Contents:

Foreword

In 1988 an international conference on the epidemiology control and prevention of Rabies and Brucellosis in eastern and southern African countries was held in Gaborone, Botswana. Representatives from many countries of the Southern African Development Co-ordination Conference (SADOC) were present at this meeting and the Proceedings of the Conference were published by Fondation Marcel Merieux. The Proceedings provided a valuable insight into the then current rabies situation of the region.

With the passage of time, new developments in rabies diagnosis and control and changes in rabies incidence have occurred. Progress has been made in areas such as diagnostic sample collection, vaccines (particularly oral vaccines) and control methodology. However, everywhere the costs of diagnosis, control programmes and research are increasing, and in many areas of the region effective control is becoming more difficult. The need for a fresh initiative on rabies was foreseen. It was appropriate to review the situation; to identify significant changes in the occurrence of the disease; to identify diagnostic problems and requirements for the standardisation of techniques; to identify achievable technical developments which could improve both diagnosis and control and to identify groups that could work towards the realisation of the developments required.

It became our task to organise a Pilot meeting which, thanks to the hospitality of the Zambian Government, took place in Lusaka during June 2-5 1992. This task was facilitated by the co-sponsorship of the Zambian Central Veterinary Research Institute, the Food and Agriculture Organisation of the United Nations, the World Health Organisation and the Office International des Epizooties. Financial support was also provided by several internationally recognised pharmaceutical companies and the grateful thanks of all participants is hereby accorded to them.

Most countries of eastern and southern Africa were represented and in addition to their papers these Proceedings contain papers from others who were unable to attend. A number of internationally recognised rabies experts agreed to present papers on topics relevent to the region and we thank them for their presentations and for permission to publish their papers.

The onerous duties of session Chairpersons and Rapporteurs were shared by different country representatives and/or speakers. it became the task of one of us (A.K.) to gather the information together and to edit these Proceedings and I thank all speakers far permission to collate information and presentations into a common format.

The need far continued effort towards rabies diagnosis and control within the region was recognised and a further meeting, to be organised by Dr. Peter Sinyangwe (Chairman) and Dr. George Bishop (Secretary) was agreed.

Peter Sinyangwe
(Meeting Convenor)

Arthur King
(Proceedings Editor)

RABIES IN EASTERN AND SOUTHERN AFRICA

A seminar organised by the Central Veterinary Research Institute, Lusaka and co-sponsored by FAO, WHO and OIE

LUSAKA, ZAMBIA, JUNE 2 - 5 1992

PROGRAMME

Tuesday, June 2 1992.

18.00 -19.00 Reception in the Ridgeway Hotel

Wednesday, June 3 1992.

09.30 Chairman: Dr. H. G. Chizyuka

Opening address: THE HONOURABLE DEPUTY MINISTER, Dr. C. KALIMA

National Reports:

10.00	11.15	Chairman:	Dr Sinyangwe	Rapporteur: Dr. Roberts
			Uganda	Dr. Illango
			Mozambique	Dr. Dias
			Madagascar	Dr. Morvan
			Zimbabwe	Dr. Bingham
			Botswana	Dr. Masupu
11.15	11.30	Coffee		
11.30	13.00	Chairman:	Dr. Rweyemamu	Rapporteur: Dr. Thomson
			Namibia	Dr. Depner
			Swaziland	Dr. Hlatshwako
			Lesotho	Dr. Khomari
			South Africa	Dr. Bishop
			Zambia	Dr. Sinyangwe

Discussion Of National Reports

13.00 - 14.30 Lunch

International Input Chairman; Dr. Wandeler Rapporteur: Dr Perry

The work of FAO Dr. Wojciechowski
The work of WHO Dr. Meslin
The work of OIE Dr. Aubert

15.45 - 1600 Tea

The Viruses Chairman: Dr. Perry Rapporteur: Dr. Gascoyne

14.30 - 15.15 African Overview and Antigenic Variation. Dr. King
15.15 - 15.45 Rabies-related Viruses. Dr. Swanepoel

Thursday, June 4 1992

Diagnosis and Post-exposure treatment

Chairman: Dr Swanepoel Rapporteur: Dr. Bingham

08.30 - 09.35 New diagnostic tools. Dr. Barrat and Dr. Bourhy

09.45 - 10.15 Human post-exposure treatment. Dr. Thraenhart

10.15 - 10.30 Mabs and post-exposure treatment. Dr. Schumacher

10.30 - 11.00 Workshop on diagnosis and post-exposure treatment

Discussion of session presentations

11.00 - 11.15 Coffee

Control of canine rabies

Chairman: Dr. Tuchili Rapporteur: Dr. Gumm

11.15 - 12.00 Control of canine rabies in Africa. Dr. Perry

12.00 - 12.15 Vaccine quality control. Dr. Rweyemamu

12.15 - 12.45 Do dogs recover from rabies? Dr. Fekadu

12.45 - 13.00 Feasibility of canine rabies control. Dr. Meslin

Discussion of session presentations

13.00 - 14.30 Lunch

Control of wildlife rabies

Chairman: Dr. Depner Rapporteur: Dr. Bishop

14.30 - 15.00 Wild dog rabies. Dr. Gascoyne

15.00 - 15.30 Control by wildlife depopulation. Dr. Aubert

15.30 - 15.45 Tea

15.45 - 16.30 Fox oral vaccination with V-RG. Dr. Brochier

16.30 - 17.15 Wildlife rabies in southern Africa. Dr. Thomson

Discussion of session presentations

19.30 - Seminar Dinner at the Ridgeway Hotel

Friday, June 5 1992

Control of wildlife rabies (continued)

Chairman: Dr. de Balogh Rapporteur: Dr. King

08.30 - 09.00 Control of jackal rabies. Dr. Bingham

09.00 - 10.00 Requirements for oral vaccination. Dr. Wandeler

Discussion of session presentations

Future Collaboration and Recommendations

Chairman: Dr. Wandeler Rapporteur: Dr. Thomson

10.00 - 12.00 Plans of work at national level

Plans of work at international level

Conclusions and recommendations

12.00 Closing address by Hr. Onder Yucer

The meeting was honoured by the presence of the Zambian Assistant Minister of Agriculture

The Honourable Dr. C. Kalima

who gave the Opening Address:

"I should like to welcome to Zambia the many international rabies experts who have given their time to address this meeting and also to welcome the representatives of eastern and southern African countries who will share with us their experiences of working with this disease.

Rabies is an ancient disease and a fearsome one; few diseases are so well known or carry the same emotional impact. In Zambia, following the establishment of the Veterinary Services in 1906 in what was the then Northern Rhodesia, rabies has been documented and efforts made to control it. By virtue of Zambia's geographical disposition there has been periodic fluctuation in the spread of disease. The objective of rabies control has always been to reduce its incidence rather than total eradication. Several interrelated factors have made it difficult to contain the disease to manageable proportions. These include insufficient rabies vaccine supplies and coverage and a vast wild game reservoir population in Zambia.

Control of rabies in Zambia includes a number of statutory legislation procedures and by vaccination programmes. A National Programme for dog rabies elimination was started in 1984. The objective of this programme was to cover over 80 percent of the canine population within a period of six years. As a result of this massive vaccination programme, the canine rabies incidence has been reduced from 57.08 percent to 21.68 percent. The government has also taken a further step to produce the rabies vaccine locally in the near future.

The pioneering work of many nineteenth century workers, culminating in the development of the first rabies vaccines by Pasteur, provided the groundwork for the modern era in the study of rabies. Since then considerable advances have been made and although effective vaccines for humans and animals exist, there is still no cure once disease symptoms develop.

I understand that the objectives of the meeting are:

(1) to review the rabies situation in eastern and southern Africa;
(2) to identify technical problems in rabies diagnosis and requirements for the standardisation of diagnostic techniques throughout the region;
(3) to identify achievable technical developments which would improve rabies control in the region and
(4) to identify groups that could collaborate and work towards the realisation of the developments required.

I congratulate the Central Veterinary Research Institute, Balmoral, in their organisation of such a venture which has received the approval and co-sponsoring of such august bodies as FAO, WHO and OIE. It is my hope that you have a successful and rewarding meeting and I look forward to seeing the results of your work in due course."

The meeting was closed by the UNDP Resident Representative, Lusaka,

MR. ONDER YUCER

who gave the Closing Address:

"Ladies and Gentlemen, distinguished delegates and scientists, it is my pleasure to close this meeting on "Rabies in eastern and southern Africa". I wish to express my satisfaction to see this regional meeting being held in Zambia. Please let me also express my gratitude to two persons whose contribution to the organisation of this international meeting has been essential for its success: this is to Dr. Sinyangwe, Director of the National Veterinary Laboratory and convenor of the consultation. Thank you very much Dr. Sinyangwe on behalf of all delegates, experts and representatives of international organisations. The other gentleman is Dr. Chizyuka, Director of the Veterinary Services and Tsetse Control, thank you very much also. We look forward to assisting you in convening more scientific consultations and seminars on subjects of common interest to the countries of the eastern and southern African region.

I know that the meeting has been very successful and that epidemiological data as well as newly available technologies have been discussed thoroughly. I noted that the rabies situation in the region is complex, involving both dogs and many wildlife species as reservoirs and transmitters of the disease. The effect on human health, livestock and endangered wildlife is far from being negligible. The various control measures taken by these countries have led to a marked improvement in the rabies problem in a number of areas.

In spite of the diversity of national situations and control strategy selected, you have identified common problems and obstacles to the implementation of your control activities. This shows that improvement could emerge in a very cost-effective manner from closer inter-country collaboration and exchange of information and technologies. This can be achieved within the framework of a sub-regional programme for canine and wildlife rabies control.

I understand that your recommendations are:

- to identify and strengthen reference centres within the sub-region, one for reference and training in rabies diagnosis and surveillance and one for veterinary rabies vaccine production.

- to assist countries of the sub-region in acquiring adequate quantities of good quality vaccine for human immunisation and until the above centre for veterinary vaccine production becomes operational countries should also be provided with animal vaccines.

- to pay special attention to and especially in areas where the dog is the main reservoir species, to strengthen operational capabilities to increase the coverage of the dog vaccination programmes.

- to strengthen research on the epidemiology of rabies in mongoose, jackal and other wildlife species, especially endangered wildlife and to initiate work on domestic dog ecology and populations.

- to study the feasibility of oral vaccination projects in the region and initiate limited-scale field trials for immunisation of wildlife and dogs.

- to elaborate comprehensive national projects for the prevention and control of rabies in man and animals.

I am very pleased to see that the meeting has been very productive and assure you that due consideration will be given to your recommendations. F.A.0. and W.H.O. should be jointly responsible for their implementation.

I wish to thank again the organisers of the meeting and the specialised U.N. agencies and other institutions which have co-sponsored it. I wish to all delegates a safe journey back to their countries and officially pronounce the closure of the meeting".

Rabies in Uganda

James Illango

Animal Health Research Centre, P.O. Box 24, Entebbe, Uganda.

Although rabies must have existed in Uganda from time immemorial, being associated with "witchcraft in dogs" or "a curse from God", the incidence and distribution of the disease in the country was not known. Laboratory diagnosis began in 1936 with the confirmation of four cases in the North-western district now known as Arua. Thereafter, knowledge of the incidence and distribution of the disease has been directly or indirectly influenced by different political regimes, which may be conveniently divided into four periods:

a) 1936-1960, pre-independence, the period of British colonial administration;

b) 1961-1970, the period of the first political government which inherited the colonial regime;

c) 1971-1980, the decade of military government of Idi Amin, and

d) 1981-1990, a period of considerable political instability which saw six different regimes.

In the pre-independence period, diagnosis was confirmed by histological and animal inoculation techniques. 49 of the 51 recorded cases were in dogs, the first wildlife case was a jackal in 1954 and the first domesticated animal other than dog was a bovine in 1956. The cases were confirmed in the districts (Fig. 1) of Arua (bordering Sudan), Mbarare and Tororo (bordering Kenya) and Entebbe, through importation by air.

Several factors may have contributed to the occurrence and distribution of rabies during this period. These include the lack of natural barriers between Arua and Sudan which facilitated the movement of stray rabid dogs across the border into Uganda; the movement of refugees with their pets and other animals (e.g. cattle) from Sudan; tribal and cultural associations of western Kenya and eastern Uganda; lack of adequate rabies control measures in neighbouring countries, particularly in Sudan where the Islamic Central Government in Khartoum put no emphasis on rabies control in the Christian-dominated southern part of the country; the role played by jackals in the maintenance of a cycle with dogs; lack of vaccination cover in the affected districts because of inadequate infra-structure and a lack of public awareness.

In the period of the first political government (1961-1970) more samples were submitted to the Central Laboratory at Entebbe, at which the laboratory services were sound. The number of positive cases increased to 225 (Table 1), of which 177 were in dogs, 3 in cats and 27 in other domesticated animal species (cattle, sheep and goats). The jackal (13) continued to be an important wildlife vector. The number of affected districts increased from 3 to 6, with the majority of cases (125 of 225) in Arua district. This was the time when political conflicts and tensions were very high in southern Sudan and Rwanda, with an influx of refugees into Arua and Mbarara districts respectively.

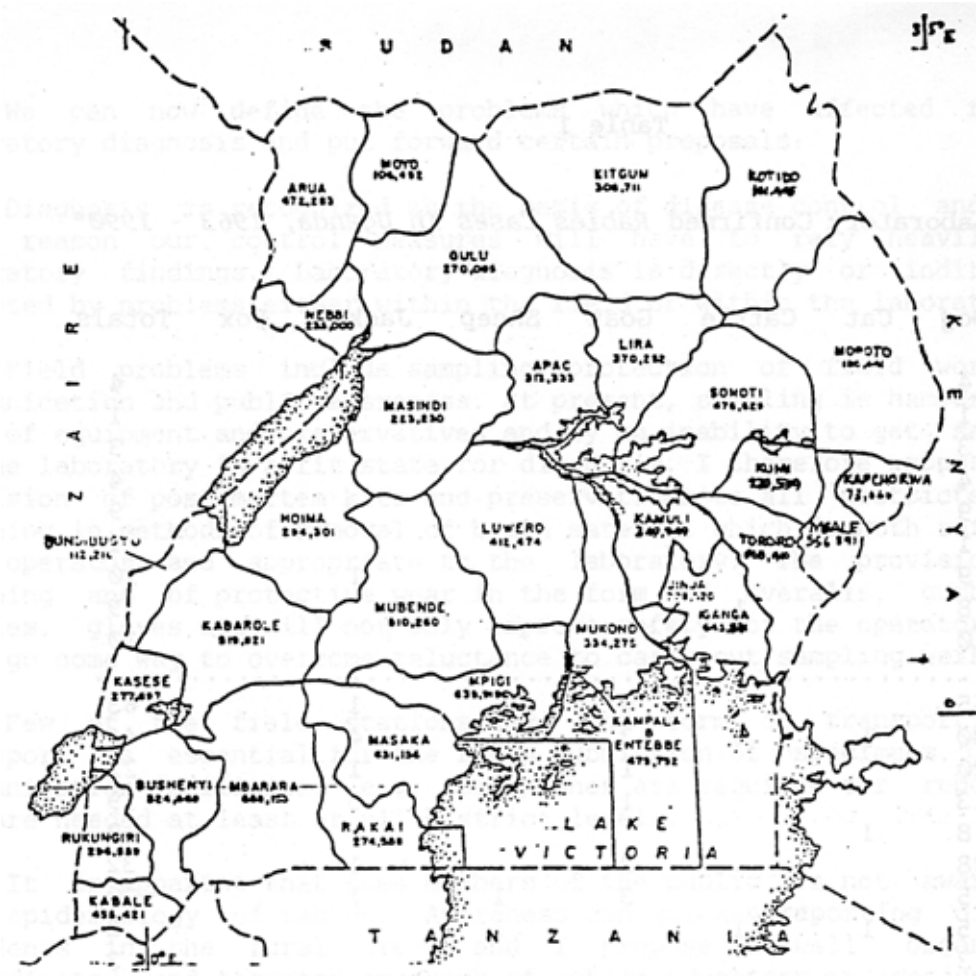

Fig. 1. Districts and populations of Uganda.

During the decade of the military government of Idi Amin, although there was a fall in the number of recorded cases to 123 (111 dog and 12 cat), more districts (17) were affected. The apparent reduction in numbers was probably associated with a breakdown of government veterinary services due to the emphasis put on defence and security, leading to a decline in disease control. Fewer vaccinations were carried out, there was an increase in the population of susceptible cats and dogs and the increased movement of people with their animals facilitated the spread of disease to other districts. For economic and security reasons the population became more urbanised and more cases were reported in urban areas (e.g. Kampala 13, Entebbe 18). With increasing economic hardship there was a breakdown of urban services including garbage collection, dog owners could not afford to maintain their pets and more stray dogs could be seen in towns and villages in which the garbage areas formed contact-points for the dogs.

The period 1981-1990 was characterised by political instability and insecurity in the first half of the decade, leading to slow recovery of the country in the second half. Submissions to the laboratory fell to 41, laboratory services were affected by the irregular supply of chemicals and reagents and the breakdown of equipment and more reliance was placed on clinical diagnosis. Now, however, there is great optimism and hope of recovery with political stability. The government has vigorously embarked upon a recovery programme which is attracting the attention of international aid donors and they are showing interest in assisting the Department of Veterinary Services.

Year	Dog	Cat	Cattle	Goat	Sheep	Jackal	Fox	Totals
1936	4							4
1937	1							1
1944	3							3
1946	4							4
1947	9							9
1953	1							1
1954	9					1		10
1956	8		1					9
1960	10							10
1961	55		2	4	1	1		63
1962	30	1	5	3	1	4		44
1963	20			1		1		22
1964	13			1		5		19
1965	8	1						9
1966	28			3		1		32
1967	15			3	1	1		20
1968	5	1	1		1			8
1969	3							3
1970	4				1	1		6
1971	14	2		2				18
1972	21							21
1973	6	6	1	1				14
1974	11			1				12
1975	9							9
1976	10	1	1	1				13
1977	21							21
1978	7	2	1					10
1979	8							8
1980	4	1		1				6
1981					1			1
1982	4			1				5
1983	2							2
1984							1	1
1985	1							1
1986				1				1
1987							2	2
1988			1					1
1990				1				1
	348	15	13	24	6	15	3	424

Table 1

Summary of Laboratory Confirmed Rabies Cases in Uganda, 1963 - 1990.
No rabies cases were reported during the 17 years of 1938-1943, 1945, 1948-1952, 1955, 1957-1959 and 1989.

We can now define the problems which have affected rabies laboratory diagnosis and put forward certain proposals:

Diagnosis is recognised as the basis of disease control and for this reason our control measures will have to rely heavily on laboratory findings. Laboratory diagnosis is directly or indirectly affected by problems either within the field or within the laboratory.

Field problems include sampling, protection of field workers, communication and public awareness. At present, sampling is hampered by lack of equipment and preservatives and by an inability to get samples to the laboratory in a fit state for diagnosis. I therefore propose the provision of post-mortem kits and preservatives to all districts and training in methods of removal of brain material which is both safe for the operator and appropriate to the laboratory. The provision of training and of protective wear in the form of overalls, gumboots, goggles, gloves etc will not only improve safety for the operator but will go some way to overcome reluctance to carry out sampling work.

Few of the field stations have any form of transport, but transport is essential for the rapid submission of specimens. Other communication facilities, e.g. telephones are required for reporting and are needed at least at all district levels.

It is apparent that some members of the public are not aware of the epidemiology of rabies. Awareness can quicken reporting of any incidents in the rural areas and I propose a well organised, co-ordinated and targeted approach of public education in conjunction with the Ministry of Health and Local Government.

Laboratory problems include safety, equipment, technical skills and training. At present, protective wear is inadequate. In addition, the protection of all rabies personnel by pre-exposure vaccination followed by establishment of antibody level is highly desirable.

Chemicals and equipment are in short supply and/or need replacement. Ideally the Central Laboratory should be diverse in its diagnostic techniques and sufficiently flexible to adjust to the methods appropriate to the condition of incoming specimens.

I propose that diagnosis should be made by one or more of the following techniques: Fluorescent antibody (FA), histopathology, animal inoculation and serology. FA diagnosis is the quickest, most sensitive, specific and reliable method and is used in most rabies laboratories of the world; it requires a fluorescent microscope and commercially available conjugate. Histopathology is the method currently in use but it is much less sensitive than FA, requires a microtome and takes several days to perform. Animal inoculation is the most expensive and time-consuming technique but it should be used where the suspect animal has bitten a human but has proved negative by FA. Serology has not been introduced in Uganda but is applicable in the monitoring of sero-conversion, for which I recommend that it is introduced.

Training of laboratory staff to improve competence and confidence and to keep abreast of new diagnostic techniques is essential. Funds are required for this and for laboratory costs which include maintenance of equipment and, for example, a mouse colony. Transport is also required, for disease investigation and collection of specimens.

In conclusion, but not of least importance, I would like to briefly detail the role of the Department of Veterinary Services in rabies control. The Department has a laid down policy and strategy which has been in force since rabies was first confirmed in 1936. The Rabies Control Act came into force in 1935 and was enacted by Parliament in recognition of the importance of the disease. Rabies is recognised as a notifiable disease of public health importance. This Act is therefore directed towards the prevention and control of Rabies, based on reporting, laboratory diagnosis, vaccination, destruction of affected species and import-regulatory measures.

There is a multi-sectoral approach involving the Ministries of Agriculture, Animal Industry and Fisheries, Health, Local Government and Internal Affairs for joint effort and co-operation. In order to co-ordinate the activities of a multi-sectoral approach a National Technical Committee was set up in 1991, amongst other things to formulate an effective preventive and control strategy for rabies. The Committee has a composition of technical personnel from the Ministries of Agriculture and Health. The major initial task of this Committee is to ensure revival and reactivation of the necessary activities which have decayed over many decades and present any modifications in the light of changing political, economic and administrative and epidemiological situations. The efforts of this Committee must, however, be paid by the seriousness, commitment and co-operation of all concerned.

Rabies in Kenya

Yatinda Binepal

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Situated on the East coast of Africa, the 564,126 km^2 of Kenya straddles the equator and thus is part of both the Northern and Southern hemispheres. About one third of the country has high agricultural potential and plantations of tea, coffee, pyrethrum, wheat, sisal and maize form the main crops; the remainder of the land is either semi-arid or arid. The majority of the estimated 25 million people of Kenya live in rural areas; the dog population is estimated to be 2.5 - 3 million.

Rabies was first reported in Kenya in October 1912 in a dog reputedly bitten by a jackal. One or two cases were intermittently reported annually until 1967 - 1974 when four to six cases were reported annually from just two of the 46 Kenyan districts (Fig. 1)
However, there was a spread towards the coast, where the disease was first reported in 1976. Another spread was towards the west of the country and districts neighbouring Uganda (Bungoma) reported the disease in 1979. Spread to the arid north led to rabies reports from Wajir and Marsabit in 1979. In March 1980 Nairobi reported its first case but it was not until March 1982 that an outbreak was observed.

In 1972 22 positive rabies cases were reported but by 1987 the yearly total had increased to 290. Today, about 200 cases are reported annually; most of these cases are from dogs but wildlife cases, although few in number, are consistently reported (Tables 1, 2).

Diagnosis

Diagnosis at Kabete is based on the fluorescent antibody test (FAT) and mouse inoculation test (M1T) and occasionally histopathological tests are performed. Material received at the Kabete Veterinary Laboratory consists of the whole carcasses, often accompanied by a history. Live animals, usually dogs and cats, are also received and are kept for observation in isolation in specially built kennels. The FAT is performed according to the W.H.O. rabies manual. The skull is opened and portions of the left and right horns of the hippocampi, cerebral cortex and medulla are dissected from the exposed brain. A 10 percent suspension of these tissues is prepared using transport medium. Drops of the suspensions are placed on microscope slides, dried at 37°C and fixed in acetone at 4°C. The slides are then flooded with anti-rabies serum labelled with fluorescein isothiocyanate for 45 minutes at 37°C. The slides are rinsed three times in PBS and air dried. Rabies positive tissues show a range of fluorescing particles from large intracytoplasmic inclusions to very fine fluorescent particles. FAT negative samples are subjected to the MIT.

Aliquots of 0.02 - 0.04ml of a 10 percent brain suspension are inoculated intracerebrally into 3 week old mice, which are then observed for 30 days. The brains of mice which die during this period are examined for rabies by FAT.

**In absentia.* Co-authors: Dr. M. J. Macharia and Dr. R. E. N. Runyenje, Dept. of Veterinary Services, Vet. Labs., P.O. Kabete, Nairobi, Kenya.

Table 1
Summary of *Rabies Cases Reported in* Kenya, 1987 - 1991.

Species	1987	1988	1989	1990	1991	Total
Human	2	1		3	2	8**
Dog	186	105	127	124	95	637
Cat	10	6	2	7	18	43
Cattle	51	72	55	50	32	260
Sheep	2	8	5	1	3	19
Goat	18	14	8	4	1	45
Horse/Donkey	7	6	2	6	3	24
Pig						
Total Domestic	274	211	199	192	152	1028
Total Wildlife*	14	1	6	5	4	30
Total Animal Positive	288	212	205	197	156	1058

* Wildlife includes fox, honeybadger, mongoose, bat, jackal, squirrel and wild dog.
** Not complete, many diagnosed in hospitals etc.

Table 2
Percentage of Samples Positive for Rabies

	1987	1988	1989	1990	1991	Total
Samples submitted	468	352	372	354	270	1816
Samples positive	288	212	205	197	155	1058
Percent. positive	61.54	60.23	55.11	55.65	57.78	58.26

Control

Control of rabies in Kenya includes vaccination of pets (dogs and cats) in an attempt to reduce the number of human cases. No effective control method is available for other domestic animals. The dog remains the primary source of rabies in domestic animals. The vaccine production unit at Kabete had been producing LEP vaccine but since 1989 no vaccine has been produced because of equipment breakdown.

Discussion

The dramatic increase in rabies cases since 1979 remains unexplained. The role of wildlife in the epidemiology of rabies in the country is unknown - a few cases in wild carnivora, bats and other wildlife are reported but an efficient and sustainable method to collect wildlife samples has yet to be established.

Since Kabete is one of only two laboratories in Kenya with responsibility for rabies diagnosis in animals it may take some time for samples to reach the Laboratory and occasionally they are then unsuitable for diagnostic work. This problem has been partly solved by the establishment of regional laboratories which have been supplied with transport medium and glycerol for the transport of brain material.

As a back-up to FAT and MIT a cell culture based diagnostic test is being adopted. Although this test using BHK-21 cells is not yet operational, attempts are being made to have it useable as soon as possible. However, the major problem continues to be the lack of equipment and its servicing or repair. The laboratory has only one FAT microscope and no back-up services. The situation is not yet desperate and with increasing communication and awareness of the general public the number of rabies cases is expected to decline.

Acknowledgement:

We wish to thank the entire staff of the Veterinary Investigation Laboratories, Kabete and KARI, Kabete, without whose support this publication could not have been compiled and also Miss Elizabeth W. Kamuyu for typing this manuscript. This paper is published with the kind permission of the Director of Veterinary Services, Kenya.

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Fig. 1. The districts of Kenya.

Rabies in Tanzania

*K. Loretu**

Animal Diseases Research Institute, P.O. Box 9254, Dar Es Salaam

Tanzania is situated on the east coast of Africa and shares land borders with Kenya, Uganda, Rwanda, Burundi, Zambia, Malawi and Mozambique. The country has an estimated human population of 23 million, dog population of 800,000 and cat population of 500,000.

Rabies was first recognised clinically in Tanzania in 1932 and dogs are now the chief vectors and reservoirs of disease, although jackals and hyenas play a similar but more limited role. A reservoir of the disease has been observed since 1956 in the Mbeya region bordering Zambia and Malawi. An almost explosive development of the disease was reported in 1980-1981 in the district island of Ukerewe and Tarime district of Mara region (Fig. 1).

The rapid spread of disease was attributed to the increasing density and mobility of the rural populations. Human deaths from the disease reached a peak in 1981 when 83 cases were recorded and a further 126 people died of the disease during the years 1986 - 1987. There are no indications that wildlife species maintain chains of infection independently from the rabies reservoir in dogs.

Diagnosis

Laboratory diagnosis is carried out at the Animal Disease Research Institute where IFA tests are employed, although a few cases are confirmed by histopathology techniques alone. The disease was confirmed in cases from ten of the twenty regions of Tanzania during the period 1987 - 1991. These cases are summarised in Table 1.

Control

Tanzania has a number of importation restrictions covering dogs, cats, monkeys, wild animals and animals brought into the country for experimental purposes. There are also tie-up Orders in areas where the disease is confirmed and Orders which permit the destruction of stray dogs. However, control of the disease is largely directed towards vaccination of the principal vector (dog) as well as cats.

Rabies control programmes have not been able to produce the desired effect chiefly because of inadequate vaccination, lack of bullets for stray dog control and underfunding. There is a need for more intersectoral co-operation (Veterinary and Medical Services), public awareness and co-operation with neighbouring countries, especially in the production of adequate supplies of vaccine.

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*In absentia

Table 1

Distribution of Rabies Cases in Tanzania 1987 -1991.

	No. of Cases					
Region	1987	1988	1989	1913	1991	Total
Arusha				1		1
Dar Es Salaam	9	2	1	2	4	18
Dodoma	3	2	2		1	8
Iringa	1					1
Kagera		1		1		2
Mbeya	2					2
Morogoro	1					3
Pwani			1			1
Tabora		2	1			3
Tanga				1	1	2
Total Animal Cases	16	9	5	5	6	41

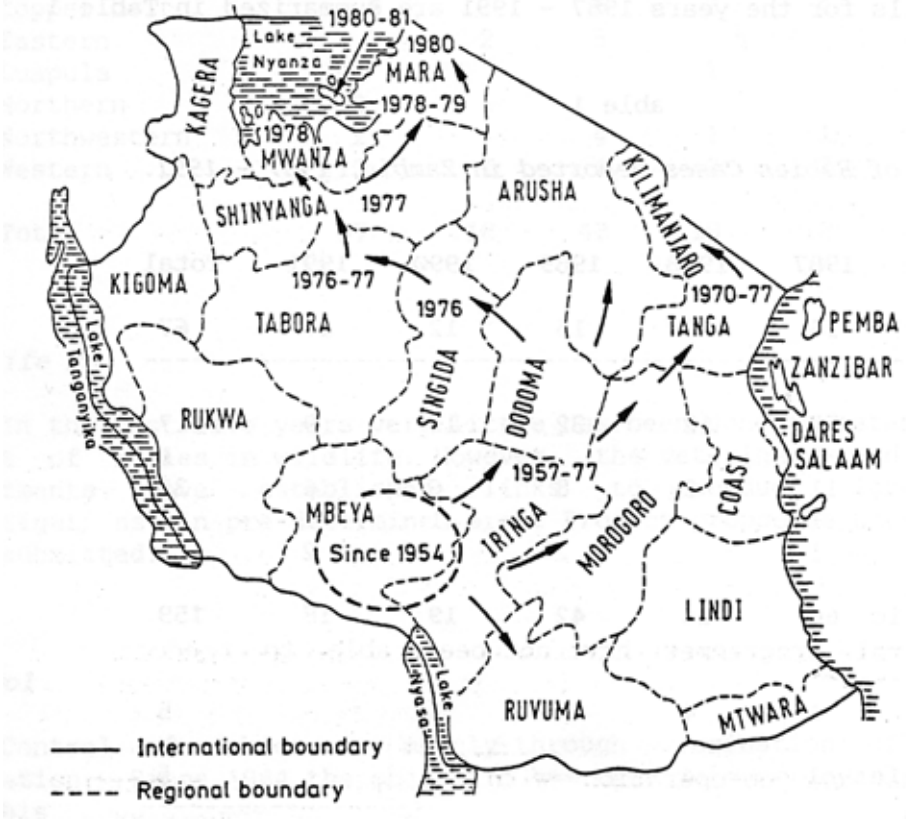

Fig. 1. Spread of Rabies in Tanzania, 1954 - 1980.

Rabies in Zambia

Peter Sinyangwwe

Central Veterinary Research Institute, P.O. Box 33980, Lusaka, Zambia.

Zambia has a land area of about 725,600 Km^2 (Fig. 1). In 1991 the human population was approximately 8.5 million and the canine population was estimated to be 700,000 dogs. Both human and animal rabies has been known to be present in Zambia since the early 1900s, the first case being recorded in 1913 when diagnostic facilities were put into place. Until 1984, the number of positive canine rabies cases exceeded that of all other domestic animal cases put together, but since 1985 when massive vaccination campaigns with tie-up orders were introduced, there has been a decline in the number of cases recorded. Several wildlife animals, mainly of the family canidae, have been found to be responsible for the transmission of rabies to man and to domestic animals. The virus has been isolated from jackal, monkey, mongoose, wild cat and hyaena. Zambia is of the more highly urbanised countries of Africa and is prone to the threat of urban and peri-urban rabies.

Diagnosis

Major diagnostic work is carried out at the CVRI in Lusaka, but three regional laboratories, at Ndola, Chipata and Mazabua, are also involved in diagnosis. Samples for diagnosis are preserved in glycerine and forwarded to these laboratories. For disease security and documentation purposes, isolation of the virus is carried out only at the CVRI. Details for the years 1987 - 1991 are summarised in Table 1.

Table 1

Summary of Rabies Cases Reported in Zambia, 1987 - 1991.

Species	1987	1988	1989	1990	1991	Total
Human	16	7	15	12	17	67
Dog	52	12	32	12	9	117
Cat	1					1
Cattle	11	2	8	6	6	33
Sheep	1			1	1	3
Goat	1		2		2	5
Total Domestic	66	14	42	19	18	159
Vulpine	1	4				5
Total Wildlife	1	4				5
Total Animals	67	18	42	19	18	164

Three. diagnostic tests are used, FAT, mouse inoculation and histopathology. Diagnostic capability is at times limited by a poor transport network, particularly in the rural areas, inadequate diagnostic reagents and a manpower drain for "greener pastures".

Human and canine rabies

The total number of samples submitted and diagnosed in both human and canine populations do not reflect the true extent of the disease. It is strongly believed, and evident, that a high percentage of cases are not reported to veterinary or medical diagnostic institutions. It is also possible to miss a few positive cases when less sensitive methods are employed to arrive at a diagnosis. The distribution of rabies cases in Zambia for the past five years is summarised in Table 2

Table 2

Distribution of rabies cases, Zambia, 1987 - 1991

	Year					
Province	*1987*	*1988*	*1989*	*1990*	*1991*	*Total*
Lusaka	12		16	6	3	37
Southern	15	8	6	5	5	39
Central	9	2	2	2	3	18
Copper belt	5	1	5	2	4	17
Eastern	7	2	5			14
Luapula				1		1
Northern	2	1				3
Northwestern	2		4	1	1	8
Western	15	1	4	2	2	24
Total	67	18	42	19	18	164

Wildlife rabies

In the past five years very little has been done to establish the extent of rabies in wildlife. However, the veterinary and wildlife departments have established links to conduct large scale investigations in pre-determined ares. Project proposals have already been submitted.

Control

Control of rabies is mainly through vaccination of the dog population. Since 1984 the objective has been to cover over 80 percent of this population, but the target has not been met due to various limitations during some years of the programme. Among the limiting factors are poor vaccine coverage in certain parts of the country and insufficient vaccine supply at times. Rabies vaccine production to meet the national requirements, using BHK cells, is in progress at the CVRI.

Tie-up orders and destruction of stray dogs, particularly in urban and peri-urban areas, are in force. Public awareness through meetings has been the major force in rabies control nation-wide. Vaccination campaigns are conducted through a co-ordinated vaccination network using the existing government infrastructure. Vaccines which have been used in the past five years include Rabin (France), lamb brain vaccine (CORI) and cell-culture adapted BHK-21 vaccine (CVRI). Vaccination coverage figures are shown in Table 3.

As in many Commonwealth countries, Zambia strongly insists upon valid importation documentation of canine and feline species from the country of origin. These are signed by authorised and competent veterinary personnel. Upon arrival in the country, animals are kept under quarantine conditions until they are proved to be negative for rabies.

Table 3
Vaccine Distribution

	Year				
	19B7	*1988*	*1989*	*1990*	*1991*
Vaccine doses;	67,375	300,950	151,200	209,150	200,000
Percent Coverage	*50.0*	80.3	69.8	78.0	76.0

F1g. 1. The Provinces of Zambia.

Rabies in Malawi

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Malawi is situated to the south-east of the African continent and shares borders to the east with Tanzania and Mozambique and to the west with Zambia. A population census in 1988 revealed that of 8 million people, almost 88 percent live in rural areas and only 12 percent are urbanised. Agriculture employs almost 83 percent of the population and the degree of contact between humans and animals, be it by accident or through people's livelihood, cannot be over-emphasised.

As is the case in many countries of Africa, canine rabies is endemic and dogs play the most important role in the transmission of the disease to humans. In the hot months of August to December a much higher number of rabies cases are confirmed at the rabies diagnostic laboratories of the country. The disease is endemic in all three regions of Malawi and although it occurs in all 24 districts, 46 percent of the cases are recorded in the two cities of Blantyre and Lilongwe. In these and other urban areas dogs have become very popular, being used as watch/guard dogs. The human-animal bond among the urban as well as the rural populations is therefore very real and close.

Although 88 percent of the domestic animal rabies cases are in dogs, cattle and cat positives are frequently found. In addition, although low in number, the disease is consistently reported in wildlife species, principally in the jackal and hyena (Table 1).

Rabies Control

Adequate canine immunisation is a major factor in preventing the spread of disease to man. In addition, the control of stray dogs plays an important role in a successful control programme. Vaccination of dogs is performed by one of three regionally based Rabies Control Teams, in conjunction with locally based Veterinary Assistants. The teams are also responsible for the destruction of stray dogs by shooting. Vaccination is usually free, but vaccine is often unavailable or in short supply and these difficulties are compounded by the lack of a cold chain in the rural and remote areas. The vaccination coverage is probably no more than 12 to 20 percent of the dog population.

In addition to the Rabies Control Teams, there are some government operated veterinary clinics, especially in the cities, that provide a vaccination service, but as these have a veterinary surgeon in attendance, a professional fee is charged, thereby acting as a hindrance to the presentation of the majority of dogs. Only ex-patriate owned pets (dogs, cats) and a very small percentage of Malawian owned dogs are vaccinated in these clinics. As it stands at present, dog vaccination as a means of rabies control in Malawi does not seem to be effective.

Although the number of recorded human deaths due to rabies is low (10 - 20 deaths per annum), post-exposure treatments, at about 3,000 per annum, take up a considerable portion of the health budget.

---

* *In absentia*

Table 1

Summary of Rabies Cases Reported in Malawi, 1986 - 1950.*

Species	1986	1987	1988	1989	1990*	Total*
Human		1				1**
Dog	134	91	149	134	132	640
Cat	6	5	2	1	2	16
Cattle	5	8	5	22	11	51
Sheep	1	2	2			5
Goat	3	2	2		3	10
Horse/Donkey				1		1
Pig	1	1				2
Total Domestic	150	109	160	158	148	725
Jackal	2	1	3	5	5	16
Civet	1					1
Duiker				1		1
Fox	1		1			2
Hyena	1	1	4	3	5	14
Njuzi			1			1
Squirrel		1				1
Unspecified			1			1
Total Wildlife	5	3	10	9	10	37
Total Animals	155	112	170	167	158	762

* Totals for only 9 months (January - September) of 1990.
** Total incomplete (see text).

Rabies in Mozambique

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The approximately 800,000 km^2 area of Mozambique stretches along the east coast of Africa and the country is bordered by Tanzania, Malawi, Zambia, Zimbabwe, Swaziland and the Transvaal of South Africa. Within the ten provinces of the country (Fig. 1) the human and dog populations are estimated to be 14 million and 650,000 respectively.

Rabies was first recorded in Mozambique in 1908 when it was shown to be enzootic in three main foci, in Tete, Angonia and Quelimane (Zambezia). The first Laboratory confirmation of the disease was performed on pathological specimens from Tete in 1950. The disease occurred in epizootic proportions in the south of the country following an outbreak in jackals along the borders with Zimbabwe and the Transvaal in 1950. The first cases in the northern part of the country were confirmed in the Nampula province in 1965.

Dogs now are the chief vectors of the disease, cats also play a minor role and other animals infected include jackals, cattle, goats, pigs and monkeys. Positive animal rabies cases are notified monthly to the Directorate of Animal Health in Maputo by the Provincial Veterinary Services. Cases reported 1987 - 1991 are summarised in Table 1.

Diagnosis

Diagnosis is carried out at four laboratories, situated in Maputo and the three provincial capitals of Chimoio, Xai-Xai and Nampula. At the Central Veterinary Laboratory (Maputo) the diagnostic techniques used are immunofluorescence. mouse inoculation and Sellers' staining; in the provincial laboratories only Seller's method is used.

Control

Animals entering the country are required to be accompanied by a rabies vaccination certificate. Dog vaccination campaigns are carried out by specialised teams, working mostly in the cities, in communal villages and in cattle-raising areas where large numbers of dogs are used to guard the herds and hunt other animals. Each province has an annual vaccination target based on an estimate of the number of dogs and the desirable minimal vaccination coverage. Vaccines used are of the Flury live virus type. LEP is used in dogs and HEP in cats and cattle. Since 1969 Mozambique has been self-sufficient in vaccine production.

The killing of stray dogs is carried out in some cities by local councils, although their resources are extremely limited. Vaccination of cattle is only employed when it is known that a herd has had contact with rabies, or after identification of a positive animal in the herd.

Post-exposure treatment is given routinely to all humans bitten by wild animals, animals known or suspected to be rabid and animals unavailable for inspection. In the case of bites by domestic animals, the animal is kept under surveillance for 10 days and treatment is commenced immediately if the animal shows signs of rabies.

Human rabies

Despite the efforts to control rabies by vaccination and the culling of stray dogs, human rabies continues to claim the lives of nearly 20 persons each year. As would be expected, the geographical distribution of human rabies shows similarities to the distribution of animal rabies. For example, Niassa province, which is the most under-populated, has the least number of cases, whereas Maputo province which borders on the capital city has the greatest number of cases of both types.

Table 1
Occurrence of Animal and Human Rabies *Cases, 1987 - 1R91*

	1987	1988	1989	1990	1991	Total
Animal rabies cases	13	18	12	5	12	60
Human rabies cases	11	19	25	10	18	73

Of the 60 cases confirmed in animals, 51 were in dogs and 2 in each of cats, cattle, goats and vervet monkeys. one case was confirmed in a donkey. Most of the cases (22) occurred in Maputo region, whilst Nampula (12), Zambezia (7) and Sofala (9) regions consistently reported the presence of rabies. The number of cases reported since 1974 shows a decline, but this is probably the result of a number of factors such as low incidence in urban rabies due to vaccination and to under-reporting due to poor veterinary infrastructure. All of the figures are likely to be an underestimate of the amount of rabies present. The reason why more human than animal cases are reported may be because rabid dogs are killed by people before diagnosis is performed and some of the cases -are the result of biting by-stray dogs. Almost all human and animal cases which were diagnosed came from provincial capitals.

Fig. 1. The Provinces of Mozambique

Rabies in Madagascar

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Although rabies has been eradicated from some islands or the world it continues to be a problem in Madagascar. The human population or Madagascar is approximately 12 million but there are no data available on the dog population. Rabies was present during the last century and the Institut Pasteur de Madagascar was established in 1898 to investigate the disease. Rabies represents a menace to public health, but economic realities have not permitted the use of all available measures to fight the problem. The disease exists primarily in canines and the problem arises from the large number of stray dogs, even in cities, and inadequate dog control.

Human rabies.

Since 1899, 124 human rabies cases have been reported. The majority of these (approximately 70 percent) were diagnosed on clinical symptoms only and there are probably other cases which are not diagnosed. Human cases are noted in all provinces of the country and all have been the result of contamination by dogs. There continues to be a few cases reported each year. The following are the figures for the past three years:

	1989	**1990**	**1991**
Laboratory confirmed dog positives	44	69	55
Human cases	0	5*	3*

* all were the result of dog bites.

The Institut Pasteur de Madagascar runs a surveillance programme according to a convention with the Madagascar Ministry of Health. All activities (diagnosis, treatment etc.) are provided free of charge by the Institut Pasteur.

Human post-exposure treatment

The Institut Pasteur de Madagascar has been responsible for rabies treatment since 1900. It assures the vaccinations for the Tananarive region, the provision of vaccine to the provincial centres and until 1990, the production of vaccine.

Because of the expense of Vero cell vaccine, brain-tissue derived vaccine is still used. Until 1990 home-produced Fermi-type vaccine was used but since 1991 this has been replaced by rabies vaccine prepared in baby mice at the Institut Pasteur de Dakar.

There are 52 Ministry of Health provincial centres of treatment in addition to the principal centre at Tananarive and the vaccine for these centres is distributed free of charge. Consultations at the Institut Pasteur over the past five years are as follows:

	Consultations	Treatments	% Treatments
1987	1745	423	24.2
1988	1674	416	24.8
1989	1983	593	29.9
1990	2515	1097	43.4
1991	2500	1185	47.4

In the past two years there has been a significant increase in the number of consultations - probably due more to better public awareness than to an increase in canine rabies, and in treated individuals - probably due to the availability of a safer vaccine. In 1991, 1331 persons were spared vaccination as a result of suspect dog observation.

Animal rabies.

Between 1959 and 1991, of 2986 animal diagnostic samples received at the Institut Pasteur, 2519 (84 percent) were from dogs. Of these, 1440 (57 percent) were positive and 23 percent of the positives were from vaccinated dogs. Of the total number of positive samples, 91 percent (1440/1573) were in dogs, 7 percent in cats, 3 percent in bovines and the remaining 6 percent were in either lemurs, rats or other species. No rabies virus reservoir amongst lemurs has been found. Of the dogs which died of rabies 85 percent were owned (and 6 percent of these were vaccinated dogs) whilst 15 percent were stray dogs. The number of cases reported is considered to be an underestimation of the situation since surveys outside of Tananarive province have been inconsistent.

Rabies Diagnosis

Specimens from all provinces are sent to the National Rabies Laboratory at the Institut Pasteur. Laboratory tests used routinely are

- rapid diagnosis using the fluorescent antibody test (FAT) on encephalitic specimens
- mouse inoculation test (MIT) performed in baby mice and completed by FAT and
- Histopathological examination of brain from both specimen and inoculated baby mice.

Recent results for 1991 - 1992 are as follows:

	No. of samples	**Positive**	**FAT+**	**MIT+**	**Histo+**
Dogs	148	124	89	123	110
Cats	5	2	1	2	1
Lemurs	5	0	0	0	0
Rats	3	0	0	0	0
Human	2	2	2	2	2

The number of specimens received each year is low, due to difficulties which include the long distances from the laboratory of many of the provincial veterinary posts and the lack of means of transport. Many samples consequently arrive in poor condition.

Rabies Control

In addition to surveillance, rabies control is effected by the regulation of animal importation, efforts to reduce the animal reservoir and the vaccination of domesticated animals.

Imported animals are not quarantined but Health Inspectors demand a certificate of good health and a certificate of vaccination against rabies.

Any animal which is known to have bitten a person is held under observation for 14 days and subjected to three veterinary inspections. Animals which develop rabies are destroyed and specimens are sent to the Institut Pasteur for confirmation of disease. Because of expense, other measures to control dogs are rarely used.

Dog vaccination programmes are not in operation, because of the costs involved. Vaccination may, however, be carried out by private or government veterinarians at the initiative of and cost to the owner. Flury LEP vaccine is used and between 1959 and 1980, 59,000 dogs were thus vaccinated. In 1988, 4,000 doses of vaccine were imported.

In conclusion

Madagascar has to face many public health problems and it is understandable why rabies is not considered as a priority amongst these problems. Rabies surveillance data generated and collected entirely by the Institut Pasteur de Madagascar does not reflect the reality of the rabies problem. These data, which are drawn primarily from the Tananarive region are sure to be an underestimation of the true rabies situation in Madagascar.

Rabies in Zimbabwe

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Zimbabwe is a landlocked country of 390,000 square kilometres. It can be divided into four general land-use types: communal areas with small scale and peasant agriculture, commercial farmland, urban areas and wildlife and forest areas. Zimbabwe has a human population of about 10 million, most of whom live in the communal areas. Since independence in 19BO Zimbabwe has been politically stable. In a census carried out in 1986, there were 1. 3 million dogs, most of which lived in the communal areas. Rabies vaccinations carried out mainly during mass vaccination campaigns achieved an overall 40% coverage at the time of this census. Jackal populations are highest in the commercial farming areas. Two species of jackals occur in Zimbabwe, the black-backed jackal *(Canis mesomelas)* in the southern regions and the side-striped jackal (*Canis adustus*) in the north.

