/RIG distribution at national depot level.

As for determining the number of unreported bovine cases I want to suggest that we move away from this terminology as it is impossible to know what is a rabies case for certain without laboratory confirmation. Diagnosis on clinical grounds is never an accurate way of diagnosing rabies. 75% of the bovine samples submitted for suspected rabies testing to our laboratory test negative and many of these cases have the typical rabies symptoms of salivation, tenesmus and bellowing. Many toxicoses, heartwater and cerebral babesiosis can imitate rabies.

I would like to propose that any animal destroyed because of a suspicion of rabies must be considered a loss due to rabies. This study will therefore determine the number clinically suspect cases that were destroyed but never send to the laboratory. Fir this reason I believe the farmer instead of the state vet or private vet should be interviewed as the farmer will not always notify a vet when he decides an animal might have rabies and destroys it.

The population frame will therefore be all cattle farmers. A list of cattle farmers in an area can be obtained from the state vet.

SOCIO-ECONOMIC IMPACT:

The term socio-economic impact is used quite often but ask somebody for a clear definition and frequently they cannot. It is easy to define in the context of the socio-economic factors leading to the presence of a disease but is not easy to define in the context of the socio-economic impact of a disease. Frequently socio-economic impact is nothing more than the economic impact of a disease on individuals or government.

The socio impact component of a disease can be defined as the effect that rabies has on people's lives. But even that is a vague statement that needs redefining.

The following might be measurable targets:

1) Firstly the number of human deaths (confirmed and suspicious)

2) Secondly, the number of people seeking treatment after being bitten because of a fear of rabies i.e. the PEP's administered. In some cases it affects several members of a household.

3) Thirdly, the ease/difficulty encountered when seeking treatment.

4) Fourthly, days post exposure that Rx was initiated

Data on the latter half can be obtained by following up on exposures to known positive cases.

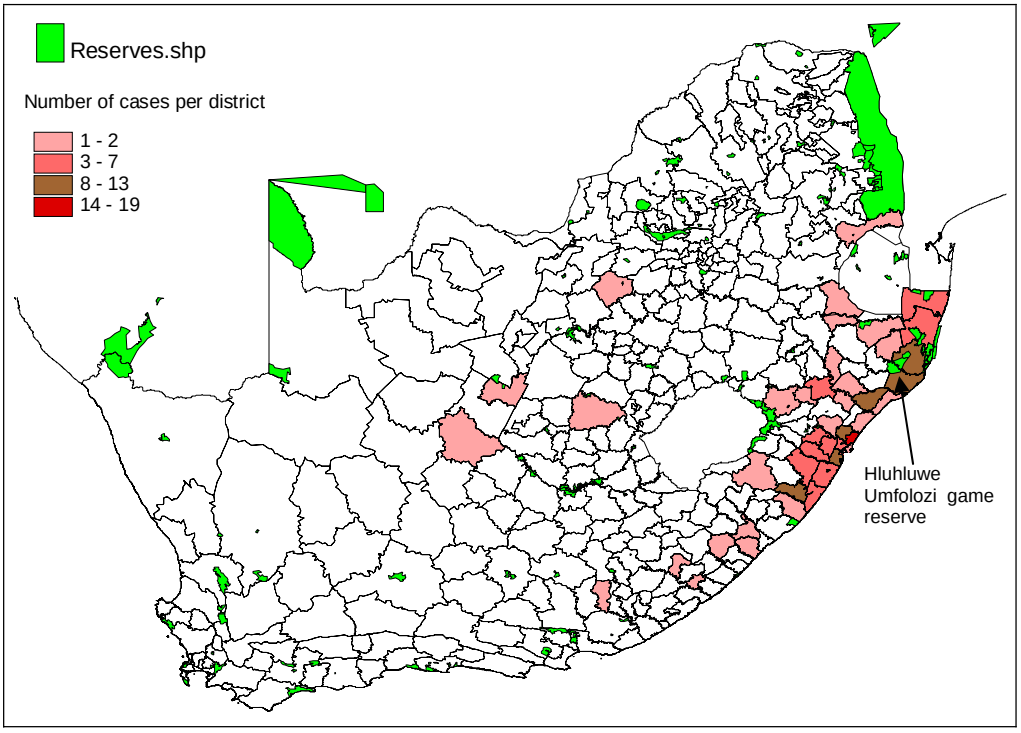
Socio-economic zones:

Statistics South Africa has divided the country into EA's (enumerator areas). Each enumerator area is a pocket sized piece of country consisting of a 100 to 200 households. Various census tables can be linked to the EA boundaries according to the users needs which makes it a powerful tool in manipulating and analyzing socio-economic data.

I therefore propose that for KZN a few villages or tribal areas are selected in severely or chronically affected areas and that the socio-economic status of the affected community is then determined afterwards using the above tables.

A preliminary survey was conducted in the 10 Tribal Authorities around Hluhluwe and Umfolozi game reserve.

Figure 1: distribution of laboratory confirmed human cases of rabies from 1987 to 2001



A veterinarian from the SPCA conducted the survey. He does house to house vaccinations in the 10 tribal authorities around Hluhluwe-Umfolozi game reserve. His method of reaching the people is to get the collaboration of the chief of each tribal area. The chief then instructs/orders his people to co-operate.

The above map is a summary of all laboratory confirmed human cases from 1987. Human cases were plotted according to the district of exposure and if the district of exposure was unknown by the district in which the person was hospitalized. As can be expected most cases occur in the dog rabies endemic area of KZN. There are certain areas that are more affected than others and the reasons for this is not clear.

The questionnaire had 10 questions.

1) Has anyone in your umuzi* ever been bitten by a dog?
   a) Y/N
   b) Who was bitten? _____
2) Where is your nearest clinic? _____
   How do you get to the clinic?
      i) Walk _____
      ii) Taxi _____
      iii) Bus _____
      iv) Own car _____
3) What is taxi cost? _____
   Does anyone earn
      i) A salary _____
      ii) A pension _____
4) Whose dog bit the person?
   a) Your own dog _____
   b) Neighbour's dog _____
   c) Strange dog _____
5) Did you think of rabies when the person was bitten?
   Y/N
6) Was one of your own dogs bitten as well?
   What has happened to the dog that was bitten?

*umuzi = zulu word for household

The primary objective of this survey was to sample dogs that had been given their first vaccinations 6 months earlier. A list was available of all these dogs. Every 14th animal on the list was selected to come up with 67 dogs, which gives a 90% confidence level and 10% precision interval.

Sixty two questionnaires were returned and the results were as follows:

- 5/62 (8%) umuzis had a member of the umuzi that had been bitten by a dog
- 3/5 were bitten by their own dog, 2/5 were the neighbour's dog, none was a strange dog.
- In 1/5 umuzis the owner's dog was also bitten, the dog was OK
- 3/5 interviewees thought of rabies at the time, 2/5 did not

All of the umuzi's new where there nearest clinic was

- 10/62 walked there, 49/62 used public transport, 3/62 used their own transport
- 43/62 received a salary or pension or both, 19/62 received no formal income.

An obvious missing question is to ask the people who thought of rabies whether they sought treatment and what the treatment was they got and why they did not seek/complete the treatment.

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