Rabies was present in Zimbabwe around the turn of the century, but was eradicated in 1913 after several years of control by dog destruction. It was re-introduced in 1950 by stray dogs separately from Botswana and South Africa and spread through the country. During the late 1950s and early 1960s it almost died out but after this remained at a fluctuating but generally high level. Canine vaccination was instituted soon after the introduction of rabies; it reached a peak during the mid-1970s and then fell to low levels due to civil disturbances during the late 1970s. Following this, the incidence of rabies reached epidemic proportions for two years. Fig. 1 indicates the relationship between dog vaccination and disease incidence. A further epidemic, resulting in a large number of cases in 1991, may now be dying out (Fig. 2). Table 1 shows species involvement over the past five years.

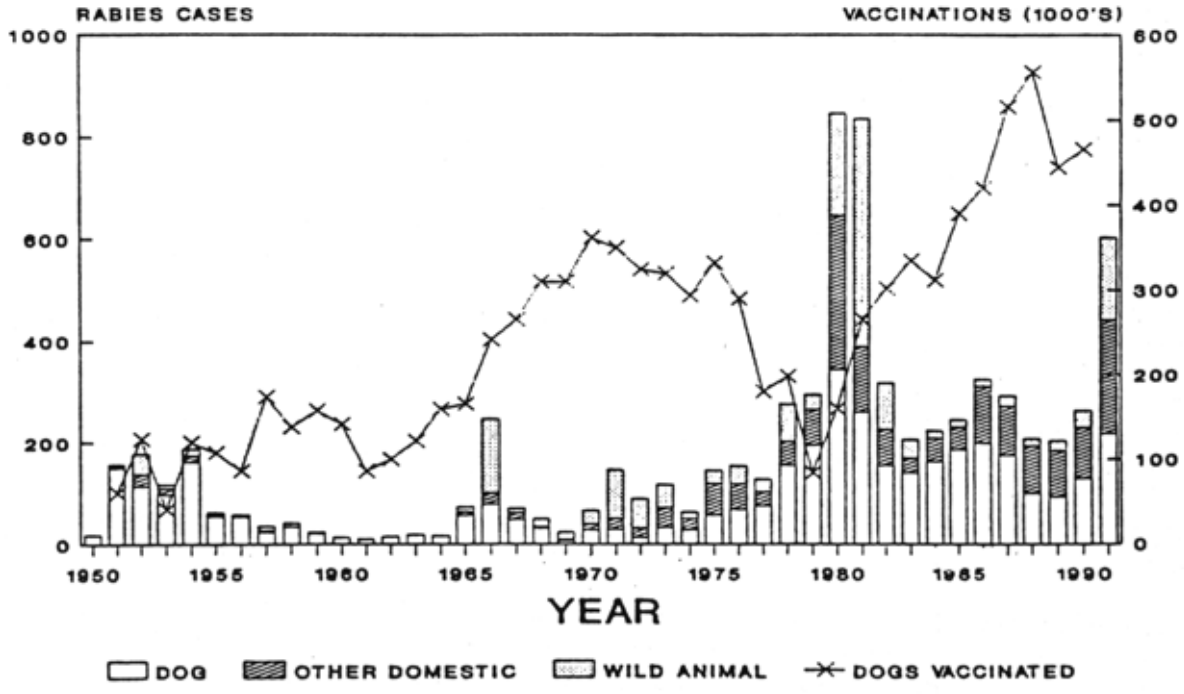

Fig. 1. Rabies Cases and Dog vaccination

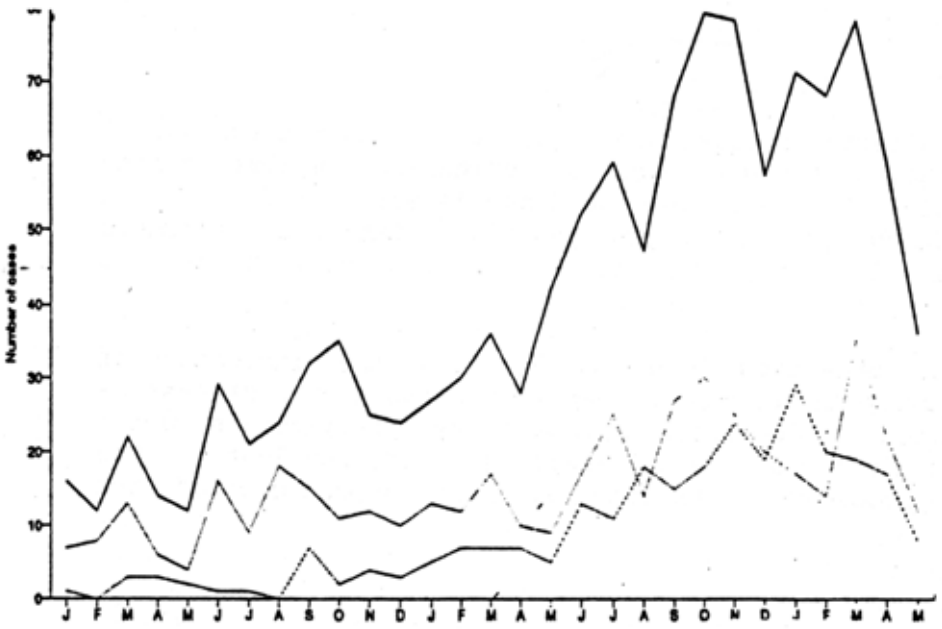

Fig. 2. Rabies Cases by Month, Jan 1990 - May 1992.

Table 1

Summary of Rabies Cases Reported in Zimbabwe, 1987 - 1991.

Species	1987	1988	1989	1990	1991	Total
Human	11	5	6	4	2	28
Dog	174	100	93	129	219	715
Cat	4	7	3	4	12	30
Cattle	68	68	71	so	183	470
Sheep	4		2	4	1	11
Goat	9	6	8	6	is	44
Horse		2	2	1	3	8
Donkey	2	2	5	4	6	19
Pig	1	2		1		4
Total Domestic	262	187	184	229	439	1301
Jackal	16	14	is	27	149	224
Wild dog	3					3
Mongoose					3	3
Civet			1	1	1	3
Aardwolf					1	1
Hyena					1	1
Honey badger	1			4	3	a
Wildcat					1	1
Antbear				1		1
Eland					2	2
Kudu					1	1
Total Wildlife	20	14	19	33	162	248
Total Animals	282	201	203	262	601	1549

The four main affected species are dogs, cattle and the two jackal species. Jackal rabies tends to occur in epidemics, whilst canine rabies is more stable. Bovine rabies incidence is associated to a high degree with incidence of rabies in jackals. Table 2 (Animals Responsible for Human Contact), shows that rabid dogs, cats and jackals all cause significant levels of bite contact.

The series of three maps (Fig. 3 - 5) shows the incidence of disease over the past year. It can be seen that although the disease is widespread, epidemics are localised. Rabies very rarely occurs in the National Parks/Wildlife areas. Jackal rabies is most prevalent in the commercial farming areas while the communal areas support most of the canine cases.

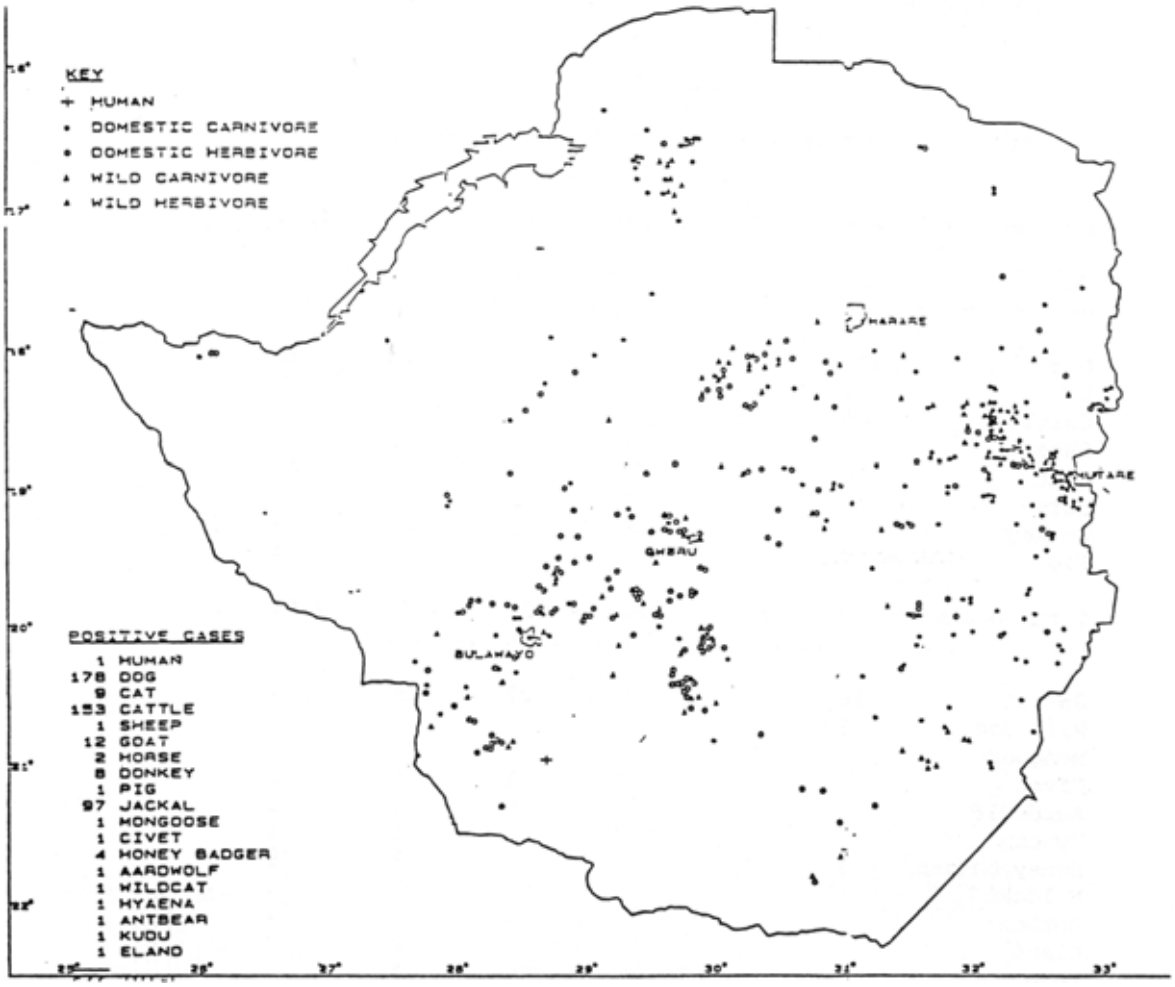

Fig. 3. Rabies Distribution, Zimbabwe. Oct. 1990 - Sept. 1991.

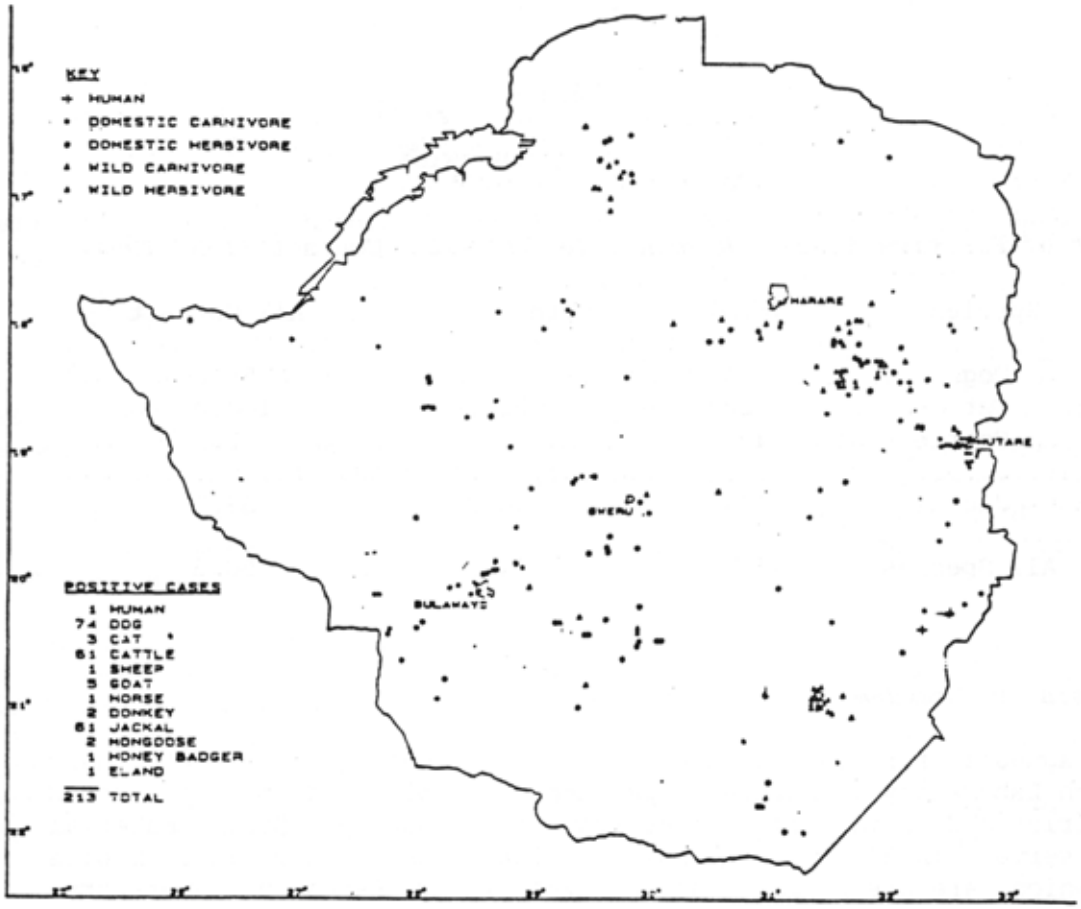

Fig. 4. Rabies Distribution, Zimbabwe. Oct. 1991 - Dec. 1991.

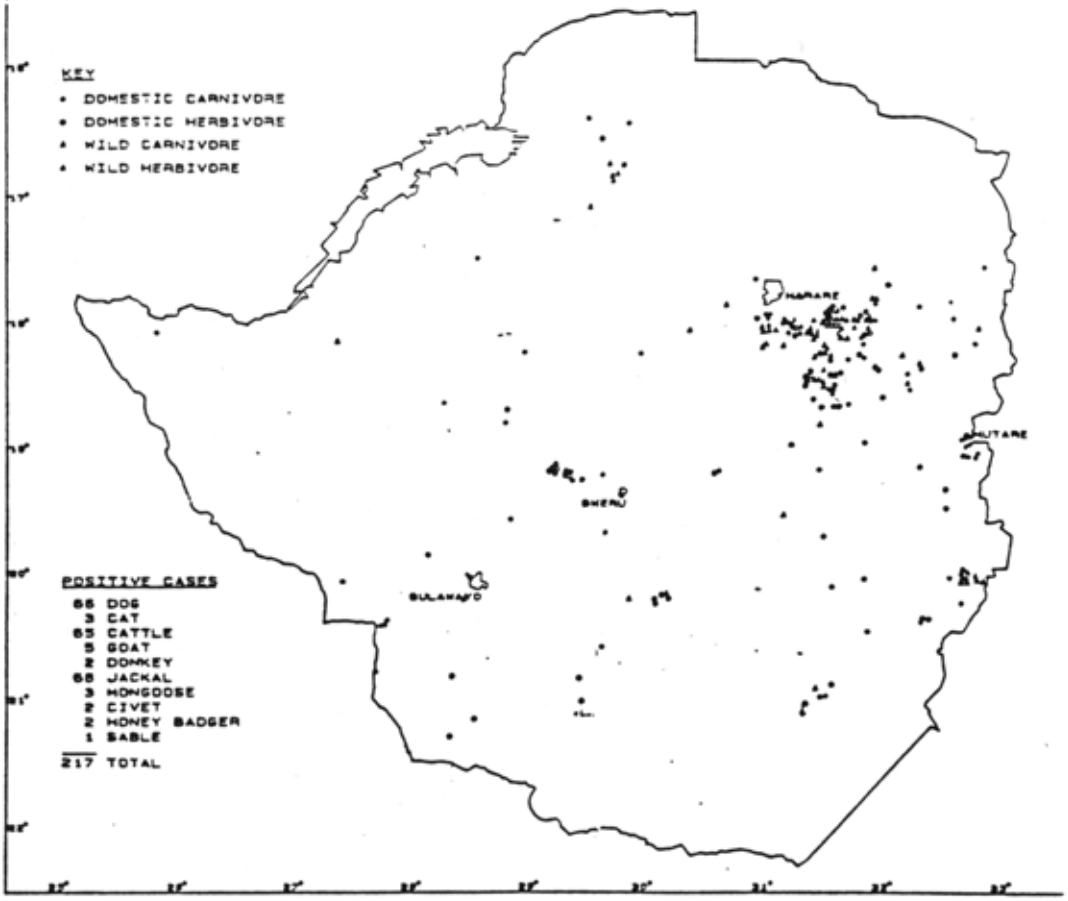

Fig. 5. Rabies Distribution, Zimbabwe. Jan. 1992 – Mar. 1992.

Table 2

Percent of Positive Animals Responsible for Human Contact (1987-1991)

Species	Total	Bite Contact	All Contact
Dog	714	39.6	58.1
Cat	30	93.3	100.0
Cattle	470		42.3
Jackal	224	26.3	39.7
All Species	**1577**	**24.5**	**50.3**

Diagnosis in Zimbabwe

Diagnosis for the country is carried out at the Veterinary Research Laboratory in Harare. Specimens are collected and despatched by district and provincial offices around the country. Brain material is preserved in 50% glycerol saline solution and packed into rabies kits which are pre-prepared at the Veterinary Research Laboratory. Specimens are transported on the railways or by express freight. Transport/communication is generally very reliable with specimens generally being processed within two days of being sent.

The main diagnostic test used is FAT. Mouse inoculation is used as a back-up in FAT negative cases.

The following are the main limitations to improvements of the diagnostic service:

1) Lack of sufficient veterinary coverage in some of the more isolated rural and extensive ranching areas.

2) Reporting of suspect cases is often unreliable. This applies particularly with human rabies cases where usually no attempt is made by hospitals to obtain confirmation and with animal cases where no human contact is involved.

3) Very high turnover of technical staff, which is caused by the national shortage of technologists combined with the considerably better working conditions offered by the private sector.

Rabies in Botswana

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The history of rabies in Botswana is ill-defined, largely due to low public awareness, poor recording and reporting and the use of the less sensitive histological diagnostic test. It is thought that the disease probably entered the country in the latter half of the 1930s. More recently, public awareness has increased and veterinary coverage of the country has improved. These factors have led to an increase in the number of submissions to the Laboratory and with the improvement in diagnostic techniques a more accurate picture of the disease situation is now available.

The number of cases reported from Botswana in humans, domestic animals and wildlife species for the period 1987 - 1991 is recorded in Table 1 and by region in Table 2. An analysis of the species involved is demonstrated in Fig. 1. Three of the six human cases resulted from contact with infected dogs, whilst the others followed exposure to infected wildlife species. The incidence of disease in domestic and wildlife species is summarised in Fig. 2 and Fig. 3 respectively.

All of the 15 veterinary districts submit reports each month and where possible send specimens to the National Veterinary Laboratory for diagnosis. The figures for animal cases in Table 1 refer only to samples which were examined at this Laboratory. The first laboratory test performed is fluorescent antibody (FAT) and specimens which give equivocal or negative results are further tested by mouse inoculation tests in which the mice are observed for 30 days.

In the past five years, 751 of 1359 (55.26 percent) of all cases submitted were positive by FAT. The majority (680 of 751, 90.5 percent) of these cases originated from Veterinary Districts North of the Tropic of Capricorn (Francistown, Maun, Gantsi, Mahalapye, Palapye, Serowe, Orapa and Selebe/Phikwe). More than 24 percent of these cases (170/664) occurred in Francistown alone, whilst a further 14 percent were from Gantsi. In the Francistown district, a fall in domestic animal vaccination in 1990 was followed by a continuous increase in the number of positive cases reported in 1990 and 1991. Indeed, this cyclical pattern of relaxation of vaccination measures followed by an increased number of positive cases has previously been observed in this district.

Throughout the five year period, the Southern Veterinary Region (Mochudi, Gaborone, Lobatse, Molepolole, Kanye, Tsabong and Jwaneng) reported a low incidence (9.5 percent of all cases), of which almost 27 percent (19 of 71) were from Molepolole alone. In this region most of the cases were confirmed in wildlife species.

Recently, an investigation into the antigenic variation which may exist amongst the rabies viruses of domestic animals and wildlife species of Botswana has commenced. The work is in its infancy and results will be the subject of a further paper.

Table 1

Summary of Rabies Cases Reported in Botswana, 1987 - 1511.

Species	1987	1988	1989	1990	1991	Total
Human	3[a]	1[b]			2[c]	6
Dog	21	10	17	25	18	91
Cat	1	1		2		4
Cattle	60	21	35	132	98	346
Sheep	3		1		2	6
Goat	21	15	21	55	40	152
Equine spp.	4		4	6	8	22
Total Domestic	140	47	78	220	166	621
Canis mesomelas	18	5	14	26	24	87
Mustelidae[d]				3	2	5
Felidae[e]	1			4	2	7
Viverridae[f]	3	1	4	1	6	15
Hyaena	2					2
Cape Fox		1				1
Bat Eared Fox		2	1	2		5
Duiker					2	2
Total Wildlife	24	9	19	36	36	124
Total Animals	164	56	97	256	202	745

a origin - cat and two dogs; b origin- unidentified wild animal;
c origin - dog and wild cat; d - Ratel and polecat;
e - caracal and wildcat; f - genet, mongoose and serval.

Fig. 1. Domestic animal, wildlife and human rabies, 1987 - 1991.

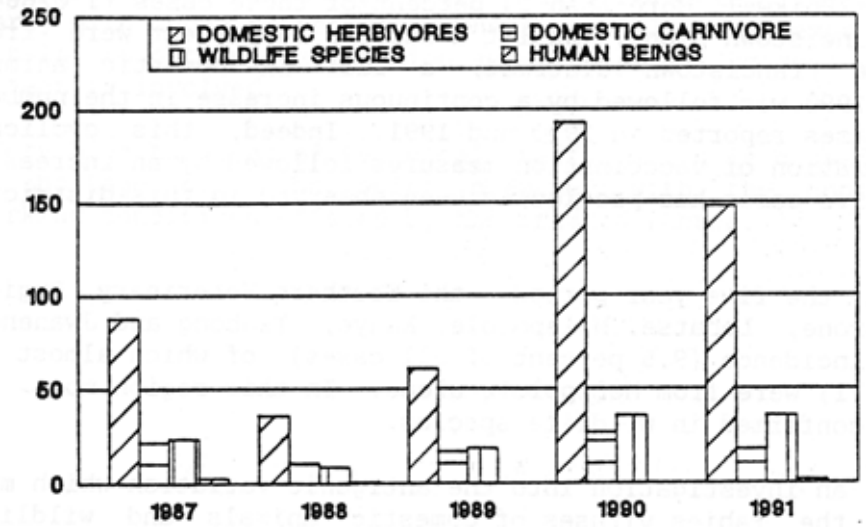
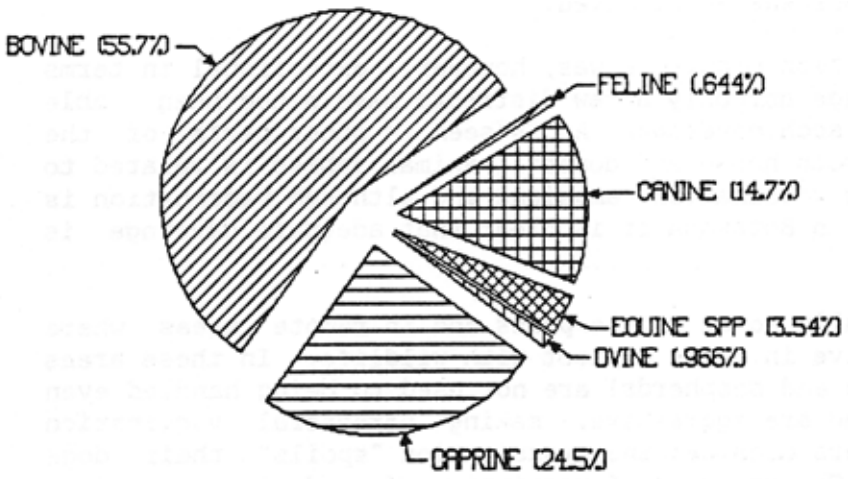


Table 2
Rabies Incidence by Region and District, 1987 - 1991.

	1987	1988	1989	1990	1991	Total
Northern Region						
Francistown	25	17	24	40	64	170
Gantsi	15	7	14	41	18	95
Maumn	27	4	5	33	18	87
Orapa	32	5	1	25	22	85
Palapye			18	34	20	72
Selebi/Phikwe	15	41	9	4	38	70
Serowe	9	5	9	52	10	85
Whalappe	1	4	5	4	2	16
Subtotal	124	46	85	233	192	680
Southern Region						
Gaborone	1					1
Jwanneng	4	1	3	3	1	12
Kanye	3	2	2	1		8
Lobatse	1	1		2		4
Mochudi	1	3	4	1	4	13
Holepolole	2	2		9	6	19
Tsabong	1	3	1	8	1	14
Subtotal	13	11	12	23	12	71
Totals Positive	137	57	97	256	204	751
Submitted	248	177	201	397	341	1359
Percent Positive	55.2	32.2	48.4	65.3	59.8	55.26

Fig. 2 Rabies incidence in domestic species, 1987 - 1991.



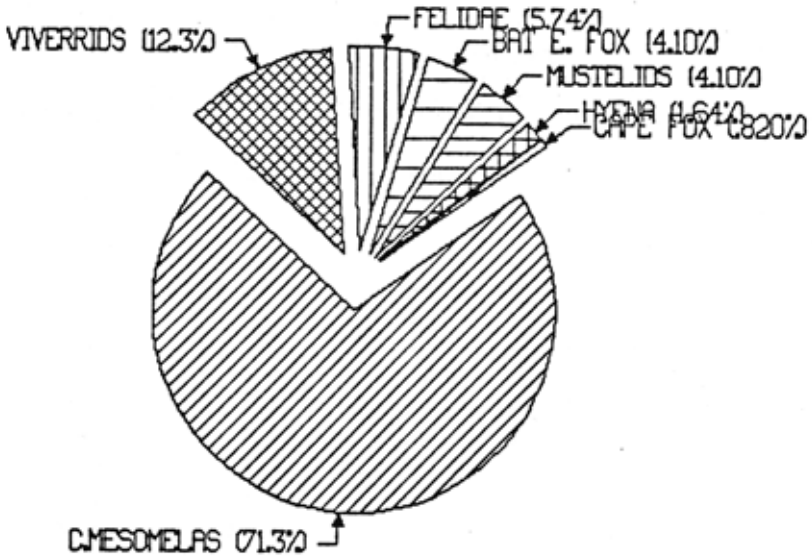


Fig. 3. Rabies Incidence in wildlife species 1987 - 1991.

Control

Rabies surveillance and control strategies have changed little since the disease was first observed in the country. Control measures have been centred upon mass immunisation, local quarantine, culling of stray dogs and jackals and campaigns of public awareness.

For example, in an attempt to reduce the weight of rabies infection in 1987, a domestic animal mass vaccination programme together with a campaign to reduce the wild carnivore population density below the threshold essential for the maintenance of rabies by (poison) baiting was mounted. Success of the programme can be measured by the reduction in the number of cases in 1988 (Table 1), although other factors may also have been brought into play. In any event the decline in case numbers was short-lived.

The 1987 vaccination programme was, however, exceptional in terms of countrywide coverage and only a few districts have since been able to attain or exceed such coverage. As is seen in other parts of the world, reduction of both human and domestic animal rabies is related to the efficiency of dog vaccination campaigns and although *vaccination is* free and compulsory in Botswana it is clear that adequate coverage is not achieved.

This is especially so at cattle posts and in remote areas where dogs and livestock live in close contact with wildlife. In these areas working dogs (hunters and shepherds) are not used to being handled even by their owners and are aggressive, making parenteral vaccination difficult. Some owners consider that vaccination "spoils" their dogs for hunting vigour. The practice of vaccinating dogs during campaigns of *vaccination against* other diseases (e.g. Anthrax), does not guarantee adequate coverage since dogs which do not accompany their owners to the crushes and dogs whose owners do not have other animals are not vaccinated.

Some culling of stray dogs occurs when a tie-up order is applied to domestic dogs and strays (and stray cats) are shot. In addition, some reduction in jackal numbers is achieved when poisoned meat is laid as knit, but this environmentally unpleasant method does not receive popular support.

In the light of the apparent increase in rabies in the past two years, I would suggest that a three-pronged approach is required to bring rabies under control.

a) A centrally co-ordinated, constantly monitored, countrywide campaign of domestic animal vaccination. During the campaign the imposition of movement restrictions and destruction of stray dogs would help to reduce the incidence of disease. The introduction of dog identification and registration in the rural communities may dissuade people from owning large packs of dogs. Identification and registration would be prerequisites for vaccination, and unregistered dogs could be impounded or destroyed.

b) The disease in wildlife should be assessed by surveillance. It may be that, as in neighbouring South Africa and Zimbabwe, the jackal is now a major wildlife vector of disease in Botswana.

c) Oral vaccination of wildlife is now a feasible proposition and work is proceeding in neighbouring countries in the assessment of baiting strategies and vaccines safe for both target and non-target species. If we are to control wildlife rabies in Botswana we should take part in these international collaborations.

Rabies in Namibia

Klaus Depner

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Namibia is a country of 823,168 km^2 with a human population approaching one and a half million and a dog population estimated to be about 80,000. The infrastructure of the country can be regarded as good.

Rabies was first recorded here in 1887. Losses amongst cattle and small stock were experienced and were attributed to the spread of rabies by rabid dogs. Until the mid-1950s rabies occurred only in the northern districts, with domestic dogs as the main victims. In this densely populated district with its large dog population the disease continues to represent a major public health hazard. In 1951 the first rabies cases in cattle in the central districts of the country were diagnosed and in 1952 the first positive diagnosis in a wild cat in the southern district was recorded *(Schneider 1977; Schneider and Hassel, 1983).*

From 1977 until 1982 an independent cycle of rabies occurred in the kudu antelope. Following initial transmission from jackals, more than 50,000 kudu died. A non-bite transmucosal transmission from kudu to kudu has been discussed. Since 1983, the epizootic has spontaneously regressed *(Barnard et al., 1982; Bassel, 1982).*

In the "post kudu rabies period" the epizootiological situation has remained more or less unchanged: dogs (urban rabies) are the main vectors in the northern districts where dogs and humans are the main victims, with jackals (wildlife rabies) in the central districts where heavy losses amongst cattle occur *(Director of Veterinary Services, Annual Reports 1983-1991).*

Epidemiology 1987 - 1991.

Of 698 laboratory confirmed cases during 1987 - 1991, 47 percent occurred in cattle, 22 percent in dogs and 13 percent in jackals (Table 1). The geographical distribution is characterised by the high incidence of dog rabies (115 of 142 positive cases) in the north of Namibia, the high incidence of cattle rabies (309 of 529 positive cases) in the central districts whilst in the southern districts only 27 positive cases of rabies have been recorded during the past five years (Fig. 1, Fig. 2 and Table 2). A total of 488 humans had contact with rabid animals.

For these statistics, only laboratory confirmed cases have been considered. However, it often happens that on a farm or in a village more than one animal is dying showing symptoms of rabies but brain material from only one or two cases is sent in for confirmation. In this connection I want to mention a case of rabies in a herd of sheep where 41 animals died. Since brain material from only three of these animals were sent in, all of which were positive, it must be assumed that the number of positive cases is higher than is recorded above.

Diagnosis

Rabies diagnosis for Namibia is carried out in the Virology unit of the Central Veterinary Laboratory, Windhoek. Because of the countrys' good infrastructure samples usually arrive in good condition. The immunofluorescence (IF) test is performed using FITC conjugate obtained from the Onderstepoort Veterinary Research Institute. Mouse inoculation tests are carried out only in human exposure cases in which the IF test result is doubtful. For antibody determination the rapid fluorescent focus inhibition test technique has been adopted.

Control

Vaccination of dogs is compulsory and is free of charge. Since 1983 RABISIN (Rhone Merieux) inactivated vaccine has been used. No attempt to vaccinate wildlife or stray dogs via the oral route has been made.

Table 1

Summary of Rabies Cases Reported in Namibia, 1987 - 1991.

Species	**1987**	**1988**	**1989**	**1990**	**1991**	**Total**
Human						
Dog	34	30	47	20	21	152
Cat	5	5	8	2	3	23
Cattle	69	73	80	59	45	326
Sheep	3	6	4	4	3	20
Goat	3	8	11	5	3	30
Horse/Donkey	1	1	4			6
Pig	1		1			2
Total Domestic	116	123	155	90	75	559
Jackal (BIB)	16	33	13	10	20	92
Jackal (silver)			1			1
Bat-eared Fox	6	7	1	3	7	24
Wild dog				1		1
Wild cat		1	1	1		3
Genet		1				1
Rooikat			2	1		3
Honey Badger	1	1		1		3
Suricate	1		1	1		3
Eland		1				1
Kudu	1		2	1		4
Red Hartebeest			1			1
Cheetah					1	1
Lion	1					1
Total Wildlife	26	44	22	19	28	139
Total Animals	142	167	177	109	103	698

Table 2
Geographical Distribution of Confirmed Rabies Cases 1987 - 1991.

	Geographical Districts			Total Cases
Species	North	Central	South	
Bovine	16	309	1	326
District Percent	5.0	94.7	0.3	
Dog	115	33	4	152
District Percent	76.0	22.0	2.0	
Jackal (B/B)	0	89	3	92
District Percent	0.0	96.0	4.0	
Other species	11	98	19	128
District Percent	9.0	76.0	15.0	
Total Cases	142	529	27	698
District Percent	20.0	76.0	4.0	

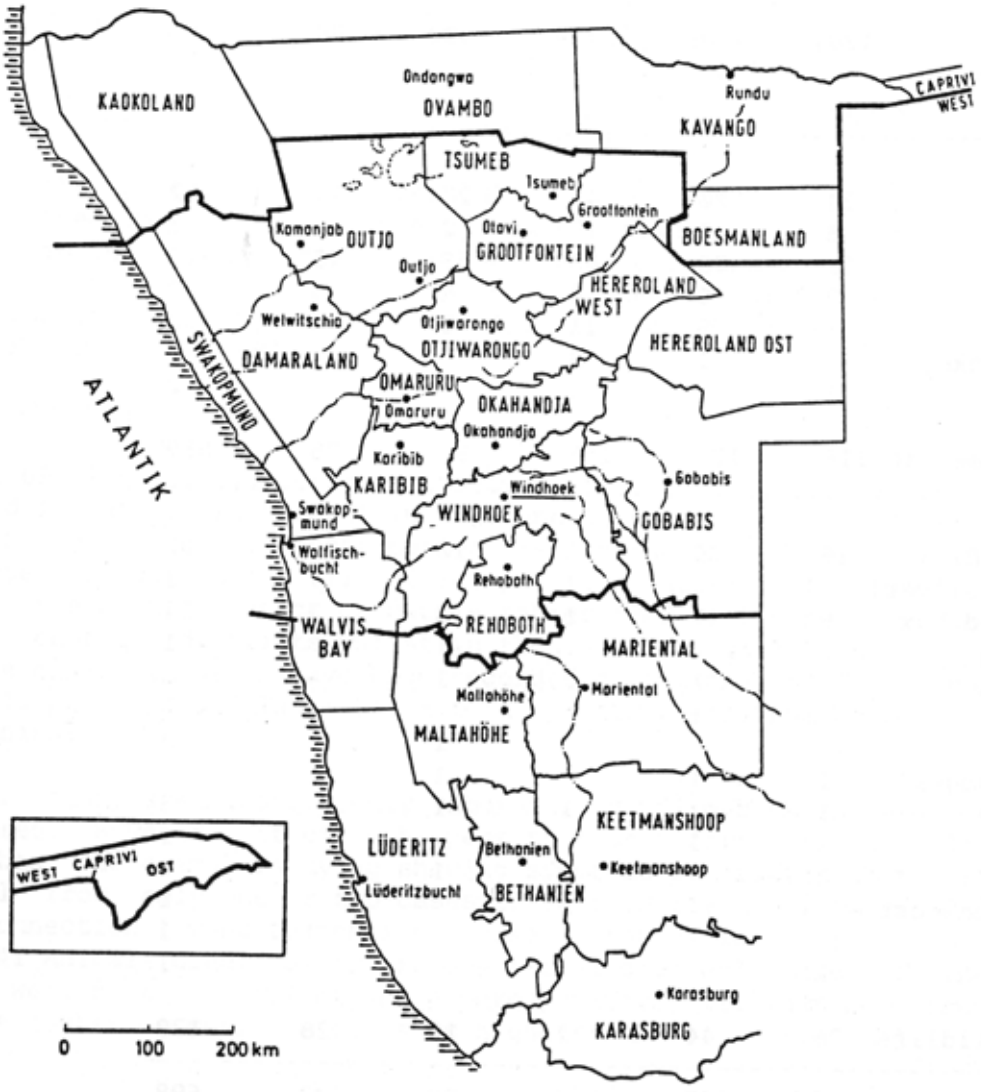

Fig. 1. Districts of Namibia

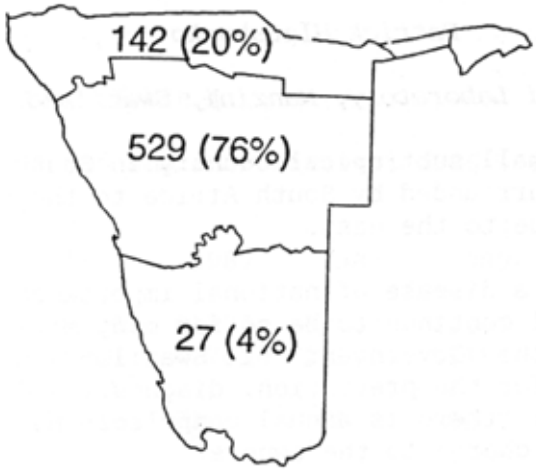

Fig. 3. Distribution of Laboratory confirmed cases 1987 -1991.

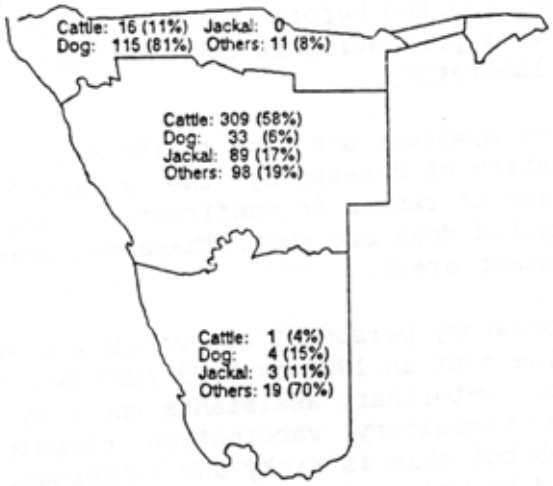

Fig. 3. Laboratory confirmed cases by species, 1987 - 1991.

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Rabies in Swaziland

Patrick Hlatshwako

Manzini Laboratory, Manzini, Swaziland.

Swaziland is a small subtropical country in South East Africa. It is landlocked and surrounded by South Africa to the north, west and south and by Mozambique to the east.

Rabies has been a disease of national importance for many years and no doubt it will continue to be so for many more years to come. Since independence the Government of Swaziland has utilised all resources available for the prevention, diagnosis and control of the disease. For example, there is annual compulsory mass vaccination of dogs which is free of charge to the owners.

Over the past five years the kingdom of Swaziland has recorded a few sporadic cases of rabies in the dog population (Table 1). All the recorded cases were diagnosed in dogs that were not vaccinated during the annual compulsory mass vaccination. Several of these cases had human contact and all those who came into contact were given medical treatment. Treatment was started before laboratory confirmation of disease in the dog. This precaution is taken in case of a false negative finding in the laboratory.

All dogs with rabies symptoms are sent to the Central Veterinary Laboratory for confirmation of disease by the indirect fluorescent antibody test. Once a case of rabies is confirmed, a dog tie-up order is issued and stray or untied dogs are shot. These measures are carried out in affected or in-contact areas.

In Swaziland all laboratory personnel are vaccinated annually with diploid cell vaccine. The cost in 1992 is R103 (US$ 50.00) per dose. Because of this high cost, veterinary assistants who play a major role during the annual mass compulsory vaccination campaign are not vaccinated against rabies but this is risky and dangerous. A cheaper human rabies vaccine would be welcomed, alternatively sponsorship for a human vaccination programme would be welcomed.

The annual compulsory mass vaccination of dogs against rabies is carried out by veterinary assistants under close supervision of a veterinarian. The media is widely used to inform the public of the vaccination centres, dates and times. All dog owners who take their dogs to vaccination centres are issued with government certificates as proof that their dogs have been vaccinated.

In centres where the number of dogs taken for vaccination is low, a second centre is arranged. One reason given by dog owners for non-attendance at the centre is that once dogs are vaccinated their hunting ability is reduced. Some dog owners have said that once pregnant bitches are vaccinated they abort or give birth to weak puppies that die after a few weeks.

Although annual compulsory vaccination campaigns cost the Swazi tax payers thousands of dollars, the number of rabies cases diagnosed each year has been reduced and the disease is less of a problem than in the past.

Table 1

Annual Dog Vaccination and Rabies Positives, 1986 - 1990.

	1986	1987	1988	1989	1990
Dog Population	66226	81137	57462	62327	66197
Dogs Vaccinated	46950	43761	22241	55154	44889
Percentage Vaccinated	70.9	71.57	38.7	88.5	67.8
Confirmed Rabid	**9**	**1**	**2**	**4**	**1**

Rabies in Lesotho

Lebohang Khomari

Department of Livestock Services, P.B. A 82, Maseru, Lesotho.

Lesotho is a mountainous country with a geographical area of 335,000 km^2 of which only 12 percent is arable. The human population is 1.6 million and the estimated dog population is 200,000.

Rabies was first diagnosed in Lesotho in 1982, after which the disease rapidly spread throughout the country and was reported in humans and a variety of domestic animal species. In 1987 the incidence reached a level which was also of concern to neighbouring countries and the government of the Republic of South Africa agreed to assist Lesotho with a rabies control policy.

A free rabies vaccination campaign for dogs was launched in 1988 and a coverage of 70 percent was attained. Annual vaccination, which is now in its 4th. year, has drastically reduced the incidence of disease despite a decline in the percentage of coverage due to allegations by dog owners that after vaccination they experienced losses from dog mortality.

The true picture of the status of rabies has always been very difficult to ascertain because of the poor reporting system and the attitude of the people to dogs. Most suspect rabid dogs are killed and disposed of before the veterinary authorities are informed. In the rural areas, which are the areas where most human and dog rabies cases occur, unrestricted dogs are common, leading to a high risk of rabies to the human population (Table 1).

Table 1

Summary of Rabies Reported in Lesotho, 1987 - 1991.

Species	1987	1988	1989	1990	1991	Total
Humam						
P-Ex. Treated	537	31	10	27		605
Death	16		1			17
Domestic Animals						
Dog	1	5	4	3	2	15
Cat				1		1
Cattle		2	1	2		5
Sheep		1				1
Goat				1		1
Equine	1					1
Total Animals	2	8	5	7	2	24

Epidemiology

Most rabies cases have involved dogs but the disease has occurred in- sheep, goats, donkeys, cattle and cats. In Lesotho there are unrestricted dogs in rural areas and semi-restricted dogs in urban areas. The occurrence of rabies in other domestic animals has led to speculation that in the absence of known dog bites, meerkats may be involved. only one meerkat, however, has been diagnosed as rabies positive.

Diagnosis

Until 1989 all brain samples mere despatched to Onderstepoort Research Institute for confirmation of disease. For one year following the purchase of our fluorescence microscope, a duplicate of each diagnostic sample was sent to Onderstepoort as a quality control.

Control

The rabies control policy of Lesotho includes quarantine, restriction of animal movement and vaccination campaigns. Dogs entering the country without valid vaccination certificates are vaccinated and kept in quarantine for 30 days; suspect dogs are also quarantined whilst under observation. A valid vaccination certificate is a prerequisite for animals which are to be exported.

The annual vaccination campaigns are nation-wide and are free of charge to the dog owner. In addition, individuals bringing their dogs to the veterinary clinics during normal working hours may have their dogs vaccinated at their own expense.

Factors which affect diagnosis and control

There is no nation-wide system of sample collection, preparation and transportation to the laboratory for diagnosis. There are not sufficient quarantine premises for dogs to be kept under observation. Although rabies is a notifiable disease, public awareness is low and suspect dogs are still being killed and disposed of without proper reporting.

In the past there was a shortage of qualified personnel and experience in vaccination. Recently there has been an improvement in the quality of vaccines and cold chain facilities.

The country is mountainous and has many rivers. In the winters, which are very cold, with snow in the highlands and during summer when the rain swells the rivers and may make roads impassable, transport is difficult and owners are reluctant to bring their dogs to centres for vaccination.

Many owners in Lesotho do not value their dogs, they would rather let them die than sacrifice a little time and money on vaccination. It is, however, normal practice for shepherds to have more than four dogs and for these people vaccination would be expensive.

Lesotho is not a rich country and therefore vital infrastructure such as transport, qualified manpower and special diagnostic facilities are at a low level.

Rabies in South Africa

George Bishop

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Time does not allow me to present comprehensive figures and data for the whole of South Africa, which comprises four main provinces (Fig. 1) and some independent States. Dr. Thomson will later present a paper on non-canine vectors and this will cover the three other provinces of the Cape, Transvaal and Orange Free State.

Natal is currently undergoing a rabies problem and during the period 1987-1991, 1062 (86.2 percent) of the 1232 canine rabies cases of this country were reported from Natal and Kwazulu alone (Fig. 2 and Tables 1 and 2). Data gathered over the years 1974 - 1989 shows that canine rabies in Natal accounted for some 25 percent of the national cases but during the period 1987 - 1991 this figure has risen to 40 percent. Two large outbreaks (in 1980 and 1984) were linked fairly closely to the process of urbanisation and a shift of many people to the larger cities because of drought. Figures (not shown) collected during the 1980s reveal that vaccination was able to suppress these two outbreaks but important social and political factors have complicated matters very considerably and the peaks in 1987 and subsequent years have chiefly been due to their influences.

The central government's Directorate of Animal Health and the Veterinary Department of Kwazulu are responsible for veterinary matters in different areas in the geographical region of Natal. Rabies does not respect political boundaries and the responsibilities and functions of the two administrative bodies have been severely curtailed and hampered by apathy, ignorance, violence, poverty, lack of education, squatting and the resistance (and often antagonism) to vaccination. Few of the people in rabies infected areas appreciate the importance of the disease and the 20 human rabies cases which were reported during 1991 were not perceived to be a real problem in comparison with deaths due to other causes such as violence and motor accidents; the needs (education, housing and finance) of the lower income groups are of a more immediate nature.

Diagnosis

There are two rabies diagnostic facilities, one at Allerton RVL in Natal and the other at the Veterinary Research Institute, Onderstepoort. Most of the rabies specimens are submitted by veterinarians (both private practitioners and state veterinarians) or their staff, animal technicians etc. Many samples are also submitted direct to the laboratory by owners or by medical practitioners. We make concerted efforts to ensure that parcels containing suspect rabies material are securely and safely packed but these efforts are not always rewarded.

At both Allerton and Onderstepoort routine diagnosis is by the fluorescent antibody test using mercury vapour and incident light illumination. In Natal, most of the specimens arrive within 24 hours of despatch and the FA results are phoned through within six hours of receipt.

Mouse inoculation and histopathology are used occasionally, particularly when a negative result has been obtained from a highly suspect case involving human contacts. Suspect vaccine breakdowns or the diagnosis of the disease in a species in which it has been infrequently reported also often lead to more intensive investigation.

At Allerton we have progressed quite far in the examination of formalin-fixed material using FA microscopy and the avidin-biotin techniques. Usually, half-brain specimens in 10 percent formalin and 50 percent glycerol-saline are submitted but entire heads or whole carcasses are sometimes received.

Table 1

Rabies Cases Reported in Natal and Kwazulu, 1987 -1991.

Species	1987	1988	1989	1990	1991	Total
Human	16	24	9	11	20	80
Dog	232	154	127	240	309	1062
Cat	6	7	1	3	4	21
Cattle	11	15	7	20	13	66
Sheep	1				2	3
Goat	3	1		4	3	11
Horse	1			1	2	4
Total Domestic	254	177	135	268	333	1167
Jackal			1			1
Meerkat	1					1
Mongoose	1	1			2	4
Genet		1				1
Bat				1		1
Total Wildlife	2	2	1	1	2	8
Total Animals	256	179	136	269	335	1175

Serological testing is carried out at Onderstepoort where use is made of a blocking ELISA system which has a multi-species capability. Onderstepoort and the National Institute for Virology in Johannesburg are always very willing to assist with more detailed investigations.

The problem of specimen transport in South Africa is a very real one because the distances are great and the summer temperatures, in many parts, very high. There has been an encouraging move towards shipping the specimens by road (courier) transport as this is usually much quicker (but more expensive) than postal services or rail. The area serviced by Allerton (mainly Natal/Kwazulu) is much smaller than that serviced by Onderstepoort (rest of South Africa) so that we have fewer problems in this regard than does Onderstepoort.

Table 2

Rabies Cases Reported in South Africa and the Transkei, 1987 - 1991.

Species	1987	1988	1989	1990	1991	Total
Human	2	4	1			7
Dog	275	203	170	264	320	1232
Cat	17	16	12	12	11	68
Cattle	114	82	69	87	66	418
Sheep	6	5	8	4	5	28
Goat	4	3	7	5	7	26
Horse	4	1	1	7	2	15
Pig	2	1		1	2	6
Total Domestic	422	311	267	380	413	1793
Canis	25	10	12	6	6	59
Cynictis	127	68	63	84	60	402
Otocyon	21	7	14	16	12	70
Felis sp.	12	1	4	11	3	31
Genetta	2	4	2	2	1	11
Suricata	10	4	8	2	3	27
Herpestes sp.	9	7	4	8	4	32
Vulpes	3		1	1	1	6
Xerus	1	1	1	2		5
Mellivora	1	3				4
Game	4	5	5	3	3	20
Procavia		1			1	2
Total Wildlife	215	111	114	135	94	669
Total Animals	639	418	381	515	507	2462

Other problems, which are frustrating at times, are the frequent lack of attention to comprehensive histories (vaccination, symptoms, human contacts etc), although our "Suspected Outbreak of Controlled Disease" forms draw attention to the importance of this history. In general, however, our diagnostic capabilities and processing of specimens meets a fairly high standard.

Control

Control of rabies is based almost entirely on dog vaccination. Control of the dog population and elimination of strays has not been applied and probably will not be in the immediate future. Vaccine is given free of charge, usually at strategically based clinics. Over the period 1987 - 1991, an annual average of 288,504 vaccinations were carried out in Natal and Kwazulu. The authorities cannot effectively enforce vaccination, however, and attempts to do so would probably be counter-productive at present. As a result, vaccination in Natal and Kwazulu probably does not exceed 40 percent of the dogs at risk and the disease, in terms of reported cases, is running away from us.

Rabies in Natal appears to be concentrated around the high population density areas such as Durban and the coastal areas to the north and south. Approximately 60 percent of the suspect rabies specimens are submitted by private practitioners and this may influence our plotting of the cases since practitioners do tend to be situated in the more highly populated areas. It is quite likely that there have been many more rabies cases in the rural areas than represented on the map but it is quite understandable that submission of suspect material from outlying areas is more difficult. For many years and for obscure reasons the number of canine cases has peaked in the month of August -it may be that breeding cycles and food scarcity play important roles in this phenomenon.

Fig. 1. The Provinces of South Africa.

Fig. 2. Rabies cases in Natal and Kwazulu, January to December, 1991.

The presentations by representatives of the regional countries in each of two sessions were followed by discussions, herewith summarised by the session Rapporteur. The issues arising from the Section on National Reports were then summarised by Dr. Rweyemamu:

Session Discussion, summarised by Dr. Roberts.

Dr. Illango (Uganda)

- What are the relationships between the Veterinary and Medical authorities in Uganda? The Veterinary and Medical authorities have a joint-committee which decides rabies policy and strategy.

- What are the figures for human rabies in Uganda? The number of human rabies cases in Uganda is not known by me.

- How many samples are submitted for diagnosis? We examined 41 in 1991.

Dra. Dias (Mozambique)

You indicated that only 20 percent of persons bitten were treated, how is selection made? Decisions are made according to the WHO recommendations, if the biting dog survives, treatment is not given.

- Of the people who died, how many were treated? Only one person died and that person was treated.

- What vaccine is used in humans? Vero cell vaccine.

Dr Morvan (Madagascar)

- How many of the 6 percent vaccinated dogs went down with rabies? 23 percent of the 6 percent developed rabies as indicated by laboratory tests.

Dr. Bingham (Zimbabwe)

- Human cases have decreased whereas cases in wildlife have increased, can you explain? No, I would have expected more human cases.

- Can you explain the 2 percent failure rate in diagnosis? The reason for failure is probably "reader" failure.

- Why has the incidence in jackals decreased? Jackal density is now below threshold level, causing the epidemic to subside.

- Is rabies regulating the jackal population? In some areas.

- Is there any attempt to control the jackal population? Yes, by poisoning, but it is probably not effective.

Dr. Masupu (Botswana)

- How do you explain the small percentage of positive dogs against the large wildlife population? It is due to vaccination coverage.

- There is a high incidence of bovine rabies, why? It is the result of bites from both wild and domestic carnivorous species.

- Could you provide details of post-exposure treatment? This is handled by the medical people, all persons bitten or those handling rabid animals are treated.

- Could you elaborate on the laboratory tests used? We use FAT and then the biological test when results are equivocal or negative.

Session Discussion summarised by Dr. Thomson.

Dr. Depner (Namibia)

- You have said that dogs are mainly affected in the northern region and dogs and jackals in the central region, do you know the history of cattle cases? Submission forms have a space allocated for the history of the case which is used to establish the background to all cases. There is often a report of a "friendly" jackal approaching a house and these animals are presumed to be the source of infection in many cases - but not all. It is my opinion that jackals are largely responsible for transmission to cattle.

- You say that the FAT and Histopathology are used for diagnosis, how sensitive is histopathology? I am not in a position to answer that question at the moment.

Dr. Matschwako (Swaziland) There were no questions

Dr. Khomari (Lesotho)

- What kind of vaccine is used in Lesotho? Inactivated vaccine (Rabisin), although formerly the Onderstepoort attenuated vaccine (Flury HEP) was used.

Dr. Bishop (South Africa) There were no questions

Dr. Sinyangwe (Zambia)

- How do you vaccinate? At a central place where owners take their dogs.

- What records are kept? The owner is issued with a certificate.

- How do you explain that in Lusaka, which has a population of about one million, only 10,000 dogs were vaccinated in 1989, bearing in mind that a dog/human ratio of about 1 : 10 usually obtains? The fact that private veterinarians immunise dogs makes this question difficult to answer.

Dr. Rweyemamu:

We can now summarise the issues arising from all of the country reports as follows:

1. Although rabies is a panzootic disease the primary level of control needs to be executed by the veterinary authorities. Best national data seem to be generated when also veterinary authorities assume veterinary responsibility for laboratory diagnosis.

2. Close collaboration with medical authorities is essential.

3. Specific emphasis needs to be developed to assess ecological significance of rabies in wildlife and wildlife/dog/domestic animal contact.

4. Analysis of species factors in rabies is required.

5. Surveillance and specific identification of rabies ecosystems do not seem to have been intense in most countries.

6. Attention to clinical, pathological or laboratory variations in reported cases of rabies is required.

7. Questions raised on the safety of vaccines used - especially live vaccines. Is guidance required on selection of appropriate vaccine?

8. Novel systems need to be devised for control of rabies in stray dogs and wild mammals.

9. Specific guidelines are needed on affordable cost-effective systems for protecting veterinary workers including field veterinary assistants.

10. How should national and regional diagnosis be organised? Is there now justification for a Regional Rabies Reference Laboratory?

International In-put

Under the heading of International Input three papers were given, by Dr. Wojciechowski (FAO), Dr. Meslin (WHO) and Dr. Aubert (OIE). These papers have been abstracted for their relevance to rabies and to southern Africa by the session Rapporteur, Dr. Perry, but because of their importance to the international community they have been reproduced in full (with permission) in the Appendices to these Proceedings.

Abstract of the paper given by Dr. Wojciechowski (FAO).

The mandate of FAO is to improve food security and it provides technical information, advice and assistance in order to achieve this. FAO priorities include sustainable development, genetic resource conservation and …… in development. For 1990/1991, FAO managed a budget of US$ 641 million for its' central programmes. In addition there were field programmes funded by donors to the extent of US$ 300 million. FAO has six departments and veterinary activities lie in the Animal Production and Health Division. There is a network of 21 reference laboratories, covering emergency diseases. Rabies is not yet included. FAO is also concerned with standardisation of reagents for disease diagnosis and control. It promotes biotechnology and the field testing of vaccines including those using recombinant antigens.

The *Pan-African Vaccine* Centre (PANVAC) was established in 1986 in Debre-Zeit, Ethiopia, with a second laboratory in Dakar, Senegal. The PANVAC will support training and the development of new diagnostic tests for those diseases affecting animal production. The PANVAC is also expected to upgrade rabies vaccine production and quality control within the region and on the international scale.

Since 1985 the FAO has been working on the concept of the Pan African Institute for Animal Health (PANHEALTH). During three Project Formulation Missions (1985, 1989 and 1990) many concepts have been developed including a) a Reference Diagnostic Centre for Africa (Kenya), b) an International Centre for Veterinary Epidemiology and Diagnostics (Zimbabwe) and c) the PANHEALTH with the core centre in Eastern Africa, two sub-regional centres and network support to forty national diagnostic laboratories.

The Pan African Rinderpest Campaign (PARC) covers 34 countries and has a network of 27 national laboratories, initially focused on rinderpest vaccine quality control. It now also works with CBPP vaccine and will expand to Newcastle Disease and rabies vaccines. it will expand to the Middle-east and South East Asia, to become a co-ordinated global programme as financial resources allow.

The FAO project in Zambia "Establishment of a Virology Laboratory at the Central Veterinary Research Institute, Balmoral" - operating near Lusaka, has potential to assist the Government of Zambia in establishing the Rabies Reference Laboratory for Zambia and its further activities for southern Africa.

Abstract of the paper given by Dr. Meslin (WHO)

WHO is involved with rabies surveillance, planning of rabies control programmes, rabies research and public education in rabies.

Co-ordinated research has been on:

1. Oral vaccination against rabies. This has included expert consultations on bait and vaccines under development, both for wildlife and for dogs. Some small projects on these subjects have been funded by WHO, for example, in Tunisia.

2. Dog population studies. These have been conducted in collaboration with Sri Lanka, Ecuador, Nepal, Tunisia and now, Zambia.

3. Vaccine potency testing. WHO is seeking in vitro tests to replace the NIH test by collaborative studies with rabies Institutes.

4. Post-exposure immunisation in humans.

5. Rabies diagnosis and surveillance. This includes a collaborative panel of laboratories and the annual rabies questionnaire distributed world-wide.

6. Planning and co-ordination of national programmes.

7. Technology transfer. WHO supports consideration to the establishment of vaccine production capacity in a country.

8. Public education. WHO has produced a film on rabies control and guidelines for the involvement and education of the public.

Dr. Meslin also reported on the WHO Expert Committee Report on Rabies. Intradermal multi-site post-exposure treatment regimens have been revised, reducing vaccine required to 0.8 ml. Dog ecology studies indicated the high susceptibility of dogs to vaccination. Also, dog elimination was shown to be ineffective as a control programme. Furthermore, the consideration of using serology as a replacement for quarantine was discussed and plans for collaborative studies established.

Abstract of the paper given by Dr. Aubert (OIE).

The Office International des Epizooties (OIE) was founded in Paris in 1924 and today this world animal health organisation has 116 member countries including 41 in Africa. It is funded by compulsory contributions from member countries and the minimum annual rate for each country (in 1991) is US$ 8.000.

The OIE has three main functions:

a) to promote and co-ordinate experimental or other research work concerning the pathology or prophylaxis of contagious diseases of livestock for which international collaboration is deemed desirable,

b) to collect and bring to the attention of governments or their health services all facts and documents of general interest concerning the course of epizootic diseases and the means used to control them and

c) to examine international draft agreements regarding animal health measures and to provide signatory governments with the means of supervising their enforcement.

Discussion of the International In-put Presentations

The expense of human vaccines was a major topic of discussion. The possible role of intradermal vaccination for post-exposure immunisation was discussed. Dr. Fekadu questioned the need for veterinarians to be pre-exposure immunised, based on the low number of reported cases in veterinarians and suggested that pre-exposure immunisation should be dealt with on a case-by-case basis. However, it was argued that people working with rabies demand immunisation before working with the virus and it is important that this service is offered.

Dr. Meslin mentioned that WHO has helped to purchase vaccine at a reduced cost. Furthermore, the high cost was associated with HDCV, but the newer vaccines are available at about US$ 10 per dose. The problem that governments will only recognise vaccines recommended by WHO (currently only HDCV) was raised.

The need for boosters was also discussed. The current WHO recommendation is that serology be done to assess the need for booster immunisation. It was commented that a long immunity results from the post-exposure immunisation regime, that may continue for up to 10 years.

African Overview and Antigenic Variation

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Overview

The standard of national presentations at this meeting has been so high that it is not necessary for me to spend too much time on giving an overview. What has emerged is that in many countries of the region the main problems appear to be less to do with rabies, its diagnosis and control, but more to do with the lack of country infrastructure, governmental support and finance.

Of the two main elements of diagnosis - getting the samples to the laboratory in a suitable condition, followed by the application of specific techniques - the former appears to represent the major problem. This suggests that, despite our best endeavours, rabies in some parts of the region is seriously under-reported, as it is in many other parts of the world.

Although it has not been our primary task at this meeting to assess the control measures that are employed in the region, we have heard several reports which have indicated that canine vaccination programmes are under severe strain - Cog vaccination programmes are not in operation because of the costs involved" or only a few districts have been able to attain or exceed former coverage" or the authorities cannot effectively enforce vaccination and attempts to do so would probably be counterproductive" - are but three of the more depressing reports that we have heard.

A striking feature is the nature of rabies within the region. In general terms, the more northerly countries report almost exclusively canine rabies, whereas further south, wildlife rabies plays the dominant role. Whether this apparent difference is real is not clear, it may be that better infrastructure and available facilities in the more southerly countries provide a more accurate reflection of the true picture. A second striking feature is the inexplicable virtual absence of rabies within national Game Reserves, although the disease may be reported in surrounding areas.

Despite the disappointments and difficulties which we all encounter in the rabies field, it is important that we maintain perspective. Science suffers in general from a lack of understanding by the public and in particular from the support of politicians and administrators. The budgets for disease control are thinly spread and, as we have heard and know all too well, rabies is not the disease problem in Africa which is at present the cause for most concern. Nevertheless, we have already witnessed at this meeting the keenness and enthusiasm with which rabies is being tackled even if resources are limited and as long as we continue in our endeavours to understand the nature and the epidemiology of the disease within the region there is hope for control in the future.

Antigenic *variation*

Taxonomically, rabies viruses belong to the Order *Mononegavirales,* Family *Rhabdoviridae* (Pringle, 1991). The *Rhabdoviridae* are characterised by a negative-sense genome of single stranded RNA and the family is divided into two genera, *Vesiculovirus,* (which includes the viruses causing vesicular stomatitis and antigenically related viruses) and *Lyssavirus,* which includes rabies and rabies-related viruses (Calisher et al., 1989). Rabies virions are bullet-shaped with an average length of 180nm and diameter of 75nm. The genomic RNA of the infectious particle contains five genes, each of which codes for a structural protein of the virion.

The G (glycoprotein) and N (nucleoprotein) proteins are the most extensively studied of these rabies proteins. The 439 amino acid region of the ectodomain G protein possesses biological functions which include attachment of the virion to cell surfaces and immunological functions which include attachment of antibody to binding sites; the region defines the virus serotype. Variation in the amino acid sequence of this region may therefore alter the pathogenic, antigenic or immunological properties of the virus (Wunner, 1991). The N protein is abundantly produced in virus infection and although its role in immunity is not clearly understood, lyssavirus group specificity is determined by its cross-reactivity and it has an important role in enabling diagnosis and virus identification by monoclonal antibody (Mabs) techniques.

Nucleotide sequences determined largely from complementary DNA (cDNA) clones of the mRNAs and genomic RNA of rabies fixed strains indicate that the sequence similarities between the deduced primary structure of the ectodomain G proteins are closely related (91 - 96 percent homology). The amino acid sequences of the N proteins are 450 residues long and they display an even higher degree (98 - 99 percent) of homology (Wunner, 1991). It is this high degree of homology between the N proteins of rabies viruses that allows differences in Mabs reaction patterns to be used as a tool in epidemiological studies.

Much of our knowledge of the ecology and epizootiology of rabies virus and the variation which occurs amongst the members of the genus *Lyssavirus* has been gained by the use of Mabs, first introduced to the rabies field in 1978 (Wiktor and Koprowski, 1978). Antigenic analysis with glycoprotein reactive Mabs (Hab-Gs) is usually carried out by a varying virus : constant antibody method using a dilution of Mab-G sufficient to neutralise 3 to 4 $\log_{10}$ of homologous virus. Analysis with nucleocapsid reactive Mabs (Mab-Ns) must be performed with the appropriate Mab-N dilution since too little antibody may lead to inconsistent results and too much antibody may fail to distinguish closely related viruses (Smith and King, 1992).

By using Mabs it has been shown that rabies isolates from a given geographical area or species have unique reactivity patterns both in the G and N protein components of the virion (Wiktor, Flamand and Koprowski, 1980). For example, only one or two major antigenic subgroups have been found in Europe, North and Central Africa, South Africa and India, whereas isolates from Madagascar, Iran and Thailand display a greater diversity (Sureau, Rollin and Wiktor, 1983).

Mab, reaction pattern analyses of isolates from an endemic area confirm surveillance observations of "compartmentation" (Bisseru, 1972) of the disease in one major host species with occasional "spill-over" to other species within the same area. The reason for such single-species involvement is not clearly understood. It may be that the amount of virus secreted in the saliva of the major host is sufficient to infect others of that host species but not always sufficient to infect an animal of a different species; it does not appear to be caused by a local disparity in the species population levels (Smith and Baer, 1988).

Most of the published information regarding epitopic variation has been gathered from the use of Mabs prepared from fixed rabies viruses. More recently Mabs panels have been constructed from the immunisation of Balb/c mice with rabies-related viruses (Bussereau, Vincent, Coudrier and Sureau, 1988; Bussereau, Vincent and Sureau, 1989 and King, 1991). These have not only revealed considerable variation amongst serotype 2 - 4 viruses, including the disparity between the serotype 4 viruses of Africa and the European Bat Lyssaviruses (EBL 1 and EBL 2) of European bats, but have also demonstrated the similarity of the viruses of wildlife origin of Africa and the Soviet Union and how these viruses differ from those of canine origin from the two continents (King, 1991).

Antigenic variation in the rabies viruses of Africa

But for an apparent upsurge in the number of rabid bats reported in Europe it is possible that the so-called rabies-related viruses of Africa may have remained confined to the history books, as few isolations have been made since 1986. 1 will not anticipate what Dr Swanepoel will say about these viruses in the next presentation, but I will give an account of how the rabies-related and serotype 1 viruses of Africa can be distinguished from each other by the use of Mab-Ns.

At my laboratory we have prepared a panel of Mab-Ns by using the rabies-related (Serotypes 2 - 4) viruses as immunogens. Briefly, the prototype strains of Mokola, Lagos bat, Duvenhage, Denmark bat and "Finman", an isolate made from a Swiss bat biologist who died in Finland of rabies believed to be of bat origin, were used to immunise Balb/c mice. Thereafter the usual fusion, cloning and RIPA techniques were employed to prepare and classify the Mab-Ns. Although no attempt was made to determine the antigenic site with which each Mab reacted, a panel of 36 Mab-Ns was constructed by testing them against a wide variety of viruses and selecting those Mab-Ns which were shown to react differently from the others.

Most of the known rabies-related viruses of Africa were obtained with the generous help of many researchers and isolates from European bats were obtained in order to compare them with the Duvenhage viruses of Africa (Table 1). In addition, a number of isolates from terrestrial animals were obtained from four southern African countries. The starting point for this latter work was as part of a jackal oral vaccination project in Zimbabwe, funded by the U.K. Overseas Development Administration, which Dr. Bingham, will describe later.

One of two techniques was used to obtain the virus isolate Mab-N reaction patterns. Either the viruses were passaged in BHK-21 cells until such time as a sufficient number of positive cells was obtained to seed Greiner 84-well "Terasaki"-type plates, or the viruses were passaged in 3-week-old mice, their infected brains were harvested and used to prepare smears on 12-well Teflon-coated 3"x1" glass slides. Prior experiments had shown that the two techniques were fully interchangeable. In addition to the rabies-related viruses, a total of 68 human and animal brains were examined from Zimbabwe (20), Namibia (3), Botswana (12) and South Africa (33) (Table 2).

Table 1.

Origins of 64 serotype 2 - 4 Viruses

Lab.Ref. (RV)	*Geographical Location*	*Year Isolated*	*Species*	*No. Tested*	*Senders' Ref.*
Serotype 2, Lagos bat viruses					
1	Nigeria	1956	*E. helvum*	1	Boulger
3	R.S.A.	1983	Bat	1	Pinetown
134	R.S.A.	1982	Cat	1	839/82
190	R.S.A.	1980	*Ep. wahlbergi*	1	687/80
133	Zimbabwe	1986	Cat	1	16302
43	C.A.R.	1974	*M. pusilis*	1	Sureau
41	Senegal	1985	*E. helvum*	1	Dakar bat
Serotype 3, Mokola viruses					
4	Nigeria	1968	*Crocidura sp.*	1	Shope
5	R.S.A.	1970	Cat	1	Umhlanga
39	Cameroon	1971	*Crocidura sp.*	1	Le Gonidec
40	C.A.R.	1981	*L. sikapusi*	1	Saluzzo
174-177	Zimbabwe	1981	Cats	4	Foggin
Serotype 4, Duvenhage (Africa) viruses					
6	R.S.A	1970	Human	1	Meredith
131	Zimbabwe	1986	Bat	1	RS16
139	R.S.A.	1981	Bat	1	1486/81
Serotype 4, European viruses, EBL 1					
154	USSR	1985	Human	1	Yuli
Various	Europe	1968-89	Bat*	41	Various
Serotype 4, European viruses, EBL 2					
8	Finland	1985	Human	1	Lumio
29	Holland	1987	*M. dasycneme*	1	47072
30	Holland	1987	*M. dasycneme*	1	47129
228	Holland	1989	*M. dasycneme*	1	92666

* Most bats were *Eptesicus serotinus* (serotines) from Western Europe but three were of unidentified species and two bats (UB1 and UB2) were from *Velpertillio murinus* (particoloured) and *Nyctalus noctula* (noctule) bats from the Ukraine. EBL = European bat lyssavirus.

Table 2.

Origins of 68 Serotype 1 Viruses from Southern Africa

Lab. Ref. (RV)	*Geographical Location*	*Year Isolated*	*Species*	*No. Tested*	*Senders' Ref.*
120,121	Zimbabwe	1986	Human	2	16300,16383
285	Zimbabwe	1990	Human	1	18769
129,2S7	Zimbabwe	1986,1990	Dog	2	15867,18775
124,125	Zimbabwe	1986	Cat	2	14034,14056
325	Zimbabwe	1991	Cat	1	18659
284	Zimbabwe	1990	Bovine	1	18765
286	Zimbabwe	1990	Jackal	1	18774
322,323,328	Zimbabwe	1991	Jackal	3	18623,18650,18914
329,330,331	Zimbabwe	1991	Jackal	3	18917,18922,18945
332	Zimbabwe	1991	Civet	1	18959
333	Zimbabwe	1991	Ratel	1	19049
130	Zimbabwe	1986	Mongoose	1	11926
132	Zimbabwe	1986	Rodent?	1	2/2/86
135,136	Namibi	1980	Kudu	2	947/80,968/80
138	Namibia	1980	Jackal	1	1070/80
390,393	Botswana	1990,1989	Canine	2	11/12/90,11/2/89
386,388	Botswana	1990,1991	Bovine	2	39/7/90,3/1/91
391	Botswana	1991	Bovine	1	8/1/91
393	Botswana	1989	Cat	1	11/2/89
396	Botswana	1990	Cat	1	13/12/90
397	Botswana	1988	Bovine	1	16/8/88
392,400	Botswana	1988	Caprine	2	8/11/88,7/12/88
389	Botswana	1990	Jackal	1	16/12/90
398	Botswana	19S9	Genet	1	1/3/89
387	Botswana	1991	Mongoose	1	2/2/91
425,426,427	R.S.A	1990	Dog	3	352,292,378
428,429,430	R.S.A.	1990	Dog	3	51,56,57
431,432,433	R.S.A.	1990	Dog	3	72,77,89
405,415	R.S.A.	1990	B.B.Jackal	2	101/90,598/90
402	R.S.A.	1990	B.E.Fox	1	20/90
416	R.S.A.	1990	W.Mongoose	1	623/90
404,421,423	R.S.A.	1990	Y.Mongoose	3	61/90,670/90,710/90
403,407,412	R.S.A.	1990	Y.Mongoose	3	57/90,257/90,466/90
413,420,422	R.S.A.	1990	Y.Mongoose	3	480/90,669/90,692/90
137,225	R.S.A.	1980	Mongoose	2	990/80,1051/80
140,141	R.S.A.	1980	Wildcat	2	1716/80,1927/80
409	R.S.A.	1990	C.Wildcat	1	298/90
419	R.S.A.	1990	G.Squirrel	1	637/90
414	R.S.A.	1990	B.B.Jackal	1	544/90
410,411,417	R.S.A.	1990	Y.Mongoose	3	332/90,416/90,634/90
418	R.S.A.	1990	G.Mongoose	1	636/90

As a first step, the Mab-N panel was tested against the prototype strains of Serotypes 1 - 4 and the results are shown in Table 3.

Table 3

Reaction Patterns of Mab-N *Panel with Prototype Viruses.*

Serotype Virus *Mab-N Reference Number**

		M					D			DB							F				
		2	5	6	7	11	1	3	9	1	3	4	9	10	11	14	1	2	3	4	5
1	CVS 11	+								+	+	+			+	+					
2	Lagos bat	+	+	+	+					+	+	+									
3	Mokola	+	+	+	+	+					+	+					+	+		+	+
4	Duvenhage Africa	+					+	+	+	+	+	+			+	+					
4	EBL 1	+						+	+	+	+	+	+	+	+	+					
4	EBL 2					+		+	+	+	+	+					+	+	+	+	+

		L															
		1	2	3	4	8	11	15	17	18	19	20	23	25	26	27	28
1	CVS 11			+	+								+	+	+		+
2	Lagos bat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Mokola		+				+	+				+	+	+	+		+
4	Duvenhage Africa		+	+					+			+			+	+	
4	EBL 1											+					
4	EBL 2								+					+			

M2 - 11 = 5 anti-Mokola Mab-Ns; D1 - 3 3 anti-Duvenhage Mab-Ns;
DBl - 14 = 7 anti-Denmark bat Mab-Ns; F1 5 = 5 anti-Finman Mab-Ns;
L1 - 28 = 16 anti-Lagos bat Mab-Ns.

As might be expected from a Mab-N panel which was constructed from a broad spectrum of immunising viruses, the reaction patterns given by the prototype viruses are easily distinguishable.

Of special interest was the distinction between the reaction patterns of Duvenhage (Africa) and the EBL 1 and EBL 2 viruses of Europe. These three virus types have been isolated from insectivorous bats of both continents but the disparity between them suggests that their relationship is somewhat more distant than was formerly supposed. Indeed, one (D1) of only three Mab-Ns prepared against Duvenhage virus does not react with the European isolates and conversely only five of twelve Mab-Ns prepared against viruses of European bat origin react with Duvenhage virus.

In addition, it is clear that there are at least two virus biotypes in European bats - only three of seven Mab-Ns prepared against the EBL 1 (Denmark bat) virus react with the EBL 2 (Finman) virus and conversely none of five Mab-Ns prepared against this latter virus react with the EBL 1 viruses.

The next step was to examine the rabies-related viruses listed in Table 1, and the results are shown in Table 4.

Table 4

Summary of Tests with 36 Mab-Ns on 64 Rabies-related Viruses.

Lab. Ref. (RV) *Mab-N Reference Number**

	M					D			DB					F						Virus group
	2	5	6	7	11	1	3	9	1	11	14	9	10	3	4	1	2	5	4	3	
1	+	+	+	+					+					+	+						Lagos bat
3,134,190	+	+	+			+			+					+	+						
133	+	+	+		+	0			0					+	+						
43	+	+	+											+	+						
41	+	+	+											+	+						
4	+	+	+	+	+									+	+	+	+	+	+		Mokola
5,39	+	+	+	+	+									+	+	+	+	+			
40,147-177	+	+	+	+	+									+	+	+	+				
6,131,139	+					+	+	+	+	+	+			+	+						Duvenhage
154,various(39)	+			V			+	+	+	+	+	+	+	+	+						EBL1
UB1,UB2	+			+			+	+	+	+	+	+	+	+	+						
8,29,20,228					+		+		+	+	+			+	+	+	+	+	+	+	EBL2

	L																Virus group
	18	1	17	3	27	8	19	4	15	25	11	23	28	2	26	20	
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Lagos bat
3,134,190	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
133	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
43		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
41				+	+	+	+	+	+	+	+	+	+	+	+	+	
4									+	+	+	+	+	+	+	+	Mokola
5,39									0	+	+	+	+	+	+	+	
40,147-177										+	+	+	+	+	+	+	
6,131,139			+	+	+									+	+	+	Duvenhage
154,various(39)																+	EBL1
UB1,UB2																+	
8,29,20,228				+						+							EBL2

+ = positive reaction; 0 = weak positive reaction;
V = variable, weak positive or no reaction;
no symbol = no reaction

The original distinctions between the rabies-related viruses of Africa, recognised by specific hyperimmune sera, were maintained in the Mab-N analyses; Lagos bat viruses were clearly distinguishable from Mokola and Duvenhage viruses. In addition the Mab-N panel recognised multiple variants within two of these virus groups.

For example, although isolates of Lagos bat virus were clearly more closely related to each other than they were to isolates of Mokola virus and vice versa, five reaction patterns in the N protein of these viruses were recognised by differential reactivity with Mab-Ns M7, D1, M11, L18, L1 and L17. Similarly, three reaction patterns of Mokola virus were observed by use of Mabs L15, F2, F5 and F4. There are too few isolates from these two virus groups, however, to permit the association of a particular reaction pattern with a particular species or geographical location. In comparison, the three Duvenhage viruses examined were defined by a single reaction pattern with 15 Mabs-Ns.

Eight Mab-Ns J25, M11, DB3, DB4, F1, F2, F5 and F4) which recognised epitopes in the EBL 2 prototype virus (Finman, RV 8) also recognised epitopes in the prototype Mokola virus (RV 4). Indeed, four of the Mab-Ns (F1, F2, F5 and F4) defined three reaction patterns within the Mokola virus serotype. The reservoir species of Mokola virus, assuming that there is one, has not been determined. The virus has not been isolated from a bat but the results above show that there is a relationship between the rabies viruses isolated from a man who died of the disease in Finland following a bat bite, bats in Holland and shrews, cats and a rodent in Africa.

Results of the examination of 68 Serotype 1 virus isolates made from terrestrial animals of southern Africa (Table 2) are summarised in Table 5.

Table5

Summary of Tests with 36 Mab-Ns on 68 Terrestrial Animal Viruses.

lab. Ref. (RV)	*Mab-N Reference Number*											
	M2	*DR1*	*DB3*	*DB4*	*DB11*	*DBl4*	*L3*	*L4*	*L23*	*L25*	*L26*	*L28*
Zimbabwe												
120,121,124,125,129,	+	+	+	+	+	+	+	+	+	+	+	+
130,132,284,285,286,	+	+	+	+	+	+	+	+	+	+	+	+
287,322,323,325,328,	+	+	+	+	+	+	+	+	+	+	+	+
329,330,331,332,333.	+	+	+	+	+	+	+	+	+	+	+	+
Namibia												
135,136,138.	+	+	+	+	+	+	+	+	+	+	+	+
Botswana												
386,388,389,390,391,	+	+	+	+	+	+	+	+	+	+	+	+
392,393,400.	+	+	+	+	+	+	+	+	+	+	+	+
387,396,397,398.	+	+	+	+			+				+	
South Africa												
425,426,427,428,429,	+	+	+	+	+	+	+	+	+	+	+	+
430,431,432,433,402,	+	+	+	+	+	+	+	+	+	+	+	+
405,415,416.	+	+	+	+	+	+	+	+	+	+	+	+
404,421,423.	+	+	+	+	+		+				+	
403,407,412,413,420,	+	+	+	+		+	+				+	
422,137,225,140,141,	+	+	+	+		+	+				+	
409,419,414,	+	+	+	+		+	+				+	
410,411,417,418.	+	+	+	+		0	+				+	

0 = weak positive

* *24* Mab-Ns were negative with all samples. These were: M5, M6, M7, M11, D1, D3, D9, DB9, DB10, F1, F2, F3, F4, F5, L1, L2, L8, L11, L15, L17, L18, L19, L20 and L27.

As mentioned above, the starting point for the examination of isolates from terrestrial animals of Zimbabwe was to investigate the likelihood of variants within them; it was felt that if the same variant was found in all species rabies might be successfully controlled or eradicated in jackal rabies areas by the oral vaccination of the target species, the jackal No variation was found in the animals tested from Zimbabwe, although it must be emphasised that the number of isolates (20) was very small, as was the number of species (8) from which they were made.

However, at the time that these preliminary tests were carried out a number of South African isolates, which had been stored frozen for almost ten years, were tested with the same Mab-N panel. Four of them (RV 137, 225, 140 and 141) from two mongooses and two wild cats respectively, proved to be different in that they reacted with only seven of the 36 Mab-Ns, five fewer than the isolates of Zimbabwe (Table 5). On checking the origin of these viruses it was found that they came from areas where rabies in mongooses was well known.

Although Mab-N work in southern Africa is in its infancy and is on-going, the results presented in Table 5 represent progress made to date. They confirm the compartmentation of the disease in major host species, with occasional spill-over to other species. For examples, the p mongoose" reaction pattern given by the virus from a mongoose (RV387) of Botswana was also found in the viruses from a cat, a bovine and a genet (RVs 396, 397 and 398 respectively) from the same country; conversely, the "canine" reaction pattern given by the viruses from dogs of South Africa was also found in RV 416, a water mongoose (*Atilax paludinosus*) from South Africa.

Foggin (1986), using the Wistar Institute Mabs panels, had earlier shown that the viruses isolated from three slender mongooses of Zimbabwe differed at the Mab-G and the Mab-N level from other serotype 1 viruses of Zimbabwe. The mongoose isolates came from geographical locations more than 200Km apart. At the time that this present work was carried out, mongoose rabies had not been reported from Zimbabwe since 1986, but in 1991 three cases were reported (Bingham, these Proceedings) and so far the isolate from one of these has been shown to be of mongoose type (unpublished data). The findings of Foggin, which deserve wider attention, together with the results tabulated here, indicate that there is a need in southern Africa to more fully examine the rabies viruses which are present, so that we may better understand the nature of rabies epizootiology within the region.

Finally, in order to examine the relationships of the Mab-N reaction patterns of all of the 68 serotype 1 rabies and 64 serotypes 2 - 4 viruses detailed in this work, and to compare them with the reaction patterns of 51 serotype 1 viruses from Eastern Europe and the Soviet Union, twenty nine reaction patterns, some of which were identical but were determined in viruses from different geographical regions, were submitted for analysis by the "Clustan" computer analysis programme (Wishart, 1987). The results are shown in Fig. 1.

Three distinctive groupings emerged. The first of these groupings was formed by the Mokola and Lagos bat viruses. This was an interesting finding in that the reservoir species of Mokola has yet to be identified and although the virus has not been isolated from a bat this may be because there have been relatively few investigations into the viruses that may be present in bats of African origin.

The second grouping was comprised of isolates of EBL1, EBL2, Duvenhage and the "wildlife" (mongoose and fox) isolates of Africa and the central region of the Soviet Union. The grouping of the bat viruses with the wildlife viruses of the two continents is difficult to explain.

The third grouping was comprised of lie "canine" viruses of both continents, together with a number of isolates of wildlife origin from Eastern Europe and the western sector of the Soviet Union. It may be that within this latter geographical area there is a coalescence of the wildlife viruses of historical times in the Soviet Union and the more recent epizootic reputed to have originated in the Volga delta at around the beginning of the second world war (Isakov, 1949) and to have spread northwards and westwards into the majority of European countries.

Prototype: serotype 1 (CVS-24) = G 11
Serotype 2 'Lagos bat) = B19
Serotype 3 (Mokola) - A24
Serotype 4 (Duvenhage) Africa = D27
serotype 4 (Duvenhage) Europe = D28
Serotype 5 (Finman) = C29

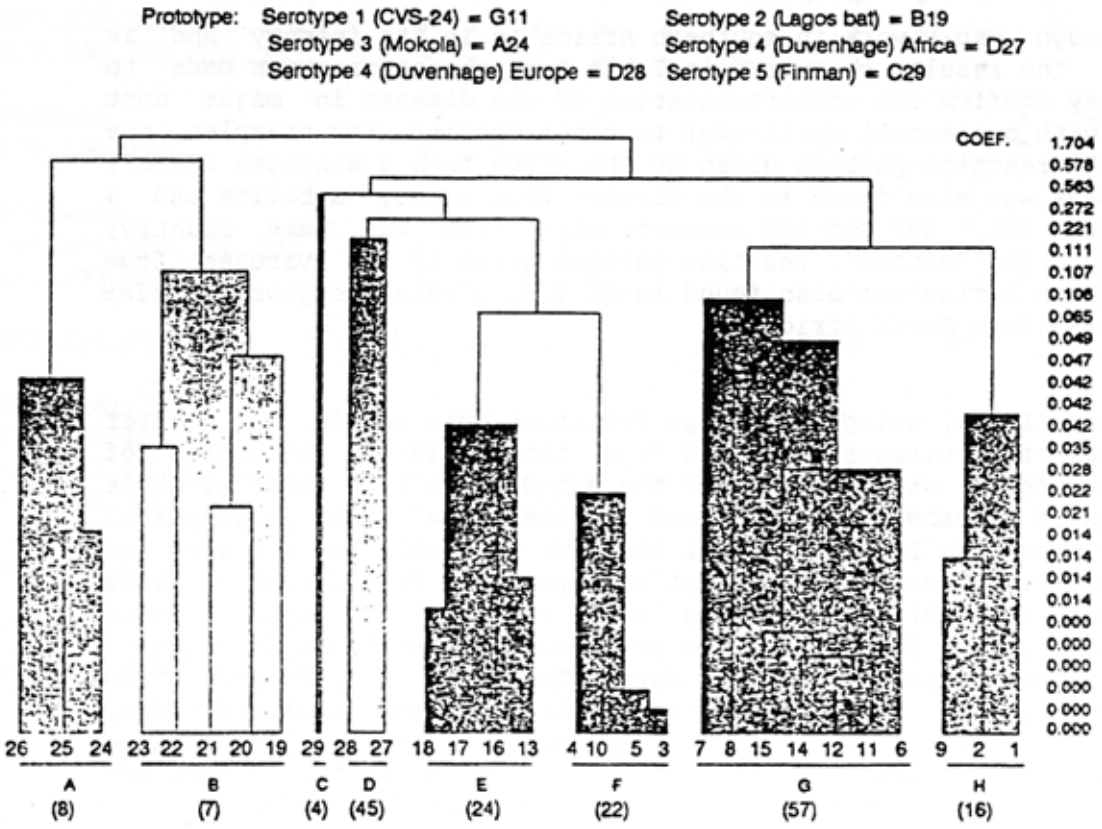

Fig. 1. "Clustan" analysis of rabies viruses with CVL Mab-Ns.

Under-column number = reaction pattern number;
( ) = number of isolates tested in group;
Group identification:
A = Mokola isolates; B = Lagos bat isolates; C = EBL 2 isolates; D = Duvenhage and EBL 1 isolates; E = African "mongoose" isolates; F = USSR wildlife isolates; G = African & USSR "canine" isolates; H = USSR and East German wildlife isolates.

The intriguing finding of isolates which could not, at least with these Nab-Ns, be distinguished from each other yet were taken from almost opposite ends of the earth, may perhaps permit a little unscientific Cite flying". Is it possible that the " wildlife" rabies viruses of the two continents are of ancient origin and that the "canine" viruses are a variant which arose many years ago and has since been carried around the world by man and his dog? We may never know the answer, but we do know that there are very many places in the world where an in-depth examination of the wildlife rabies viruses has yet to begin.

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Rabies-related viruses

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The history of the rabies-related viruses had its origins in the discovery of bat-associated rabies in the Americas, where the viruses which occur in insectivorous and vampire bats have all proved to be biotypes of rabies virus proper. Findings in the Americas aroused world-wide interest, and Lagos bat virus was discovered in 1956 in Lagos in Nigeria, when it was isolated from fruit bats in a deliberate attempt to determine whether bat-associated rabies also occurs in Africa. The virus was subsequently isolated in surveys from bats in 1974 in the Central African Republic (one isolation) and in 1985 in Senegal (two isolations). There was no indication that the bats tested in deliberate surveys behave abnormally.

During an epidemic of dog-rabies in Natal in 1980 and 1981, however, positive (cross-reactive) rabies immunofluorescence was recorded in several fruit bats which behaved abnormally, i.e. were caught by pet dogs and cats during daylight, or were found dead in gardens or swimming pools. Three isolations were made from such bats and all proved to be Lagos bat virus. A further isolation of Lagos bat virus was made from a fruit bat which was apparently found dead by dogs in a garden in Durban in 1990. Furthermore, Lagos bat virus was isolated in 1982 from a cat in Stanger, Natal, and in 1986 from a cat in Dorowa, Zimbabwe.

Suspicion that the cats were infected with a rabies-related virus was aroused by the facts that both cats had been vaccinated, that the cases occurred remote from any other known cases of rabies at the time, that fluorescence was weak with anti-rabies conjugate and that the signs of illness in the cat in Zimbabwe (lethargy and paresis), were not typical of rabies. Presumably the cats became infected from contact with bats.

Mokola virus was first isolated from shrew in Nigeria in 1968 (4 isolations) in the course of a survey for arthropod-borne viruses in small mammals in Mokola Forest, near Ibadan. Isolation of the virus from the cerebrospinal fluid from a young girl with nervous disease in Ibadan, Nigeria, was tentatively reported in 1969, but for a variety of reasons this isolation is no longer regarded as valid. An isolation mad: in 1971 from the brain of a girl who died in Ibadan of nervous disease, is regarded as valid.

Mokola virus was subsequently isolated in surveys from a shrew in 1974 in Cameroon and a rodent in 1983 in the Central African Republic. In 1981 and 1982, the virus was isolated from six cats and a vaccinated dog in Bulawayo, Zimbabwe, which were tested for suspected rabies. Suspicion that a rabies-related virus was involved was aroused by atypical signs of illness and weak fluorescence with rabies conjugate.

The incident in Zimbabwe prompted re-examination of a stored virus obtained in 1970 from a cat in Umhlanga, Natal, which had fluoresced atypically, and this also proved to be Mokola virus. It is notable that Mokola virus has never been obtained from bats, and it is surmised that the cats and dog gained infection from shrews or rodents -possibly there was an epizootic in small mammals in Bulawayo in 1981-82.

Duvenhage virus was first isolated in 1970 from an adult male living in Warmbaths district, Transvaal, South Africa, who died from rabies-like disease five weeks after being bitten by an insectivorous bat. The only other isolations of the virus came from an insectivorous bat caught in daylight by a cat in Louis Trichardt, Transvaal, in 1981 and another caught in a survey in southern Zimbabwe in 1986.

Prior to 1985, there were 12 isolations of "rabies" from bats in Yugoslavia, Turkey, Germany and Poland (only insectivorous bats occur in Europe) and one from a human who died in Russia in 1977 after being bitten by a bat. Most of the isolations were made in surveys, but some of the bats had behaved abnormally, e.g. were active during daylight. The viruses were initially identified as "rabies" virus in tests which cannot distinguish rabies from the related viruses, and most were discarded, but three of the early isolates from Germany were preserved and subsequently found to be closely related to Duvenhage virus.

At this stage there was speculation that Duvenhage virus may have been imported into Europe in bats, as for instance in ships carrying fruit from South Africa. In 1985, however, there was a further isolation of virus from a bat, which attacked a woman in Denmark, another from a bat zoologist who dies in Finland, as well as an isolation from a person who died after being bitten by a bat in Russia.

These isolations prompted the conducting of surveys in Europe, with the result that during the remainder of 1985 and over the ensuing few years there were over 450 isolations from bats in Denmark, Poland, Germany, Netherlands, Spain, France and Czechoslovakia. Many of the bats from which isolations were made behaved abnormally, particularly in Denmark, and it seems probable that an epizootic situation existed in Europe in 1985 and the following years. The number of isolations made in surveys currently amounts to about 40 per annum in Europe.

As mentioned in the talk by Dr King, the results of monoclonal antibody studies indicated that there are two subtypes of European bat virus, associated with serotine and myotine bats, and that these are distinct from Duvenhage virus which occurs in southern Africa. Unpublished studies with monoclonal antibodies at several reference laboratories in Europe, failed to identify rabies-related viruses among thousands of isolates from terrestrial vertebrates, apart from the human isolates mentioned above.

Studies conducted in South Africa (National Institute for Virology) with panels of monoclonal antibodies received from Dr King at Weybridge, and from the Wistar Institute in Philadelphia, and the Centres for Disease Control in Atlanta, failed to reveal rabies-related viruses among 120 isolates from humans and domestic and wild animals in South Africa, apart from the bat and cat isolates mentioned above, thus confirming observations made by Dr King on southern African isolates. The position in Africa appears to be similar to that in Europe: although rabies-related viruses could theoretically adapt to terrestrial vectors and spread to cause problems similar to rabies, this does not appear to have happened yet.

It has been suggested that diagnostic laboratories should include tests with a selected small panel of monoclonal antibodies in their screening of field specimens in order to detect rabies-related viruses. However, misleading results may be obtained with abbreviated panels of monoclonal antibodies, and some laboratories in Africa have not yet mastered or instituted adequate routine diagnostic procedures.

Moreover, the low incidence of infection with rabies-related viruses likely to be encountered in diagnostic laboratories, does not seem to justify the extra work and expense involved in screening all specimens with a large panel of monoclonal antibodies. In those rare instances where rabies-related viruses are encountered in diagnostic laboratories, there are usually clues which assist in arriving at the correct diagnosis, such as the involvement of an unusual host (e.g. bat, shrew), or a history of the affected animal having been bitten by such a host, atypical signs of illness, the occurrence of the case in isolation from other known cases of rabies and the appearance of atypical or weak fluorescence in tests with anti-rabies conjugate. it makes more sense to apply the monoclonal antibody tests only in instances where there are features which suggest that rabies-related viruses may be involved, and the tests on such viruses are probably best conducted at regional or international reference laboratories.

The monoclonal antibody tests on rabies isolates in South Africa referred to above, also confirmed the finding of Dr King that separate biotypes of rabies virus circulate in dogs and mongooses in southern Africa.

EXPERIMENTAL DIAGNOSIS OF RABIES.

ADAPTATIONS TO FIELD AND TROPICAL CONDITIONS

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Introduction

Clinical signs of rabies are generally not characteristic and may vary greatly from one animal to another, depending on the areas of the brain in which the virus is replicating and on individual responses to infection. As clinical observation may only lead to a suspicion of rabies, the only way to confirm the disease is to look for the presence of rabies virus or its components in samples collected from the suspected animal.

Several laboratory techniques may be used to detect rabies virus infection. These techniques include either histological or cytological methods to detect viral replication, or viral antigen, or the virulence and pathogenicity of non-inactivated virus, or viral nucleic acid.

This paper deals with sampling and shipment techniques and methods used for the routine diagnosis of rabies. Emphasis will be placed on techniques particularly applicable to hot or tropical countries. The subjects covered will be:

Collection of samples: occipital foramen and retro-orbital routes

Shipment of samples

Histological Techniques

Immunocytochemical Techniques

Immunoenzymatic Techniques

Virus isolation Techniques: souse inoculation and cell culturre

Diagnostic Quality Control

Experimental diagnosis on preserved samples and

Choice of preservation techniques

Where relevant, attention will be given to the equipment required, technical training needs, test sensitivities, equipment costs and costs of individual tests.

Collection of samples

Brain sampling by opening the skull is hazardous when practised outside of laboratory or when technicians are not regularly trained. However, in field conditions, for example for epidemiological studies, one of two techniques may be used to collect brain samples without opening the skull:

A. Occipital foramen route brain sampling: *(see Fig. 1. page 79).*

Brain samples may be taken through the occipital foramen (Barrat and Blancou, 1988) by using a drinking straw (5mm in diameter) or a 2ml disposable plastic pipette (Bourhy and Sureau, 1990). The steps of this procedure are successively:

- cut the skin and neck muscles over the joint between the occipital bone (condylus occipitalis) and atlas vertebrae
- bend the head forward to give access to the occipital foramen
- pass a straw through the foramen and screw it into the brain, heading in the direction of an eye. This way the straw pierces the rachidian bulb, the base of the cerebellum, the Ammon's horn and the cortex.
- pinch straw between fingers and gently withdraw it from the head.

B. Retro-orbital route brain sampling: (Montano Hirose et al., 1991):

- push the eyeball to one side
- use a trocar to make an entry through the posterior wall of the eye socket
- introduce through this hole a straw or a 2ml disposable plastic pipette, screwing it in, in the direction of the occipital foramen
- pinch straw between fingers and gently withdraw it from the head

The cerebral tissues sampled here are the same as those sampled via the occipital foramen, but they are taken in reverse order.

Shipment of samples

Ideally, the head or the entire animal should be kept cool during rapid transport to the laboratory. When these conditions cannot be followed, different preservation techniques may be used; the choice is governed by the laboratory techniques that will be used for diagnosis.

Formalin solution inactivates rabies virus. Virus isolation tests cannot then be used, but diagnosis can still be made by a modified fluorescent antibody test and by histology.

Glycerin solution does not inactivate rabies virus. It is a preservative that temporarily inhibits the growth of contaminants. As the virus is not inactivated, all laboratory techniques (including virus isolation tests) may be used on samples in glycerin.

Laboratory techniques used for diagnosis

The techniques cited here are detailed in the OIE "Recommended Techniques and Requirements for Biological Products" and in the WHO monograph (Kaplan and Koprowski, 1974). Therefore only improvements or adaptations to special conditions are detailed.

Histological Techniques

In 1903, Negri described eosinophilic inclusions in the cytoplasm of brain cells of rabid animals. Although these inclusions, which correspond to aggregates of viral proteins, are specific for rabies virus infection, the staining techniques used are not specific since they merely detect affinity for acidophilic stains.

Staining techniques The two most frequently used are:

Sellers' technique, used on smears. Results can be obtained within one hour of sample receipt. With autolysed samples, even at the first stage of autolysis, interpretation of the results may be difficult.

Mann's technique involves tissue embedding in paraffin and section cutting. Results take longer but can be obtained within 4 days of sample receipt; interpretation of results is easier.

Equipment required

Both techniques require an incandescent-light microscope. In addition, the classical histology equipment for embedding and cutting is needed for Mann's technique.

Training of Technicians

Regular training is necessary to achieve good sections of embedded samples and their staining.

Test Sensitivity

The sensitivity of histology is closely related to the preservation of the sample: the result is more reliable when samples are fresh than when autolysis has begun. Sometimes non-specific inclusions may be observed and these are difficult to distinguish from Negri bodies. Depending on the freshness of the sample, Mann's technique detects 60 - 95 percent of positive samples.

Cost of histology techniques:

Equipment for Mann's staining:		
Microtome	8800	us $
Light microscope	4000	US $
Embedding automatic system	12000	US $
65°C incubator	1100	US $
Miscellaneous	1500	US $
Storage of slides	1300	US $

Test costs:

Sellers' staining	0.5 US $
Mann's staining	2.2 US $

Immunocytochemical Techniques.

These are used to detect viral antigen whether or not the virus it inactivated.

Direct immunofluorescence:

Immunofluorescence was introduced to rabies diagnosis by Goldwasser and Kissling in 1958. Fluorescent conjugates may be prepared from either polyclonal or monoclonal antibodies. Polyclonal conjugates are prepared from antisera to native virus or purified nucleocapsids, which form the most abundant antigen in infected cells. Monoclonal conjugates, usually prepared against the nucleocapsids (Mab-Ns) may contain a single antibody but a mixture of Mab-Ns is usually employed.

Equipment required

Specimen examination requires blue-light fluorescence as fluorescein is excited by 490nm wavelength light and emits a greenish light of wavelength 510nm. This light may be obtained either with a halogen 12V 10OW lamp or mercury vapour lamp. Both lamps have a life-time of about 200 hours, but the mercury vapour lamp is 5 to 15 times more expensive than the halogen lamp.

Training of **technicians**

Regular training in specimen examination is of particular importance. This is even more important when the sample is autolysed.

Test Sensitivity

When used by regularly trained persons, the direct immunofluorescence test may detect 97 - 99% of positive samples. Results may be obtained within three hours of sample receipt.

Cost of fluorescence techniques

Equipment costs:

Light microscope	4000 US $
Fluorescence equipment	5500 US $
370C incubator	1100 US $
Refrigerator and freezer	1200 US $
Miscellaneous	700 US $
Storage of slides	1300 US $

Test costs:

The cost of one test is 1 US $. Several tests may be used for a single diagnosis (confirmation of a negative result).

Conclusion

The fluorescent antibody test is the preferred technique for rabies diagnosis. It alone may provide a result and it may also be used to reveal the replication of rabies virus after cell culture or mouse inoculation. It may also be used on brain samples preserved in glycerin solution after washing in PBS, or, after enzyme treatment, on samples preserved in formalin solution.

Immunoperoxidase on smears

Immunoperoxidase may be used instead of immunofluorescence. Both techniques have the same sensitivity (Genovese and Andral, 1978), but as peroxidase tests take longer to perform and are more expensive, they are no longer used in routine laboratory diagnosis.

Immunoenzymatic Techniques

An ELISA technique has been developed (Perrin et al, 1986). This Rapid Rabies Enzyme Immuno-Diagnosis (RREID) detects the presence of rabies nucleocapsid in brain samples. In kit form, the RREID is commercially available and is distributed by Diagnostic Pasteur (ref. no. 72201). Plates may be read by eye, or more easily by the utilisation of a spectrophotometer. Results can be achieved within 4 hours of specimen receipt. The correlation between fluorescent antibody test and RREID is 96 - 99%.

Training of technicians

If the technician does not know any other ELISA technique, a few days training will be required. The interpretation of the test does not need any special training. Since positive and negative controls give clear-cut results, the threshold is easy to determine.

Cost of Immunoperoxidase techniques:

Cost of Equipment:

Spectrophotometer	7300	US $
Titerplate washer	3700	US $
370C incubator	1100	us $
Refridgerator	550	us $
Multichannel pipette	750	US $
Miscellaneous	750	US $

Test costs

The cost of one test is 4.5 US $ if it is made in duplicate. it is possible to use one single well per sample.

Conclusion

This simple and rapid technique is ideal for epidemiological studies. The immunocapture phenomenon allows easy and reliable diagnosis, even on autolysed samples.

The RREID has been improved by the creation of two new tests:

- RREID-biot which includes an amplification step using biotin-avidin complex
- RREID-lyssa which is also amplified and directed against PV, Mokola and EBL strains

Virus Isolation Techniques

These techniques detect viable rabies virus. They may be used in vivo (by mouse inoculation) or in vitro (on neuroblastoma cells).

Virus isolation in mice:

5 to 10 adult OF1 germ-free mice or 10x 1-2 day old new-born mice are inoculated intracerebrally. The mice are inspected every day for 28 days and the brains of any mice which die are checked for rabies by FAT. Because of the variability of rabies virus incubation periods, results may not be quickly obtained. For example, the incubation period for street fox rabies virus is 9 - 14 days.

The waiting period may be shortened by the sacrifice of one mouse every 3 days from day 5 onwards, followed by the *examination of* its brain by FAT. The correlation between FAT and mouse *inoculation tests* is good, both diagnostic methods detect rabies in 92 - 99 percent of samples from rabid animals.

Equipment required

A special room must be reserved for animals and an immunofluorescence laboratory is required to check for rabies in the brains of animals which die.

Training of technicians

Technicians must be used to taking care of animals and be able to perform immunofluorescence tests.

Cost of mouse inoculation:

Cost of Equipment:

The cost of the mouse room depends upon its degree of sophistication and the price of manpower. These costs begin at 450 - 650 US $ and can reach up to 44,000 US $ - 54,500 US $ for 2,000 mice in insulated boxes. To be added are the costs of an immunofluorescence laboratory.

Test costs:

The cost of five adult mice, their food and of the products needed for diagnosis is 14 US $ per specimen. If one uses new-born mice, the price is then 34 US

Conclusion

The mouse inoculation test does not give rapid results, but it has the advantage of being a simple and reliable method for the isolation of rabies strains for further typing.

Virus isolation in cell cultures

A suspension of the specimen is used to inoculate a cell monolayer. Most often, the replication of rabies virus in neuroblastoma cells does not induce any cytopathic effect, the presence of virus is revealed by a fluorescent-antibody test.

Equipment required

The cell line used here is a neuroblastoma cell line of murine origin obtained from the ATCC (catalogue no. CCL 131). For rabies diagnosis by cell culture a separate culture room with immunofluorescence facilities is required.

Training of technicians

The person who makes rabies diagnosis by virus isolation in cell cultures must be used to the manipulation of cells and the performance of fluorescent antibody tests.

Test Sensitivity

Tests performed in Malzéville show that after incubation for 48 hours, N2a cell diagnosis detects 96 - 97% of positive samples (Barrat et al., 1986). One cycle of virus replication in these cells takes at least 18 hours.

Cost of cell culture virus isolation techniques:

Cost of equipment;

Inverted microscope	5500	US $
C02 incubator	9000	US $
Refrigerator and freezer	1300	US $
Vertical Laminar flow hood	5500	US $
Refrigerated centrifuge	12000	US $
Liquid nitrogen cylinder	1400	US S
Miscellaneous	1700	US $
Fluorescence	see above	

Test cost:

One diagnosis costs 2.2 US $ per sample.

Conclusion

Diagnosis by cell culture techniques is at least as sensitive as the mouse inoculation test. Results are obtained more quickly (usually 3 days) and the tests are cheaper to perform.

Laboratory diagnosis quality control

In a study by the CDC, Atlanta, of the quality of experimental diagnoses made in different US laboratories, 129 laboratories were sent 10 test slides. 41 of the laboratories gave erroneous results. These laboratories were sent a further group of 10 slides from other cases and 6 laboratories again made interpretation errors.

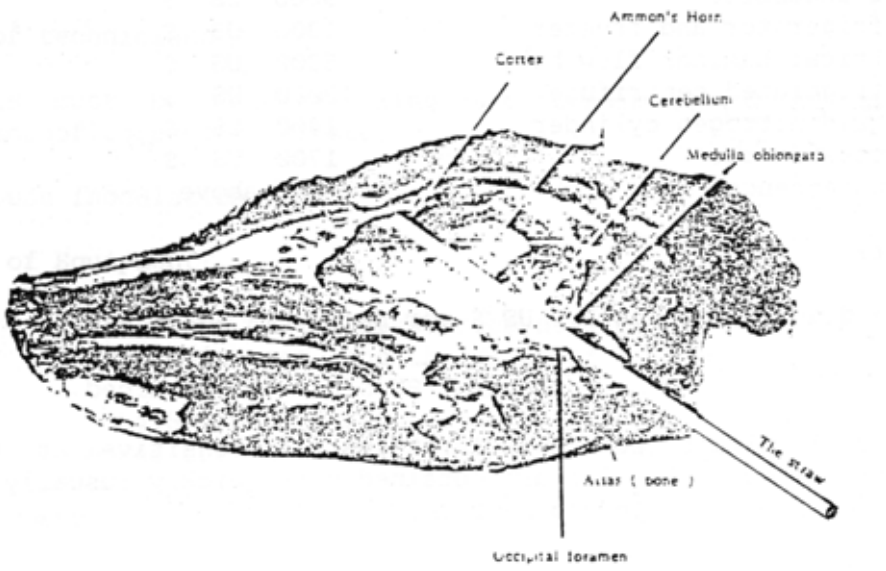
These results were correlated with the number of diagnoses performed in the different laboratories. The most important factor was related to the number of diagnoses made in a laboratory - the more samples that were tested the fewer the test errors.

The five main sources of error were:

1. technical procedure not followed
2. no quality control, no control slides
3. incomplete or partial technical procedure
4. inadequate microscopes
5. irregularly trained technicians

This study emphasises the great importance of regular training in and practice of FAT. It also highlights the need for strict quality control. in fact, these two points indicate that diagnosis should be under a centralised structure.

Fig. 1. Occipital foramen route brain sampling.



Experimental diagnosis on preserved specimens

As stated above, if it is not possible in field conditions to keep samples cool and send them rapidly to the diagnostic laboratory, they may be preserved by using either formalin or glycerin preservation.

Treatment of formalin preserved specimens:

Histology (Mann's stain for example) may be performed directly on a portion of the already fixed sample.

Alternatively, FAT may be indirectly performed on formalin preserved samples. Treatment with proteolytic enzymes is necessary (Umoh and Blenden, 1981; Barnard and Vosges, 1982); pre-treatment with a trypsin digestion step is required (Barrat, 1986).

The samples to be treated consist of small pieces of brain (3 mm cubes) stored in a 10% formalin solution. The following procedure is used:

1. grind one piece in 5ml of Ca-free and Mg-free PBS pH 7.5
2. Centrifuge the tube and discard the supernatant fluid
3. resuspend the deposit in 5 ml of a 0.25% trypsin solution
4. keep the tube refrigerated (+4°C) overnight
5. centrifuge the tube and discard the supernatant fluid
6. wash the pellet twice in Ca-free and Mg-free PBS
7. make smears from the pellet and perform FAT

In our laboratory we have tested the capacity of this technique to recover positive samples after up to 20 days at ambient temperature (i.e. 20 - 25°C).

On day 0, naturally infected rabid foxes were selected by FAT. The FAT positivity of fresh samples was estimated for the quantity of fluorescence on a 5 point scale. Different Ammons' horn samples, as for fresh FAT, were fixed in formalin.

On days 2, 5, 8 and 20, a piece of fixed brain was treated according to the procedure described above and the results were recorded according to the defined scale.

Results:

	Days in 10% formalin at 20-25°C			
	2	5	8	20
with grinding	100% N=98	97% N=98	98% N=98	91% N=98
without grinding	92% N=52	75% N-=	85% N=71	62% N-=

Fluorescence was more abundant in samples which had been ground. This indicates that grinding allows better contact with trypsin, leading to improved test sensitivity.

Histological examination

Histological examinations were performed at days 2, 5, 8 and 20 using Mann's technique on other samples taken from the same foxes.

Tests were performed on 50 samples. Histology recovered 50 positive samples after 2 and 5 days in formalin and 49 after 8 and 20 days.

Sensitivity of diagnosis using both FAT and histology

The overall sensitivity of techniques available for formalin preserved samples was determined on 50 naturally infected rabid foxes. The results were as follows:

	Days in formalin at 20-25°C.			
	2	5	8	20
FAT alone	50	so	so	48
Histology alone	50	50	49	49
FAT + Histology	50	50	50	50

Conclusion

The use of both FAT and histology allows identification of 100% (N=50) of positive samples after up to 20 days in formalin solution at ambient temperature.

Occipital foramen sampling and formalin preservation

Following trypsin treatment of 107 rabid fox brains fixed in formalin and held at room temperature for 8 days, 98 percent were FAT positive. Of 107 straw samples from the same brains held in formalin at room temperature for the same 8 day period, 102 (95.3 percent) were positive following trypsin treatment. However, only 14 of 24 (58.3 percent) of straw samples from these foxes which were held in formalin at room temperature for 8 days were positive following trypsin treatment of the entire sample instead of only a small 3 mm, cube (or 50 microlitres of the pellet obtained after the first washing step).

Conclusion

The use of formalin fixation with the straw technique, followed by trypsin treatment, considerably improves the accuracy of diagnosis from specimens which have not been kept cool and rapidly transported to the laboratory.

Treatment of glycerin preserved samples

Glycerin has an antiseptic action, but it also softens the brain tissues and makes their handling difficult. If animals are sampled with straw or pipette, whatever is the route, the sample must be kept inside the straw so that glycerin softens only those small parts of the sample at the opened ends of the straw.

In order to eliminate as much glycerin solution as possible, the samples are then washed in PBS before any other treatment. After washing, the glycerin preserved sample is processed according to the classically described techniques.

Influence of temperature on diagnosis in glycerin preserved samples

On day 0, 130 foxes received for diagnosis were sampled twice with a straw through the occipital foramen and diagnosis was performed classically on a fresh sample after opening of the skull. The straw samples were preserved in glycerin solution.

When the normal diagnostic test was positive, one "straw" sample was kept for 8 days at ambient temperature (i.e. 20°C) and the other straw was kept in an incubator at 37°C.

FAT and cell culture tests were performed on both fresh and preserved samples. Fluorescence quantity was recorded on a 5 point scale (fluorescence in 0%, 12.5%, 25-50%, 50-75% and 75-100% of fields).

Preservation out of the straw

After 8 days preservation in glycerin solution with sample outside the straw, 18% of the samples were negative. Fluorescence intensity was estimated on 10 samples during this kind of storage and the average fluorescence decrease was 12.5%.

After 8 days at 37°C, FAT was negative for 2 samples and cell culture for 1 of these same 10 samples; but no sample was negative to both FAT and cell culture. Fluorescence decrease was 50% in these conditions.

Preservation within the straw

All 130 samples which were kept within the straw in glycerin for 8 days at 20°C were positive by both FAT and cell culture. The average decrease of fluorescence was measured in 35 samples and was less than 12.5%. At 37°C also both tests were positive for all samples. The average decrease of fluorescence was measured on the same 35 samples and was less than 25%. The same comparison was made between fluorescence in cells when samples were preserved at 20°C and at 37°C. The average decrease of fluorescence was 12.5%.

Conclusion

When samples are to be preserved in glycerin solution, it is better to keep them protected within the straw. After 8 days in glycerin solution with the brain sample inside the straw, the recovery rate was 100% for positive samples determined on fresh specimens. The result was obtained whatever the temperature, 20°C or 37°C.

Choice of a preservation technique

If samples cannot be rapidly sent refrigerated to the laboratory, for example in the context of epidemiological field studies, they must be preserved. Two possibilities exist, each of which allows the use of different laboratory techniques. If samples are preserved in glycerin solution, they must be left inside the sampling straw. If samples are preserved in formalin solution, they must be pushed out of the sampling straw or pipette with a cotton swab or air by use of a rubber bulb.

The two preservative solutions offer the same recovery rate of 100% after 8 days at ambient temperature or even at 37°C. The choice of preservative therefore depends both on available laboratory techniques and on transport conditions.

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Evaluation of the molecular diversity within the Lyssavirus Genus

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Significant advances in the techniques available for rabies diagnosis have taken place over the past thirty years. The traditional method of histological examination for Negri bodies in the brain sections has been largely replaced by immunofluorescent staining methods for the detection of nucleocapsid antigen in brain smears (fluorescent antibody test: FAT) (Dean and Abelseth 1973); in many laboratories the accurate but time-consuming technique of virus isolation by mouse inoculation has been replaced by a cell-culture technique (rabies tissue culture infection test: RTCIT) (Portnoï, et al. 1982) which is equally as accurate but which can be used to detect the products of virus replication in days rather than in weeks (Bourhy, et al. 1989). Considering that not all laboratories possess equipment for immunofluorescence or cell culture, an enzyme-linked immunosorbent assay (Rapid Rabies Enzyme Immuno Diagnosis = RREID), based on the immunocapture of rabies ribonucleoprotein, was developed (Perrin, et al. 1986). This was particularly adapted for routine diagnosis and for epidemiological studies of large numbers of specimens. The results obtained with RREID correlate well with those obtained with FAT and RTCIT (Bourhy, et al. 1989; Bourhy and Sureau 1991). Nevertheless, RREID shows a low sensitivity for rabies-related viruses. We then modified the RREID by using biotinylated antibodies, streptavidin conjugate and a mixture. of monospecific polyclonal antibodies against ribonucleoprotein from Pasteur virus (lyssavirus serotype 1), European Bat lyssavirus subtype 1 (EBL1, unclassified) and Mokola virus (lyssavirus serotype 3) (Perrin, et al. 1992). The modified technique (RREID-lyssa.) was used for the detection Of ribonucleocapsid of different lyssavirus serotypes. The threshold of detection of the modified technique (RREID-lyssa) was lower (0.2 ng of viral nucleocapsid whatever the serotype) and the sensitivity and the specificity was identical to the RRE1D. Consequently, RREID-lyssa. can be a useful tool for diagnostic laboratories that receive specimens infected by rabies-related viruses.

Today, the need is not only for fast accurate diagnosis but also to find methods for typing isolates and for appreciating their variability throughout the world. To meet these requirements a progressive polymerase chain reaction (PCR) technique for rabies recently has been developed (Sacramento, et al. 1991). Already it is a promising alternative tool for diagnosis, a very efficient means of virus typing and a useful tool in molecular epidemiological studies (Tordo. et al. 1992).

Although in this paper we have insufficient time to discuss the fine details of the PCR technique, the basic principles can be outlined. Since PCR is a highly sensitive technique in which contamination through sample-handling must be avoided at all costs, high quality sample collection is an absolute prerequisite. As has been demonstrated during this meeting, internal brain sampling without autopsy by the introduction of a disposable plastic pipette via the occipital foramen (Barrat and Hallek 1986) or via the retro-orbital route (Montano Hirose, et al. 1991) is simple and these sampling methods are ideal for PCR. Although where possible we used the original infected brain, on some occasions nucleic acid extraction for PCR was performed on suckling mouse brains infected with the original virus at the lowest passage number available. The brain is homogenised in an extraction buffer using a plastic pestle adapted to the Eppendorf tube. Up to four successive protein extractions from the lysate (with phenol, then twice with 50/50 phenol/chloroform and finally with chloroform) may be performed by the addition of an equal volume of solvent, mixing vigorously and then separating the aqueous from the organic phase by centrifugation at room temperature. Sodium acetate (pH 5.2) is added to give a final concentration of 0.3 M and the total RNA is precipitated by the addition of two volumes of ethanol. Following further centrifugation, the pellet is washed twice in 70% ethanol, dried, then resuspended in pyrolysed water at 1 µg/ml (absorbance at 260nm) (Sacramento, et al. 1991).

Comparison between the sequences of the Mokola virus (serotype 3) (Bourhy, et al. 1993 Bourhy, et al. 1989) and the PV strain (serotype 1) (Tordo, et al. 1988), two lyssaviruses representative of the most divergent scrotypes according to antigenic, studies allowed the delineation of conserved regions within the lyssavirus genomes that can be useful targets for primers. The cDNA synthesis and amplification of the viral transcripts by PCR may then be carried out as has been described (Sacramento, et al 1991). Primers allowing the amplification of the whole nucleoprotein (N), glycoprotein or pseudo-gene were defined (Sacramento et al. 1992; Bourhy, et al. 1992; Tordo, et al. 1992). Each of these three target genomic regions possess their own purposes: the N gene can be used for diagnosis, taxonomy, typing, epidemiology and immunological studies; the glycoprotein gene is useful for epidemiology and for immunological studies; the pseudo-gene is interesting for typing and epidemiology. Other primers were used for direct sequencing. They were produced with a direct synthesiser and used after detritylation and alkaline deprotection. It is imperative to include in every PCR test series a positive (infected) and a negative (uninfected) brain control, as well as a reaction where the nucleic acid sample is replaced by water, which serves as a very sensitive method for the detection of contaminant either in materials or in solutions.

For diagnosis purpose, the PCR products were diluted, heat denatured for 10 min, chilled on ice and then filtered on to a nylon membrane using a multi-well vacuum filtration unit. The nylon membranes used in these blotting techniques are air dried before covalent binding of the nucleic acids by UV illumination to 312 nm for three min. (Sacramento, et al. 1991). For virus typing, 2-10 µl of the PCR products are digested by a panel of selected restriction enzymes and separated by electrophoresis on a convenient agarose gel in buffer containing ethidium bromide. The pattern is analysed on a print of the gel viewed under UV illumination at 312 nm (Bourhy. et al 1992; Sacramento, et al 1991).

The purpose of our study was also to provide the first complete analysis of the genetic diversity of the lyssaviruses by sequence comparison of representative isolates of all the reported serotypes and unclassified European bat subtypes. The first open reading frame of the lyssavirus genomes, the N gene, was selected for two reasons - for comparative purpose, because the N protein was the main target antigen in taxonomic studies (Dietzschold, et al. 1988) and to approach the molecular basis of the antigenicity and cross-protection between serotypes, since the N protein is the second viral antigen involved in protective immunity (Celis, et al. 1990; Tollis, et al. 1991). The sequences of the N protein of a fox isolate from France, Lagos bat virus, Duvenhage virus and two isolates of EBL 1 and EBL 2 obtained by PCR, were aligned with Mokola virus and rabies PV strain. Analysis of AA and nt similarity between the nine isolates established that members of the Lyssavirns genus can be divided in at least 6 genotypes (figure 1) (Bourhy, et al. 1992; Bourhy, et al, 1993). Genotypes 1 - 4 are concordant with their respective serotypes and EBL1 and BBL2 should be considered as independent genotypes, 5 and 6. In addition, our study shows that genotyping is not only consistent with classification by serotyping but is quicker and more sensitive. Intergenotypic relationships show that genotype 2 (Lagos bat virus) and genotype 3 (Mokola virus) are the most phylogenetically distant from the vaccinal and classical rabies virus of genotype 1. Genotypes 4 (Duvenhage) and 5 (EBL1) are closely related to each other. The threshold of similarity below which a new genotype should be defined is in the interval given by the lowest percentage of similarity found within one genotype (97.1%) and the highest percentage of similarity found between two genotypes (93.3%). The amino- and carboxy-terminus of the N protein are more variable than the central part. Nevertheless, the overall AA similarity of the nine N proteins is 77% and the putaive phosphorylation site mapping to serine in position 389 (Dietzschold, et al. 1988) is conserved in all the isolates. These data established that the AA sequence of the nucleoproteins was preserved, within the Lyssavirus genus during evolution.

The analysis of the other open reading frames of the Mokola genome and their comparison with the PV strain established the rate of divergence of each protein (Bourhy, et al 1993) When closely related strains from serotype 1 (i.e. PV and SAD B19) are compared, the percentage similarity of the N, MI, M2 and G proteins are 99.1, 97.3, 90.6 and 96.4 respectively. With less closely related strains (i.e. PV and AVO1), the conservation decreases more sharply, from the N to the G proteins, from 97.6, 91.6, 91.1 to 88.5%, respectively. When viruses of different serotypes are aligned, such as the PV strain (serotype 1) and Mokola virus (serotype 3), the conservation falls to 79.8, 47.4, 76.3 and 58.4% respectively. This indicates that the rate of divergence of protein is varying according to the evolutionary distance of the viruses. The nucleoprotein > matrix protein > glycoprotein > phosphoprotein conservation order in lyssaviruses is consistent with the vesiculovirus findings and seems more general in the Order Mononegavirales (Bourhy, et al. 1993; Tordo, et al. 1992). Nevertheless, whatever the protein N, M1, or G a weak conservation of the AA sequence of the antigenic sites in the rabies virus was noticed in Mokola virus (Bourhy, et al. 1993; Tordo, et al. 1993). The mutations observed in these immunogenic regions explain the lack of cross-protection between vaccinal strains and Mokola virus.

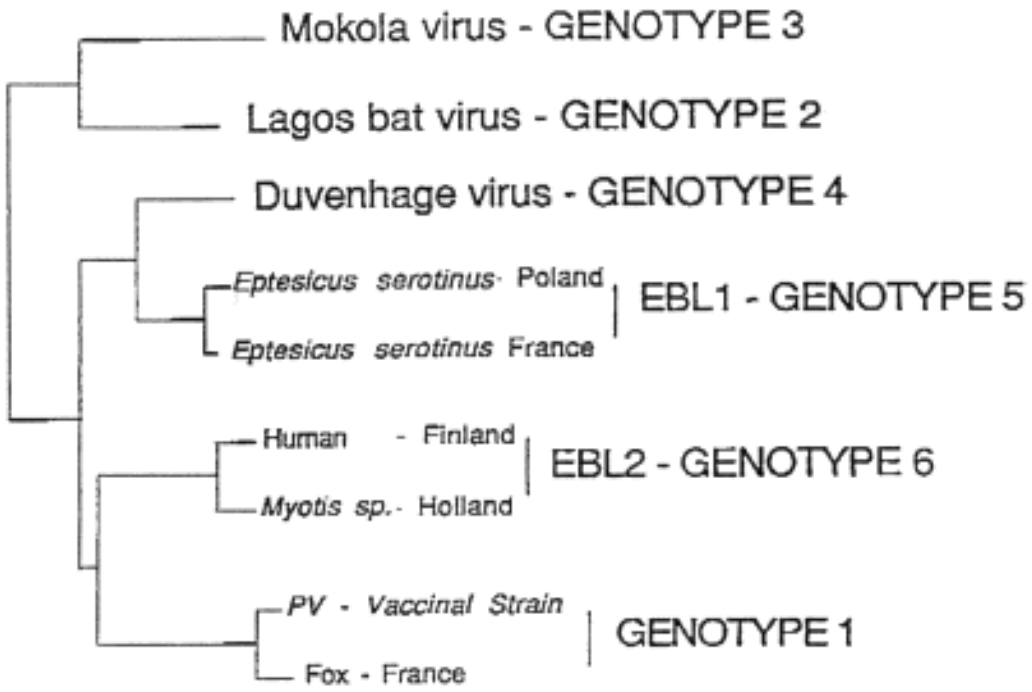
In the non-protein coding regions, the structure of the presumed leader (+) gene and the 5' start site and 3' stop signal of the Mokola virus mRNAs have been reported (Bourhy, et al. 1989; Bourhy, et al. 1993). The non protein-coding regions of the nucleoprotein and phosphoprotein mRNAs of other 10 rabies and rabies-related viruses were studied. All the lyssavirus mRNAs start with the consensus (AACANNNCT) and ends with the consensus polyadenylation signal (WGA7) (Bourhy. et al. 1993) and both of these sequences are consistent with the canonical transcription signals of all the rhabdoviruses thus far studied, be the host species mammals, fish or plant.

Thus our study shows that rabies-related viruses share the same genomic organisation as the rabies virus. Within this common scheme, only slight differences in the length of genes and intergenic sequences are observed. The comparison of the nucleoprotein sequences established that the Lyssavirus genus can be divided in at least 6 genotypes and that the molecular diversity of this genus was lower than that of the Vesiculovirus genus. Furthermore, we described two diagnosis methods the PCR and the RREIDlyssa that allow the detection of any lyssavirus in an infected specimen.

Acknowledgements

We are deeply indebted to Arthur King for his help in preparing this manuscript. This is worthy of a bottle of old Napoleon.

Figure 1: Phylogenetic tree of lyssaviruses based on the alignment of the nucleoprotein sequences. The horizontal branch lengths are indicative of the evolutionary distances generated by the ClustalV package of multiple alignment programs (Higgins and Sharp. 1989). The vertical lines are for clarity.



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Discussion of presentations on Diagnosis and Post-exposure treatment' were summarised by Dr. Swanepoel:

New Diagnostic Tools (Dr. Barrat and Dr. Bourhy)

Can straw sampling be carried out on large animals? Yes, one method which is practiced is to drive a six-inch nail in first.

How can autolysed material be handled in the field? This depends upon the importance of the specimen, whether there is human contact, if these samples comes from an endemic area etc.

What is the source of conjugate? There are now different types available, polyclonal and mixed monoclonals.

The sensitivity of immunofluorescence is said to be good but what about the rabies-related viruses? The Pasteur conjugate picks up all rabies-related viruses.

What about false positives? Training in immunofluorescence techniques is important.

What is the reason for false negatives? Again, training is important, but negatives should be repeated and if possible different parts of the brain should be examined.

In response to a question, Dr. Bourhy confirmed that PM virus was not included in his dendrogram of the rabies viruses.

Human Post-exposure Treatment (Dr. Thraenhart)

Indications are that doubling or even trebling of doses is prevalent in Africa, does this mean that doubling or trebling must be used? Interjection by Dr. Meslin (WHO): doubling or trebling of doses is NOT a WHO recommendation.

Why is there a double dosage rate of horse immunoglobulin? Decay of heterologous immunoglobulin is faster, there is a shorter half-life, 3 - 4 days maintenance of half concentration.

Nabs and Post-exposure Treatment (Dr. Schumacher)

Following comments (by Dr. Thraenhart and Dr. Fekadu) that monoclonal antibodies did not protect monkeys and that in similar experiments involving short and long incubation periods in mice no protection was given, Dr. Schumacher said that the vaccine given must be potent, the balance with antibody was important and that WHO recommendations must be followed.

Discussion of presentations on control of canine rabies were summarised by Dr. Gumm:

Control of canine rabies in Africa (Dr. Perry)

- A comment was made that the extrapolated figures given for vaccine cover of some countries may be slightly misleading. If, in fact, as has been seen or mentioned as in Natal, South Africa, vaccination is focal and strategically applied in one spot then the cover is not as bad as it looks.

- The business of making dogs liable to vaccination at three months was based on the use of Flury vaccine, but it actually worked better at 6 - 8 months. With the new inactivated vaccine is there any thought to decreasing that age? Yes, it can be used at less than three months in non-vaccinated dams. In others it might be possible to go down to four weeks.

Vaccine Quality Control (Dr. Rweyemamu)

- Do you propose to establish different potency tests for rabies vaccines? No sir, what I am saying is that we would like to participate actively in the debate on standards for veterinary vaccines, not merely copy standards set for human vaccines.

- This is what was meant, that there should be primers for veterinary vaccines? - FAO should not be excluded from this debate. If you look at the design of the NIH test, which is dependent upon micro-injection, the question is whether it is appropriate to the vaccine in use, which is a single dose then going back a year later. Also, the antigen quantification must take into account that there is adjuvant, it is not a simple transposition of a human vaccine, you have to take into account that adjuvants do have an effect on the dose response.

Do dogs recover from rabies? (Dr. Fekadu)

There were no questions or comments on this paper.

Feasibility of canine rabies control (Dr. Meslin)

Only one comment (by Dr. Rweyemanu) was made. If there was any *indication in* the paper which I presented that I was trying to override WHO recommendations, this is not the case. On regional vaccine production, trade barriers must be removed and monopolies resisted - it must be a free market.

Discussion of presentations on control of wildlife rabies were summarised by Dr. Bishop:

Wild dog rabies (Dr. Gascoyne).

- Were the four Frankfurt wild dogs challenged? Certainly not!
- Did not some of the vaccinated wild dogs subsequently die? In the Masai Mara two dogs vaccinated a year previously with Rabisin developed rabies. Apparently this was due to a breakdown in the vaccine cold chain. Also, vaccination sero-conversion had not been measured. A year after vaccination in the Serengeti there were further deaths - the cause was not established but they might have been from Distemper.

Control by wildlife depopulation (Dr. Aubert)

There were no questions following this paper.

Human Rabies and post-agposure treatment

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Rabies is a public health problem in human medicine, in so far as annually approximately 50,000 humans die from the disease. Most of the persons at risk live in 90 countries with a population of 2.4 billion, where the rabies reservoir is the dog. In this area more than 95 percent of human rabies cases are transmitted by dogs. Consequently, vaccination *of* dogs considerably reduces the risk to man, as has been shown in Europe and the USA.

Vaccines prepared from brain tissues are still used in some parts of the world. Their use should be discontinued as soon as possible, because these brain tissue vaccines are of uncertain potency and may induce severe nervous illness by the myelin content in the vaccine. Therefore the WHO Expert Committee on Rabies (anon., 1992) has re-iterated its recommendation "to limit or abandon completely - where economically and technically possible - the production of encephalitogenic brain-tissue vaccines".

On the other hand, human rabies can be efficiently prevented by the use of concentrated, purified, safe and immunogenic vaccines prepared from cell cultures. Administration of such vaccines in vaccination schemes is recommended by the World Health Organisation. The vaccine is effective for pre- and post-exposure treatment (PET). More than 10 million people annually throughout the world receive PET.

The age distribution of human rabies cases in Ethiopia was reported by Ayalew (1985) and shows that 51 percent of the victims were younger than 20 years of age. Addy (1985) found that in Ghana, the prevalence of human rabies depended in the season of the year: 68 percent of 257 cases occurred during the dry seasons, i.e. May to June and October to November.

Human rabies is a deadly disease which leads to death after onset of symptoms. The incubation period depends upon the site of the bite, as was shown for 257 cases in Ghana (Addy, 1985):

Seven persons with bites on the head or neck had an incubation period of 14 - 46 (average 31) days, 44 persons with bites on the upper limbs died of rabies after 35 - 100 (average 68) days, whilst 28 patients with bites on the lower limbs had an incubation period of 40 -160 (average 100) days.

In 21 cases with bites at more than one site, an incubation period range of 22 - 80 (average 51) days was recorded. Although the incubation period in general may be up to 3 months, incubation of up to 7 years have been observed (Smith et al., 1990).

Table 1 shows the intervals from bite to death of 707 cases in Thailand (Wilde and Chutivongse, 1989).

Table 1.

Interval from bite to death of 707 cases of rabies in Thailand

Interval	*Percentage of deaths*
7 - 10 days	3.2
10 - 20 days	41.9
21 - 28 days	25.8
1 -3 months	16.1
3 -6 months	3.2
6 - 12 months	3.2
0 year	6.5

The range of clinical symptoms recently has been summarised by Fishbein (1991) and is shown in Table 2.

Table 2.

Summary of clinical rabies symptoms

Clinical Status	*symptoms*
Incubation Period	None.
Prodromal	Fever, anorexia, nausea, vomiting, headache, malaise, lethargy, anxiety, agitation, depression, pain or paresthesia at the site of the bite.
Acute neurological phase	Hyperventilation, hypoxia, aphasia, paresis, paralysis, hydrophobia, pharyngeal spasms, incoordination or other signs of the CNS, confusion, delirium, hallucination, anxiety, agitation, depression and marked hyperventilation.
Coma	Pituitary dysfunction, hypoventilation, Apnea, hypotension, cardiac arrhythmia, cardiac arrest, coma.
Death	From pneumothorax intravascular thrombosis, secondary infections.

Vaccines

The WHO Expert Committee on Rabies met in September 1991 and updated their recommendations, which were first laid down in the Technical Series No. 709 (anon., 1984).

Cell-cultured rabies vaccines combine safety with high immunogenicity. Several types of cell cultures may be used:

i diploid cells,

ii primary cell cultures or

iii continuous cell lines

In addition a highly purified duck embryo vaccine offers the same immunogenicity and safety as modern cell culture vaccines.

Diploid cell-culture vaccines, which are produced on WI-38 or MRC-5 cells, are the "golden standard". Whereas the production of these vaccines are very expensive, vaccines produced on primary chicken fibroblast cells (PCEC vaccine of Behring Werke, Marburg, Germany) or on the continuous cell line Vero (PVRV vaccine of Institut Merieux, Lyon, France) are cheaper. These vaccines should have a potency of at least 2.5 IU/dose.

Rabies in humans can be prevented in nearly all cases by the following measures:

i Prevention of bite ("Cave Canem")

ii Prevention of infection in humans by

- Prophylactic vaccination of animals

- Pre-exposure vaccination of humans

- Post-exposure treatment of humans:
  wound treatment
  active (+ passive) immunisation.

i The prevention of a bite by walking with a thick wooden stick as a weapon against offending animals proved to be effective in reducing human rabies in a field trial in Tanzania.

ii For prophylactic treatment with cell-cultured vaccines, the vaccine is administered on days 0, 7 and 28. For adults, the vaccine should always be administered in the deltoid region. For young children, the antero-lateral aspect of the thigh is also acceptable. The gluteal area should never be used for vaccine injections, since administration in this area results in lower neutralising antibody titres.

Pre-exposure immunisation should be offered to persons at high risk of frequent or unrecognised exposures, such as laboratory staff working with rabies virus, veterinarians, animal handlers and wildlife officers. Periodic vaccine booster doses are recommended for persons at continuing risk of exposure to rabies.

PET should be performed according to the 3 categories of infection risk. Which were defined by the WHO Expert Committee on Rabies. The details are shown in Table 3.

Table 3

Post-exposure Treatment
(WHO Expert Committee on Rabies 1992)

Category of infection risk	Type of contact	Post-exposure treatment
I	- touching or feeding of animals - licking of intact skin	No treatment if reliable case history available.
II	- nibbling of uncovered skin - superficial scratch that does not result in bleeding - licking over broken skin	Administer vaccine immediately Stop treatment if: 1. animal (only dog or cat) is healthy throughout observation period of 10 days or 2. animal is euthanized and found FA negative.
III	- single or multiple transdermal bites or scratches - Contamination of mucous membrane with saliva (i.e., licks)	Administer immunoglobulin and vaccine immediately. Stop treatment if: 1. animal (only dog or cat) is healthy throughout observation period of 10 days or 2. animal is euthanized and found FA negative.

The combination of local wound treatment, passive immunisation and vaccination is recommended for all severe exposures (category III) of rabies. In all cases the most important first measure is local wound treatment. Health authorities should train the people in how to perform first-aid treatment which reduces the risk of infection. The treatment is shown in Table 4.

Rabies immunoglobin should be applied as follows:

i for all category III exposures,

ii. irrespective of the interval between exposure and the beginning of the treatment,

iii 20 IU/kg body weight of human or 40 IU/kg body weight of purified equine immunglobulin,

iv as much as possible to be infiltrated around the wounds,

v The rest to be given i.m. into the gluteal region in a single dose followed by

vi a complete course of vaccine.

Table 4

Local mound treatment
(WHO Expert Committee an Rabies 1992)

a) First-aid treatment by layperson

- Immediate washing and flushing with soap and water, detergent, or water alone

- Application of alcohol (70%), tincture or aqueous solution of iodine

b) Treatment by Physician

- Treat as in (a) and then

- apply anti-rabies immune globulin by careful instillation into the depth of the wound and by infiltration around the wound (and the rest i.m. into the gluteal muscle).

- postpone suturing of the wound; if suturing is necessary, use immune globulin locally

- inject anti-tetanus, antibiotics etc., where indicated to control infections other than rabies.

The application of the cell-culture vaccine is performed as in Table 5, which contains those vaccination schedules recommended by WHO.

Table 5

Post-exposure treatment against rabies with cell-culture vaccines

i) intramuscular application

a) Essen - schedule

one dose given at days 0, 3, 7, 14 and 30
into the deltoid region only

b) "abbreviated 2 - 1 - 1 Schedule"

Two Doses given at day 0 in
the right and left arm,

One Dose given at days 7 and 21 into
the deltoid region only

ii) intradermal application

0.1ml on day 0, 3 and 7
at 2 sites

0.1ml on days 30 and 90
at 1 site either fore or upper arm.

Table 6 gives some hints which schedule should be selected.

Table 6

Hints for the selection of a WHO recommended post-exposure treatment schedule

"Essen-schedule"

- most experience with all cell culture vaccines, for categories II and III.

"2-1-1-schedule":

- especially useful for category II, i.e. vaccination without immunoglobin

"intradermal-schedule":

- only recommended for centres specialised in i.d. injection.

  Use for category II and III.

Although cell-culture vaccines are of high potency for effective immunogenicity, it is necessary to maintain their potency by the following measures:

- store the vaccine from import to use in the refrigerator (+4 to +8°C): cold chain!

- USE LIQUID VACCINE AS SOON AS POSSIBLE after reconstitution of the lyophilised vaccine;

- if some reconstituted vaccine is not used immediately, it can only be used later if:

  it is stored at +4 to +8°C and

  it is used within the same day of reconstitution.

Due to the advances made during the last decade it is possible to prevent human rabies by the use cell-culture rabies vaccine, one of the most immunogenic and safe vaccines known. To obtain this goal it is necessary to solve the local problems of availability of vaccine and immunoglobulin. Only economical difficulties prevent the world-wide use of cell-culture rabies vaccines.

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Treatment of Rabies with House Anti-rabies Monoclonal Antibodies.

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Effective postexposure treatment of rabies as recommended by the World Health Organisation includes the prompt use of human or equine anti-rabies immunoglobulins (HRIG or ERIG respectively) together with the administration of rabies vaccine. Animal experiments have demonstrated that treatment with vaccine alone does not prevent lethal rabies virus infection in post-exposure situations (Koprowski et al., 1950; Sikes et al., 1971). Anti-rabies antibodies appear to be an essential component in the treatment of rabies, although the precise role of such antibodies in postexposure treatment is unclear.

Because of the adverse effects ranging from local reactions at injection sites to anaphylaxis connected with the use of ERIG, only HRIG is ideally recommended for the postexposure treatment of humans. However, because of the high cost of HRIG and the high incidence of human rabies exposures, there is often insufficient amounts of HRIG available to complete the full recommended postexposure treatment. Limited access to HRIG is probably a major contributing factor in the increasing number of postexposure treatment failures (anon., 1988; Wilde et al., 1989).

We investigated the potential usefulness of anti-rabies monoclonal antibodies (Mabs) of murine origin in postexposure treatment of laboratory animals. Based on the knowledge that anti-rabies antibodies play a significant role in protection against rabies, five Mabs of the Wistar Mabs collection were selected according to the following criteria:

1. Mabs should belong to the IgG-isotype, since IgM-antibodies show no in-vivo activity (Turner, 1978).

2. The majority of the Mabs should recognise the rabies glycoprotein and neutralise a broad spectrum of rabies and rabies-related viruses.

3. The anti-glycoprotein antibodies should differ in their epitope specificity to prevent the escape of neutralisation resistant viruses (Lafon et al., 1983).

4. Some Mabs should recognise the rabies nucleoprotein because anti-N antibodies have been shown to regulate the immune response of T-lymphocytes (Celis et al., 1985) and

5. The activities of the Mab-cocktail should be similar or identical to the ones found in human rabies immune globulin (HRIG).

Five characterised Mabs, produced as previously described (Schumacher et al., 1989), were mixed to give a Mab-cocktail as shown in Table 1.

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Table 1.

Characteristics of Mabs used in this Study

Mab No.	Isotype	Antigenic and epitope specificity	Specific Virus-neutralising activity IU/mg	Virus strain specificity
509-6	IgG2a	G site I	1,667	Neutralisation of all Serotype 1 viruses* except some virus isolates from bats.
1112-1	IgGl	G site IIc	61	Neutralisation of all Serotype 1 viruses and Duvenhage strains 1-6.
523-11	IgG2a	G site IIb	8,242	Neutralisation of all Serotype 1 viruses except some street virus isolates from bats and foxes, neutralisation of Mokola 3.
802-1	IgG2b	N site II	0	Binding to all Serotype 1 virus strains and some rabies-related viruses.
502-2	IgG2a	N site 111	0	Binding to all rabies and rabies-related viruses.
Mab-cocktail		G, N	1,376	ND

*all fixed and street rabies virus strains.

The Mab-cocktail represents a mixture of 112.5IU/ml of Mab 509-6; 10IU/ml of Mab 1112-1; 800IU/ml of Mab 523-11; 0.04mg/ml of Mab 802-1 and 0.6 mg/ml of Mab 502-2, to give a total of 1,376 IU/mg.

The protective effectivity of these murine Mabs was determined in six-week-old female white Swiss mice. Various dilutions of individual Mabs or of the Mab-cocktail were inoculated intramuscularly, followed by a lethal dose of the Challenge Virus Strain (CVS) rabies virus at different times.

Whereas pre-treatment of mice with N-specific Mabs did not increase survivorship as compared with untreated controls, administration of glycoprotein-specific Mabs and Mab-cocktail 24 hours before challenge effectively prevented a lethal rabies infection. The biological activity of the antibodies varied greatly and in vivo activity did not correlate with in vitro activity. This suggests that protection is not simply conferred through neutralisation of extracellular virus, but mechanisms such as complement-mediated lysis of rabies virus infected cells or antibody-dependant cellular cytotoxicity might also play a role (Davis and Metzger, 1983).

This theory is supported by the observation that removal of the constant region of Mab 523-11 reduces the in vivo activity of the F(ab')2 fragment 14-fold in comparison with the intact antibody, while the in vitro neutralising capacities remain comparable (Table 2).

Table 2.

Passive Protection of ICR mice with G-protein specific Mabs

Mab dose (IU)	Mortality* MAD 1112-1	Mab 509-6	Mab 523-11	$F(ab')_2$ 523-11
100	-	-	0/10	2/11
20	-	1/10	2/11	7/10
4	0/10	3/10	8/10	10/10
0.8	1/10	7/10	10/10	10/10
0.16	0/10	-	-	-
0.032	2/10	-	-	-
ED_{50}	<0.03	2.0	2.36	34.00

Mice were challenged intramuscularly with 5 x 10^6 $MICLD_{50}$ of CVS 24hrs. after Mab treatment.

When mice are immunised with vaccine, the immune system responds at both the cellular and humoral levels (B-cell antibodies, T-helper cells, cytotoxic T-cell, Interleukins, Natural Killer cells, TX, etc.). However, even today little is really known about the mechanisms which lead to such immunity. The site of virus inoculation greatly affects the dose of the Mab-mixture necessary to protect mice against a lethal challenge. For example, five times the amount of Mab was required to protect mice challenged by inoculation into densely innervated tissues such as footpads, as was required to protect mice against the same challenge inoculated into the popliteal muscle (data not shown).

Experimentally, two peripheral injections of vaccine (i.e., active immunisation) induced an immune response which protected 6 of 7 mice against a lethal intracerebral challenge (Table 3). The titre (GMT) of neutralising antibody required to achieve this protection was 768. In contrast, when the Mab-cocktail was injected (i.e., passive immunisation), leading to a circulating neutralising antibody titre (GMT) of 3992, only 1 of 7 mice was protected from the same lethal intracerebral challenge (Table 3). This again indicates the complexity of the immune response to rabies, most likely involving humoral as well as cellular factors.

Table 3.
WA Titres and Mortality Rates After Immunization
with BPL-inactivated ERA Virus or with Mab Treatment.

Immunization with *BPL-ERA*[1]		*Mab treatment*[2]	
VNA Titre[3] (GMT, n=7)	Mortality	VNA Titre[3] (GMT, n=7)	Mortality
768 (90 - 3,125)	1/7	3,992 (2,430 - 7,290)	6/7

1 Mice were immunised with 2ug of BPL-inactivated ERA virus on days 0 and 7, and intracerebrally challenged on day 14 with 5×10^4 $MICLD_{50}$ of CVS.

2 Mice were treated with 200 IU of Mab-cocktail and 24hrs later challenged intracerebrally with 5×10^4 $MICLD_{50}$ of CVS.

3 Mice were bled 2 hrs before challenge.

Post-exposure treatment with Mabs was rendered difficult by the short incubation period (5 - 6 days) of the mouse adapted challenge virus strain CVS. In contrast, hamsters with an incubation period of 10 - 14 days and raccoons (minimum 21 days) were effectively protected against a lethal intramuscular infection when treated 24 hours after challenge (data not shown). Post-exposure protection was antibody-dose and serum-titre dependant and correlated directly with the biological half-life of the antibodies.

When we combined the injections of Mabs with the application of inactivated rabies vaccine in a post-exposure-like fashion we observed a suppressive effect of the circulating Mabs on the generation of an active humoral immune response to vaccine in mice and rabbits. As this effect was dose dependant, did not affect the T-helper cell compartment and could not readily be overcome by multiple booster vaccination we attributed this effect to the formation of antigen-antibody complexes, which exert a tolerizing effect on the immature B-lymphocyte population and therefore prevents the maturation of antigen-specific antibody secreting plasma cells (Schumacher, 1992). In true post-exposure treatment a similar phenomenon has been observed and was reported to be avoidable by combining protective doses of antibody preparations with highly immunogenic vaccines (Turner, 1980).

In conclusion it can be stated that because of their high specificity and efficacy, their purity and consequently their low risk of side effects, the possibility of product standardisation and quality control, the permanent availability and the lack of contaminating agents as compared with human immune globulin preparations (HIV, Hepatitis B), murine Mabs could be useful for post-exposure treatment, provided a cost effective way of production can be found.

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THE EPIDEMIOLOGY OF DOG RABIES AND ITS CONTROL IN EASTERN AND SOUTHERN AFRICA

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Introduction

Dog rabies has been reported with apparent increasing incidence in the eastern and southern African region over the last twenty years, and many countries are currently documenting record numbers of confirmed cases. Furthermore it is generally agreed that the disease is grossly under-reported, both in dogs and in man, and the extent of underreporting may also be on the increase due to a variety of factors. If rabies is to be effectively controlled in the region, it is important that its occurrence is well-documented, its epidemiology is well-understood, and its impact is carefully quantified in order to attract the appropriate level of financial and logistical support from governments, donor agencies and local communities. This paper reviews the main epidemiological features of dog rabies recorded in recent studies in the region, reviews the efficacy of control measures currently in use, and proposes some needs for the future to ensure more effective control of this most severe of zootic diseases.

Epidemiological features of dog rabies

Role of the dog

Numerous of the authors of country reports in this Proceedings have documented the importance of the dog compared to other species with regard to confirmed rabies cases in different species in their countries; the reported proportion of confirmed dog rabies cases diagnosed in the region is shown in Table 1. In general it appears that dogs make up a higher proportion of rabies cases in eastern Africa that in southern Africa; in southern Africa various wildlife species, notably the black-backed jackal *(Canis mesomelas),* the side-striped jackal *(C. adustus)* and the yellow mongoose (*Cynistis penicillata),* play a significant role in certain areas. This may be a real phenomenon, dependent upon factors such as the differing wildlife population densities and land-use systems in the two sub-regions. However, it may be an artificial phenomenon, reflecting better dog rabies control in southern Africa than eastern Africa, and thus a greater relative contribution of wildlife to the total numbers of cases recorded in southern Africa. In Europe and North America, wildlife species became increasingly important in the epidemiology of the disease following the control of dog rabies (Steck and Wandeler, 1980; Perry, 1987). The apparent difference between the two sub-regions could well be a combination of the two factors.

Furthermore, differences in the proportions of dog rabies cases in different countries should be interpreted cautiously due to the different times over which these observations have been made. Some authors listed in Table 1 report results of long-term surveillance, while others report short-term studies.

For example, the proportion of dog rabies cases assessed over the period of 1950 - 1991 in Zimbabwe is 52% (Foggin, 1988; Bingham, 1992), but this proportion was lower during the jackal rabies epidemics of 1979-82 (Foggin, 1988) and 1991-92 (Bingham, 1992). A similar change, in this case an increase in the proportion of dog cases, has recently been reported from South Africa, due to the dog rabies outbreak currently occurring in Natal (Bishop, 1992).

Table 1.
Proportion of total reported rabies cases occurring in dogs in eastern and southern Africa.

Eastern Africa		Central Africa		Southern Africa	
Ethiopia	90%[1]	Malawi	80-87%[2]	Zimbabwe	53%[3]
Kenya	65%[4]	Mozambique	89%[5]	Botswana	16-41%[6]
Tanzania	89%[7]	Zambia	78%[8]	South Africa	55%[9]
				Namibia	20%[10]

For refs. See : 1(Fekadu, 1982); 2(Msiska, 1988); 3(Foggin,1988); 4(Anon,1991); 5(Lopes Pereira et al, 1988); 6(Mosienyane,1988); 7(Machuva,1988); 8(Hussein et al., 1984); 9(Gummow and Turner,1988) and 10(Schneider, 1985).

Incidence of rabies

There has been an increasing incidence of rabies, and in particular dog rabies, reported from many countries of the region over the past twenty years, and this is perhaps typified by the incidence figures reported for Kenya over the period 1958-90 (Figure 1).

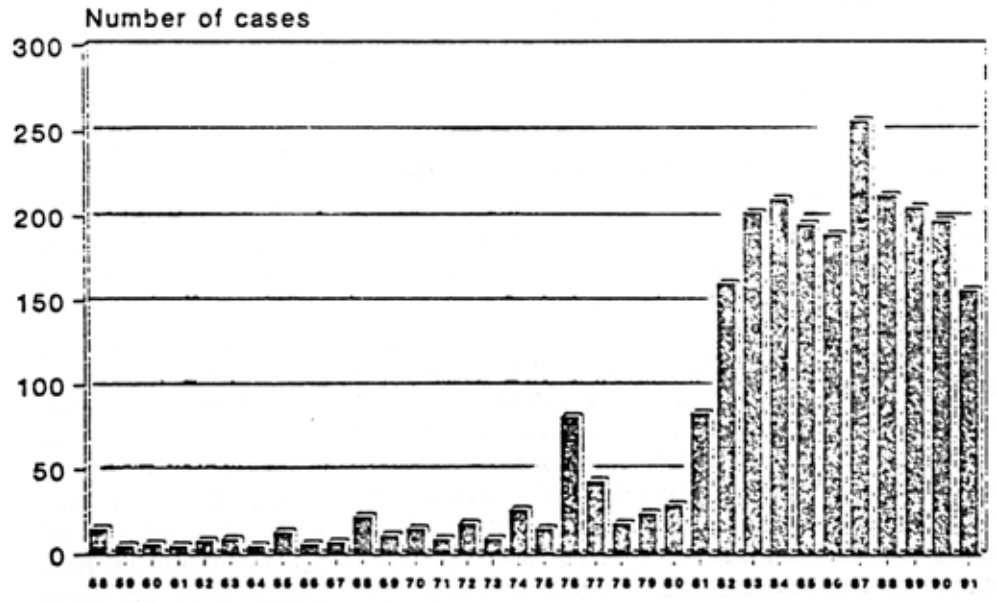

Fig. 1. Incidence of rabies (all species) in Kenya 1958 - 1990

However, incidence figures are influenced by several factors, not just increasing numbers of cases per unit of population, and many of these have been operating in the region. It is thus important to differentiate between real changes in incidence, and apparent changes which are the results of other changing factors, such as changes in the population size or structure and changes in diagnostic efficiency.

True incidence rates are a function of the dog population at risk of rabies, (incidence is the number of new cases divided by the (dog) population at risk (Thrustield, 1986; Martin et al., 1987), but for most countries of the region the size and structure of the dog population is not known or even estimated. For countries there dog population surveys or censuses have not been carried out, estimations may be made on the basis of the ratio of dogs to humans, as human census data and population projections are usually available. Bögel et al. (1982) estimated dog:human ratios of from 1:8 to 1:11 under different circumstances and conditions in the world, although clearly these ratios vary widely both within and outside these ranges depending on numerous factors.

In his census of the dog population of Zimbabwe in 1986, Brooks (1990) estimated the ratio to be 1:6.3, and comparing this with dog population figures published by Adamson in 1954, Foggin (1988) calculated a rate of growth of the dog population over those 32 years to be 5.15% per annum, considerably greater than the 2.94% growth of the human population of Zimbabwe over that period. Clearly throughout the region the dog population has increased dramatically over the last 30 years, and this must be taken into consideration carefully when assessing apparent changing disease incidence rates.

Apparent changes in rabies incidence are also caused by changes in sample submission rates or policies, and by changing diagnostic capacity. This was most dramatically illustrated in the Uganda country report of these proceedings, where recorded rabies cases over the three decades of 1961-70, 1971-80 and 1981-90 were 226, 132 and 12 respectively. This apparent decline in rabies incidence is in total contrast to the marked increase in cases seen in neighbouring Kenya in the latter period (Kariuki, 1988), and is reportedly the result of both decreased sample submission due to national insecurity, and to declining diagnostic capacity due to lack of reagents and equipment (Illango, 1992).

Other epidemiological features

Perhaps the most comprehensive study of dog rabies epidemiology in Africa was that recently carried out by Foggin (1988) in Zimbabwe, and the reader is referred to this work for extensive discussion of rabies in southern Africa. Among other things, he analysed data accompanying rabies submissions over the period 1950 - 1986, in which he included an analysis of data on age-specific incidence, incubation period, clinical signs and the interaction of rabid dogs with other species. He reported that almost 76% of confirmed rabies cases were in dogs over 12 months old, and only 4% of cases were under three months of age. Relating the age-specific incidence to the results of the Zimbabwe dog census performed in 1986 in which 20% of dogs were found to be under three months of age (Brooks, 1990), he concluded that the low incidence of rabies in dogs of this age group, a group that is not legally required to be vaccinated in that country, is unlikely to pose a significant threat to the control of dog rabies.

Incubation periods were calculated from case-history forms accompanying 77 confirmed cases where the contact which resulted in infection was confirmed, and these are presented in Table 2. They fall within the range known for dogs.

Table 2.

*Incubation period of rabies in 68 dogs in Zimbabwe.**

Incubation period (weeks)	No. of dogs
<2	4
2 - 3	39
4 - 5	14
6 - 7	5
>7	6

Mean incubation period 3.6 weeks, Range: 10 days - 12 weeks.
*(From Foggin, 1988).

Foggin (1988) classified the clinical signs reported in rabies positive submissions into three main groups; those showing aggressive behaviour, those showing predominantly paralytic signs and those not fitting into either of the first two groups (Table 3). The mean reported duration of signs was 5.7 days (range 2 - 12 days). The proportion of paralytic rabies was lower than that generally recorded in dogs, and Foggin suggested that this might have been due to his classification as furious any dog that attempted to bite at any stage during the clinical course.

Table 3.

*Signs shown by vaccinated and unvaccinated dogs with confirmed rabies in Zimbabwe**

Sign	Vaccinated	Dogs with rabies (unvaccinated)	Percent of total
Typical furious rabies or tendency to bite	83	2298	75.9
Paralytic rabies without marked behavioural changes	57	180	7.6
Signs not conforming to either group	39	327	11.7
Signs unknown or not recorded	7	145	4.8
Total	**186**	**2950**	**100.0**

*(From Foggin, 1988)

Of 687 rabid dogs for which data were gathered for the period 1982-86, 193 were observed attacking and biting other dogs. Foggin (1988) suggests that this observation of one dog in five biting at least one other dog is probably a gross underestimate of the number actually exposing other dogs, as few would have been under close supervision during the entire course of the clinical disease. Very few of these observed rabid dogs (about 3%) exposed other domestic animals.

The control of rabies

The WHO Expert Committee on rabies describes five basic elements of dog rabies control (WHO, 1992). These are:

Epidemiological surveillance
Community education and participation
Immunisation
Dog control
Organisation and implementation

In this paper, I will discuss two of these elements, immunisation and dog control, and review methods for evaluating their efficacy.

Immunisation

i Vaccines used

Many countries of the region have developed the capacity to produce rabies vaccines for dogs, and these include Kenya, Mozambique, South Africa and Zambia. Vaccines produced in these countries have generally been the low egg passage (LEP) and high egg passage (HEP) vaccines; the lamb brain vaccine was produced in Zambia. However, with the greater international availability of well-tested and relatively cheap vaccines for dogs from commercial sources, there has been an increasing trend to use these rather than prepare vaccines locally, and it is understood that LEP vaccine is no longer produced in Kenya and South Africa and the lamb brain vaccine is no longer available in Zambia. Some countries, such as Zimbabwe, have relied entirely on the importation of rabies vaccines since the egg-adapted vaccines became available in the early 1950s.

Rabies vaccinations of dogs are generally provided by government veterinary services free of charge to the majority of the dog owning population, although some countries, such as Kenya, levy a nominal charge (for Kenya it is KSh.2, or about 6 US cents). Some urban residents of the region obtain rabies vaccinations of dogs through private veterinarians at commercial prices.

A number of rabies vaccines, both attenuated and recombinant, have induced neutralising antibodies when given to dogs by the oral route, but to date none of these has been field tested (WHO, 1992). At least two bait systems for delivering oral vaccines to dogs have been tested in Africa, one in Zimbabwe (Perry et al., 1988) and one in Tunisia (Kharmachi et al., 1992). Oral rabies vaccination provides an alternative technology for the future to supplement traditional immunisation procedures, particularly for the neighbouring dogs, but strategies for delivering these must be carefully designed and tested. The WHO (1991) has developed protocols for such studies.

ii Targeting vaccine delivery

The delivery of rabies vaccine to dogs is generally conducted by Veterinary Departments, the frequency and logistics of which vary from country to country. In many countries vaccination is carried out on an annual basis, and for rural populations this is often delivered in conjunction with other livestock vaccinations at cattle dip tanks or vaccination centres. In general, the onus is on the dog owner to present dogs for vaccination, and little or no targeting at specific segments of the dog-owning populations is carried out.

The WHO has recommended the use of a classification system for dogs, based on their level of dependence on humans for food, shelter and companionship, and on the level of restriction or supervision imposed. This system is designed to provide the classification that can be used for the improved targeting of rabies control measures to specific components of the dog-owning population (WHO, 1988). This classification is shown in Figure 2. For example, the restricted dogs and the family dogs are highly accessible for immunisation, but may also be the target for dog removal if not immunised in a vaccination programme. True feral dogs are removed if possible. The term stray dog, commonly used in the past, should be used to describe a dog not in compliance with local regulations (WHO, 1988). A straying dog may be a feral dog, an abandoned or lost animal or merely a free-roaming family dog.

	FULL RESTRICTION *Dog is physically separated from the rest of the population on a permanent basis*	SEMI-RESTRICTION *Dog has access to the rest of the population some of time*	NO RESTRICTION *Dog has free access to the population at all times*
FULL-DEPENDENCY Dog given all of its essential needs intentionally by humans	RESTRICTED DOG	FAMILY DOG	
SEMNI-DEPENDENCY Dog is given a proportion of its essential needs intentionally by humans		NEIGHBOURHOOD DOG	NEIGHBOURHOOD DOG
NO DEPENDENCY Dog is given none of its essential needs intentionally by humans			FERAL DOG

Fig. 2. Classification of the dog-owning population.

In addition, it has also been shown that knowledge of dog population ecology can enhance rabies control programmes and study methodologies have been developed and tested, (WHO, 1990). Of particular importance air total population size, proportion of dogs accessible for immunisation by a given vaccination strategy, and population turnover. However, very few such data have been gathered within the eastern and southern Africa region. An exception to this is the study of Brooks (1990) in Zimbabwe, and the results of this work will provide a useful baseline for future comparative studies in other countries of the region. A brief summary of his findings are presented in Table 4.

Table 4.

*Summary of a dog census in Zimbabwe**

Parameter	Value
Average age	2.3 years (female:2.0, male:2.5)
Life expectancy	4.6 years (female:4.0, male:5.5)
Surviving puppies /female/year	1.4
Dog density	
-by province	1.4 - 6.7 per sq.km
-by land use	
commercial	0.6 per sq.km
commercial	6.0 per sq.km
urban	20.0 per sq.km
Dog:human ratio	1:8 to 1:4.7 (by province)

*(From Brooks, 1990)

The total dog population size of Zimbabwe in 1986 was found by Brooks to be 1.3 million, providing an overall dog: human population ratio of 1:6.3. Although this is a slightly larger ratio than that discussed by Bögel et al. (1982), the close comparability illustrates the validity of using such ratios in estimating dog population sizes. Table 5 shows the estimated and projected human population of Zimbabwe for the years 1969, 1982 and 1986 (World Bank, 1990) and compares the derived estimated dog population figures for those years, using the ratios proposed by B69el et al. (1982), with the 1986 dog census data.

Table 5.

Estimated human population of Zimbabwe 1969 – 1990* *and calculated dog population based on dog:human ratios and dog census figures for* 1986.

		Estimated dog population		
Year	Human population	1:8	1:11	Dog census (Brooks,1990)
1969	5.1	0.64	0.5	-
1982	7.5	0.94	0.7	-
1986	8.5			1.3
1990	10.0	1.25	0.9	

*Figures derived from World Bank, 1986, in millions.

iii Assessing vaccination coverage

Several authors have suggested that dog vaccination coverage levels of 60-80% are required to effectively control rabies, and WHO proposes a target for vaccination coverage of 70% of the dog population (WHO, 1987). Foggin (1988) proposed that a sustained vaccination coverage of 50% of dogs would be sufficient to keep dog rabies to an acceptable and possibly controlled level. These coverage levels are not based on results of controlled studies, but although effective vaccination coverage levels will depend on numerous factors, including potential contact rate between infected and susceptible dogs and thus dog densities, these figures provide pragmatic and practical targets for local and national campaigns.

Clearly, it is important to be able to assess vaccination coverage levels at both national and sub-national levels. Five possible methods for assessing *vaccination coverage* are presented and discussed.

a) Vaccine doses used as a proportion of dog population

A useful rough estimate of coverage may be derived on the basis of the number of doses of vaccine distributed as a function of the estimated dog population. If reasonably accurate data on dog population size are not available, these may be calculated on the basis of estimated dog:human ratios, and the values of from 1:8 to 1:11 proposed by Bögel et al. (1982) provide a good starting point. Using human population estimates of the World Bank (1986), and vaccine issue statistics derived from Luusah (1988), Machuva (1988) and Msiska (1988), I have calculated rough dog population sizes and estimated vaccination coverage for Kenya, Tanzania and Malawi, and these are illustrated in Table 6. The highest value calculated by this method is for Malawi in 1980 (12.9%), and the lowest value rate was for Kenya in 1979 (2,4%).

Clearly these are crude estimates, but they do raise interesting questions relating to the efficacy of rabies control programmes. In Zimbabwe, using dog census data and vaccine doses issued, Brooks (1990) calculated a national vaccination coverage of 40% for 1986,and using a similar method to that described here, Bishop (1992) estimated a coverage of about 35% in South Africa for 1991. It is significant to note that all of these fall well below the 70% level advocated by WHO (1987), and the 50% level proposed by Foggin (1988). In Kenya for example, current vaccine issue is approximately 300,000 doses annually (Gituma, M.G., personal communication, 1992). Coverages of 70% or 50% in 1992 for that country would require 1,610,000 and 1,150,000 doses respectively, based on an estimated dog:human ratio of 1:11 and projected human population statistics derived from the World Bank (1986).

Table 6

Estimated human and dog population sizes and dog vaccination coverage for three countries in the region

		Kenya	**Tanzania**	**Malawi**
population (millions)				
1969		11.0	12.3	4.0
1979		15.3	17.5	5.6
1990		25.0	27.0	8.0
Estimated dog populations (millions)				
1969	1:8	1.4	1.5	0.5
	1:11	1.0	1.2	0.36
1979	1:8	2.0	2.2	0.7
	1:11	1.4	1.6	0.5
1990	1:8	3.2	3.4	1.0
	1:11	2.3	2.5	0.73
Estimated rabies vaccination coverage, based on dog: human ratio of 1:11				
1969		4.3		
1978			5.3	
1979		2.4		
1980				12.9
1987				5.0
1988			3.9	

b) Vaccinal status of rabies submissions

The only recorded use of this method in Africa has been reported by Foggin (1988), who analysed the reported vaccinal status of dogs submitted for rabies examination during the period 1983 to 1986. He found that 14.3% of the 1206 dogs submitted had been vaccinated according to the legal requirements of Zimbabwe, and in a further 8.8% the vaccination had expired. The level is considerably lower than the 40% vaccinal cover calculated and reported by Brooks (1990) for 1986, but it is of course to be expected when examining dogs suspected of rabies, a sample biased in favour of being unvaccinated.

c) Antibody prevalence

Antibody prevalence data have been used, along with other indicators, to assess the vaccination coverage afforded by a mass vaccination campaign in Lima, Peru (Choel et al., 1987). These authors sampled 616 dogs chosen randomly in four age cohorts, and serum was tested by the rapid fluorescent focus inhibition test. One year after vaccination, 97% of sampled dogs had titres of greater than 0.IU/ml, although the prevalence of titres greater than 5IU/ml declined from 64% at 2-3 months after vaccination to 40% at 12 months after vaccination. In the eastern and southern African region, Foggin (1988) carried out a serological study to evaluate the effects of the breakdown in animal health services during the independence war in Zimbabwe. In 1981, one year after the end of the war, 1,806 dogs were bled in seven areas of the country, and tested using the rapid fluorescent focus inhibition test. Overall, 11.8% of sera had neutralising antibody, but there was considerable variation by area, ranging from 0% - 22%.

d) Comparison of vaccine use with rabies incidence

The comparison of rabies vaccine doses issued or used with reported dog rabies incidence offers a potential tool for assessing the efficacy of vaccination procedures, but it has rarely been used effectively in the region for several reasons. Firstly, vaccine quantities issued to the field are not necessarily an indicator of demand, but often an indicator of the availability of government resources, often in foreign exchange, to purchase vaccines, and of the priority given to rabies control. Secondly, if less than 15% of the dog population are being vaccinated, as estimations presented earlier in this paper suggest may be occurring in the region, vaccination is unlikely to have any measurable effect on the numbers of rabies cases reported at national level. Thirdly, the efficacy of this technique will also depend on the long-term consistency and efficacy of the rabies diagnostic service.

Msiska (1988) provided data on the recorded rabies cases and dog vaccinations for Malawi over the period of 1978 to 1987, and commented that "there is no direct relationship between the number of positive cases and the number of animals vaccinated". Approximate calculations presented earlier in this paper suggest that vaccination coverage in Malawi may have been in the order of 13% and 5% for the years 1980 and 1987 respectively (Table 5).

When Bleakley (1987) compared the number of reported dog rabies cases in Zimbabwe with the number of free vaccinations carried during the latter stages of the independence war, a period when animal health services were severely disrupted, she demonstrated a definite inverse relationship. During the period 1968-72, Foggin (1988) estimated a vaccine coverage rate of 59%. These observations suggest that a clear relationship between dog rabies occurrence and vaccinations carried out can only be demonstrated where vaccination levels have been sufficient to affect national rabies incidence rates.

e) Marking vaccinated dogs

The technique of estimating population size by capturing animals, marking them, releasing them and recapturing them, either visually or in traps, has been used extensively with wild animal populations (Seber, 1973; Davies, 1982). The technique has been adapted to dog populations in urban and rural settings (Beck, 1973; 1979).

Recently, Perry et al (in preparation) used a captive/recapture method to estimate the proportion of dogs vaccinated in a high-density suburb of Nairobi, Kenya. In a five day vaccination programme, 433 dogs were vaccinated and each vaccinated dog was fitted with a nylon collar. One week after vaccination, a team traversed the high-density suburb observing and counting collared and uncollared dogs. Of 339 dogs observed, 242 had collars. Using the Lincoln Peterson method (Seber, 1973), the dog population size for the area was estimated to be 580-635 (with 95% confidence), and the vaccination coverage was thus calculated as 68-75%. This technique offers a simple but effective method of estimating vaccination coverage that has been used in several countries outside the region.

Dog population control

The WHO (1992) describes four methods of dog population control that may be used as adjunct procedures to control rabies. These are confinement, habitat control, reproductive control and removal. Although confinement measures, in the form of tie-up orders, have been used in the past in most of the region, they are rarely applied now, and the only measure in fairly widespread use is dog removal. However, dog removal is rarely applied effectively. To be effective, it should be timed appropriately, ideally following a comprehensive vaccination programme in which vaccinated dogs are identified and marked and the unmarked dogs are subsequently removed efficiently and humanely by shooting or poisoning.

Furthermore, the unmarked dogs, which will generally be neighbourhood dogs, should be reduced to a level at which rabies transmission is interrupted, and this level (which is highly density dependent) should be maintained (WHO, 1992). If this is not achieved, dog removal is likely to be ineffective, and even counter-productive. Where dog removal is used in the absence of an effective immunisation programme, it often removes dogs which are easily accessible for immunisation, and provokes public antipathy to rabies control programmes.

Probably the best illustration of effective dog removal, although unlikely to be reproduced in the future, is from Zimbabwe, where during the rabies outbreak of 1902-13, 60,000 dogs were killed over a two-year period (Sinclair, 1922). It is likely that the total dog population of the country at the time was under 100,000!. Current levels of dog removal are considerably less. Machuva (1988) reported figures for dog removal in Tanzania for the period 1978-88. If the World Bank (1986) human population estimates for the country during those years and the 1:11 dog to human ratio used earlier are taken, crude calculations suggest that annual dog removals have not exceeded 1% since 1981.

Significantly, that 1% of dogs represents over 22,000 animals, and the valiant effort in removing the number of dogs undoubtedly drew on not inconsiderable human and financial resources of the veterinary department. Although it is dangerous to generalise from broad statistics such as these, it is possible that given the rapid turnover rate of dog populations in Africa, the resources used to destroy 1% of the dog population might be more effectively allocated to the vaccination or surveillance aspects of rabies control programmes.

Some selected needs for the future

There are clearly numerous requirements to enable effective rabies control programmes to operate throughout the eastern and southern African region. I have selected four needs that I consider to be crucial.

i An improved understanding of dog ecology

The dog population of the region has grown considerably over the last 30 years, and with it the socio-economic status of its owners or keepers has diversified, making access to dog populations for immunisation a much more complicated process. An effort is required to review dog ecology in the region with the aim of determining the approaches and techniques necessary to reach the desired proportion of the dog population with the desired frequency. Numerous sets of guidelines have been prepared as to how to achieve this (WHO, 1987; 1988; 1990; 1992) and studies based on these have been carried out in several countries outside the region, including Ecuador, Tunisia, Sri Lanka and Nepal. Studies within the region are currently underway in Machakos, Kenya and in Lusaka, Zambia.

ii Co-operation with others at all levels

Rabies control in the region has traditionally been in the hands of the government veterinary authorities, with, in many cases, limited collaboration or communication with the government health authorities. This centralisation of implementation responsibility is unlikely to be sustainable for much longer. Veterinary departments throughout much of the region have undergone considerable changes during the past 30 years. Departmental budgets have declined dramatically in real terms, the numbers of professional staff have increased substantially, and in many countries extremely limited funds remain available for operational activities.

Furthermore, the livestock-owning community has increased in size and diversified, making veterinary services more difficult to deliver under a department umbrella. For the control of diseases that affect livestock productivity, many countries in Africa are moving towards cost-sharing in government-delivered services, and in the development or enhancement of private veterinary services. However, this trend is unlikely to include the control of rabies, a zoonotic disease for which governments will continue to bear a considerable portion of the control responsibility. It is thus crucial that to be effective, the load of responsibility within governments is spread to include departments of health and local government, and the delivery of rabies control is carried out through organised community participation to improve the adaptability and sustainability of dog vaccination programmes.

iii Objective appraisal of problems, resources and appropriate control measures

Rabies control measures in the region have changed very little for much of this century, and certainly since the introduction of the egg adapted vaccines in the early 1950's. However, the dog population, the epidemiology of the disease, and the human and financial resources available to control it have changed dramatically. In many parts of the region, a fresh look at rabies and its control by veterinary authorities in the light of current conditions may well be justified, in order that limited resources can be appropriately allocated to activities that are important and achievable.

iv Put rabies in context

Having suggested that rabies control will continue to be largely a governmental responsibility, it will be important to ensure that appropriate funding levels are available to achieve this. Both veterinary and health authorities of the region have an increasing number of demands on their services, and often a decreasing level of resources to meet these demands. It is therefore of great importance that rabies control is put in context with the other demands on veterinary and health services, and this is done by means of socio-economic studies that include benefit: cost analysis. Throughout the region, studies are required that calculate and document the cost of the disease (in financial and human terms), the cost of control programmes, and the benefits to be derived from effective control. The results will allow disease control planners and others to put rabies in a more appropriate context within the broader concerns of animal disease control, livestock production and human health.

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PATHOGENESIS OF RABIES VIRUS INFECTION IN DOGS:

DO DOGS RECOVER FROM CLINICAL RABIES?

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Introduction

Dogs are the principal transmitter of rabies to man and animals in most countries of Africa, Asia and South America, where they are responsible for over 95% of the human rabies cases reported world-wide (WHO, 1989). The clinical course of rabies in dogs and that it could be transmitted by bite has been known for over 4000 years (Tierkel, 1975). After the virus is introduced to the body by bite or inoculation, it is assumed to remain at the site of entry for a long time (Baer and Cleary, 1972). The virus has been shown to replicate locally, prior to its centripetal transport to the brain (Baer and Cleary, 1972; Murphy and Bauer, 1974; Baer et al., 1968). During the transport period, specific rabies antibody is not detected before signs of illness appear, apparently because insufficient viral antigen is presented to key organs to trigger the immune system.

Pathogenesis in the dog

Incubation period

The incubation period of canine rabies varies from 7 days to many months. The length of incubation period apparently depends on several factors, including the site of exposure (inoculation), the infecting dose and the virus strain (Fekadu et al., 1982).

In dogs experimentally infected with various doses of dog rabies virus strains to simulate natural infection and the incubation periods ranging between 7 and 125 days, depending on the dose and strain used, dogs were observed for at least 2 years to register unexpectedly long incubation periods (Fekadu et al., 1982). Some researchers reported that dogs died 8.5 months after challenge (Tierkel et al., 1975). Although these findings show that the incubation period is mainly dose-dependent, implying that the long incubation periods observed in naturally infected animals may be attributable to exposure to very small amounts of virus, the variation in incubation periods in naturally infected animals remains to be documented.

Clinical signs

1. Prodromal phase

During the prodromal phase, the dog's behaviour may change. Aggressive and high-strung dogs may become more affectionate than usual, and ordinarily friendly dogs may become shy and seek secluded areas or become snappy and irritable. The dog's temperature may rise slightly, the pupils may dilate and the nictating membrane may cover the eye. The dog may also salivate excessively.

2. Excitable phase (furious rabies):

Sighs of the disease are most easily recognised during this phase. The dog becomes severely agitated and restless and sometimes gets an urge to roam. The dog is most dangerous at this stage because of its urge to bite anything it encounters. In most cases, an altered phonation (a characteristic high pitched bark) develops, caused by paralysis of laryngeal muscles. The dog has difficulty swallowing because of spasms and paralysis of the pharyngeal muscle, causing the animal to drool. If the dog does not die during one of the characteristic convulsive seizures, the disease usually progresses to muscular incoordination, paralysis, coma, and death.

3. Paralytic phase (dumb rabies):

Dumb rabies occurs when the excitable phase is extremely short or absent. The most characteristic sign is the "dropped jaw" caused by paralysis of the masseter muscles. The animal often makes choking sounds as if a bone were stuck in its throat. Attempts to remove this "bone" often result in owners scratching their hands on the dog's teeth and being exposed to the disease.

The clinical signs of rabies in humans and animals have been known for centuries, but our knowledge of the pathogenesis of rabies in the principal vector, the dog, is still very limited. To elucidate some of the factors involved in the virus-host relationship in this animal, we inoculated dogs intramuscularly with varying doses of representative strains of street rabies virus from dog salivary gland suspension from rabies endemic areas of the Americas and Africa, to simulate natural infection (Fekadu et al., 1982; Fekadu and Shaddock, 1984).

The incubation and morbidity periods in these experimentally infected dogs, ranging from 9 - 125 days, depended on the dose rather than on the virus strains used. Seventy percent of the experimentally infected dogs developed the dumb form of rabies, 12 percent the furious form and 18 percent died without showing any signs of disease. In this experiment it was shown that the intramuscular lethal dose for a dog was 32 mouse intracranial lethal doses, not as high (86,000 $MICLD_{50}$) as previously reported (Fekadu and Shaddock, 1984; Vaughn et al., 1965).

Excretion of Virus

The particular time of salivary virus excretion *before* sickness is crucial, since transmission may occur when the animal appears normal and no preventive measures are taken. The failure to appreciate the significance of such normally acting but infective animals can result in delayed diagnosis and possible fatal results in those persons exposed. Rabies virus is usually present in the saliva when clinical signs appear, but in some studies prior to 1970 rabies virus was also demonstrated in the saliva of dogs 3 - 6 days before clinical signs appeared (Vaughn et al., 1965).

Frequency of salivary gland infection

The frequency of salivary gland infection in experimentally or naturally infected rabid dogs has been reported to vary from 61 percent to 75 percent. In one experiment in 1982, however, in which dogs were inoculated peripherally with graded doses of canine virus (to simulate natural dog-to-dog transmission) the excretion of rabies virus in their saliva depended not only on the strain but also on the dose of inoculum (Fekadu et al., 1982). At necropsy, 67 - 83 percent of dogs with positive salivary glands excreted virus in their saliva before and during illness and 25 - 100 percent of the dogs in each group had virus in the salivary glands at death, depending primarily on the dose of inoculum.

Pre-symptomatic excretion time

The pre-symptomatic excretion time of the experimentally infected dogs ranged from 1 to 14 days. Of the 39 inoculated dogs that died, 37 percent excreted virus in their saliva 1 - 14 days before onset of illness, and an additional 10 percent excreted virus after onset. However, 64 percent of rabid dogs had virus in their salivary glands, depending on the incubation periods and dose of inoculum.

Almost all (95 - 100 percent) of the dogs inoculated with a lower dose (30 - 300 $MICLD_{50}$) had virus in the salivary glands, while dogs inoculated with high doses rarely did. The canine rabies virus strains used in this experiment seem to be excreted long before onset, unlike the fox or dog strains used earlier (Vaughn et al., 1965; Fekadu et al., 1982).

The possible transmission of rabies from a rabid animal to a human usually depends on the presence of the virus in the salivary glands (at death or at sacrifice) and its possible excretion in the saliva. However, the failure to demonstrate virus in the salivary glands of rabid animals at death does not always exclude the possibility of virus having been excreted in the saliva; rabid animals may on rare occasions excrete virus in the saliva, yet have no virus in the salivary gland and brain at death (Fekadu et al., 1983).

Carrier state

Apparently healthy dogs in the field have been reported to intermittently excrete rabies virus in saliva. These observations in naturally infected dogs have recently been confirmed in an experimentally inoculated dog that recovered without any supportive treatment, then intermittently excreted virus in its saliva for up to 305 days after recovery (Fekadu et al., 1981). The excretion of virus in the saliva of such apparently healthy dogs may play a role in perpetuating the virus in nature and transmitting the disease (Fekadu et al., 1983). Tonsils may also play an important role as the sequestration site and source of excretion.

PATHOLOGY

1. Light microscopy

Despite the striking clinical manifestations of rabies in both humans and animals, rabies shows little or no grossly visible pathologic changes. Meningeal vessel congestion is often the only visible abnormality and it may be quite marked, but subarachnoidal haemorrhage is rarely seen. Cerebral oedema, common in most viral encephalitides, may be mild or absent.

The most common changes observed by light microscopy are mild to severe perivascular infiltration (cuffing) with lymphocytes, few plasma cells, and macrophages; focal and diffuse lymphocytic and granulocytic infiltration of meninges, as well as focal concentrations of lymphocytes and plasma cells beneath the ependyma and in the stroma of choroid plexus. Neuronal degeneration varies from minimal to severe; satellitosis and neurophagia of isolated neurons as evidence of early necrosis of these cells are rarely observed (Baer, 1975; Jubb and Kennedy, 1963; Tangchai et al., 1970).

These changes are common in many viral encephalitides, however, and are not specific to rabies. The only pathognomic lesion characteristic of rabies is the cytoplasmic inclusion body described by Adelchi Negri (Negri, 1903); through the immunofluorescent technique (Goldwasser and Kissling, 1958), the Negri body was documented to contain rabies nucleocapsid antigen.

The degree of inflammation of the brain and, less commonly, the spinal cord, is directly proportional to the length of the incubation and morbidity periods. Intracytoplasmic inclusion bodies are present in the pyramidal cells of the hippocampus and occasionally in the Purkinje cells of the cerebellum. Other areas where Negri bodies are readily found are the brain stem, pons, cerebral cortex and cerebral part of the spinal cord. Sixty to 100 percent of experimentally infected dogs have Negri bodies, depending upon the virus strain and the dose of inoculum (Fekadu et al., 1982).

2. Electron microscopy

At the ultrastructural level the pathological changes in the CNS also vary, ranging from moderate to severe. Inflammatory infiltrates and focal disruption of myelin are common. Some neurons contain from small, granular, finely fibrillar, viral matrices to numerous matrices, varying in size and shape. These are accompanied by prolific numbers of virus particles budding from membranes of the rough endoplasmic reticulum and occasionally from the outer lamella of the nuclear envelope and the plasma membrane (Fekadu et al., 1982).

The eosinophillic ground substance (inner body) of the Negri body observed by light microscopy was also shown to be identical to the matrix seen by electron microscopy, corresponding to the site of virus replication (Matsumoto and Miyamoto, 1966; Matsumoto, 1975).

3. Peripheral distribution

Rabies virus spreads from the site of infection to the CNS and back to the peripheral organs via the nerves. The dissemination of virus in peripheral tissues depends on the dose of inoculum and the length of incubation periods. A large inoculum produces a short incubation period and a rapid course of illness, leading to death before spread of virus throughout the brain. After long incubation periods, virus is distributed in many parts of the body.

The ultimate distribution of viral infection depends upon the virus strain and the dose of inoculum used. The amount of antigen demonstrable in tissues varies markedly, depending on the dose of inoculum. Virtually every organ examined may have viral antigen, confirming previous reports in experimentally infected rodents. Viral antigen was occasionally demonstrated in most internal organs including the kidney, intestine, and bladder. Virus from the gastrointestinal mucosa, pancreas, or liver, may possibly be excreted but would most likely be inactivated by digestive enzymes. The most important source for rabies transmission is, therefore, the saliva and the oro-nasal secretions (Fekadu and Shaddock,1984).

More specifically, virus was detected in salivary glands in only 25 percent to 40 percent of dogs inoculated with a large dose, compared with almost all dogs inoculated with a small dose. In naturally infected dogs the presence of virus in salivary glands ranges from 75 percent to 100 percent, suggesting that the amount of virus introduced by bite is low.

The most important factor in the transmission of rabies is the presence of virus in the saliva and salivary glands, especially during the period before detectable signs of disease appear. Previously, virus excretion in the saliva of dogs was considered to occur during or just before the appearance of signs, but in a recent experiment, we reported that dogs inoculated intramuscularly with canine strains of street rabies excreted virus in their saliva up to 14 days before signs appeared. In one dog that recovered after inoculation with an Ethiopian street rabies virus strain, rabies virus was excreted intermittently in its saliva for 305 days after recovery (Fekadu et al., 1981; Fekadu and Baer, 1980).

Excretion of virus in saliva depends on its presence in the salivary glands. Although tonsils have been shown to play an important role as the sequestration site and source for rabies virus excretion, in one dog that intermittently excreted virus in its saliva for months after recovery, the only tissue from which live virus was isolated at necropsy was the tonsils.

Tonsils from other animals experimentally infected with various isolates of rabies virus were examined for the presence of viral antigen in the tonsils in relation to salivary glands and brain. The findings show that tonsils are infected at higher frequency than the salivary glands (Table 1). In dogs inoculated with either insectivorous bat or with dog street virus strains, up to 20 percent recovered without supportive treatment; these recoveries were neither dose nor virus strain dependent (Fekadu, 1988).

Table 1.

Detection of rabies virus in tonsils compared with other tissues in different animals inoculated with street rabies virus strains

Animal		*Virus*	*Positive Tissues*				
Species	*No.*	*Strain*	*Tonsils*	*Saliv.Gld.*	*Muscle*	*Skin*	*Brain*
Dogs	14	Dog	12	9	5	5	14
Foxes	12	Fox	12	11	8	7	12
Raccoons	10	Raccoon	5	5	0	1	10
Skunks	6	Skunk	4	4	NT*	3	6
Monkeys	16	Fox	16	is	13	15	16

* NT = not tested

Resistance to challenge

Dogs that resisted street rabies virus challenge without signs of disease and failed to develop a virus neutralising antibody (VNA) titre, were challenged after a 2 year observation period. They developed a rather high anamnestic serum VNA response and resisted challenge, indicating that factors other than VNA induced by rabies G protein play a role in protecting dogs from rabies infection (Fekadu et al., 1992). It has also been reported that animals with WA titres succumbed when challenged, whereas others which had no detectable amounts of antibody survived (Lodmell et al., 1969).

In addition, the role of cell-mediated immunity has been noted in recovery from attenuated rabies virus infection in mice, in which T cells were stimulated by both rabies G and N proteins (Miller et al., 1978). VNA, therefore, may not be the only factor involved in recovery from rabies.

The mechanism of such recoveries, however, is still not well understood. Recovery from rabies has been reported in only a limited number of humans and animals since Pasteur's time. Pasteur was the first to report that dogs occasionally recovered from rabies, and he considered subsequent resistance of these dogs to reinfection as a strong indication of previous abortive infection.

The most commonly used criterion for detecting non-fatal rabies infection is the isolation of virus from saliva, brain, or other tissues of animals that recover after sickness. Brain biopsy specimens have even been taken from humans for virus isolation tests to confirm a diagnosis of rabies when apparent recovery from rabies had occurred.

However, a high VNA titre in the brain or the CSF has been shown to be the only definitive diagnostic test for demonstrating recovery from CNS rabies infection in humans and animals (Bell et al., 1966).

Sickness and recovery

In order to elucidate the role, especially in sickness and recovery, of the different rabies virus proteins in the pathogenesis of rabies in dogs, we inoculated dogs with preparations of various rabies virus proteins. Dogs were vaccinated with the rabies G-protein alone, a combination of G and the rabies nucleoprotein N, or the N protein alone, prior to peripheral challenge with a street rabies virus (Fekadu et al., 1992).

All dogs vaccinated with rabies G protein or G plus N proteins developed VNA titres. Dogs vaccinated with N protein alone, however, had no detectable VNA titre prior to challenge, and the levels of antibody directed against N protein were low (Tables 2 and 3).

Table 2

Serum VNA titres of dogs vaccinated i.d. with either Vaccinia virus recombinant expressing rabies N protein, G protein or G plus N proteins or Vaccinia virus

		*Serum MA titre (WIC) at a given week post vaccination**						
Group		*1*	*2*	*5*	*8***	*9*	*10*	*12*
I	(N)	<0.1	<0.1	<0.1	<0.1	0.3****	1.5	1.5
II	(G)	0.3	1.5	1.5	1.5	37.0	37.0	37.0
III	(G + N)	<0.1	1.5	1.5	1.5	37.0	37.0	37.0
IV	(Vaccinia virus)	<0.1	<0.1	<0.1	<0.1	<0.1	0.3	NT*****

* = all titres were <0.1 at day 0. ** = challenge date
*** - one of the seven dogs in Group I had VNA 1 week after challenge
**** - Not tested.

Table 3

Rabies virus N antibody titres in dogs vaccinated with a vaccinia virus recombinant expressing the rabies virus N protein determined by ELISA against rabies R protein

	Absorbency determined at 1:100 scrum dilution	
Dog No.	*Pre-challenge*	*Post-challenge*
27	0.639	2.391
84	0.573	2.141
366	0.555	1.436
24	0.490	1.891
36	0.660	1.586
40	0.651	1.875
65	0.490	0.761

Five of seven dogs vaccinated with N protein alone developed clinical rabies 11 to 14 days after challenge. The incubation periods in these dogs were 3 to 7 days shorter than those of the control dogs (Table 4).

Table 4

Sickness and recovery in dogs vaccinated i.d. with Vaccinia recombinants expressing either rabies virus N protein, G protein, G and N proteins simultaneously or vaccinia virus.

Group	Incubation period (days)	Sickness	Death survival	Recovery
I (N)	11-14	5/7	2/5	3/5
II (G)		0/5	0/5	
III (G + N)		0/5	0/5	
IV vaccinia virus	14-21	6/6	6/6	0/6

The difference in incubation periods between controls and those vaccinated with N protein was statistically significant ($p < 0.02$). All control dogs died. of the seven dogs vaccinated with N protein, only two died of rabies (although five had sickened). Viral antigen was demonstrated by FA in the brain tissue of all the dogs that died. The remaining three dogs in group I gradually recovered without supportive treatment, after a morbidity period ranging from 7 to 12 days. Recovery was confirmed by the presence of VRA titres in the CSF collected after the disappearance of clinical signs (Table 5), whereas none of the dogs vaccinated with the G protein, G + N proteins or the controls developed any detectable amounts of VNA in the CSF.

All dogs that were vaccinated with G or G + N had a high booster response 5 days after challenge, but only one of the dogs vaccinated with N developed a VNA titre 8 days after challenge. A similar VNA titre was also detected in one of the unvaccinated controls (Table 5).

Table 5.

MA titres of dogs vaccinated with vaccinia virus recombinant expressing rabies virus N protein and then challenged with street rabies virus

Dog No.	EMA titres (IU/ml)1 Post-challenge serum	Post-recovery serum	CSF
27	8.2	14.1	12.9
84	0.1	7.3	1.4
366	0.6	12.9	10.1
24	<0.1	<0.1	<0.1
40	0.6[2]	NT[3]	NT
65	<0.1[2]	NT	NT

1=Pre- and post- vaccination serum titres were <0.1 in all cases
2=Dogs that died 3NT - not tested

Protection, sickness or survival of dogs vaccinated with the N protein was not dependent on the titre of VNA or antibody against N protein prior to challenge, as dogs with detectable amounts of antibody died whereas others that had no VINA survived challenge (Table 5).

Our study shows that all dogs vaccinated with G protein were fully protected and developed a high booster response after challenge. Five (71 percent) of the seven dogs vaccinated with the N protein survived challenge with or without signs of rabies. Two (28.5 percent) of the seven N-protein vaccinated dogs that did not sicken tailed to develop VNA after challenge, unlike dogs vaccinated with G protein, indicating that priming dogs with N protein may not induce higher VNA response, contrary to previous reports (Dietzschold et al., 1987).

Three of five N-protein vaccinated dogs (60 percent) sickened and recovered without supportive treatment and developed high VNA titres both in the serum and CSF, indicating that N protein may not only be involved in induction of T cell response (Miller et al., 1978; Mifune et al., 1981), but also in sickness and recovery (Fekadu et al., 1992).

The specific role of N protein in sickness and recovery in dogs is not clear. The mechanism of protection against rabies challenge in the absence of virus neutralising antibody could be attributed to the induction of cytolytic T cells as well as T helper cells that support the activity of virus-neutralising antibody-producing B cells; or by promoting the attachment of anti-N antibody via Fe receptor to phagocytic cells, which are then stimulated by the infecting (challenge) virus to produce cytokines that inhibit viral replication.

Conclusions

Most dogs experimentally infected with street rabies virus developed clinical signs of rabies before death, but up to 18 percent of our dogs died without showing detectable signs of illness. In those showing signs, rabies was not invariably fatal. Up to 20 percent of dogs recovered without any supportive treatment.

The pathological changes observed depended mainly on the inoculum dose; small virus doses produced longer incubation periods and resulted in more pathological changes.

Some dogs inoculated with dog strains excreted virus in their saliva up to 14 days before signs appeared. There was no relationship between the time of virus excretion in the saliva and the morbidity period, or the titre of virus in the salivary glands at death.

One dog that recovered from clinical rabies intermittently excreted rabies virus in its saliva for 305 days after recovery. The carrier state in rabies may possibly play a role in perpetuation and survival of the virus and may become a source for rabies outbreaks whenever a new population of rabies-susceptibles reaches the critical density.

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Rabies and African wild dogs *Lycaon pictus*

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Introduction

This paper describes an outbreak of rabies in an endangered species, the African wild dog (*Lycaon pictus*) and discusses the significance of the disease from the perspective of wildlife conservation.

The African wild dog is among the most endangered large carnivores in Africa. The species formerly ranged widely throughout Sub-Saharan Africa but now, in 19 countries, they have disappeared entirely (Ginsberg and Macdonald, 1990). Where they can still be found, they exist at very low densities. In the entire 30,000 km^2 region of the Serengeti in Tanzania, there are probably fewer than 100 African wild dogs, a population which is becoming increasingly confined to the protected wildlife area of the National Park. Such a small, isolated population faces numerous threats to its survival (Goodman, 1987).

Disease is an extreme form of environmental variation or catastrophe that can have devastating consequences on persistence for small populations. For the Serengeti African wild dog population, disease poses one of the major threats to its survival, not only because their numbers are so low but also because the species is highly social. The entire population exists in only three or four packs. Within these packs all individuals interact frequently, for example by mouth-licking and by regurgistrating food to pups (Estes and Goddard, 1967; Schaller, 1972) which is likely to facilitate transmission of any infectious pathogen.

Rabies in Serengeti Region, Tanzania

Between 1954 and the late 1970s, no cases of rabies were reported in the Serengeti area of Tanzania (Rweyamanu, 1973; Magembe 1985). However, in the late 1970s an epidemic spread north through the country, reaching the Serengeti region in 1978 (Magembe, 1985). In this outbreak over 90 percent of cases were reported in domestic dogs, with sporadic cases in wildlife. It was not until the late 1980s that there was laboratory confirmation of rabies as a cause of death in wildlife in the Serengeti National Park, with mortality in bat-eared foxes *(Otocyon megalotis)* (Maas, in preparation).

Subsequently, in the Masai Mara Game Reserve in Kenya, part of the Serengeti ecosystem, rabies was confirmed in a pack of wild dogs. This outbreak occurred in 1989, with three confirmed cases and mortality in 20 out of 22 dogs in one pack (Kat and Richardson, per. comm.). In the following year rabies was isolated from a Serengeti wild dog carcass in a pack that subsequently disappeared.

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In this case in the Serengeti, one adult and six pups found feeding on the carcass of a dead adult appeared lethargic and weak, with hind-limb ataxia. The adult was followed for 24 hours and showed clinical signs including restlessness, progressive hind-limb paralysis, abnormal tail and ear carriage, frequent yawning and one episode of salivation. He exhibited abnormal behaviour, such as lying out in the sun in the middle of the day and chewing persistently on skulls he encountered. No signs of aggression were observed. The adult, which had previously been fitted with a radiocollar, went underground the same night. Despite extensive aerial and ground radiotracking over the next two days, he could not be found and he is presumed to have died underground. The pups and other pack members have not been seen again.

Rabies Diagnosis

Brain stem samples were collected from the adult wild dog carcass by inserting straws through the occipital foramen, using World Health Organisation (WHO) collection kits (Barrat and Blancou, 1988). Samples were preserved in 50 percent glycerol saline and sent to the WHO Collaborating Centre for Research and Management in Zoonoses Control, Malzeville, France for diagnosis.

Diagnostic tests included direct immunofluorescence, mouse inoculation (Kaplan & Koprowski, 1973) and inoculation of murine neuroblastoma cells (Barrat et al., 1988). All tests were positive for rabies. Typing of the isolated virus was carried out at the Central Veterinary Laboratory, U.K., using a panel of antinucleocapsid monoclonal antibodies (King, 1991). The virus was identified as Serotype 1 with a reaction pattern closely similar to canid-associated rabies virus from southern Africa (King, 1991).

Vaccination of Lycaon pictus in Serengeti

Hall and Harwood (1990) suggest that the following guidelines should be followed when vaccinating wildlife:

i) assessment of potential effect of disease on the population

ii) the overall aims of the vaccination programme

iii) the availability and trial status of a possible vaccine

iv) assessment of associated risks

v) the feasibility and design of a vaccination programme.

With respect to these points, Tanzania National Parks considered the following information to be relevant when making the decision to implement a rabies vaccination programme in the Serengeti wild dog population:

i) Based on data from the Masai Mara, part of the Serengeti ecosystem, the potential impact of rabies in wild dogs was known to be severe, causing high mortality in packs.

ii) The aim of vaccination was to reduce mortality in an endangered population.

iii) For safety reasons, only an inactivated (dead) vaccine was considered acceptable for an endangered species. A trial vaccination programme was carried out on seronegative captive African wild dogs in Frankfurt Zoo using an inactivated vaccine (Madivak; Hoechst) known to induce an antibody response in foxes (Neukirch et al., 1978).

Following intramuscular injection of 1 ml of vaccine in this trial, no adverse effects were recorded in any individual. Five weeks after vaccination, serum samples were collected from four wild dogs and serological analyses were carried out at the Veterinäruntersuchungsamt, Frankfurt using an ELISA (Mebastion et al., 1989). All four animals had seroconverted, three individuals having rabies neutralising antibody titres of 2 International Units (IU) /ml and the other with an antibody titre of 4 IU/ml (Frost, unpubl. data). No challenge experiments were carried out as the Frankfurt Zoological Society considered this to be unjustified in an endangered species.

iv) In the Serengeti, it was considered feasible to inoculate each animal by administering vaccine in a projectile dart. Possible risks included dart impact injuries, injection site reactions and disruption of the pack. These were considered to be acceptable risks in comparison with the threat of rabies infection.

V) The known surviving population of wild dogs in the Serengeti comprised 38 individuals in two packs and it was considered feasible to vaccinate every individual within these packs. Radiocollars had been fitted to two members of each pack, allowing the group to be located. Wild dogs can be approached to within 10 metres in a vehicle and dart inoculation of individuals was considered a feasible method of vaccine administration.

Vaccination protocol

Two dogs from each pack were anaesthetised for fitting or removing radiocollars. 62.5 mg xylazine (Rompun; Bayer), an average dose rate of 2.5 mg/kg and 50 mg ketamine (Vetalar; Parke Davis & Co.), an average dose rate of 2.0 mg/kg were administered using air-pressurised darts (New Softy, Distinject) fired from an air-powered dart gun (K1inke Model 3/2). Blood samples were taken from the saphenous vein and serum was separated and frozen within a few hours. 1 ml inactivated rabies vaccine (Madivak; Hoechst) was administered by intramuscular injection in the quadriceps femoris. Intravenous injection of 0.5 mg of the alpha2 -antagonist RX821002A (Reckitt & Colman) was administered to reverse anaesthesia.

1 ml vaccine was administered to all remaining dogs in the pack older than 12 weeks by darting into the shoulder muscle mass. Pups in one pack were vaccinated after 12 weeks of age.

Neither pack was greatly disturbed or disrupted by the darting procedure. The observed effect on each individual was minor and fairly consistent. Individuals usually jumped and occasionally yelped when hit by the dart and then ran about 10 metres. In a few cases they did not flinch. Once the dart had fallen out or was pulled out, individuals settled down quickly.

The packs were closely observed after vaccination, each animal being checked for signs of injection site reaction, lameness, injury or systemic illness. No adverse effects were recorded in the immediate 48 hour period after vaccination.

Attempts were made to locate and monitor each pack on an approximately monthly basis by aerial and ground telemetry. Between 1 and 5 months, one vaccinated adult disappeared. At eight months after vaccination, at least four vaccinated adults died (confirmed by retrieval of radiocollars). Although the skeletons of these carcasses were found, no soft tissues could be retrieved and a laboratory diagnosis could not be determined.

Serological Analysis

Pre-vaccination serum samples, collected from 12 dogs from several packs, were analysed to measure baseline rabies virus neutralising antibody titres. These samples included sera from dogs anaesthetised for fitting, replacing and removing radiocollars during the previous two years, using the same protocol as described above. Between 28 and 59 days after vaccination, three individuals were anaesthetised for collection of post-vaccination blood samples to assess antibody response to vaccination. One of these dogs had been vaccinated by intra-muscular injection and two had been vaccinated by darting.

Serological analysis for rabies virus neutralising antibody was carried out at the Central Veterinary Laboratory, U.K. Rabies virus neutralising activity was measured using a modified rapid fluorescent focus inhibition test (Smith et al., 1973). Titres were expressed in IU/ml determined by comparison with a standard antiserum.

Results

The results of the serological analyses are shown in Table 1. Five out of 12 individuals, from several packs, had titres of rabies neutralising antibody >0.5 IU/ml. With the exception of dog DHPM, who has never been seen since the serum sample was collected, all seropositive individuals were alive a minimum of five months after the blood samples were taken.

Post-vaccination samples were all greater than 1.0 IU/ml, a level that is considered to be indicative of successful rabies immunisation in foxes (Blancou et al., 1986). In the 2 paired serum samples (dogs M and SF), increased antibody titres were seen subsequent to vaccination. The increase was substantially greater in the hand-vaccinated animal than in the dart-injected animals.

Table 1
Serum neutralising antibody titres to rabies virus in African wild dogs in the Serengeti National Park, Tanzania

Wild dog Ref.	*Pack*	*Date sampled*	*Pre-vacc. titre(IU/ml)*	*Post-vacc. titre(IU/ml)*	*Days post-vacc.*
LIMP	Naabi/Salei	14.5.88.	1.1		
LEGS	Naabi/Salei	22.5.89.	1.1		
MBILI	Naabi/Salei	1.9.90.	0.37		
SF[1]	Salei	16.1.90.	0.37	1.1	28
M[2]	Salei	1.9.90.	1.1	10.0	28
D583	Ndolha/Ndutu	17.7.89.	<0.21		
VY	Ndoha	11.9.90.	<0.21		
FLEUR	Ndoha	11.9.90.	0.64		
N685[1]	Ndoha	9.11.90.		1.92	59
N188	Ndoha	19.1.91.	0.37		
DHPM	Hill	26.2.90.	0.64		
DBGM	Border	17.2.90.	<0.21		
DMDM[3]	Mountain	24.5.90.	<0.21		

Salei pack was vaccinated on 1.9.90 and the Ndoha pack on 11.9.90.

1 Inoculated by dart
2 Inoculated intramuscularly by hand
3 Radiocollared male who showed clinical signs described here.

Discussion

Wildlife is a valuable resource in many African countries. Although the many National Parks throughout the continent protect wildlife from human persecution and habitat loss, small endangered populations are still clearly threatened by diseases, such as rabies. In order to identify disease threats and to implement appropriate control measures, there needs to be increased surveillance of disease in wildlife. This necessitates close liaison between National Parks staff and Veterinary officers and the establishment of effective protocols for submitting wildlife samples to diagnostic laboratories.

Of many potential pathogens threatening Lycaon and potentially other endangered canids, rabies is of particular concern. Firstly, the disease results in high mortality and secondly, the virus is a generalist pathogen. Although the density of the Serengeti African wild dog population is undoubtedly too low to maintain rabies independently within the ecosystem, the disease has the potential to infect a wide range of species.

Thus, *Lycaon is* continually at risk from spill-over transmission from the principal host species, either directly or indirectly Although spill-over infections in dead-end hosts are not epidemiologically important in terms of rabies transmission and maintenance, the consequences for an endangered species may be catastrophic.

Domestic dogs have comprised by far the majority of reported rabies cases in the Serengeti region (Magembe, 1985) and are the most likely source of infection for *Lycaon.* The virus isolated from the Serengeti wild dog, a Serotype 1 canid-associated virus, was furthermore indistinguishable from the virus isolated from a domestic dog in an area adjacent to the Serengeti National Park (King, unpub. data). The contact rate between *Lycaon* and domestic dogs is not known, however the potential exists for rabies transmission between the two species, either by direct contact or indirectly via other carnivores. With the growth and expansion of human populations in the Serengeti region, domestic dog numbers are likely to be increasing and contact rates between domestic dogs and wild carnivores are probably rising. The Serengeti *Lycaon* vaccination programme was implemented as a short-term solution to a critical situation. However, for long-term regional control of rabies to safeguard wildlife and benefit local communities, control measures need to be targeted at the principal host species, which in Serengeti is probably the domestic dog population.

Vaccination of wildlife is clearly a controversial issue, particularly where endangered species are involved. Although the post-vaccination titres recorded here are associated with protection in other species, it is not known with certainty what degree of protection is conferred in *Lycaon.* Without clear evidence for vaccine failure in this population, however, challenge experiments to determine vaccine efficacy may not justified.

The relative paucity of data presented here reflects some of the logistical and ethical problems associated with investigating disease in wild populations and in the management of endangered species. In this case, for example, it was not considered acceptable to anaesthetise a large number of wild dogs after vaccination for the sole purpose of collecting a blood sample. It was also considered unjustifiable by the conservation organisations involved to carry out challenge experiments on either captive or free-ranging African wild dogs, in order to confirm vaccine efficacy. However, if an intervention involves some risk to individuals, such an intervention may only be justifiable in an endangered species when the beneficial outcome can be confirmed. In this case, given that inactivated vaccines are safe and that the darting procedure interfered with packs to only a minor degree, the potentially beneficial effects of vaccination are likely to have outweighed possible harmful impacts.

In the Serengeti, mortality was confirmed in at least four animals eight months after vaccination. However, no samples could be retrieved and no diagnosis was possible. This clearly demonstrates that disease surveillance and long-term monitoring following any intervention, such as vaccination, is essential in endangered species management.

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CONTROL OF RABIES BY WILDLIFE DEPOPULATION

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In Europe at the beginning of this century, long before any large scale vaccination of dogs, dog depopulation, when applied with compulsory muzzling and movement restraint, was proven to be an efficient method of rabies control. Until recent years, depopulation of other reservoir species was the only feasible measure to break the transmission chain of rabies in wildlife. The intention of control by depopulation is to decrease the density of the reservoir species to a level at which the transmission chain is broken. But the question remains as to the efficiency of depopulation measures. We can divide this question into two:

1. To what extent do depopulation measures decrease the level of target population?

2. How does a reduction of the density of the reservoir species affect rabies epizootics?

Surprisingly, scientific papers dealing with the first question are not numerous. Large scale depopulation measures chiefly have been organised by administrative and veterinary authorities more interested in the final results regarding rabies incidence than in intermediate results such as the level of reservoir population. Evaluating the level of free ranging populations requires specific approaches which were unavailable to early pathologists. Some examples of reduction of carnivora populations can be found outside the field of rabies and from several papers I will summarise the most frequently cited:

Beason (1974) conducted intensive short-term predator removal as a game management trial in South Texas. Over 9 square miles, coyotes, bobcats, raccoons and other carnivora were removed by trapping, *poisoning and* hunting. For the first six months of the years 1971 and 1972, a total of 40,300 trap nights, 12,000 strychnine baits of various kinds and 250 hunting hours were employed by one or two men. In the experimental areas, coyote and bobcat tracks declined until approaching or attaining zero. But when the predator removal programme terminated at the end of June, both species began an immediate increase; before November, experimental areas maintained numbers similar to those of control areas. The predator removal enhanced reproductive success of some game species, but it also enhanced the successful installation of immigrating predators.

On the other hand, when applied in isolated conditions, the removal of predator species produces an effect lasting several years: on two islands in the Gulf of Bothnia (Bergön: 1,800 ha and Ranön: 2,350 ha), Marcstöm et al., (1989) removed foxes and marten, then obtained low densities of both predators for three and seven years after the last removal operations - i.e. at least until the end of the observation period.

In South Dakota over an area of 1,100 square miles, four wildlife control methods for all predator species were employed: bait poisoning (January - February), aerial locating of dens then gassing (May), shooting and trapping (all months) (Trautman et al., 1974)
Interestingly, a by-product of this trial was that among all predator species (fox, badger, raccoon and skunk), the fox was the least reduced, -63%, not very different from the result obtained with raccoons (-68%), but less severe than the reduction of skunks (-81%) and badgers (-88%). (Note: these results were re-calculated from the original data given by the authors).

Control of introduced foxes has also been carried out where it threatens indigenous species. In South Western Australia, Kinnear et al., (1988) monitored five endangered rock-wallaby (*Petrogale latexulis*) populations over a six year period. A fox control programme was maintained for four years over two areas, with the result that the resident rock-wallaby population doubled or tripled, despite the quick replacement of killed foxes, while rock-wallaby population in sites not subjected to fox control declined or did not significantly vary.

Also in Australia, dingoes (*Canis familiaris*) are economically significant predators of domestic livestock and aerial baiting with sodium fluoroacetate (the "1080" compound) has been used for many years for poisoning dingoes. There is some evidence that this method is not very effective compared with the number of baits distributed, partly because of the removal of baits by non-target species (foxes and birds), and partly because of the dogs' preference for natural prey (McIlroy et al., 1986). Within three years after an aerial baiting over 940 km^2 of North Western Australia, the dingo population increased to about its former level (Thomson, 1986).

The main points that can be drawn from these few examples are:

1. Control measures can have a real impact on carnivora populations, but this impact is generally difficult to assess except in small areas.

2. The depressing effect on the level of the population is rapidly compensated by immigration, except in isolated (insulated) populations.

3. In some examples, even when rapidly compensated, the predatory removal can reach its targets (protection of game or endangered species), but results are obtained at the cost of a heavy removal pressure, which also affects non-target species. As far as the more efficient methods of poisoning are concerned, they are also the least specific.

Rabies control mobilises all the available methods: trapping, shooting (by day or by night), gassing dens, poisoning - which may be performed by trappers, hunters, landowners and farmers, with or without the direct support of authorities by bounties or payment of professional teams. (Extended bibliographies can be found in Lewis J.C., 1975 and Debbie J.G., 1991).

The intentional distribution of pathogens other than rabies (for example of canine distemper) specific for carnivora, though conceivable, has never been carried out as it is has no public appeal and is practicably difficult to establish and monitor (Winkler, 1975).

Fox depopulation was reported to have been successfully used in the Dijon area of France during the years 1921-1928. High mortality of foxes was preceded by numerous records of rabies cases in domestic animals. Direct observations of foxes attacking domestic animals which later developed the disease and laboratory diagnosis of rabies in domestic animals and foxes testified to the role played by the fox (Barbier, 1929). Foxes were poisoned with strychnine baits and the last case of sylvatic rabies (a dog bitten by a fox), occurred in 1928. The very detailed paper of Barbier allows a critical study of this epizootic:

- the only baiting campaigns which are recorded were performed in 1926 - i.e. 2 years before the end of the rabies cases in foxes. Whether other poison campaigns were organised after 1926 is not stated.

- as elsewhere in Europe at the time, this region was under the regime of dog enzootics. In addition, just at the beginning of the fox enzootic, the number of rabid dogs had doubled (Fig. 1).

The Dijon epizootic in foxes appeared as a direct consequence of dog enzootics. It can be assumed that the dog virus strain, although highly invasive in the fox population, was not sufficiently adapted to persist in this host.

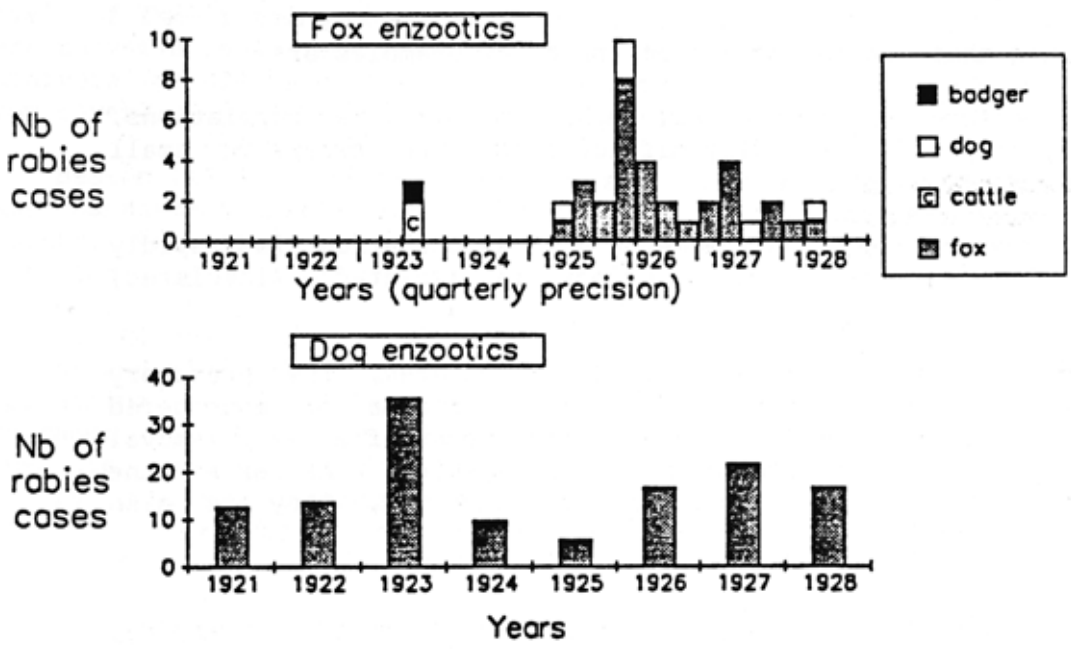

Fig. 1. Rabies outbreak in Dijon area (France) 1921 - 1928.
Above: Fox or fox mediated cases (laboratory confirmed).
Below: Dog cases. Interpreted from Barbier, 1929.

Another claim for the success of fox depopulation is given in reports of the 1945-46 epizootics in Corsica. Likely related with the landing of troops from North-Africa, the first cases occurred in dogs. Then, a great number of foxes were found dead and subsequently rabies affected livestock. Three measures were undertaken: poisoning with strychnine baits, bounties and cartridges, a supply of which was strongly appreciated and appeared to be more inductive than money at this time of restrictions (Janjou, 1948). No definitive argument allows us to conclude if fox rabies was eradicated by artificial depopulation or by rabies itself. It is interesting to remark that, in this situation also, the rabies virus originated from dogs.

A general feature of ancient reports is that although they are very convincing due to the accuracy and the number of testimonies, they do not afford comparative data on the density of wild carnivora before and after the control. Nowadays, the problem remains the same as far as large areas are concerned.

In Alberta, the arrival of fox rabies in 1952 was tackled with large scale operations (Ballantyne, 1958) - over forest areas by trapping and poisoning (6,000 coyote "getters", 429,000 strychnine baits) and over rural areas by poisoning (75,000 cyanide capsules, 163,000 strychnine baits). The mortality was tremendous: 105,000 coyotes, 50,000 foxes, 7,500 lynx, 4,200 wolves, 1,850 bears, 500 skunks and 64 cougars. These huge numbers have been regarded as an example that nobody should follow (Macdonald, 1980). Alberta was freed from rabies until 1971, when skunk rabies arrived.

At least Ballantyne had the honesty to publish not only records obtained of the target species, but also the records of game or rare species destroyed. What was more possible to put before the public at the time and also the only known cure, is more questionable today. Nevertheless, after 1956, the culling campaigns elsewhere in North America or in Europe were even more severe, exceeding largely the rate of 0.17 wild carnivora killed per square kilometre per year reached in Alberta. The Alberta success is another fact which supports our view that a host population infected with an heterologous strain of rabies virus will only ensure an unstable enzootic cycle, i.e. a cycle weak enough to be interrupted by a population reduction programme: during the Alberta episode, rabies largely involved coyotes after the initial importation of the disease by foxes (Ballantyne and O'Donaghue, 1954).

On the other hand, the late settlement of rabies in skunks in the same province was impossible to thwart by similar programmes, although they were able to alleviate its prevalence by comparison with the limited statistics of the neighbouring Saskatchewan (Rosatte et al., 1986).

In Europe, Denmark, taking advantage of its peninsular situation, set against successive outbreaks intense reduction programmes:

- at the first outbreak (1964-65), the spread of rabies was halted 30km north of the southern border after intense gassing. Two protective belts were maintained: a first belt where fox populations were reduced by gassing and shooting until reaching not more than 20% of initial level and a second belt where shooting only was practised.

- the second outbreak of 1969-70 crossed both belts but by extending the gassing belt, rabies was halted 60 km north of the southern border (Müller, 1974). The protective belts were maintained until 1975.
- a third outbreak which occurred in 1977 gradually decreased under the pressure of gassing and ended in 1982. Since then, Denmark, no more under threat, has remained free from terrestrial rabies with no fox control (Rabies Bulletin Europe, 1977-1991, WHO Collaborative Centre, Tübingen).

In Belgium intense gassing operations of fox dens by special teams using cyanhydric acid produced transient reductions of the disease (Marchal et al., 1984). These operations were claimed as successful by the authorities, but debated by others (Collet, 1978) in that gassing was unable to prevent successive invasions of the country (Fig. 2). However, these results should be analysed through the scale of the continent to which they belong.

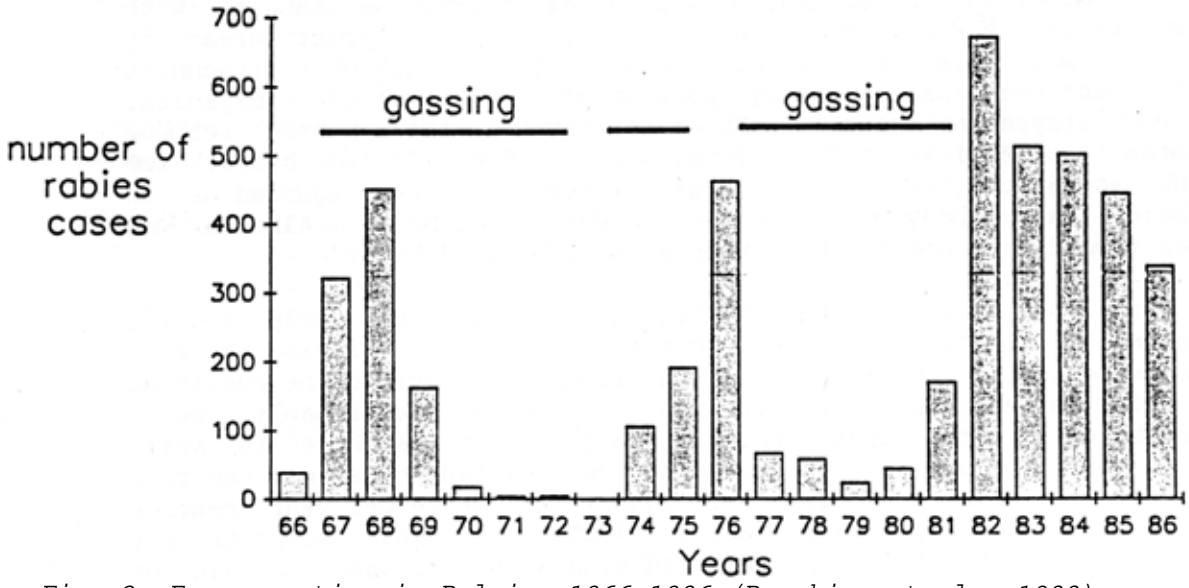

Fig. 2. Fox enzootics in Belgium 1966-1986 (Brochier et al., 1988). Intense gassing led to a decrease in rabies cases but did not prevent new contamination of the area.

In neighbouring France, data collected from administrative units (the "départements") of the same size as the infected Belgian area, although using similar methods, reported differing conclusions. Local authorities which obtained mild success asked for the reinforcement of this policy. Other authorities, not less willing at the beginning but obtaining no conclusive results, became doubtful or discouraged. Figs. 3 to 5, (dotted line indicates limit of enzootic), illustrate the unpredictability of rabies incidence when limited data is considered.

In some départements everything seemed as if the strenuous culling of foxes had entailed longer lasting enzootic episodes compared with départements where fox populations had been left untouched and were actually more efficiently exterminated by rabies itself! This observation was later proved perfectly sound by the mathematical simulations of Smith and Harris (1989): the duration of a rabies incident is increased when 50% of a fox population is culled, compared with no culling at all.

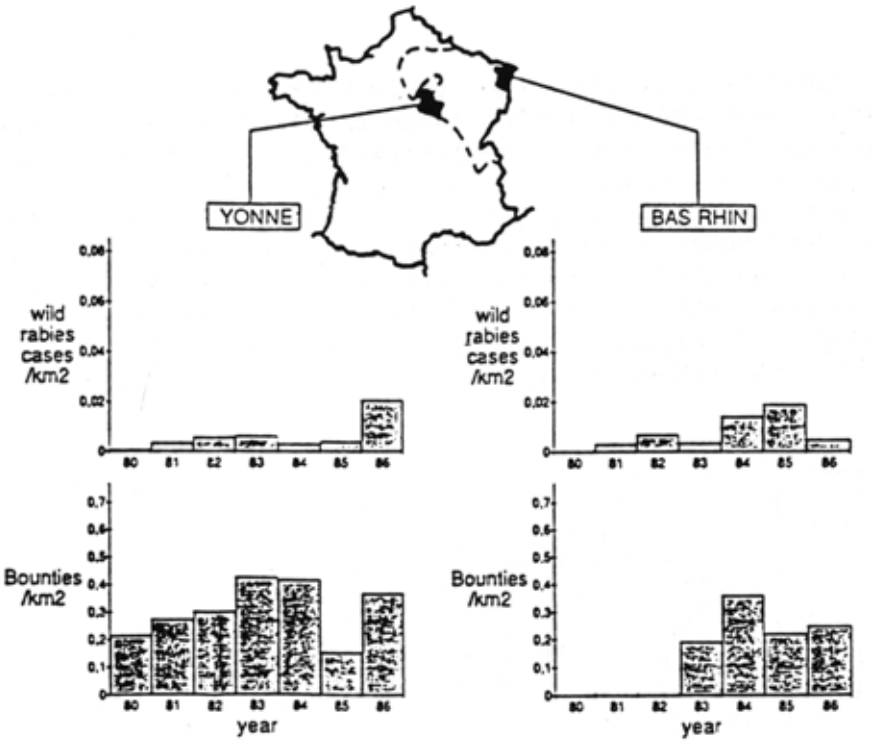

Fig. 3. Rabies cases in wild *species and bounties given.*
In Yonne, the high number of foxes killed did not prevent the disease entering, but the lessening of fox reduction in 1985 was followed by a higher rabies incidence in 1986. In Bas-Rhin, fox destruction was followed by a decrease in rabies incidence after three Years.

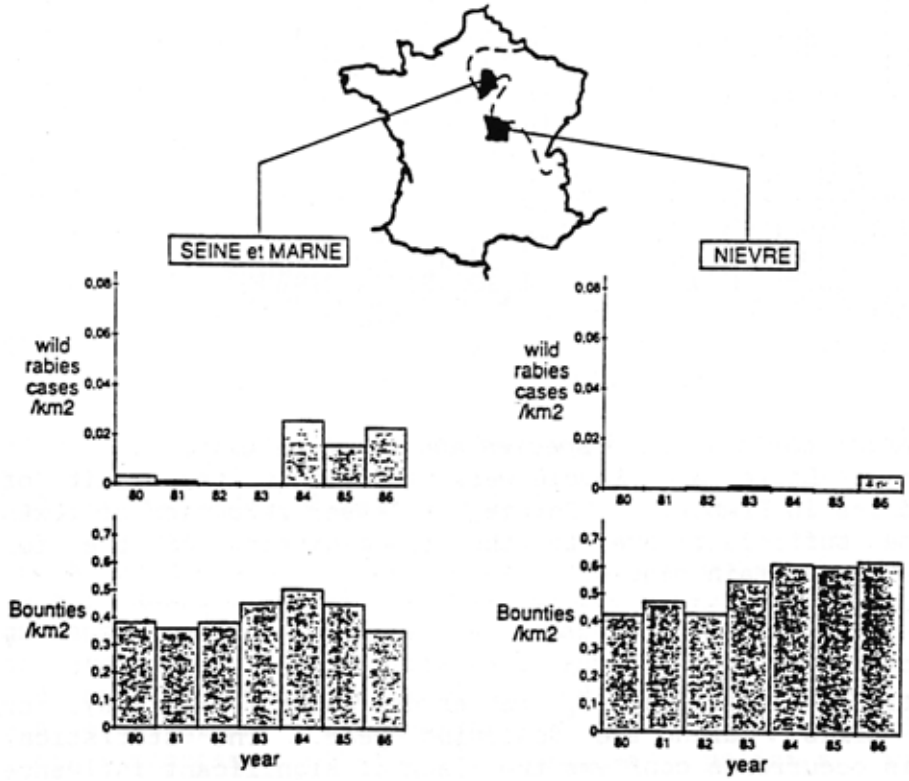

Fig. 4. Cases in wild species and bounties given.
In Seine-et-Marne, fox destruction halted the spread of rabies in 1980-81, but the area was heavily infected from 1984. In Nievre, fox destruction led to a comparable scenario.

Fees (bounties) were paid for the tails of foxes, as a proof of their destruction. Some years, French authorities decided to enforce fox depopulation in some areas, concentrating all of the money available for bounties in strategic départements, while in others the bounty system was temporarily cancelled. We compared the evolution of rabies in the départements after one year with or without bounties. We recorded that on 12 occasions the cancelling of bounties in a département had been followed one year later by a decrease of the rabies cases among wild species in the same département. In 20 cases, however, wildlife rabies increased the following year.

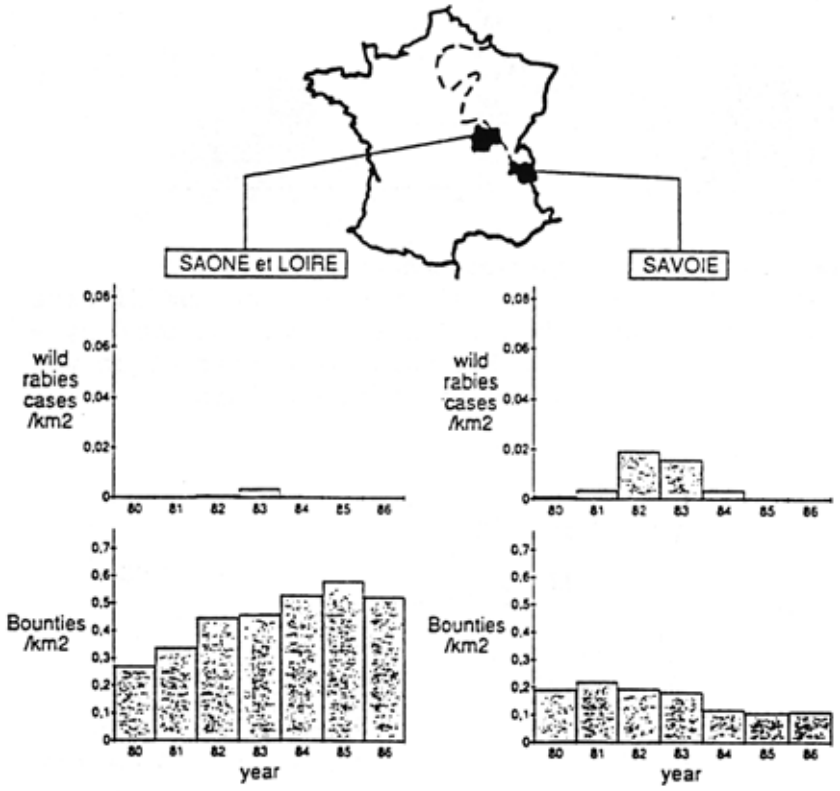

Fig. 5. Rabies cases in wild species and bounties given.

Both Saone-et-Loire and Savoie were situated at the limit of extension of rabies in France. In Savoie, a lesser reduction of foxes appeared to be sufficient due to the fragmentation of the fox population by high mountain ranges.

When bounties were attributed, rabies decreased (74 times), or increased (79 times) during the following year. The statistical analysis of this occurrence confirms the lack of significant influence of bounties on the future of the epizootics (Chi^2 test = 1.25) or at least the lack of predictive value of bounty statistics. Consideration of more reliable data (rabies cases in domestic cats only, because they are seldom vaccinated), did not change the conclusion (Aubert, 1988).

In the infected area of France, taken as a whole, no clear relationship could ever be established between the bounties and rabies incidence. The annual ratio, number of bounties/infected area, is fairly consistent, unlike the ratio of the number of rabies cases/infected area (Fig. 6). Because of high birth and natural death rate, fox population is essentially an annual crop (Davies and Wood, 1959). Wherever bounties were used, they entailed some problems: a proportion of foxes were falsely claimed to have been killed in areas under control and in North America, where trapping is traditional, it encouraged the stealing of animals from traps (Debbie, 1991).

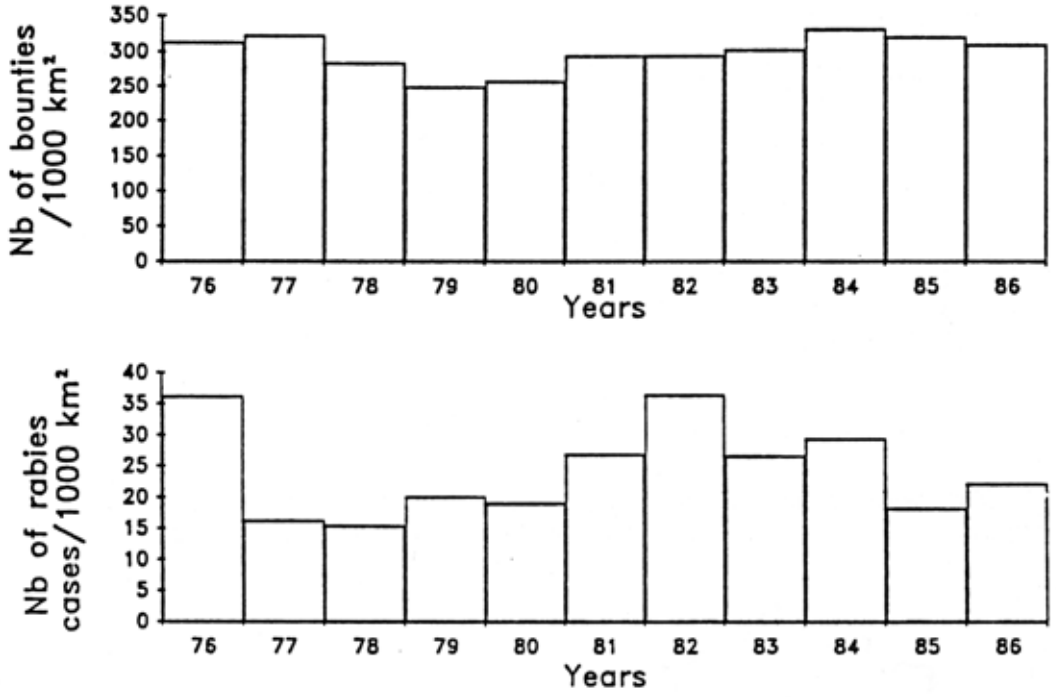

Fig. 6. Number of rabies cases and number of bounties given for the destruction of foxes in France, from 1976 until 1988. Both parameters are restricted to the infected areas.

MATHEMATICAL MODELLING OF RABIES ENZOOTICS.

In a mathematical simulation of a spatial model of fox rabies, Molison and Kwulasmaa (1985) found that the disease maintains itself through wandering foci, mostly well separated from each other, so that numbers are only held down locally at any one time. In fact rabies achieved by itself the more dramatic reductions - locally leading to its own eradication. The analysis of all data published on the transitory success of fox depopulation policies in Europe demonstrates that re-infection of areas swept by rabies was only delayed by organised reduction, and this goal was more easily achieved in areas isolated by natural barriers where immigration is hampered, as in Alpine valleys of France (Fig. 5), Italy (Irsara et al., 1990) and Switzerland (Wandeler et al., 1974).

In this context, the positive regression of the frequency of animal rabies according to the number of foxes shot per surface unit per year described by Steck and Wandeler (1980), only means that when foxes are scarce, rabies is less frequent, not more. Particularly it does not mean that the depopulation measures were necessary or efficient to reduce foxes (in order to limit rabies). If that had been the case, hunting records and rabies cases would have been negatively correlated.

The depopulation of foxes never produced such convincing results of its efficiency as did oral vaccination of foxes (Fig. 7). Such oral vaccination is efficient even when their population increases, according to our observations in the French Alps (Fig. 8).

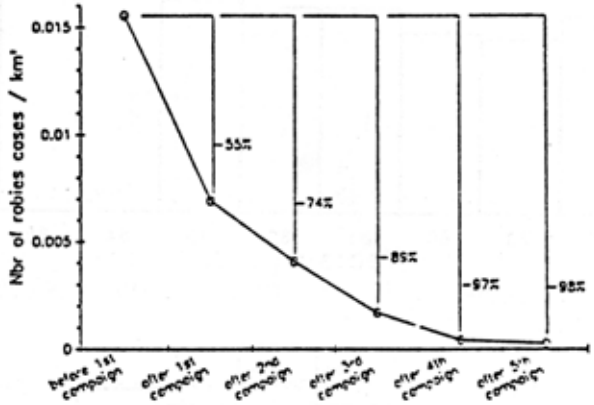

Fig. 7. Decrease in the density of *rabies cases* in the area where campaigns of fox oral *vaccination were* carried out in France.

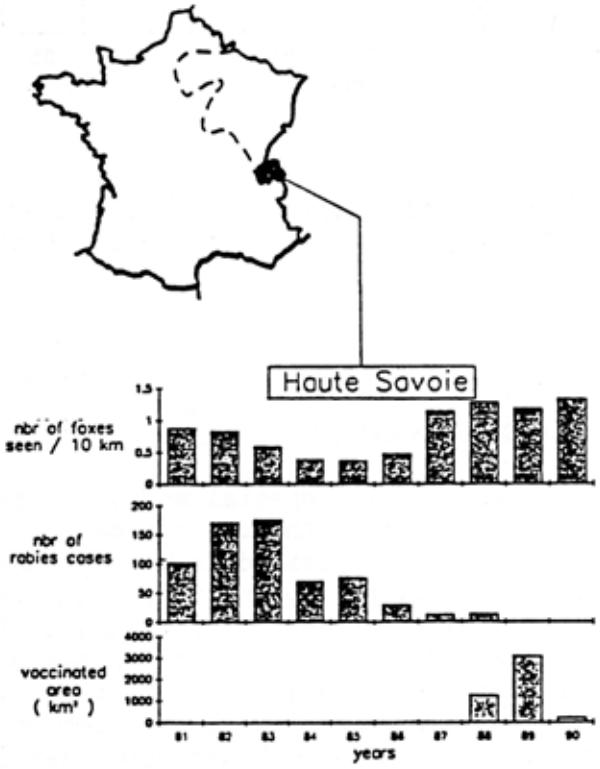

Fig. 8. Fox density, *rabies cases and size of vaccination area 1981-90.* The high density of foxes did not hamper the efficiency of oral vaccination which eliminated rabies within 1 year.

Mathematical modelling affords a useful tool to interpret data: it makes it more obvious that some interpretations of the past were perfectly sound. Several examples have been given above and elsewhere (Aubert and Artois, 1988; Aubert, 1989). The logistic curve applied to fox rabies by Anderson et al., (1981), is a mathematical representation of facts simple to admit (Fig. 9):

- the growth of an animal population is not unlimited: it depends on the carrying capacity of the milieu,

- when the density is high, near its limit, the growth rate is low (mortality is high and even in some situations, the birth rate can also decrease),

- when the density is low, both sexes are so far from each other that the growth rate is low,

- in intermediate densities, the growth rate is maximal.

Fox population reduction aimed to decrease the density threshold below which rabies could not be sustained. Several years of attempting to reach this threshold proved that the threshold was situated in the intermediate part of the curve, where the growth rate rapidly compensates the culling.

On the other hand, the vaccination of foxes moves the enzootic threshold upwards in a zone of the curve characterized by a low growth rate. To reach this new threshold, a mild culling is sufficient and will not be rapidly compensated.

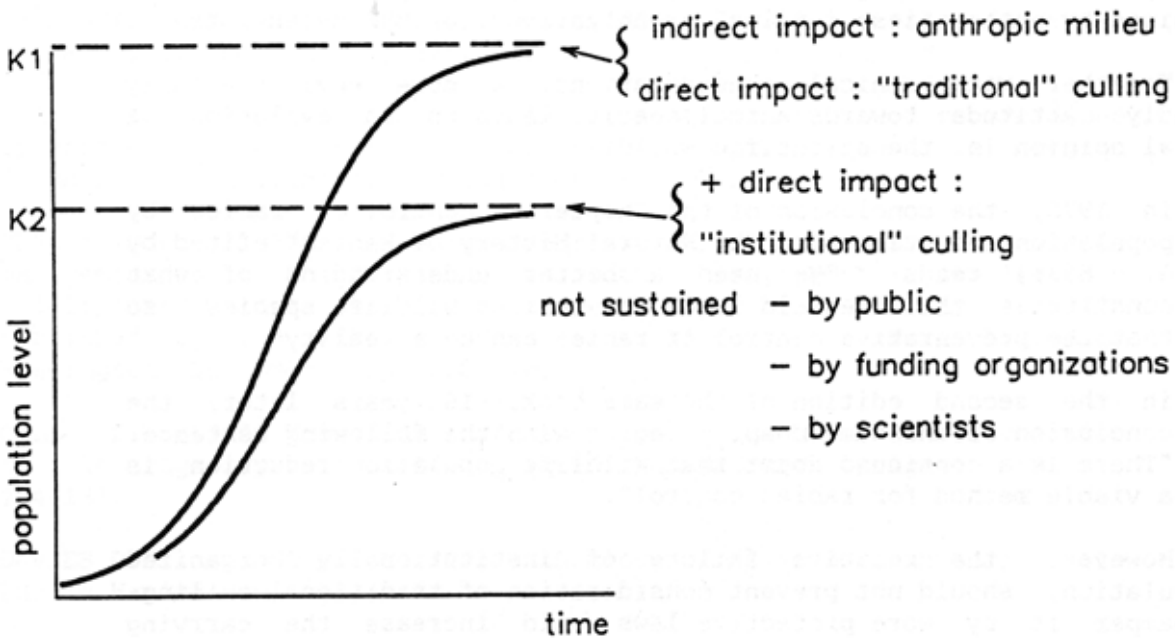

Fig. 9. Potential growth of a fax population: the logistic curve.
Maximum values of density are limited by the carrying capacity of the milieu, K1 or K2. By adding institutional culling to other limiting factors (traditional culling included), the carrying capacity K1 is reduced to K2, but the lower curve obtained is unstable as far as the institutional culling cannot be sustained for a long time.

DISCUSSION

It is important to remember that Man definitively intervenes in the determination of the carrying capacity of the milieu. The occupation of the milieu with high human rural density which reached an acme by the middle of the 19th. century in Europe (Pouthas, 1956; Agulhon et al., 1976), brought high densities of dogs (which itself supported the predator of dogs, the wolf), but contained the fox, considered as a competitor for animal proteins. Logically, dog rabies was a component of the scenery but, unlike the experience of the 20th. century, fox rabies outbreaks were isolated in time and places.

The human drift from the land during the 20th. century, the disappearance of family poultry, the transformation of gardened landscapes into unlimited fields on one hand, or fallow lands on the other, benefited the fox. The historical outbreaks of wildlife rabies during disruptions, such as that caused by the Napoleonic wars, illustrates a contrario the efficiency of the human pressure on wildlife in the rural societies (Steel and Fernandes, 1991).

It means that the carrying capacity is the resultant of indirect actions of man (actions on the milieu), but also direct action (hunting). The cultural hunting habits are durable, at least more stable than the institutional measures taken by authorities; they are also cheaper and more efficient: in France, organised night shooting never killed more than 1 fox for 5.4 foxes killed by traditional hunting (Aubert et al., 1988). Taken as artificial, institutional depopulation measures are close to being regarded as cruelty. Even when justified by reasoning, they are no longer sustained by authorities, funding organisations, or by scientists.

Together with ethical considerations, a more environmentally friendly attitude towards animal species leads to an evolution of general opinion in the scientific world:

- in 1975, the conclusion of the Chapter on control of rabies by population reduction (in "The Natural History of Rabies" edited by G. Baer) reads: "We need a better understanding of what constitutes the threshold level for various wildlife species, so that the preventative control of rabies can be a reality"

- in the second edition of the same book, 16 years later, the conclusion of the same Chapter begins with the following sentence: "There is a continued doubt that wildlife population reduction is a viable method for rabies control".

However, the relative failure of institutionally organised depopulation, should not prevent consideration of traditional culling: to hamper it by more protective laws would increase the carrying capacity of the milieu for the fox. The traditional control of the fox by hunting ensures the feasibility and the efficiency of oral vaccination campaigns.

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ORAL VACCINATION OF FOXES USING A VACCINIA-RABIES

RECOMBINANT VIRUS

Programme of sylvatic rabies control in Belgium

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In Belgium, as in other countries of Western Europe, the red fox is the vector and reservoir of the current endemic of sylvatic rabies. Since rabies control by fox population reduction was not achieved, oral immunisation of foxes, by the distribution vaccine-baits, has been experimentally assessed and subsequently engaged in the stole rabies infected area of Belgium.

A. NATIONAL PROGRAMME OF SYLVATIC RABIES CONTROL

The rabies infected area covers 10,000 km^2 in the southern part of the country and is bordered by a river and four neighbouring countries: the Netherlands, Germany, Grand Duchy of Luxembourg and France. This region was heavily infected before the campaigns of fox vaccination -840 animal rabies cases were recorded in 1989. That represents a mean rate of 1 case per 10 km^2. In Fig. 1, rabid foxes are shown by black dots and white dots show the rabid domestic animals, mainly cattle and sheep, which are responsible for most human infections.

Fig. 1. Location of 841 rabies cases in Belgium, 1989.

Fig. 2 shows the yearly evolution of the number of rabies cases in the infected area, since 1981 to the first semester of 1992. Rabies has been endemic' since 1981 and its incidence remained high, especially during the year 1989.

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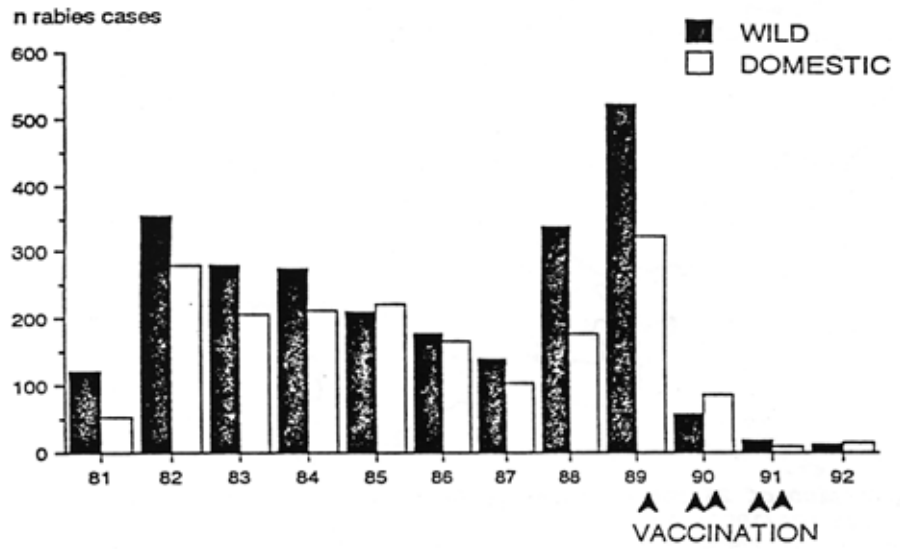

Fig. 2. Evolution of the number of rabies cases since 1981.

In the autumn of 1989, the first campaign of fox vaccination was carried out in the whole of the infected area. Subsequently, similar full campaigns were performed four times: two in the spring and autumn of 1990 and two in the spring and autumn of 1991. Campaigns performed in 1989 and 1990 induced a drastic decrease of the rabies incidence in the country (Fig. 2). Notification of rabies in domestic animals is mandatory in Belgium and thus the prevalence of rabies in livestock and pets provides a reliable indicator of disease prevalence in wildlife.

The number of rabies cases reached a very low level but rabies persisted in 1991 in spite of two more campaigns. This epidemiological situation can be explained by consideration of the spatial evolution of rabies. The following maps present the epidemiological results obtained after each campaign of vaccination (Fig. 3-7). As shown by examination of the results from the second semester of 1991 and the first of semester 1992, the geographical pattern or rabies has changed.

Fig. 3. Location of 118 rabies cases in Belgium, 1st. semester. 1990.
1st. campaign (10/1989); 10,000km^2; 15 baits/km^2; 56% of 193 foxes TC+

Fig. 4. Location of 26 rabies cases in Belgium, 2nd. semester.1990. 2nd. campaign (04/1990) 10,000 km^2; 15 baits/Km2; 50% of 176 foxes TC+.

Fig. 5. Location of 16 rabies cases in Belgium, 1st. semester 1991. 3rd. campaign (11/1990); 10,000 km^2; 15 baits/km^2- 74% of 165 foxes TC+

Fig 6. Location of 13 rabies cases in Belgium, 2nd. semester 1991. 4th. campaign (04/1991; 10,000km^2; 15 baits/Km^2; 62% of 192 foxes TC+

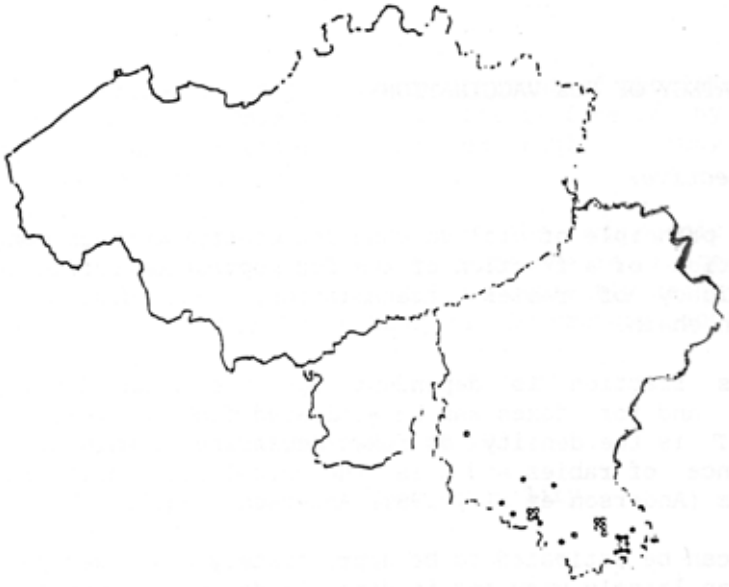

Fig. 7. Location of 27 cases in Belgium, 1st semester, 1992.
5th campaign (10/1991); 10,000 km^2; 15 baits/km^2; 69% of 71 foxes TC+

Since April 1991 until now that is to say for more than one year, all fox cases recorded were within 20 km of the border with France and more than 70 percent were less than 5 km from the border. Rabies seems to have disappeared from the majority of the initial infected area.

France reported numerous rabies cases in the contiguous area and hence the rabid foxes found in Belgium , could have been either "foreign" foxes in their dispersal period or unvaccinated foxes infected by these "foreign" rabid foxes.

Regarding the current epidemiological situation, a new spatial strategy for bait dispersal has been planned for 1992. To determine the size and limits of the new vaccination area, we have considered all rabies cases recorded for one year both in Belgium and in neighbouring countries to a depth of 50 km around Belgium. Then, for each rabid fox, a potential movement of 30 km has been estimated (Fig. 8). All these data have permitted the construction of a vaccination area which forms an immune belt along political borders. A first "defence" campaign was carried out in April 1992 and another campaign will be carried out in the autumn.

Fig. 8. Formation of a vaccination area to give a rabies-immune belt.

B. STRATEGY OF FOX VACCINATION

B1 Objective:

The principle of oral vaccination control methods consists of the immunisation of a fraction of the fox population sufficient to reduce the efficacy of rabies transmission, thus disrupting the viral infection chain.

This fraction is dependent upon the mean density of animal targets, and for foxes can be estimated from the relation p >1-KT/K where KT is the density of foxes necessary to maintain the endemic persistence of rabies and K is the actual fox density in the absence of rabies (Anderson et al., 1981; Anderson, 1985).

KT can be estimated to be approximately 0.4 foxes per km^2. The K value can largely vary and is directly dependent upon the biotope and the season: the density can vary from 0.1 in poor biotic areas to 4 and more in some suburban areas (Harris and Rayner, 1986). The density is also lower in winter than in summer.

It is consequently very difficult to determine precisely the "p" value in a given infected area. One to two foxes/km^2 is an overestimation of the mean density in Europe. Thus, for such a value, the proportion of the fox population (p) which must be vaccinated varies from 0.6 to 0.8. if the threshold is not reached, rabies could be "controlled" by inducing a decrease of the number of rabies cases, but the virus would persist in the fox Population. On the other hand, if the threshold is reached, the rabies virus infection chain should be disrupted and the disease eradicated.

B2 Oral vaccine:

The first step for achieving such an objective consists of choosing a good vaccine. Until recently, when considering the oral route as the only one applicable for vaccination in the field, protection against rabies could be conferred only by attenuated live virus vaccines.

The three main requirements of a live virus vaccine to be used in the field are efficacy, safety and heat stability. In western Europe, three attenuated live virus vaccines are currently in use. One such classical attenuated strain of rabies virus is SAD B19, produced in Germany (Schneider and Cox, 1983). This vaccine is efficacious for immunising foxes but remains pathogenic for some non-target species such as rodents, which are very attracted to the baits. The SAD B19 strain has also been shown to be pathogenic for the striped skunk, a target species in North America. Furthermore, since attenuated strains of rabies virus are comparatively heat-sensitive they do not resist some climatic conditions (and they must be stored frozen).

Thus, as far as innocuity and heat-stability are concerned, there was a need to improve vaccines to be used in the field. Therefore, two new-generation vaccines were developed.

One of these, the SAG_1 vaccine, is an apathogenic mutant of the SAD Bern strain (Leblois et al., 1990). This vaccine so far is free of any residual pathogenicity but, as with other strains of rabies virus, remains comparatively heat-sensitive.

The other, a recombinant vaccinia virus expressing the immunogenic glycoprotein of rabies virus, has been developed by Transgene in France (Kieny et al., 1984). This vaccine, so-called VR-G, is currently being used on a large scale in France, the Grand Duchy of Luxembourg and Belgium.

Table 1
Oral Vaccines Used in Western Europe

Vaccine	Efficacy (for fox)	Safety	Heat-stability
SAD B19	+		- +
SAG	+	+	- +
VR-G	+	+	+ +

The development of V-RG has been performed by four groups of collaborators: Rhone Merieux and CNEVA in France, the Wistar Institute in the USA and the University of Liege in Belgium.

Efficacy

Several experiments have demonstrated that 10^7 $TCID_{50}$ of V-RG, administered by the oral route, can protect 100% of foxes against a rabies challenge. The duration of protection is at least 18 months and similar results were obtained when the V-RG at 10^8 $TCID_{50}$ was enclosed in a machine-made bait (Blancou et al., 1986; Brochier et al., 1988).

Safety

Since only rabies glycoprotein is represented in the vaccine, it is clear that VR-G is free from risk as far as rabies virus infection is concerned. However, the safety of the attenuated vaccinia virus (which is used as an expression vector) needed to be evaluated.

1. Absence of residual pathogenicity

Many experiments carried out by several laboratories have given the following results: regarding the target species, we could attest that VR-G is completely apathogenic whatever the route and dose of administration (Blancou et al., 1986; Brochier et al., 1988 and Blancou et al., 1989).

Extreme doses, such as 10^2 and 10^{10} $TCID_{50}$ remain safe. Furthermore, 10^8 $TCID_{50}$ of VR-G may be administered without inducing any adverse effect by the following routes: oral, subcutaneous, gastric, intranasal, intra-ocular and by scarification.

The absence of pathogenicity also needed to be verified in non-target animal species, notably those which are likely to eat vaccine-baits in the field.

The safety of V-RG was proven in target wildlife species, in five laboratory animal species, in five domestic animal species and finally, in several wildlife non-target species, of both European and American origin and especially those species which compete with the target species in bait uptake (Wiktor et al., 1984; Wiktor et al., 1985; Soria-Baltazar et al., 1987; Blancou et al., 1988; Brochier et al., 1989b; Desmettre et al., 1990 and Artois et al., 1990). More recently, the absence of pathogenicity of the V-RG has also been demonstrated in two species of primates: the squirrel monkey and the chimpanzee (Rupprecht et al., 1992).

2. Absence of viral excretion and transmission

For testing the excretion and horizontal transmission of the V-RG, newly vaccinated animals have been held in close contact with unvaccinated controls. When using this assessment method, no transmission of immunising amounts of V-RG has been observed in the red fox, dog, cat, cattle, ferret, badger and wild boar (Blancou et al., 1986; Brochier et al., 1988; Blancou et al., 1989; Brochier et al., 1989b and Desmettre et al., 1990). Similar results were obtained with chimpanzees (Rupprecht et al, 1992).

Furthermore, several techniques of viral detection failed to detect any virus in the saliva, salivary glands, the blood and internal organs such as the brain, the spleen and lymph-nodes of newly inoculated foxes. The highly sensitive method of polymerase chain reaction could detect the VR-G at very low levels in tonsils, buccal mucosa and soft palate only (Thomas et al.,1990). Thus, rabies immunisation appears to occur after a restricted multiplication of the VR-G.

3. Genetic stability

The vaccinal virus should not revert to virulence and must therefore have a good genetic stability. The genetic stability of V-RG has been verified after serial passages in vitro in cell lines, as well as in vivo in the laboratory mouse and the red fox (Desmettre et al., 1990 and Thomas et al., 1990).

4. Absence of pathogenicity in immuno-suppressed animals.

In this sense, the VR-G has also been shown to be apathogenic for immuno-suppressed athymic nude mice (Desmettre et al., 1990).

5. Absence of emergence of asymptomatic carriers

Experimentally, it has been shown that vaccination with V-RG of foxes which were incubating the disease did not preclude death of these animals from rabies (Brochier et al., 1989a).

Stability

Heat stability is an important attribute of a vaccine for use in field conditions. V0G can resist environmental conditions, especially climatic factors such as heat, temperature fluctuations and U-V light. V0G has been shown to retain its capacity to immunise for one month in

field conditions, a period which corresponds to the delay of uptake that many baits may undergo in the field. This point is the more important since foxes are likely to hide their food for storage before eating. In addition, a stable vaccine offers flexibility for planning a temporal strategy of bait dispersal. Since V-RG does not need to be stored frozen, remaining viable at +4°C, planning is facilitated.

In a field trial VR-G heat-stability was tested under local climatic conditons (Brochier et al., 1990). After baiting, the minima and maxima of environmental temperature were recorded daily. Fig. 9 shows the changes in environmental temperature during the first month. Despite the alternate natural freezing and thawing, the virus titre remained quite stable.

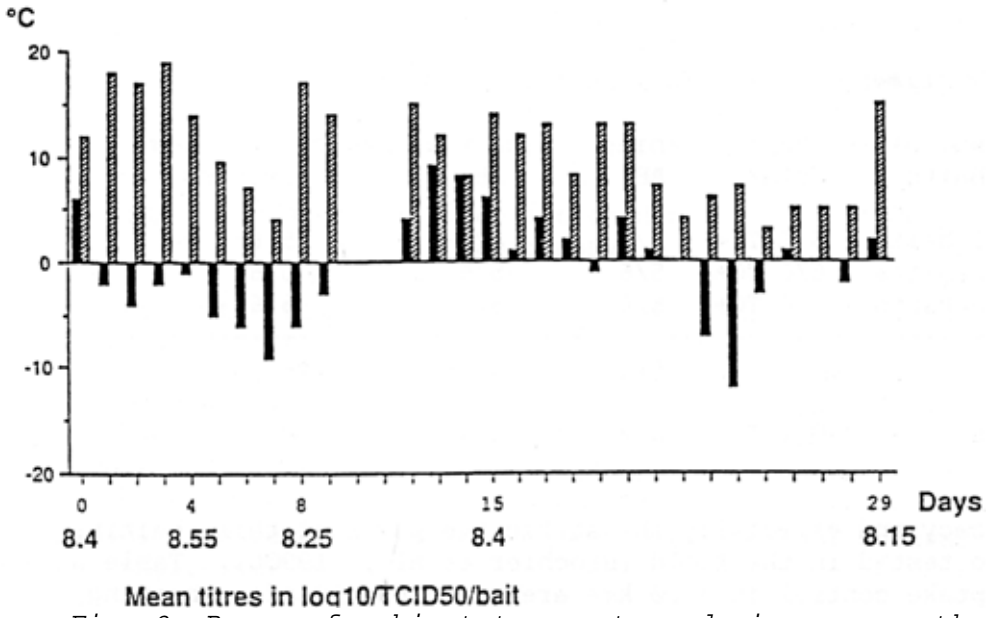

Fig. 9. Range of ambient temperature during one month.

B3 Vaccine bait system

The development of an efficient baiting system is important since an attractive bait permits the self -vaccination of the target species.
A bait containing the V-RG is currently commercially produced by Rhone-Merieux. A suspension of V-RG at 10^8 $TCID_{50}$ is contained in a plastic sachet. This is then enclosed in a fox-attractive mixture consisting of plant and animal proteins and fish oil aggregated by a synthetic polymer. Tetracycline, which has been introduced into each bait, serves as a bio-marker of bait uptake and can be detected in bones by using fluorescence microscopy.

This vaccine-bait system presents the following characteristics :

- it is attractive to the fox (but also to *non-target species)*
- it can be stored without freezing
- because of its mechanical resistance it can be dropped by air
- it is heat stable and does not melt; this allows time for uptake
- it is machine made and thus easily available

- it allows good delivery of the liquid vaccine to the oral cavity (the appetant mixture/vaccine liquid ratio is good and the plastic sachet is easily perforated by the teeth).

The efficacy of this vaccine-bait system has been tested in captive foxes (Brochier et al., 1990b). Each fox of three experimental groups was fed one, two or three baits containing 10^8 $TCID_{50}$ of V-RG. As shown by the incorporation of tetracycline, all the foxes voluntarily ingested at least one bait. one month after baiting, 15 of 18 foxes developed rabies antibodies, 14 of 15 developed vaccinia antibodies and 16 of 18 foxes resisted a lethal rabies challenge (Table 2).

Table 2.
Experimental V-RG oral vaccination of foxes

No. of Foxes	No. of Baits	Bait Uptake	Rabies AB	Vaccinia AB	Rabies Challenge survivors
6	1 bait	6/6 TC+	5/6	4/6	5/6
6	2 baits	6/6 TC+	5/6	5/6	6/6
6	3 baits	6/6 TC+	5/6	5/6	5/6
		18/18	15/18	14/18	16/18
4 controls		0/4 TC+	0/4	0/4	0/4

The efficacy and especially the attractive power of this baiting system was also tested in the field (Brochier et al., 1990b). Table 3 shows a bait uptake control in a 20 km^2 area. On day 30 after baiting, 94 percent of the baits had disappeared, either eaten or at least carried off by animals.

Table 3
Removal of 238 baits over 20 km^2 area

Day 0	Day 4	Day 8	Day 15	Day 30
0	65	128	163	223
	27%	54%	68%	94%

As far as ecology is concerned, tetracycline as a biomarker allowed the identification of non-target species which competed with the fox for bait uptake. When using this assessment method, the ingestion of baits could be demonstrated in domestic cats and dogs, wild boars, stone martens, polecats, woodmice and crows.

B4 Bait distribution

The third and last step for the achievement of the objective is to plan a strategy of bait distribution in the field. This strategy should be considered at both spatial and temporal levels. As far as the spatial pattern is concerned, there are four main questions:

- what are the size and limits of the vaccination area

- what is the mean density of baits to be distributed

- is there a need for specific target habitat baiting

- should baiting be uniform, or should bait stations be created

For response to these questions, three groups of factors need to be considered:

Epidemiological factors rabies spread and incidence

- Geographical factors natural, artificial and political borders

- Ecological and biological factors

1. target species:
   - movement
   - social and spatial organizations
   - population density
   - demography

2. competitor species:
   - population density
   - demography

As far as the temporal pattern of bait distribution is concerned, we must respond to the following questions:

- How many campaigns, how often

- What is the best time of the year for vaccination

Again, for response to these questions, several natural factors must be considered:

- Epidemiological factors - rabies spread and incidence

- Climatic factors - vaccine bait resistance to heat, U-V light freezing, vegetation, snow

Ecological and biological factors

1. target species
   - turnover of the population
   - movement patterns
   yearly evolution of the population density

2. competitor species - yearly evolution of the population density (wild and domestic)

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WILDLIFE RABIES IN SOUTHERN AFRICA

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There is circumstantial evidence that rabies existed in the interior of South Africa prior to the first recognised introduction of canine rabies at Port Elizabeth in 1892 (Culver, 1927; Du Toit, 1929; Neitz and Thomas, 1932; Henning, 1956). Furthermore, the fact that from 1916 onwards yellow mongooses (*Cynictis penicillata*) were frequently afflicted by rabies (Henning, 1956) and that the virus associated with this species did not transmit easily to, or between, dogs (Alexander, 1952; Du Toit, 1929) suggests that the virus prevalent in the hinterland of South Africa prior to 1950 was maintained by wild animals and was poorly adapted to domestic dogs.

Canine rabies did not become a common disease in South Africa until after 1950 when it entered northern Transvaal from Botswana, having originated in northern Namibia or southern Angola (Alexander, 1952). The canine rabies epizootic has continued to move down the east coast of South Africa to the present day and is currently responsible for a serious problem in Natal (see G. Bishop, these Proceedings).

The predominant role which the yellow mongoose has played in wildlife rabies in South Africa is demonstrated in Table 1, which shows that from 1916 onwards the number of cases in this species has made up more than 70 percent of the total number of cases of rabies diagnosed in wildlife. Conversely, rabies in bat-eared foxes (*Octocyon megalotis*) only appeared after 1950 and also first became common in black-backed jackals (*Canis mesomelas*) in Zimbabwe and South Africa after that date (Alexander, 1952). It is thus tempting to conclude that rabies in wild canids only became established in southern Africa after canine rabies became enzootic.

Rabies has also historically been associated with the small spotted genet (*Genetta genetta*) in the Vryburg and Mafeking districts of South Africa (Du Toit, 1929) and cases in the African wild cat (*Felis lybica*) have occurred in the same approximate area since early in this century (Table 1). However, relative incidence of rabies in these two species seems to have declined since reaching a peak in 1961-70 (Table 1).

Table 1

Incidence of rabies diagnosed in wildlife vector species: 1929 -1991

Species	*1929-1947 (18)*	*1952-1960 (9)*	*1961-1970 (10)*	*1971-1900 (10)*	*1981-1910 (10)*
Mongoose	*116 (76)*[2]	*126 (78)*[1]	*727 (77)*[1]	*1034 (75)*[2]	*1146 (69)*[2]
Small spotted genet *G. genetta*	*18 (12)*	*12 (7)*	*56 (6)*	*41 (3)*	*40 (2)*
black-backed jackal *C. mesomelas*	*1 (1)*	*11 (7)*	*57 (6)*	*108* (8)	*100 (6)*
African Wild Cat *F. lybica*	*6 (4)*	*9 (6)*	*68 (7)*	*14 (1)*	*13 (1)*
Bat-eared fox *0. megalotis*	*- (0)*	*2 (1)*	*16 (2)*	*71 (5)*	*181 (11)*
Others	*11 (7)*[3]	*2 (1)*[4]	*21 (2)*[5]	*109(8)*[6]	*191 (11)*[7]
Total	*152*	*162*	*945*	*1377*	*1671*

( ) = percent of cases for that period;
1= all mongooses;
2 = *Cynictis penicillata* or probably C. *penicillata;*
3 = *7 spp.*
4 = *2 spp.*
5 = *5 spp.*
6 = *8 spp. plus 30* non-specified felids;
7 = 14 spp.

Amongst the other potential wildlife vectors of rabies (ie. carnivores) in which the disease has been diagnosed in southern Africa (Table 2), some are regularly although infrequently affected, eg. suricates, small grey mongooses, water mongooses, honey badgers (ratels), striped polecats and caracals. Whether any of these species are important in the maintenance of rabies remains to be established.

Table 2

Potential vectors of rabies in which the disease has been confirmed in Southern Africa

FAMILY	*COMMON NAME*	*SCIENTIFIC NAME*
Viverridae	[1]Yellow mongoose	*Cynictis penicillata*
	[2]Small-spotted genet	*Genetta genetta*
	[2]Suricate	*Suricata suricatta*
	[2]Small grey mongoose	*Galerella pulverulenta*
	[2]Water mongoose	*Atilax paludinosus*
	Slender mongoose	*Galerella sanguinea*
	Banded mongoose	*Mungos mungo*
	Dwarf mongoose	*Helogale parvula*
	Civet	*Civettictis civetta*
	Large grey mongoose	*Herpeste ichneumon*
	Large-spotted genet	*Genetta tigrina*
Canidae	[1]Bat-eared fox	*Otocyon megalotis*
	[1]Black-backed jackal	*Canis mesomelas*
	[2]Side-striped jackal	*Canis adustus*
	Cape fox	*Vulpes chama*
	Wild dog	*Lycaon pictus*
Mustelidae	[2]Honey badger (ratel)	*Mellivora capensis*
	[2]Striped polecat	*Ictonyx stiatus*
	Striped weasel	*Poecilogale albinucha*
	Otter	*Aonyx sp. or Lutra sp.*
Hyaenidae	Aardwolf	*Proteles cristatus*
	Brown hyaena	*Hyaena brunnea*
	Spotted hyaena	*Crocuta crocuta*
Felida	[2]African wild cat	*Felis lybica*
	[2]Caracal	*Felis caracal*
	Small spotted cat	*Felis* nigripes
	Lion	*Panthera leo*
	Leopard	*Panthera pardus*
	Cheetah	*Actinonyx jubatus*
	Serval	*Felis serval*

1 = species regularly affected
2 = species fairly often affected

The relatively recent appearance of rabies in bat-eared foxes in Namibia and South Africa is interesting because, certainly so far as South Africa is concerned, it is not clear whether it arose from a virus population already present in the country or arrived via the spread of infection in this species through Namibia and/or Botswana. It could not have moved south from East Africa where rabies in bat-eared foxes also occurs (Irvin, 1970) because there is a discontinuity in the distribution of the species between East and southern Africa (Skinner and Smithers, 1990).

The first two diagnosed cases in southern Africa occurred in the northern Transvaal in 1955 and the next three in the central Cape Province between 1964 and 1966. In 1969 there was an isolated case in the Etosha National Park (Namibia), whereafter rabies in this species was widely recognised in the western and central Cape Province, the western Orange Free State and throughout Namibia (Fig. 1).

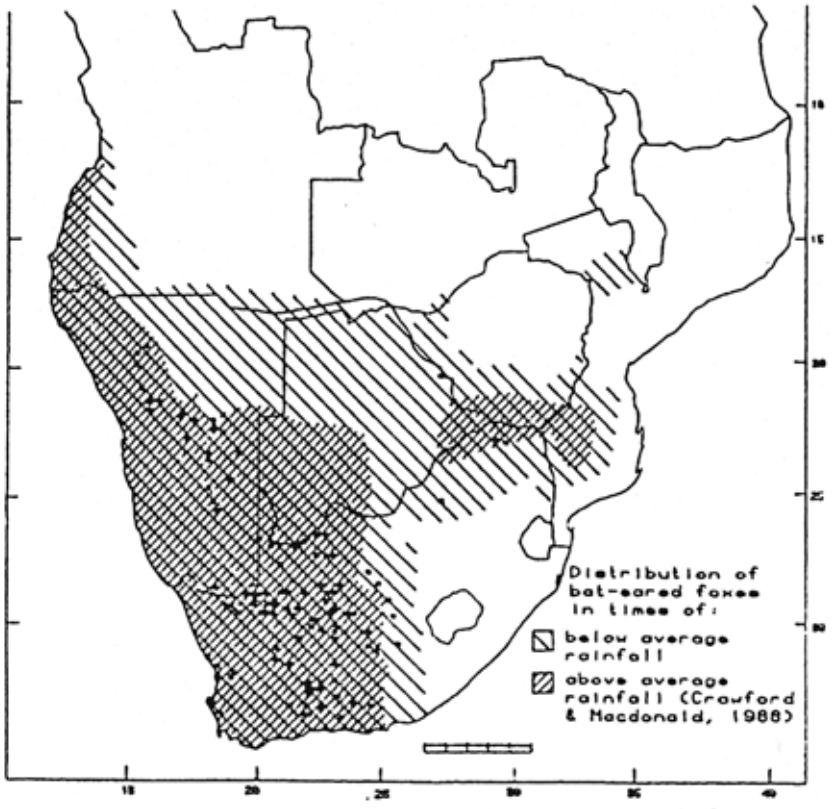

Fig. *1. Distribution of bat-eared foxes.*

The finding that three of the bat-eared fox isolates so far examined using panels of monoclonal antibodies are "canine type" (A. King, 1992; personal communication) indicates that "canine type" rabies is not only distributed along the eastern seaboard of South Africa in dogs but is also established in bat-eared foxes in the drier western regions of South Africa and in Namibia.

There is also a possibility that rabies in bat-eared foxes is responsible for the marked "population crashes" which have been observed to occur in this species periodically (A. Mackie, 1992; personal communication). One such crash was observed in the Orange Free State in 1982/3 and it is interesting to note that from 1980 onwards there was an appreciable rise in the number of rabies cases diagnosed in bat-eared foxes in South Africa (Records of the Onderstepoort Veterinary Research Institute).

"Canine type" rabies is also associated with black-backed jackals in South Africa and Zimbabwe (where side-striped jackals [*Canis adustus*] are also involved) (A.King, 1992, personal communication; J. Bingham, these Proceedings). The virus type is clearly antigenically different from most viverrid isolates made in South Africa, particularly those from yellow mongooses (A.King, 1992, personal communication). Hence, indications are that there are at least two antigenically and biologically different rabies viruses in South Africa.

In the light of the long-standing involvement of other species such as genets and African wild cats it is possible there may be more than two virus "types" in southern Africa and that a situation analogous to the species compartmentalisation which exists in North America may also prevail in southern Africa. This will only be elucidated by more extensive monoclonal antibody studies on local virus isolates and/or sequence analyses of viral genomes. This latter approach is in progress at the Onderstepoort Veterinary Institute.

One of the observations regarding wildlife rabies in southern Africa which has resulted in considerable speculation is that rabies within wildlife reserves is a rare disease. Only in the Etosha National Park (northern Namibia) among the major wildlife reserves in southern Africa is rabies regularly diagnosed (principally in black-backed jackals and bat-eared foxes). In reserves such as the Kruger National Park (KNP) and the Hluhluwe/Umfolozi Game Reserve Complex (South Africa) rabies in wildlife is almost unknown (there was a case in a civet in the KNP in 1980). This is despite the fact that on a number of occasions rabid dogs have been found wandering in these reserves and the diagnosis confirmed by laboratory investigation.

It has been argued that in reserves managed for species diversity no single species is able to attain population densities sufficient to sustain the infection. There is, however, no data to support this contention and, furthermore, most carnivores do not compete directly within a given habitat.

Rabies in yellow mongooses

The only free-living species in southern Africa in which rabies has been studied in any detail is the yellow mongoose (Fig. 2). This is a semi-social species of mongoose that lives in smallish colonies (mean of 4 individuals but extending to 20 or more) which use burrows for shelter. Their diet is largely termites and other insects although they are opportunistic carnivores. They hunt singly or at most in pairs and are diurnal or crepuscular in their activities although in summer they may hunt at night.

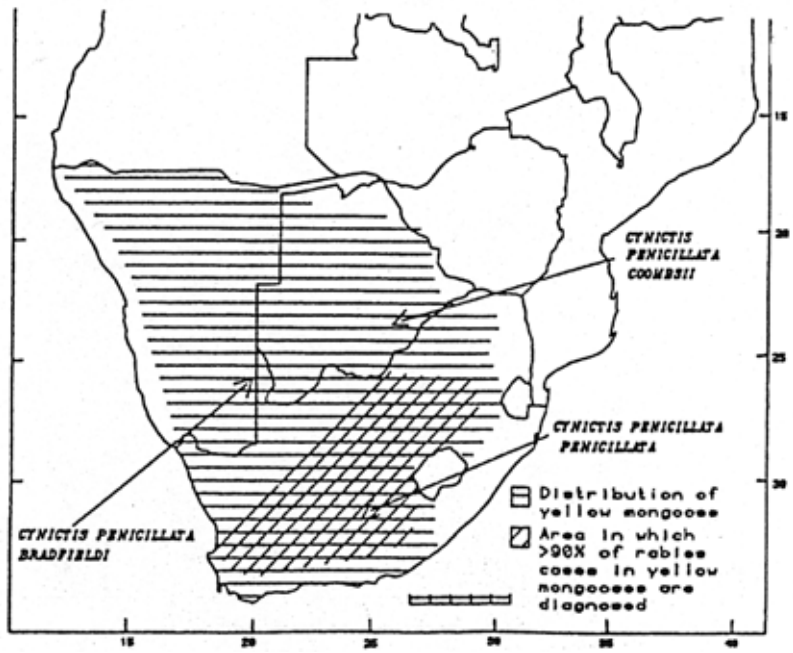

Fig. 2. Distribution of Yellow mongoose.

Diagnostic data show that the incidence of rabies in yellow mongooses, reaches a peak in August/September each year. In the summer rainfall area, where the yellow mongoose is mainly distributed, this corresponds to the dry period in early spring prior to the onset of the rainy season.

Various theories have been advanced to account for this. Snyman (1940) speculated that the seasonal occurrence of rabies was possibly due to the mongoose home range increasing at such times but concluded that seasonal variation in incidence was probably more apparent than real. This he explained on the basis of better detection of rabid mongooses when vegetation cover was sparse and short. However, it can be shown that the incidence of rabies in cattle in yellow mongoose areas also rises in late winter and early spring (Fig. 3) which indicates that the phenomenon is not merely apparent. Zumpt (1976) suggested that this rise in incidence could be explained by the young from the previous breeding season being ejected from the colony prior to the September-October birth period. This explanation may also be questioned in the light of recent findings which indicate that the " yearlings" of the previous generation often remain in the colony as "child minders" (Wenhold, 1990; A. Raza, 1991. Personal communication).

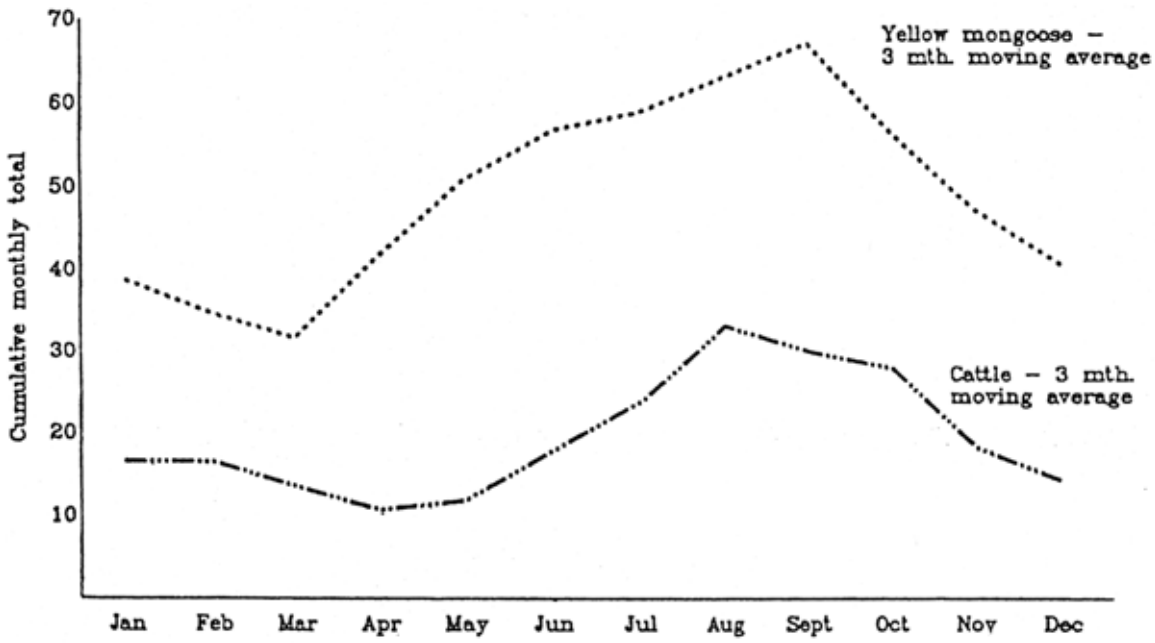

Fig. 3. Seasonal incidence of mongoose and cattle rabies.

The incidence of rabies in yellow mongooses in South Africa since 1952 is shown in Fig. 4. The pattern of incidence is not easy to explain and certainly does not appear to be directly related to the quasi - 18 year oscillation cycle in southern African rainfall described by Tyson (1986).

Perhaps the most interesting feature of rabies in yellow mongooses is that the disease does not generally occur in the whole of the area over which *Cynictis penicillata* is distributed (Fig. 4). This may be related purely to differences in population density. Alternatively, recent morphological and genetic studies on yellow mongoose populations raise the possibility that there may be differences with respect to susceptibility of three subspecies of *C. penicillata* which have recently been proposed (Taylor et al., 1990).

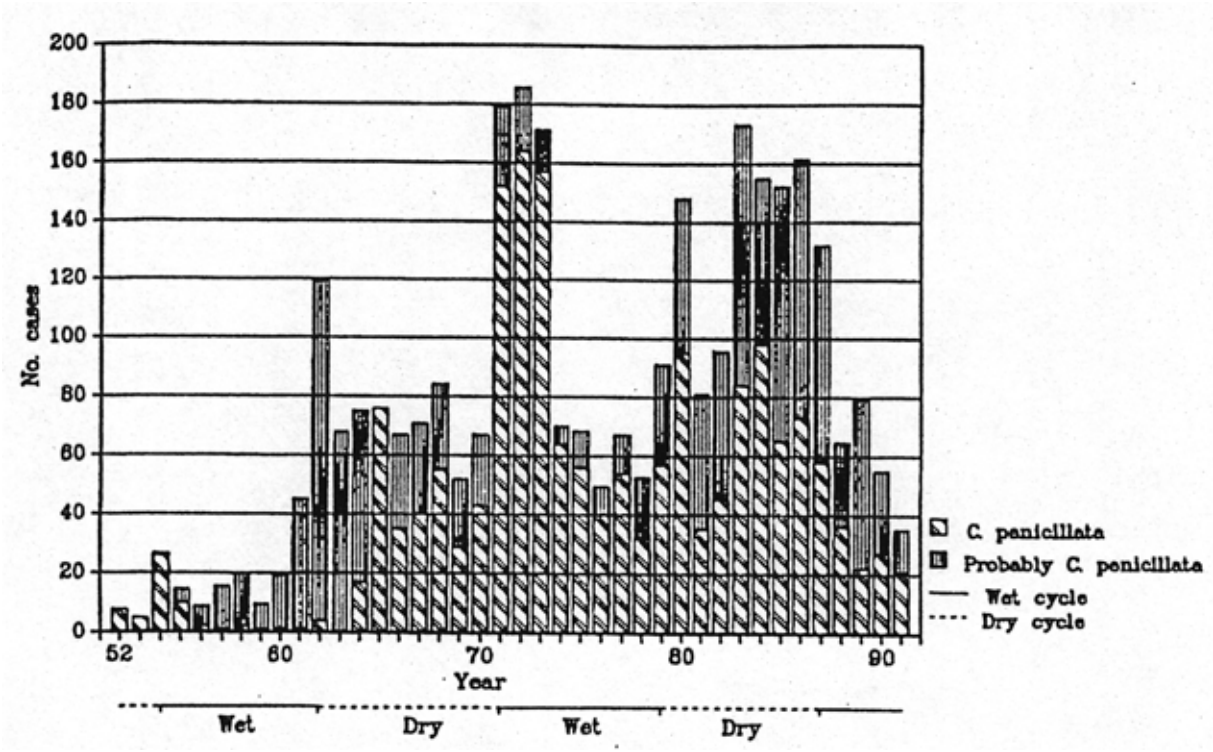

Fig. 4. Incidence of rabies In the yellow mongoose since 1952.

Unfortunately, almost no experimental studies on the interaction between *Cynicitis penicillata* and rabies virus have been conducted. This is something we are in the process of trying to rectify.

Rabies in victim species

Although losses in cattle in the Northern Transvaal due to jackal-transmitted rabies has been a problem at times in the past (Brückner et al., 1978) and has led to routine vaccination of cattle in that region, rabies in farm animals in South Africa due to either sylvatic or domestic animal vectors is not generally a problem.

As far as wildlife species are concerned only sporadic cases are encountered in a variety of species, one of the commonest being the ground squirrel (*Xerus inauris*) which frequently occupies the same burrow systems as yellow mongooses and suricates.

The most noteworthy outbreak of rabies in a "victim" species was that in Kudu (*Tregelaphus strepsiceros*) in Namibia in 1978 - 1985, when more than 50,000 animals are thought to have died. It has become generally accepted that this epizootic was not due to transmission from a "vector" species but due to "transmembrane transmission" between infected and susceptible kudu browsing on thorny Acacia trees and shrubs (Barnard and Hassel, 1978).

However, this was never proved and the fact that there was a concomitant rise (Fig. 5) in the incidence of rabies in cattle (which are generally not browsers) raises the probability that the kudu epizootic in Namibia has yet to be satisfactorily explained.

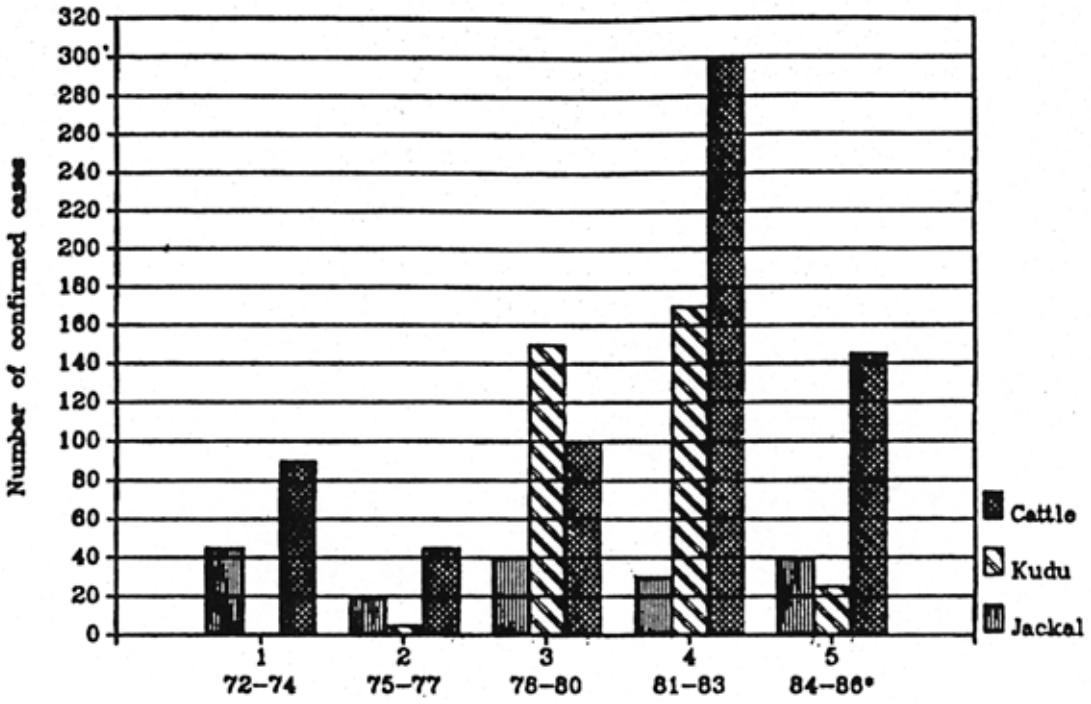

Fig. 5. Rabies in cattle, kudu and jackal.

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MODIFIED LIVE VIRUS ORAL VACCINATION IN ZIMBABWE

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The two target species were:

Black-backed jackal (*Canis mesomelas)* and

Side-striped jackal (*Canis adustus)*

In the future, the dog may become a further target species.

There are three components in developing an oral vaccination system:

1) Testing the efficacy of vaccine in the target species.

2) Testing the safety of vaccine in non-target species.

3) Testing the bait delivery system to the target species.

The efficacy of SAD (Berne) vaccine in jackals

Methods

- Vaccine (Berne Type IV) was instilled by syringe or given in a vaccine sachet attached to a chicken head.
- Three vaccine dose rates were used
- Instillation was done without anaesthesia or under light sedation.
- The baits were loaded with 2ml of vaccine at $10^{7.7}$ $TCID_{50}$/ml.
- To test for local virus replication, salivary swabs were taken from all jackals at 1, 3 and 7 days after vaccination and tested by intracerebral inoculation into weaned mice.
- All jackals were bled at the time of vaccination and at monthly intervals to test for serum neutralising antibody. All jackals had nil antibodies at vaccination.
- Half the jackals were challenged at one month and the rest at one year after vaccination with 2000 $MICLD_{50}$ of jackal salivary gland isolate.
- The jackals were observed for six months after challenge. The one-year challenge group is still under observation.

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Results

- All the vaccinated jackals developed significant rabies neutralising antibody (Table 1).

- All vaccinated jackals have so far resisted challenge while all controls developed rabies.

- All salivary swabs were negative for virus.

Table 1.

Serum Titres of Vaccinated Jackals

				Reciprocal Titre (Months)		
Jackal	*Species*	*Dose*	*Challenge*	*1*	*6*	*12*
41	SS	6.3	Month	25	125	
57	SS	6.8	Month	56	125	
53	SS	7.7	Month	280	125	
3	SS	7.7	Month	125	280	
42	SS	6.8	Year	125	125	25
1	SS	7.7	Year	280	125	125
2	SS	7.7	Year	125	280	125
14	BB	6.3	Month	25	25	
6	BB	6.8	Month	125	280	
8	BB	7.7	Month	125	280	
10	BB	7.7	Month	625	280	
13	BB	Bait	Month	125	280	
is	BB	6.3	*	125	>625	
5	BB	6.8	Year	280	125	125
12	BB	7.7	Year	125	125	125
4	BB	7.7	Year	125	625	125
7	BB	Bait	Year	125	625	125
52	BB	Nil	Month	Died:	FAT Positive	
54	BB	Nil	Month	Died:	FAT Positive	
16	SS	Nil	Year	Died:	FAT Positive	

SS - Side-striped jackal
BB = Black-backed jackal
* Died during the eighth month. No challenge given.

Safety trials of SAD (Berne) in non-target species

Only chacma baboons *(Papio ursinus)* have been tested to date.

- Wild-caught adult baboons were used.

- 2mls of "field strength" vaccine ($10^{7.7}TCID_{50}$/ml) were instilled orally under light anaesthesia.

- Baboons were observed for four months after vaccine instillation.

- No virus could be isolated from saliva taken 1, 3 and 7 days after instillation.

- Two of four baboons died of rabies after incubation periods of 11 and 13 days.

- Rabies in these two was confirmed by FAT and mouse inoculation.

- Monoclonal antibody studies showed that the virus was indistinguishable from SAD.

- No virus was isolated from their salivary glands.

- The highest antibody titre obtained from the two surviving baboons was 1:25.

Reported in Bingham et al., 1992.

Baiting of jackals

Baiting systems are variable and need to be modified according to local conditions.

The following general principles have been found to be helpful in maximising bait uptake by jackals:

- The bait must be attractive and "chewy". Meat-type baits are the most attractive to jackals, however, chunks of muscle or offal are often swallowed whole. Chicken heads are ideal.

- The area around the bait should be scented with attractant to draw jackals from a distance. A liquid putrefying mixture of offal and eggs works well.

- Baits should be laid strategically, e.g. along roads or paths.

- Baits should be laid under vegetation or cover to minimise detection by scavenging birds and to minimise exposure to sun.

- Baiting should take place preferably during the winter months and during the afternoon, as this is most favourable for vaccine stability.

A baiting system for the Lowveld areas of Zimbabwe

Baits (chicken heads with tetracycline) were broadcast from a vehicle in clusters of 3 at intervals of 500 metres along every road.

Bait density = 6.3/km^2 or = 6.5/km of road; Total area = 265 km^2

Up to 72% of jackals will consume at least one bait.

The major non-target bait-consuming species are wart-hogs, civets, honey badgers and baboons.

Up to 70% of baits are taken during the first night by jackals, although this depends on the time of year, food availability, etc.

Survey of background Tetracycline levels in jackals

- 33% of the jackal population has bone fluorescence indistinguishable from tetracycline. This will mean that tetracycline will be unsuitable for use as a biomarker.

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Requirements for Oral Vaccination Against Rabies in Africa

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In recent decades enormous progress has been made in our understanding of biology, pathogenesis, epidemiology, and control of rabies. Molecular biology has led us to the threshold of a new era in disease prevention. Nevertheless the number of human rabies deaths in the world has not diminished accordingly. The majority of these fatalities occur in developing countries.

Rabies Control: Goals and Targets

The ultimate purpose of rabies control is the protection of man from both infection and economic losses. The occurrence of rabies in man can be controlled by prophylactic vaccination and post-exposure treatment, by reducing the risk of human exposure, or conclusively by disease elimination. The easiest way to reduce the incidence of human infection is by prophylactic immunisation of those domestic animals which are the most common source of human exposure. A far more ambitious task is the elimination of rabies in its main host.

Over large areas of Africa, Asia, and Latin America, dogs make up the vast majority of all rabies cases diagnosed in animals. in many places no wildlife rabies cycle is recognised. Where dog rabies is independent of wildlife rabies, it should be possible to eliminate the disease by proper mass vaccination of dogs. This has indeed been achieved. Mass vaccination campaigns of dogs, as early as 1920 in Japan and later in other parts of the World, eliminated canine rabies in these areas (Bögel et al., 1982; Baer and Wandeler, 1987; Larghi et al., 1988). In the 1980s, dog rabies control programmes were very successful in Latin American cities, but unfortunately not in other areas with canine rabies.

Wildlife rabies control by decimating host populations has been attempted in nearly all host species. However, their resilience to persecution and reproductive potential together with high carrying capacities of rural and urban habitats, often render control efforts unavailing. More promising is the mass vaccination of the main hosts, although immunisation of free-living wild animals is not an easy task. The wild mammal has to be lured by some trick into vaccinating itself. This is possible when oral vaccines are included in baits targeted at the principal rabies host species. The methods have to be simple and efficient, so that it becomes technically and economically possible to establish the level and distribution of herd immunity required to eliminate rabies.

The goal of wildlife rabies control by oral immunisation should be local elimination of the disease, or control of its spread to uninfected areas. It is necessary to know the host species in order to direct control efforts properly. For example in Europe, efforts have to be directed toward red foxes *(Vulpes vulpes),* since they alone are responsible for the maintenance and spread of the disease. It would not make sense to immunise or control European badgers (*Meles meles)* and stone martens *(Martes foina).*

However, as long as there is wildlife rabies in an area, it is also important to have dogs, cats and eventually other domestic species vaccinated, as they can occasionally become infected through contact with foxes and transmit the disease to man.

The species of the order Carnivora recognised as main hosts of rabies in different parts of the World are: the red fox (*Vulpes vulpes*) in subarctic and northeastern North America, in temperate zones of Asia, and in Eastern and Central Europe; the arctic fox (*Alopex lagopus)* in arctic regions of America and Asia; striped skunks *(Mephitis mephitis)* in the American Midwest and in California; raccoons *(Procyon lotor)* in Eastern North America; different species of mongoose *(Herpestidae)* in southern Africa, in the Caribbean, and possibly also in South Asia; and jackals (*Canis aureus* and C. mesomelas) in Africa and parts of Asia (Blancou, 1988). They all are able to support initial epidemics of high case density and thereafter an oscillating prevalence over many years. They all are small to medium-size omnivores, scavenging, and preying on rodents, other small vertebrates, and invertebrates.

Several other carnivore species, besides those mentioned above, may also function as main hosts of rabies, but the role of other common carnivores in Africa, Asia, and Latin America is not, as yet, well understood. Findings with monoclonal antibodies indicate that a number of specific African rabies strains are associated with different African host species (see King, this symposium). However the link between rabies strain specificities and population biology and behaviour of hosts has not yet been examined fully. Populations of a number of bat species in the Americas, in northern Europe, and perhaps also in Asia and Africa also maintain independent epidemics. The rabies strains circulating in bats are transmitted to terrestrial mammals (including humans) only rarely, with the exception of bovine paralytic rabies transmitted at high frequency by vampire bats in Latin America.

All these observations point to the importance of defining the purpose of rabies control campaigns. The epidemiology of the disease needs to be understood. If dogs alone are responsible, disease elimination is an achievable goal. If wild carnivores are recognised as the "reservoir", it might be easier to protect humans by immunising their pets rather than by attempting to eliminate the disease. This strategy was adopted by many States of the USA between 1945 and 1955 with the effect that both dog rabies and human rabies cases declined sharply, in spite of persisting wildlife rabies (Baer and Wandeler, 1987).

If disease elimination by wildlife vaccination is envisaged (as in parts of Canada and many European states), it becomes essential to identify the responsible target species. Their ecology and population biology should be reasonably well documented, so that effective bait-distribution strategies can be designed, and the ecological impacts of vaccination campaigns can be assessed.

Oral Vaccination against Rabies: Preconditions

A vaccine to be used for oral immunisation of freeliving wild animals should comply with a number of requirements (Wandeler, 1991).

1. The vaccine should immunise the target animals orally. There are marked species differences in the magnitude of the immune response following exposure to oral vaccines. For example: red foxes and probably also species of jackals can be immunised easily with a number of modified live virus vaccines (SAD and SAD derivatives). Arctic foxes, raccoons and domestic dogs require higher doses for effective immunisation, while other species, e.g. skunks, cannot be immunised by the oral route with these live attenuated vaccines. Genetically engineered vaccines may have completely different species specificities. Only a few potential target species have ever been tested thoroughly with the available poxvirus (e.g. V-RG) and adenovirus (Ad5-RG) recombinant vaccines.

2. The vaccine should be apathogenic for man, for the target species and for other species eating the bait. Unfortunately complete apathogenicity for all species (including immunocompromised individuals) may be an unattainable goal for any live-virus vaccine. SAD, which was instrumental in the elimination of rabies from large parts of Europe, has a residual pathogenicity for practically all rodent species tested, for immunocompromised individuals of numerous species, and as recently documented, also for a primate species, Papio ursinus (Bingham et al., 1992). There are several ways to modify the genetic make-up of a vaccine virus in order to reduce its pathogenicity. The escape mutant SAG was selected from SAD using a monoclonal antibody directed against a pathogenicity factor (Flamand et al., 1988). The recombinant vaccinia rabies-glycoprotein,(V-RG) vaccine has a much reduced residual pathogenicity due to the elimination of the thymidine-kinase gene (Buller et al., 1985).

3. The vaccine should not be excreted. Again, this is a requirement which is difficult to fulfil completely. All live vaccines, whether live attenuated or recombinant, must replicate in the host species to elicit an immune response. A future generation of genetically engineered vaccines may have the ability to introduce an antigen gene into host cells without producing infectious progeny.

4. The vaccine should not easily mutate to higher pathogenicity.

5. It should be free of contaminants.

6. It should not rapidly lose immunogenicity during storage.

7. It should be stable at environmental temperatures for several days, but not for prolonged periods.

8. It should be easy to produce and inexpensive.

9. It should bear at least one genetic marker.

If a safe, efficacious, and sufficiently thermostable vaccine is available, then a suitable bait needs to be selected. The most important qualities baits should have for proper vaccine delivery are that they should be:

1. attractive for the target species,

2. eaten by the target species rather than stored (cached),

3. avoided by other species (including man). All baits tested so far have been picked up not only by various domestic and wild carnivores, but have been taken up by ruminants and rodents as well, the animals most vulnerable to the residual pathogenicity of SAD strain,

4. available to a large proportion of the target species population,

5. not harmful to the vaccine,

6. designed to release the vaccine into the oral cavity, or bring enterically coated or acid-resistant vaccines into the small intestine,

7. permissive to the incorporation of a biological marker (e.g. tetracyclines),

8. easily available, inexpensive and storable,

9. able to maintain their integrity and attractiveness in the field for at least as long as the vaccine remains active.

In the event that vaccine and bait are found to be suitable, then the next goal is a vaccine delivery system that assures mass immunisation of target species. This requires temporal and spatial bait distribution strategies (for details see Wandeler, 1991). When deciding on these strategies it is important to take into consideration technical resources (vaccine storage facilities, airplanes, vehicles, cold chain), administrative structures, and manpower needs, as well as constraints imposed by safety requirements, terrain, climate, etz.

Oral Vaccination against Rabies: The State of the Art

The control of wildlife rabies by immunisation became an attainable target in the early 1970s when it was found that attenuated rabies vaccine immunised foxes orally (Black and Lawson, 1970; Baer et al., 1971). This discovery suggested the possibility of administering an oral rabies vaccine to wild carnivores by bait. It was found that the SAD and ERA strains and their derivatives fulfilled the majority of the requirements to immunise foxes. The first field trial in foxes was carried out in Switzerland in 1978, followed by additional campaigns. These rapidly freed the major part of Switzerland and later large areas of other European countries and Canada from rabies (Wandeler, 1991). More recently, genetically engineered recombinant vaccines have been prepared with great potential for wildlife rabies control (Blancou et al., 1986, Rupprecht et al., 1986). A vaccinia-rabies glycoprotein recombinant (V-RG) has been used successfully in Belgium (see Brochier, this symposium; Brochier et al., 1991), Luxembourg, and in parts of France; and first limited field trials are being carried out in raccoon rabies areas of the eastern USA.

The most important conclusion to be drawn from the field applications of oral vaccination in Europe and in Canada is that it is possible to immunise enough foxes by bait in order to stop the spread of the disease into rabies-free areas and to eliminate the disease from enzootic areas. In areas freed of fox rabies the disease also disappeared from all other species (not from bats). The disease did not re-appear spontaneously from an undetected reservoir after fox vaccination campaigns were discontinued, but rabies was occasionally able to re-invade a fox population from infected contiguous areas.

European and North American fox rabies can be eliminated by oral immunisation with live attenuated vaccines and with V-RG, and there is good reason to assume that raccoon rabies in eastern North America could be controlled with V-RG (Hable et al., 1992). However very little progress has been made so far in controlling other wildlife rabies epizootics in other geographic areas. Since it is possible to vaccinate jackals by the oral route with the vaccines currently available, present efforts to test their safety in African non-target species, to develop jackal-specific baits and bait distribution schemes, may soon lead to testable vaccination systems for these species (see Bingham, this symposium). Unfortunately, other important rabies vectors in Africa, such as mongooses, have not been well investigated.

It might be tempting to use oral vaccination to protect endangered African carnivores like wild dogs (*Lycaon pictus*) from rabies. But it is clearly contra- indicated to use any live attenuated or genetically engineered vaccine not tested for immunogenicity and residual pathogenicity in the target species. In the case of the African wild dogs it is not even certain that rabies is responsible for all of pack die-offs. If distemper or other immunocompromising diseases are involved, the application of live oral rabies vaccines may have disastrous effects.

Oral Vaccination against Rabies: Safety and Environmental Impact

A great number of concerns have been expressed about the release of genetically modified organisms into the environment. Many of them relate to the degree of predictability of the epidemiological behaviour of the live vaccine once released. Regal (1986) has made an attempt to categorise these concerns in a number of theoretical models. In discussing these models Regal concludes that all of them have some deficiencies, and that each case needs to be assessed individually (see also Tiedje et al., 1989).

Concern number one about the release of genetically modified virus vaccine is that the vaccine virus itself (e.g. SAD in rodents) or a variant (mutant, recombinant) of it may cause mortality in target or in non-target populations. Differences of vaccine pathogenicity for different species --(e.g. SAD: carnivores - rodents; Ad5-RG: Mus - Sigmodon) are not always predictable. Deletions in the viral genome created for accommodating the insert may alter the species specificity and pathogenicity. Horizontal transmission could lead to adaptive evolutionary changes of vaccine virus properties. Thus far, the laboratory studies with SAD and V-RG, and subsequent field investigations, have produced no evidence that vaccine virus spreads among target or non-target species or that they change host specificity and pathogenicity (Wandeler et al., 1982; Wandeler, 1j91).

Another popular concern relates to the altered selection pressure on the rabies virus itself. Street rabies virus may change antigenic properties in an immune host population. Some of the new recombinant vaccines give only partial protection and set the scenario for the selection of escape mutants of street rabies virus. No such events have been observed in monitored oral vaccination zones of Central Europe and Ontario. It does not seem likely that such mutants have properties leading to horizontal transmission.

The purpose of oral wildlife vaccination is the elimination of rabies, in other words the elimination of an important mortality factor. The target species is a carnivore embedded in a particular ecosystem, with its own predators, competitors, etc. No doubt, its abundance is of significance in this complex, interactive, multispecies system. Any alteration of target species population dynamics will bring changes for the species they prey on and for their competitors.

All the above considerations indicate that careful evaluations are necessary before a live (attenuated or genetically engineered) vaccine is applied in the field. The preconditions (vaccine efficacy and safety) formulated above need to be rated for the species occurring in the target area. Population parameters of the species influenced should be compiled. A good system of surveillance needs to be established. Information needs to be collected on the actual impact of rabies, on the prevalence of naturally occurring rabies antibodies, and on the prevalence of viruses related to those used in the recombinant vaccine. Even when all laboratory studies and subsequent field investigations have not produced any evidence that unforeseen events may result, scrupulous surveillance of every wildlife vaccination experiment is still indicated.

Dogs are not Wildlife

Dogs are kept and/or tolerated in very high numbers in most human societies. Their abundance is not explained by their limited economic usefulness. Cultural practices determine the level of supervision of their social interactions and access to resources (food, water, shelter, mates). It is assumed that high density dog populations permit the occurrence of enzootic canine rabies, but this is not very well documented. We suspect that the disease in dogs may not always exist independently from wildlife rabies. There is, however, no doubt that rabid dogs are the major source of human infection. The widespread occurrence of human rabies is not only due to the frequency of exposures, but also to failure in applying proper treatment after bites from rabid animals.

There are comprehensive guidelines from WHO and WHO/WSPA for dog rabies control and dog population management. These documents give detailed guidance on the planning and management of control programmes, on legislation, and on technique, in local programme execution. Where dog rabies is independent of the occurrence of the disease in wildlife, elimination should be the goal rather than a temporary reduction of the incidence rate. The most economical way to achieve this goal is by mass vaccination. Unfortunately, this target is often not reached (see Perry, this symposium).

There may be many reasons for failures for not reaching the required herd immunity in dog populations: inadequate logistics, insufficient community participation, large numbers of ownerless dogs, etc. It is often thought that a majority of these problems could be solved with an oral vaccine for dogs. While oral wildlife immunisation allows for efficient bait distribution by airplane (Johnston et al., 1988), baiting systems for domestic dogs require different approaches.

Baits containing vaccine may be spread in areas where many ownerless dogs concentrate, e.g. garbage dumps, but with this measure only a minority of a total dog population will be reached. Owned, but poorly supervised dogs may be fed baits on the road as they are encountered, or baits may be distributed to owners with instructions to feed them to their dogs (Frontini et al., 1992). All approaches would require considerable logistic efforts, which may be as great as the efforts required for a campaign using traditional parenteral immunisation. Whatever the bait distribution system is, the chances for human exposure to the vaccine is much higher than in oral wildlife immunisation programs.

Oral vaccines for dogs are under development, but none is ready for field application. Limited trials (mostly unpublished) of oral immunisation of dogs with a number of candidate vaccines produced acceptable results when high titered vaccine was instilled directly into the oral cavity. The vaccine virus titres needed to be higher than for oral fox immunisation. Even higher doses of virus were required when vaccines were given in baits. The real handicap is safety. Dogs are very closely associated with humans, especially with children, in a majority of cultures. The following scenario is a possibility: a dog picks up a bait, releases the vaccine into its oral cavity, enters his owners' house and licks the baby's face. The baby may have a flu or other immunocompromising disease ...

It is therefore imperative that oral dog vaccines meet higher safety standards than those presently used for oral wildlife immunisation. Killed oral vaccines, and recombinant vaccines using vectors incapable of complete replication in mammalian cells, or which do not have humans as potential hosts should, be given preference. Escape mutants derived from traditional modified live virus and recombinant vaccines using vectors with deletions should be shown to be innocuous for primates. Traditional modified live virus vaccines (SAD, ERA) should not be used for oral vaccination of domestic dogs because of their well documented, residual pathogenicity.

Conclusions

Rabies elimination by oral immunisation of wildlife is possible. But the technologies developed to control wildlife rabies in Europe and North America need adaptation to African conditions. Efficacy and safety concerns need to be addressed; aspects of logistics need to be analysed. In order to design an effective rabies control program it is essential to be well informed about the epidemiology. There is a long list of African carnivora possibly involved in maintaining rabies. The required herd immunity can only be established in a population when both of the following conditions are met: 1. the vaccine is safe and potent for field application, and 2. the vaccine delivery system assures the mass immunisation of the target species.

Oral vaccination of domestic dogs might facilitate canine rabies control in future. There is no field tested system available yet. However, dog rabies control can be achieved today by well planned and executed parenteral vaccination campaigns.

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Appendix I

FAO ACTIVITIES RELATED TO RABIES CONTROL IN AFRICA

We fully agree with the description given by Crick[1] (Gibbs, 1981): "Rabies is not a major disease of food animals, and in general, its direct economic effect on the farming industry is small". From the FAO point of view complexity of animal rabies control, its zoonotic character as well as nearly totally mortal outcome after occurrence of the disease's symptoms may have a negative impact on:

- the general disease situation within the country (territory)
- relevant ecosystems
- through engaging: veterinary, medical and municipality services and using limited financial resources on important food animal diseases control.

In the chapter of the "Virus diseases of food animals" (also Gibbs 1981) Rweyemanu classified rabies within the Category A and quoted rabies as a fatal infection both in domestic and wildlife hosts.

The International Conference "Rabies in the Tropics" held in Tunis in October 1983, and organised by the WHO reviewed: current knowledge of rabies and rabies related viruses and information on rabies vaccines for man and animals, epidemiology of rabies in the tropics and outlined requirements for animal rabies control including participation of medical and veterinary services. The present Pilot meeting on Rabies in southern Africa should address the similar issues with regards to the Sub-region.

As formulated by the WHO Expert Consultation on Rabies (Geneva 1991), on a global scale the most dangerous reservoir of rabies is the dog population. Transmission from dogs represents over 99% of all human cases of rabies reported world-wide. The distribution of rabies (percentage) in endemic areas in Africa gives the following figures (Turner 1976, cited by Crick 1981):

Area	Dogs	Cats	Farm Animals	Wildlife
North	88	7	5	0.1
Central	98	1	1	-
South	25	3	24	48

The data surely requires updating (which is one of the objectives of the present meeting). Please note the high percentage (48%) of wildlife reported to be associated with rabies cases in southern Africa.

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1 Crick, J. (1981). In: Virus Diseases of Food Animals. R. E. P. J.Gibbs, Ed. Vol.II. Disease Monographs. Academic Press, pp469 - 516

The proportion of human population living in urban areas of Africa[2] will increase by nearly 20% by the year 2025. Dog population control in urbanised areas may become even more important in the future and may have to be carried out on a larger scale than at present..

The FAO direct and indirect involvement in rabies control included:

1. Until 1980:

   Latin America: Project on control of vampire bat rabies in cattle Bolivia (1960s - 1978).

2. Since 1980s.

a) Participation in the WHO Meeting on Rabies 1983, 1991 - FAO experts participated in the WHO Expert Committees on Rabies, 1992 (Geneva), and the OIE Ad Hoc Meting on Rabies Control, Paris.

b) Requests from developing Countries:

1983. Lesotho: requested vaccine for control of human and dog rabies.

1989. Yemen Arab Republic: project formulation mission: "Human and Canine Rabies control" was implemented by the consultants: rabiologist and dog ecologist. The increased number of human cases (20-10 per year) since 1982 and the fact that 98% of exposures were caused by dogs (mostly urban) were reported. The FAO stressed the necessity to reduce the stray dog population by almost 300,000 in the big cities. The project intended to assist the country to control rabies in man and animals by decreasing the number of stray dogs, informing the public opinion, training personnel, establishing a rabies monitoring system and extending post-exposure treatment. The formulated project has not been finally approved for implementation by the Government of Yemen due to changed priorities. Also the intended dog population management and control were not fully acceptable to medical, veterinary and municipality departments to be involved.

1990. Sultanate of Oman: the country remained free from rabies from 1979 until 1990 when the first cases of human, domestic animals and wildlife rabies were reported. The mission of a rabiologist was launched to ascertain the epidemiological and epizootiological situation of fox and human rabies and to prepare outlines of project proposals covering:

- prevention and control of the disease in both human and animal populations,

- realistic programme for fox rabies control by oral immunisation,

- investigations of the availability of national resources, laboratory facilities for rabies diagnosis and technical know-how of the staff.

1991 (June), Thailand: request was received to strengthen a cell culture vaccine production in Pak Chong;

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2 "Prospects of World Urbanisation" Population Studies No. 112, UN, Dept. of International Economic and Social Affairs, 1988 (ST/ESA/SER. A/112, New York).

1991 (October), Ecuador: request was received to prepare the sub-regional project on prevention, control and eradication of sylvatic rabies in the countries of Andean Sub-region;

1991 (November), WHO/VPH requested to assist a consultant in his visit to Tanzania to investigate the outbreak of human and canine rabies in Unguja Island;

1992 (March), Oman: consulted FAO on veterinary vaccine to be used in animal rabies control.

1991 to date. The FAO considered preparation of the fact finding, project formulation mission to the Middle East to be launched early 1993 in order to prepare the regional programme/project proposals on animal rabies control in arid and semi-arid ecosystems. The mission will be co-ordinated with the VPH/WHO.

A. Meetings:

1980 Expert Consultation on Control of Emergency Diseases, Rome, June.
1981 Inter-Governmental Consultation on Development of Facilities for Veterinary Education in Southern Africa, Lusaka, March.
1982 FAO/OAU Expert Consultation on Improvement of Diagnostic Services in Africa, Nairobi, November.
1982 Workshop on Emergency Disease Control, Limassol, Cyprus, November/December
1983 Séminaire sur 1'Epizootiologie et les aspects économiques de la santé animale, Niamey, Niger, Janvier.
1983 Stage sur la production des vaccins, Dakar, Octobre/Novembre.
1984 Workshop on Emergency Disease Control, Bangkok, November.
1984 Expert Consultation on Veterinary Education in Africa, Nairobi, December.
1986 Expert Consultation on Improvement of Animal Health Services in CILSS Countries.
1986 Expert Consultation on Biotechnology for Livestock Production and Health, Rome, September.
1988 Expert Consultation on the Application of Biotechnology in Livestock Production and Health in Developing Countries, Havana, September.
1990 Expert Consultation on Cost/Benefit Analysis for Animal Health Programmes in Developing Countries, Rome, September.
1991 Expert Consultation on Veterinary Vaccine Quality Control in Developing Countries, FAO, Rome, gave specific recommendations on strengthening of rabies vaccine Q.C. as well as applying wherever it is possible the joint production and quality control of medicines and veterinary vaccines. The Q.C. of rabies vaccines was recommended to be strengthened on two levels: by a manufacturer and by national control authorities, December.

B. Publications:

- Reports of the above-mentioned meetings.
- "Emergency diseases of livestock", Vol. 1: "Diseases and diagnoses", Vol. 2, pp. 147. Contingency planning for emergency diseases and emergency actions, FAO Rome, 1984.

C. Activities of the Collaborating Centres:

Biotechnology (information networks on molecular virology), The Institute of Virology, University of Rome, Italy.

Veterinary diagnosis, Central Veterinary Laboratory, New Haw, Weybridge, UK.

Veterinary diagnosis (tropical veterinary medicine), Centre Veterinary Studies, Roslin, Midlothian, Scotland, UK.

Veterinary epidemiology and informatics, Instituto Zooprofilattico Sperimentale, Teramo, Italy.

D. Activities of the FAO/WHO and WHO/FAO Collaborating Centres:

Research and Training in Veterinary Public Health, Indian Veterinary Research Institute, Izatnagar, India.
Istituto Superiore di Sanita, Rome, Italy.

E. Projects:

The FAO project in Zambia "Establishment of a Virology Laboratory at the Central Veterinary Research Institute, Balmoral" - operating near Lusaka, has potential to assist the Government of Zambia and its future activities for southern Africa.

F. Pan African Veterinary Vaccine Centre (PARVAC)

The PANVAC has been developing since 1984/85 under the FAO/0AU/UNDP project initially under the name of the FAO/0AU/URDP Regional Vaccine Quality and Training Centre. Two PANVAC centres have been established in the hosting national veterinary institutes of Dakar, Senegal and Debre-Zeit, Ethiopia, to strengthen vaccine production, quality control and relevant equipment maintenance in 23 countries. In Africa, 23 veterinary vaccines are being produced on different technological levels. Veterinary anti-rabies vaccines are produced in nine countries: Cameroon, Chad, Ethiopia, Kenya, Madagascar, Mozambique, Nigeria, Senegal and Zaire. The development objective for PARVAC is to make Africa self sufficient for the essential veterinary vaccines through:

1. Information quality control of priority vaccines.
2. Promotion of biological standardisation and control in Africa.
3. Vaccine technology transfer to Africa including adaptation or development to suit local conditions.
4. Training and support services to African vaccine producers including technical backstopping of the repair and maintenance of vaccine equipment.

The priority vaccines have been defined by the Directors of the National Vaccine producing laboratories participating in the Pan African meeting (Nairobi, September 1990) and endorsed by the Directors of Veterinary Services of OAU Member States as: Rinderpest, Contagious Bovine Pleuropneumonia (CBPP), Newcastle Disease, Rabies, Foot and Mouth disease (Eastern and Southern Africa) and a bacterial vaccine (to be defined on regional basis).

As the quality of the tissue culture rinderpest vaccine has been a most important issue for the ongoing Pan African Rinderpest Campaign (PARC) covering 34 countries, the major emphasis during the first year of PANVAC operation was QC of that product manufactured in 12 countries. Later, (1991) a continental quality testing of the CUP vaccine started.

PANVAC has gained the confidence of African vaccine manufacturers providing continuous technical backstopping and training. It has been acknowledged internationally that strict and comprehensive vaccine testing for PARC significantly contributed to a dramatic decrease of rinderpest infected countries in Africa. Out of 18 countries declaring rinderpest in the early 1980s, in 1991 only 3 countries showed the disease, mostly in inaccessible areas affected by civil disturbances.

Regular training seminars and workshops run by two PANVAC Laboratories as well as bench training and carefully targeted consultancies help vaccine producers on different levels. Meetings with African Vaccinologists as well as information provided through the regularly published 'PANVAC Bulletin' also contributes to dissemination of scientific and technical information. The PANVAC was started as the first international institution integrating vaccine production manufacturers and technologists and preparing computerised data base on the institutions, technological issues and manpower. An internationally recommended institutionalisation of the PANVAC has started as the first international institution integrating vaccine manufacturers and technologists and preparing computerised data base on the institutions, technological issues and manpower. An internationally recommended institutionalisation of the PANVAC has started and the relevant project proposals have been submitted to potential donors. The expected outputs will cover:

- Expansion of the programme for international vaccine quality control of the six priority vaccines (including rabies)
- Establishment of a network of six sub-regional QC laboratories
- Creation of a depository of characterised reference vaccine materials,
- Establishment of internationally recognisable QC criteria
- Promotion of the Principles of Good Manufacturing Practices (GMP)
- Establishment of a practically orientated process development unit within PANVAC
- Setting up of an effective PANVAC Documentation and Vaccine Data base
- Technical back stopping of vaccine producers in repair and maintenance of laboratory equipment.

The PANVAC is expected to upgrade rabies vaccine production and quality control within the Region and on the international scale.

G. Pan African Institute for Animal Health (PANHEALTH)

Since 1985 the FAO has been working on the concept of the Pan African Institute for Animal Health (PANHEALTH). A need has been recognised for a centre in Africa for technology transfer and development as applied to animal disease diagnosis and control which would: identify, test under African conditions, further develop as necessary and eventually suggest application for technologies, providing

training for African scientists in the methods, techniques involved and advise and assist governments directly or indirectly in their application. While the centre would be primarily orientated to African disease problems, it would also provide a base for scientists from developing countries to work on and contribute to the transfer of the new technologies to meet the needs of the region.

During three Project Formulation Missions (1985, 1989 and 1990) many concepts have been developed including:

a) Reference Diagnostic Centre for Africa (Kenya).
b) International Centre for Veterinary Epidemiology and Diagnostics (Zimbabwe).
c) the PANHEALTH with the core centre in Eastern Africa, two sub-regional centres wad networking support to forty national diagnostic laboratories.

The Project's mandate of PARREALTH included:

- transfer of new diagnostic and disease control technologies to Africa and standardisation of existing diagnostic procedures for field application

- Training in current and new diagnostic procedures to African animal health and laboratory personnel

- development of new diagnostic tests or packages that can be applied readily at field laboratory and farm level

- production and distribution of diagnostic reagents, biologicals and kits for the tests which prove to be cost effective

- application of modern technologies and concepts to the research on those important diseases or disorders of animals in Africa that impose an important economic constraint on livestock developments

- research on those animal diseases which originate in Africa and which may have an economic and/or sanitary importance for other continents

- application of modern concepts of epidemiology, including economics of disease, in order to define their patterns and the most cost effective control strategies

- acting as the continent-wide IBAR/FAO reference centre for tropical animal diseases

- strengthening of the national capability in disease diagnosis and control including emergency back-up through visits of PANHEALTH specialists and/or consultants. In 1990 the Project Document was prepared for 10 year programmes and establishment of a core and two sub-regional centres. The above-mentioned mandate and network approach cleared by QAU and FAO was suggested (total US$ 36m). Eight African countries, Zimbabwe, Ethiopia, Kenya, Uganda, Tanzania, Mali, Senegal and Cameroon expressed their offers to host the core centre or subcentre. The concepts have been discussed at technical and political level.

The final version, due to the high budget requirements and a necessity of establishing a core centre and sub-centres has recently been redrafted due to difficulty in finding donors, and some doubts about the designed institution's cost effectiveness and prospects for a sustainability. Since March 1992 the FAO HQs has been preparing the modified concept of the mobile task force institution (network) to serve the same purpose, but to be based on already existing institutions and partly networks. Finalisation of the modified project is expected during 1992.

The PANHEALTH would definitely contribute to strengthening of rabies diagnosis verifying the existing status and level of diagnostic testing available and their standardization and upgrading.

The organisation of African Unity/International Bureau for Animal Resources OAU/IBAR has been very supportive on the issues of PANVAC and PAN HEALTH. A similar support to be already existing and well functioning structures of PANVAC has been expressed on different international technical and political fora such as the OAU, OIE, Conferences of Ministers and Meetings of Directors of Vaccine Production Laboratories in Africa. The FAO initiative on international strengthening of veterinary vaccines and disease control methods (including: diagnostics, technology transfer and modern epidemiology) in Africa has been monitored by other geographical Regions and Organizations and there are attempts to create similar structures, i.e. in Asia. Both PANVAC and PANHEALTH are relevant to international attempts strengthen rabies control in southern Africa.

H. FAO Networks on Biotechnologies

The AGAH has been developing the following major networks:

1. The FAO Technical Co-operation Network including laboratories in Argentina, Brazil, Columbia, Chile, Mexico and Uraguay (Latin America) and China, India, Indonesia, Malaysia (S.E. Asia). The network deals with biotechnologies related to veterinary diagnosis and vaccines (including rabies).

2. Computer Assisted Network on Nucleic Acid and Protein Sequencing in Latin America (CANAPS).

In order to assist molecular biologists from 12 countries of the Region: Argentina, Bolivia, Brazil, Columbia, Costa Rica, Cuba, Chile, Guatemala, Mexico, Peru, Venezuela and Uraguay, nucleic acid and protein sequencing, the FAO since 1989190 has been organising the CANAPS network aiming at development of new vaccines and specific diagnostic tools (nucleic acid probes). The new techniques will be useful tools for a control and eradication of infectious and some parasitic diseases of livestock and poultry.

About 60 laboratories using personal computers will be interlinked through modems/telephone lines with two powerful VAX computers (nodes) located in: the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, and the Department of Biotechnology, University of La Plata, Argentinia, and Laboratorio Technologico del Uruguay. Apart from the assistance in genetic manipulations the network will be useful for an information exchange among scientists involved and biological 'on line' search. The network will be co-ordinated through the FAO Collaborating Centre

on Biotechnology (Information networks on molecular virology) located in The Institute of Virology, Rome. It is anticipated that in early 1993 this FAO sponsored network will be linked to the European ones (nodes: Heidelberg and Trieste) and later with other continental networks of that type.

3) Eastern/Central European Network on Biotechnology

It is foreseen that in future similar networks will be developed in Africa inter-linking i.e. vaccine producing and diagnostic laboratories and related structures. This would facilitate planning for an integrated rabies control strategy in the Area.

Appendix II

THE WORK OF WHO IN RABIES

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Rabies still represents a significant problem in both developed and developing countries. In the former group of countries rabies reservoirs are constituted by wildlife species (e.g. foxes, raccoons, skunks, raccoon dogs etc.) and the burden for the health sector is essentially due to a relatively large number of costly rabies treatments applied to exposed people (e.g. 100 to 150 persons per million inhabitants). Most of the estimated 35,000 human deaths due to rabies occurring at the global level are reported in about 90 countries where about 2.4 billion people live. It is estimated that between 150 and 250 million dollars are spent annually in human post-exposure treatment and activities for the control of rabies (i.e. dog vaccination and dog seizing operations).

Since its inception WHO has been active in the rabies field. The first veterinary public health activities of the Organisation in 1948 focused on rabies and were the expansion of work carried out internationally by the "Societe des Nation" (SDN) during the 1930s on the safety and efficacy of human rabies post-exposure treatment. Very rapidly WHO, in order to provide Member States with a practical review of current and most modern techniques used in the Laboratory, published for laboratory workers of both developed and developing countries a book entitled "Laboratory Techniques in Rabies". An up-date of the third edition published in 1974 is being prepared and should become available in 1993.

During the past 45 years, WHO was the convenor of eight meetings of the Expert Committee on Rabies. The last such Committee meeting was held in September 1991 and the Report* should soon become available from WHO. This Committee endorsed the use of economical reduced post-exposure regimens using modern cell-culture vaccines applied either intra-muscularly or intradermally. It also proposed an alternative strategy to long quarantine for the transfer of dogs and cats to rabies-free countries and additional simple tools and techniques for implementing dog rabies control activities. At the global level, WHO collects information on rabies and issues on an annual basis the World Rabies Survey.

As can be seen, WHO is the international organisation leading work in all aspects of human and animal rabies. During the past 25 years, WHO in addition co-ordinated research on the oral vaccination technique (OVT) and the feasibility of its application over large areas of Europe.** WHO also promoted inter-country collaborative projects for surveillance and control of fox and animal rabies in Europe. One of the outcomes is the Rabies Bulletin for Europe prepared by the WHO Collaborating Centre for Rabies Surveillance and Research in Tubingen, Germany.

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* WHO Expert Committee on Rabies, Eighth Report. Geneva. World Health Organization, 1992 (WHO Technical Report Series No. 824).

** see article "Control of Rabies in Wildlife" by W. G. Winkler.

More recently, WHO promoted research on baits and baiting techniques and surveillance of OVT in various animal species present in continental USA and neighbouring countries (e.g. mongooses in some Caribbean islands).

During the past ten years major WHO activities have, however, focused on dog mediated rabies which remains the cause of nearly all human rabies deaths recorded world-wide. The VPH Unit of WHO, to assist Member States, issued in 1983 and updated later on, the Guidelines for Dog Rabies Control, which provides detailed and practical information on appropriate techniques for planning and management of dog rabies programmes, for conducting dog population studies, on proper technologies for rabies diagnosis and rabies vaccine production.

WHO headquarters also co-ordinated an inter-regional programme sponsored by AGFUND (Arab Gulf Fund for United Nations Development Programmes) and Radda Barnen (the Swedish branch of Save the Children) involving pilot areas located in developing countries with different geographical, social and cultural backgrounds (i.e., Ecuador, Sri Lanka and Tunisia).

The project led to the elaboration of a simple strategy for conducting mass vaccination campaigns of dogs, the accumulation of comprehensive information on the dog populations in urban and rural areas of each pilot zone, as well as on the dog-human bond in these countries.

The project also allowed the development of simplified techniques for gathering basic information on the target (dog) population prior to or during the implementation of control activities. Research on the feasibility of oral vaccination of dogs was also performed within the framework of this project. A film on human and canine rabies in developing countries (with English, Arabic and Spanish commentary) was made with AGFUND support and is now available from WHO. Models of aids for public information/education were also developed under the WHO/AGFUND project.

WHO also organised, during the ten years under review, a series of consultations dealing with specific topics such as (a) rabies vaccine potency tests including the evaluation of in-vitro techniques as potential replacement of the mouse protection test (NIH test); (b) the evaluation of the efficacy of reduced post-exposure regimens for humans; (c) the use of monoclonal antibodies for rabies diagnosis and research and (d) the safety and efficacy requirements for rabies vaccines for the oral vaccination of dogs.

WHO's work is carried out thanks to its network of Collaborating Centres located in Europe, North America, India and Thailand, as well as associated reference laboratories. The WHO Regional Office for the Americas deserves special mention as it initiated in 1983 a regional programme for canine rabies control in major urban centres of South American countries. The programme, which aims at eliminating urban rabies by 1995 has already contributed to largely reducing the number of human deaths in this part of the world.

For the years to come, WHO, with its collaborating centres and regional offices, will continue to promote rabies research and to support national programmes for rabies control and elimination. Oral vaccination of dogs will be one of the main topics for WHO co-ordinated research, together with the development of new rabies diagnosis tools, cheap products for human and, whenever advisable, animal post-exposure treatment.

Regional or sub regional meetings, similar to this one, where country representatives express their country's interest in rabies control activities and demonstrate the ability of local health and veterinary services to cope in many areas with most aspects of the problem should help development, with WHO assistance and other agencies, of effective inter-country programmes for dog rabies elimination.

Appendix III

THE ROLE OF THE OFFICE INTERNATIONAL DES EPIZOOTIES IN WORLD HEALTH

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The need for an international organisation to inform governmental veterinary services of the spread of diseases was recognised as early as 1871 (World Veterinary Meeting in Vienna). However, it was the spread of rinderpest from Pakistan to Belgium in 1920 which prompted the foundation of the Office International des Epizooties - the OIE -in 1924 in Paris. Today, this world-animal health organisation has 116 member countries.

Objectives: The three main functions of the OIE as defined by the International Agreement of 1924 which created the organisation are:

a) to promote and co-ordinate experimental or other research work concerning the pathology or prophylaxis of contagious diseases of livestock for which international collaboration is deemed desirable.

b) to collect and bring to the attention of governments or their health services all facts and documents of general interest concerning the course of epizootic diseases and the means used to control them and

c) to examine international draft agreements regarding animal health measures and to provide signatory governments with the means of supervising their enforcement.

Co-operation between member countries is organised to:

- prevent the spread of contagious diseases of animals
- assist the development of animal production through improved health information
- contribute to development of health by sharing scientific progress
- ensure that international trade in animals and animal products is governed by technically justified health considerations (particular emphasis has been placed on this point by the GATT who have sought collaboration with the OIE on this matter) and to
- provide veterinary services, which are the instrument of this co-operation, with the means of operating efficiently.

The activities and budgets necessary to carry out the objectives of the OIE are decided by the OIE International Committee, which receives advice and proposals from the Administrative, Regional and Specialist Commissions.

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*Paper prepared by Dr. J. Blancou, Director General of the OIE, from documents of the OIE including PJB publications 1990.

The Director General is required to implement the decisions of the Committee, and is responsible for the administration of the OIE. Assistance to the Director General is provided by the Central Bureau in Paris, a regional bureau in Tokyo and possibly in Washington. The International Committee meets every year for a General Session, attended by representatives from all member countries.
Budget: The OIE is funded by compulsory contributions from member countries, set at rates according to a three-tier system - each country opting for the rate it wishes to pay (minimum in 1991: 8,000 US$ per year).

Other (Voluntary) contributions usually add between 10-15% on top of the regular budget.

World-wide organisations: One of the OIE's strengths is its global coverage. All major countries are members and those countries which are not members are aware of the OIE's existence and activities. The OIE can bring together countries with various political systems to discuss common animal health related problems enabling the organisation to adopt policies aimed at improving disease control.

In particular, the OIE has an important role to play in warning national governments (and regional authorities) not to become complacent about diseases which may seem to have been eradicated: no country, however advanced, can be totally sure that it is safe from diseases that exist beyond its frontiers.

Information system: The basis for the OIE's activities in disease control is the dissemination of information on the presence of diseases which pose a threat to livestock, in order that emergency measures may be taken when necessary at a national and international level.

The OIE defines two lists of animal diseases:

List A - communicable diseases of considerable severity that are highly contagious. The diseases are capable of extending beyond national boundaries and result in serious socio-economic and animal losses, upsetting international trade.

List B - the communicable diseases which are considered to be of health importance on the national scale, and which may adversely affect international trade.

OIE member countries are obliged to report immediately the occurrence or re-occurrence of any disease of List A, and any new development in a disease (List A or not), which is sufficiently important epidemiologically to justify informing other countries.

The "warning subsystem" enables countries adjoining a country in which an outbreak of a disease has occurred to be alerted and any other country which requests such information. All disease information is also communicated immediately to the FAO (Food and Agriculture Organisation of the United Nations). the WHO (World Health Organisation), the IICA (Inter-American Group for Cooperation on Agriculture) and the European Commission.

Sanitary rules applied to international trade of animal and animal products: this major activity of the OIE is described in a special (joint) document.

Other activities: Many other activities are presently carried out by the Commissions or the Working Groups of the OIE, concerning notably:

a) Aquaculture

The OIE has devoted considerable efforts to the study of fish diseases since the 1960s. The Fish Diseases Commission has been strengthened in recent years - in line with the explosion of aquaculture in areas such as Asia - and has extended its area of competence to crustacea and molluscs. The commission is preparing health regulations and standards for international trade in fish.

b) Biotechnology

A "Biotechnology Working Group" was formed in 1989 and has produced documents on the use of biotechnology-based products, diagnostic reagents and veterinary vaccines. It publishes a review *(Veterinary Biotechnology Newsletter)* of recent achievements in biotechnology and veterinary science.

c) Veterinary Drug Registration and Biological Standardisation

Another important area where progress has been made in the 1980s - and an important area for the future - is veterinary drug registration, harmonisation of registration world-wide and biological standardisation.

The OIE Expert Group on Veterinary Drug Registration (established in 1986) works in close collaboration with the "International Technical Consultation on Veterinary Drug Registration" and the "Codex Alimentarius Committee on Veterinary Drug Residues in Food" (FAO).

The OIE has established a code of practice for the registration of veterinary drugs (at the request of the Codex) and has produced three volumes of a Manual of recommended diagnostic techniques and requirements for biological products".

New trends and priorities for the 1990s

During the years 1989 to 1990 a survey was carried out in the five OIE Regional Commissions to determine which OIE activities were considered by member countries to be the most important for the next ten years. The results of the survey showed that the office should give priority to the following:

Veterinary standards for international trade

Promotion of technical co-operation for the control of animal diseases

Training in management and administration

Veterinary drug registration

In 1990, a Working Group or "Task Force" specially created by the Office, proposed that these priority objectives should be achieved by:

- Developing an information strategy
- Increasing emphasis on international trade standards
- Developing collaborating centres
- Providing an emergency response fund
- Promoting projects with national veterinary services

These objectives and strategies were approved by the International Committee of the OIE during the 59th General Session in May 1991 and they now constitute the official guide for all activities undertaken by the OIE until year 2000.

Fox oral vaccination with V-RG (Dr. Brochier)

- Is the V-RG vaccine licenced for use in Europe? The licencing is still under consideration and vaccine use is still experimental.
- What is the efficiency of bait vaccination in other species? The answer to this question will be given in a further paper tomorrow.

Wildlife rabies in southern Africa (Dr. Thomson)

- What is the effect of rabies on mongoose populations? We have no data on that but we do have evidence that from time to time there have been bat-eared fox population crashes.
- Why are there no reports of rabies in National Parks, is it because of a lack of specimens, because the disease is not detected or easily detectable? These could be the reasons, it is difficult to prove or to disprove.

Control of jackal rabies (Dr. Bingham)

- A comment by Dr. Aubert was that on the basis of the results of the baboon pathogenicity trials in Zimbabwe the use of SAD vaccine has been discontinued in France.
- What is the density of jackals in the Lowveld area? The density has been estimated at 1 per square kilometre.
- What is the significance of the high tetracycline rates in the areas of sugar estates? I do not know the answer to this question.

  Dr. Wandeler commented that tetracycline-like compounds have been found in Namibian Mummiver and therefore there are other compounds in Africa which mimic tetracycline fluorescence. Treatment of livestock with antibiotic preparations will not result in significant levels in scavenging jackals, except for the use of pessaries.

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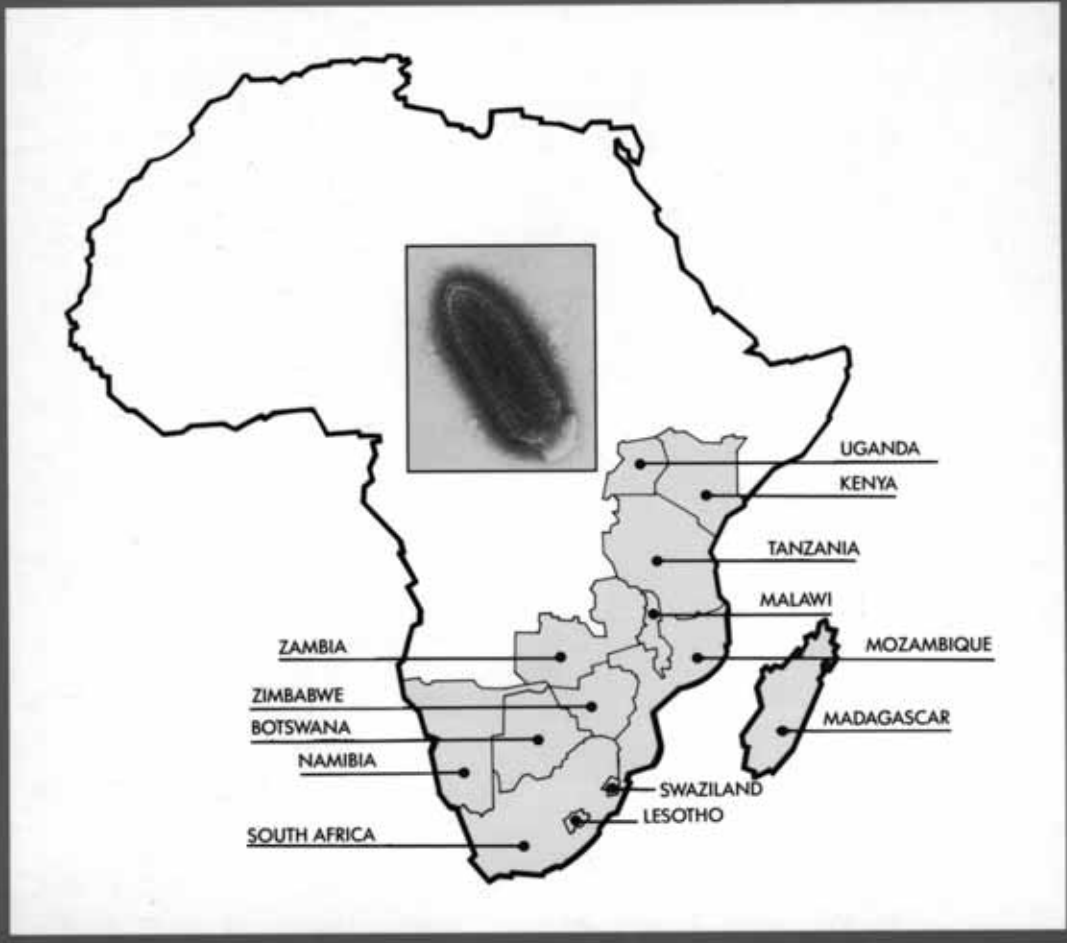

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